STUDIES ON THE RECONSTITUTION AND RECOMBINATION OF MILK CONSTITUENTS AND MILK PRODUCTS

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SUMMARY

The aim of this work was to investigate the possibility of using recombined or reconstituted milk as a substitute for fresh fluid milk.

The studies were carried out by examining the organoleptic characteristics of pasteurised recombined, or pasteurised reconstituted milk and pasteurised fresh milk. The recombined milk was produced from skim milk powder (SMP) and three different milk fat sources (anhydrous milk fat (AMF), unsalted butter and cream). The reconstituted milk was prepared from whole milk powder (WMP). Sensory panelists tasted five samples comprising: 100% pasteurised recombined or reconstituted milk, 100% pasteurised fresh milk and 75:25, 50:50 and 25:75 blends of the pasteurised recombined or reconstituted milk and the pasteurised fresh milk. Results of the organoleptic tests indicated a preference for the pasteurised fresh milk over pasteurised recombined or reconstituted milk. Blending the recombined or reconstituted milk with pasteurised fresh milk after processing seems to increase their preference by the taste panel. The results of the present work support the hypothesis that recombined milk is an acceptable product and can be used as a substitute for pasteurised fresh milk. In fact, the 25% recombined or reconstituted milk samples were nearly accepted by the panel as much as the 100% fresh milk samples. The overall acceptability of pasteurised fresh milk was 5.9, while the mean value of overall acceptability of the samples comprising recombined or reconstituted milk and 75% fresh milk was 5.5 on an eight-point hedonic scale ranging from 'Like extremely' (8), to 'Dislike extremely' (1). The organoleptic results showed no significant differences between the recombined milks prepared with different milk fat sources and the reconstituted whole milk.

The effect of storage on the quality of WMP, SMP and AMF:

The effects of different storage temperatures (-18°C, 4.5°C, 11.5°C and 22.5°C) for a period of 20 months on the chemical, physical and microbiological quality of SMP, WMP and AMF were studied. The effect of storage of these raw materials (SMP and AMF) on the organoleptic characteristics of recombined milk prepared from them were also examined. The results indicated that there were no microbiological changes in the SMP, WMP or the AMF during

storage and defects were purely chemical and physical in nature. Storage of the SMP resulted in an increase in its moisture content, in the level of scorched particles and brought about a decrease in its dispersibility. The moisture content of the WMP increased during storage as did the solubility index. The dispersibility and wettability decreased. These changes were more pronounced as storage time and temperature increased. The peroxide values of AMF increased at storage temperatures of 4.5°C, 11.5°C and 22.5°C. The acid values of the AMF showed very little increase at 11.5°C but a more pronounced increase at 22.5°C. The organoleptic characteristics of the recombined milk produced from the stored ingredients showed a significant deterioration in the odour of the recombined milk. This was more pronounced when using ingredients which has been stored at 22.5°C for 20 months. The detectability of fattiness and rancidity in the recombined milk were also increased significantly with this storage treatment. These changes in odour, fattiness and rancidity would arise from the lipolysis and the auto-oxidation of the AMF. The detectability of chalkiness in the recombined milk was increased as a result of the chemical and physical changes in the SMP during the storage.

The auto-oxidation stability of AMF with and without antioxidants:-

The fat stability test was used to study the accelerated auto-oxidation of AMF. This test was also used to study the effect of various antioxidants in controlling the degree of auto-oxidation in AMF. Finally, a fat stability test was used to select the kind of antioxidants and their levels of addition for a further experiment which involved the storage of AMF at various temperatures for a period of one year. Two types of antioxidants were used. One was based on naturally-occurring compounds as ascorbyl palmitates, DL-α-Tocopherol, Ronoxan A. Ronoxan A is a commercial mixture of ascorbyl palmitate (min. 25%) with DL-α-Tocopherol (min. 5%) and lecithin. The other kind was based on synthetic materials not found in fats and oil such as the commercial products Embanox BHA (Butylated hydroxyanisole), Embanox BHT (Butylated hydroxytoluene), Embanox 2 (18% BHA, 20% BHT and 62% vegetable oil) and Embanox 7 (67% BHA and 33% Dodecyl gallate). The ascorbyl palmitate had a significatn effect in retarding the auto-oxidation of the AMF.

This effect was more pronounced at higher levels of this antioxidant (200 ppm). The DL-α-Tocopherol behaved as a pro-oxidant and this behaviour was more pronounced at higher concentration (200 ppm). Ronoxan A had a very highly significant effect in retarding the auto-oxidation of the AMF particularly at levels of 1000 and 2000 ppm. These two levels of Ronoxan A were chosen to be used in the next project. The maximum permitted level (UK) of 200 ppm of BHA and BHT in AMF showed reasonable antioxidant activities. This effect was more pronounced by having the advantage of the marked synergistic action of BHA and BHT in the formulation of Embanox 2. The maximum permitted level (UK) of Embanox 7 (300 ppm) showed a better antioxidant activity than Embanox 2, BHA and BHT, and was chosen to be used in the next project.

The effect of storage on the quality of AMF with and without antioxidants:-

An experiment was designed to study the effect of storage conditions and antioxidants on the quality of AMF and the organoleptic characteristics of recombined milk made from it and in doing so to investigate the suitability of the International Dairy Federation's recommended maximum temperature for the transport and storage of AMF which is 10°C. The defined storage temperature of 6, 8 and 10°C were selected to investigate chemical and organoleptic changes of the AMF. Parallel investigations were made at 32°C to represent non-refrigerated storage conditions in regions such as the Middle East, which import and utilise AMF for recombined milk manufacture. A storage temperature of 55°C was also included in the study to represent a peak storage temperature condition which would result in accelerated deterioration. The antioxidant Ronoxan A at a level of 1000 ppm was used to study its effect on selected chemical and organoleptic changes in the AMF at all storage temperatures. The antioxidants Ronoxan A at level of 2000 ppm and Embanox 7 at a level of 300 ppm were used only at 32°C and 55°C storage temperatures. Three major analysis were made of the AMF with and without the antioxidants during storage:-

1. Peroxide value
2. Acid value
3. Quantitative measurement of individual free fatty acids (FFAs) in AMF.

The initial pattern of FFA in AMF was dominated by oleic acid ($C_{18:1}$) and palmitic acid (C_{16}). The amounts of short chain FFAs (n-butyric acid, n-hexanoic acid, n-octanoic acid) in the AMF was very low at the beginning of the trial. The level of FFA in the AMF showed no significant change at 6, 8 and 10°C during storage. The presence of the antioxidant Ronoxan A in the AMF increased the level of palmitic acid, the extra amount of palmitic acid being derived from ascorbyl palmitate. The storage temperatures of 32°C and 55°C caused significant increases in the amounts of all FFAs in AMF. These increases were more pronounced at a storage temperature of 55°C. The results showed a slight development in auto-oxidation of untreated AMF stored at 6, 8 and 10°C. The antioxidant (Ronoxan A) at a level of 1000 ppm reduced the initial peroxide value of AMF from 0.104 mEq O_2/kg to zero level. However, the antioxidant-treated AMF also showed a slight development in auto-oxidation at storage temperatures of 6, 8 and 10°C but the peroxide values remained below the original value of AMF (0.104 mEq O_2/kg) after one year. The development of auto-oxidation in AMF was more pronounced at the higher storage temperatures of 32 and 55°C. The presence of the antioxidants in AMF were effective in retarding the auto-oxidation of AMF. However, these antioxidants did not show the ability to stop completely the auto-oxidation process in AMF.

The quality of the AMF had a great influence on the acceptability of the recombined milk. The recombined milk produced from control AMF (i.e. stored at -18°C) showed a 100% of acceptability. This acceptability was reduced slightly in the recombined milk produced from AMF stored at low-storage temperatures (6, 8 and 10°C) either as a result of auto-oxidation development in AMF or due to the presence of antioxidant in AMF. The acceptability of the recombined milk was reduced as a result of lipolysis and fat oxidation in AMF stored at 32°C and 55°C.

Relationship between the level of FFA in AMF and the acceptability of pasteurised recombined milk:-

Adding synthetic fatty acids to AMF was carried out to investigate the relationship between the level of FFA in AMF and the acceptability of recombined milk made from it. This study showed

a significant decrease in the acceptability of the recombined milk as a result of increasing the level of FFAs in AMF.

The lipolysed flavour of recombined milk was detected firstly by a sharp smell, followed by a strong soapy sensation on the tongue. The taste panelists differed in their recognition and response to lipolysed flavour in recombined milk. The gas chromatographic method for analysing the FFAs in AMF showed a high recovery and reliability for estimating the original and the added FFAs in AMF. The quantitative determination of the amounts of individual FFAs could be the key to overcoming the variation of the acid values between different methods.

ABBREVIATIONS

about	ca
American Dry Milk Institute	ADMI
anhydrous milk fat	AMF
atmosphere	atm
British Standard	B.S.
Bureau of Dairy Industry	BDI
butylated hydroxyanisole	BHA
butylated hydroxytoluene	BHT
centigrade	°C
centimetre	cm
cleaning-in-place	CIP
colony-forming units	cfu
degree(s) of freedom	df
Embanox 7 (at level of 300 ppm)	Emb 7
ethylenediaminetetraacetate	EDTA
Fahrenheit	°F
Food and Agriculture Organisation (United Nation)	FAO
free fatty acids	FFA
Gas Chromatography	GLC
gram	g
hour	h
International Dairy Federation	IDF
kilogram	kg
laboratory	Lab
litre	l
mean square = $\frac{ss}{df}$	M.S
Mega. Newton. metre^{-2}	M.N.m^{-2}

micro (10^{-6} x)	μ(prefix) e.g. microgram = μg
Milk fat globule membrane	MFGM
milliequivalent	mEq
milligram	mg
millilitre	ml
minimum	min.
minute	min
mol/litre	M
normality	N
number	no.
part per million	ppm
peroxide value	PV
poly acrylamide gel electrophoresis	PAG
pound	lb
Pro-milk difference	PMD
revolution per minute	r.p.m.
Ronoxan A at level 1000 ppm	1 RA
Ronoxan A at level 2000 ppm	2 RA
second	s
skim milk powder	SMP
standard error of difference of means	SED
sum of squares	ss
temperature	Temp
treatment	Treat.
Ultra-high-temperature treatment	UHT
undenaturated milk-serum protein	UMSP
undenaturated milk-serum protein nitrogen	UMSPN

undenaturated whey protein nitrogen	WPN
variance ratio = M.S (effect)/M.S (residual)	F
weight	wt
Whole milk powder	WMP
World Health Organisation ((United Nations)	WHO

1. The Progress of Recombined Milk

Production from season to season and from area to area led to the introduction of drying technology to preserve surplus milk for use when and where it was in short supply. Within the last thirty years there has been a steady increase in the amount of dried milk sold to areas of the world where the demand for liquid milk exceeds the available supply. Dried milk is also particularly useful in countries where the warm climate and difficulties in transportation militate against supplying a fresh product to the consumer. Initially, whole milk powder was reconstituted directly but problems were encountered with the storage of the powder leading to a liquid milk with a stale flavour. The early whole milk powders also gave a reconstituted milk with a high level of sediment and foaming was encountered during reconstitution. Subsequent developments led to the use of recombination processes in which skim milk powder was reconstituted and standardised with cream, unsalted butter or anhydrous milk fat. Barnes (1971) reported that in 1968 the Food and Agricultural Organization (F A O) listed 19 countries in Asia, Africa, South America and the West Indies in which recombination plants were in operation on a commercial scale. It was estimated that upwards of 20,000 tons of butter oil and 60,000 tons of spray-dried skim milk powder were being used annually in the production of recombined dairy products. In 1969/70 the Australian Dairy Produce Board supplied 4,000 tons of butter oil and 19,000 tons of skim milk powder to the five "joint venture" recombined milk plants which they had helped to establish in South-East Asian countries.

In 1975 the New Zealand Dairy Board's trade with Iraq was running at around $10 million annually (Anon, 1975) for dairy products used for the manufacture of processed cheese and recombined milk and milk products.

Thuraisingham (1982) reported that the Asian countries imported annually over US $250 million worth of milk and dairy products. These imports were mainly in the form of full cream milk powder, skimmed milk powder and butter oil which were subsequently recombined or reconstituted locally.

Kieseker (1975) reported that the Seventh edition (1973) FAO/WHO of

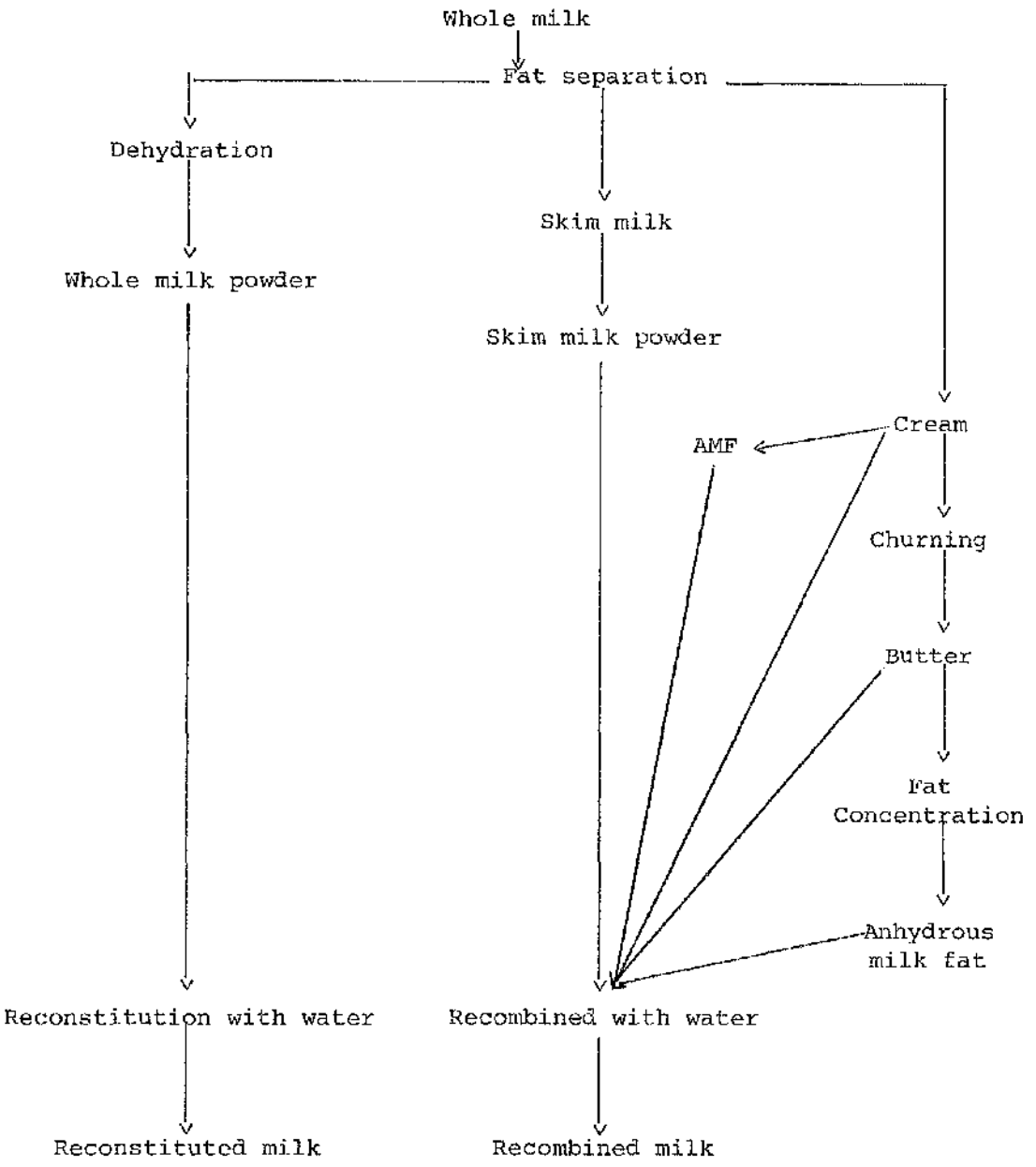


Figure 1: Reconstituted and Recombined milk

the Code of Principles concerning Milk and Milk Products clarifies terminology for the processes of reconstitution and recombining as follows:

> Reconstituted product: is the milk product resulting from the addition of water to the dried or condensed form of product in the amount necessary to re-establish the specified water-solids ratio.
>
> Recombined product: is the milk product resulting from the combining of milk fat and milk solids-not-fat in one or more of the various forms with or without water.

This combination must be made so as to re-establish the product's specified fat to solids-not-fat ratio and solids to water ratio.

In reviewing the early work of the reconstitution of dry milk solids, Gibson (1952) reported at that time some countries relied almost solely on dry milk solids as a source of milk, and in some of the larger centres in Western Canada, as much as 40 per cent of the daily requirements of milk for fluid sales was made up from reconstituted dry milk. Terms of solubility, dispersibility and wettability and the recommended laboratory methods were all discussed widely in his review. Flavour, appearance and lack of experience for reconstituted whole milk powder affected the quality of the product as a beverage for the armed forces, which led to a study of the possibility of using dry butter fat and low heat non-fat dry milk solids for more satisfactory reconstituted milk. The results of the study has led to the establishing of seven reconstitution plants in Japan, Okinawa and Guam, by the U.S. Army Quartermaster Corps. Two methods of reconstitution of dry skim-milk powder, butter fat and water were discussed in relation to giving a good powder hydration and proper fat dissolving to achieve a body and flavour of finished product comparable to fresh fluid milk.

In two cities in Canada, it was estimated that on a yearly basis for 1951, 10 to 20 per cent of the total fluid milk sold was reconstituted, mostly from spray dried skim-milk and fresh sweet cream (White, 1953). According to the author's experience it would be difficult to distinguish between the reconstituted and fresh milk supplied to a number of city markets. The use of the highest quality of ingredients for reconstituted milk was a major factor for a satisfactory product for fluid milk purposes. Spray skim milk

powder was successfully reconstituted and the fat content standardised with sweet cream. Unsalted butter or butteroil could be used with satisfactory results. The United States military specification in 1950 for anhydrous milk fat was presented in the article. Oxidative deterioration was the main restrictive factor in using whole milk powder for reconstitution commercially by dairy plants in Canada.

Superiority in flavour of recombined milk compared with reconstituted milk from whole milk powder was due to the severe pre-heating used in producing whole milk powder to prevent the oxidation of fat which resulted in a "heated flavour" product from which the recombined milk is free (Anon, 1956). According to the author a commercial company from the USA installed a recombining plant in Yokohama producing 16,000 gallons of recombined milk and commercial plants were in operation or under installation in Hong Kong, Manila and Bangkok. At the same time he discussed the possibility of breaking down the high fat content of water buffalo milk in India by the reconstituted milk to produce a so-called toned milk. Specification of all reconstituted milk ingredients and the equipment of reconstitution were discussed in the same article.

The means of processing the recombined milk in poor countries from raw material sent by food aid programmes was the main aim in producing a simple blender built to be driven with either a universal electric motor or a small 2-cycle gasoline engine. The motor (or engine) turns the homogenizing head at approximately 10,000 r.p.m. An ordinary milk can was used as the container. Recombined milk from skim milk powder and butter has been produced successfully by the above machine (Holland, 1963).

The advantage of better flavour and lower cost of recombined milk using skim milk powder and butter oil comparing that with milk reconstituted from whole milk powder was discussed, along with the work of some other workers on means of improving the flavour of recombined milk, by Loftus Hills (1964). He also mentioned the attempt to supply the Asian population with plain and flavoured recombined sterilised milk. Two New Zealand sponsored plants were established in Hong Kong and Singapore around 1964. A similar Australian sponsored plant was also installed in Singapore shortly

afterwards. Sweetened condensed milk and evaporated milk produced from recombined milk with special attention to the raw material was also discussed in the same article.

A unique sea-going dairy plant was developed by the USA for an aid programme to underdeveloped countries of the world. The plant was designed to occupy a space no larger than a family garage. Distilled sea water from the ship's distilling plant was used for recombination of milk in a production of 1000 gallons daily. The milk was packaged in one-third quart cartons. Supplies for the nine-month cruise programme included 150,000 lbs (68027 kg) of non-fat milk and 60,000 lbs (27211 kg) of milk fat (Anon, 1964).

Local milk production is not considered in Kuwait to be expedient because of the hot and dry climate; shade temperatures reaching some 50°C and the difficulties of providing feed for the animals. In 1964 a joint enterprise with shares held by Kuwaiti businessmen and Federation of Danish Dairy Associations started the use of Danish skim-milk powder, butter fat and purified sea water to provide recombined liquid milk, plain, flavoured yoghurt and ice cream. The capacity of the plant was in the region of 8000 litres of milk an hour. The processing was highly mechanised and automated (Anon, 1965).

Inhabitants of Gibraltar had been accustomed to either tinned milk or small quantities of fresh milk which came from Spain. In 1953 Gibraltar received its own recombination plant from the USA (Shaw, 1965). The plant produced between 700-1000 gallons of recombined pasteurised and homogenised milk daily. The plant was a very compact two-room lay out. Australian dried milk powder with low foaming characteristics during the mixing and filling of cartons was supplied in 56 lb bags, in sufficient amount for three months production.

A large project established in a south-east Asian country was reported by Twomey (1966). The total capital involved was around £1,000,000 with the plant producing one million cases of recombined sweetened condensed milk a year, using nearly 3,500 tons of skim milk powder and 1,500 tons of milk fat. The product was made from a mixture of skim milk powder, anhydrous milk fat and unsalted butter,

sugar and water to give a composition of 30% milk solids, 44% sugar and 26% water. The quality of the product met with ready approval.

Sanderson (1968) investigated the following aspects of the production of recombined town milk in New Zealand with a view to determining:

1. The most suitable ingredients for the manufacture of recombined milk.

2. What levels of recombined milk can be added to fresh milk without affecting (a) the flavour of the milk, (b) the general keeping quality, and (c) the domestic usage.

3. The possibility of producing a recombined milk which can be added to fresh milk without reducing the cream-line of the milk as compared with fresh milk.

Producing a cream-line recombined milk to be a satisfacotry source of town milk was achieved by homogenising the fat as cream then added to the reconstituted skim milk, and an appreciable cream-line can be obtained on fresh milk containing 40% of above recombined milk. On the other hand, flavour or domestic usage of the milk was not affected by adding up to 60% of the recombined milk.

At the same time Wallace (1968) found that if 40% of autumn and winter supply of fresh milk in New Zealand was substituted by recombined milk a saving of 712,000 New Zealand dollars could be achieved plus the revenue generated from the sale of surplus milk to manufacturers could reduce the cost of "town" milk by 0.57 cents/ pint.

The level of exported dairy products from New Zealand and Australia to the Far East countries in 1968-1969 was reported by Patrick (1970) as follows:

Butter	7,000 tons
Milk fat	14,000 tons
Milk powder: skim	120,000 tons
other types	37,000 tons

Which was mostly used as a raw material in their local processing and distribution industries for production of recombined milk and its products.

Lamar (1970) mentioned that there are some reconstituting plants in Saudi Arabia supplying milk to the people who live in the cities.

Recombined milk plants in Indonesia, Cambodia and in other developing countries have proved the feasibility of replacing imported milk and milk products utilising milk powder and butter oil imported from dairying countries. This kind of substitution will introduce a new industry to the developing countries with the opportunity of more jobs as well as saving in foreign exchange, Barnes (1970).

Recombined milk is a very important key in solving the problem of malnutrition in developing countries by producing additional milk from imported raw material and also encouraging local milk production by providing essential processing and distribution facilities, Barnes (1971).

The production of recombined sweetened condensed milk from skim powder, sugar, butter oil and water in South East Asia was reported by Sayce & Park (1971). Raw material specification, plant design and the manufacturing process were reported in detail. Recombined milk, butter and cheese manufactured from skim milk powder and butter fat were mentioned briefly.

An increase in the general availability of dairy products in the Philippines due to the establishment of recombined milk plants was mentioned by Kieseker (1975). A survey of South East Asia in 1972 has shown there were some thirty plants producing recombined, filled or concentrated milk with an estimated capacity of 680,000 tonnes as concentrated milk per year. Specification of raw material and description of the manufacture of sterilised, sweetened condensed, and evaporated recombined milk were explained in the same article.

Recombined milk plants in Asian countries were the main influence in increasing the intake of milk by the people who are traditionally not milk consumers (Thuraisingham, 1982).

That recombined milk plants which depend on imported raw materials act as a very positive catalyst in the development of indigenous milk production in countries having a small milk production was reported by Bosworth (1982) with special reference to the Indonesian trail.

Cherrey (1982) commented that recombined milk is an important product for both "dairy" countries and "non-dairy" countries. Soft curd type cheeses in France have used milk powder as a substitute in winter when fresh milk is in short supply. At the same time establishing recombined plants in many non-dairy countries resulting in:

- encouraging the consumption of milk and milk products as a habit,
- improving the health of the population,
- facilitating the increase in the number of dairy farms.

2. Processing and Engineering

In small scale processing, involving mixing whole milk powder with water as is the case in camps or schools, portable hand or electrically operated mixers are suitable for preparing batches in milk cans. In a dairy plant a reasonably satisfactory job may be done using the standard dairy equipment such as a double jacketed vat which has a good agitator to dissolve the powder in water.

The American Dry Milk Institute (1947) recommended the use of a labour saving device based on having a standard milk pump connected to a storage vat by a suitable pipe line and a hopper fitted to the pipe line by which the dry milk is added. The vat is filled with the required volume of water and the dry milk is added through the hopper while the water is circulated by the pump. Later, the same system was described by Gibson (1952).

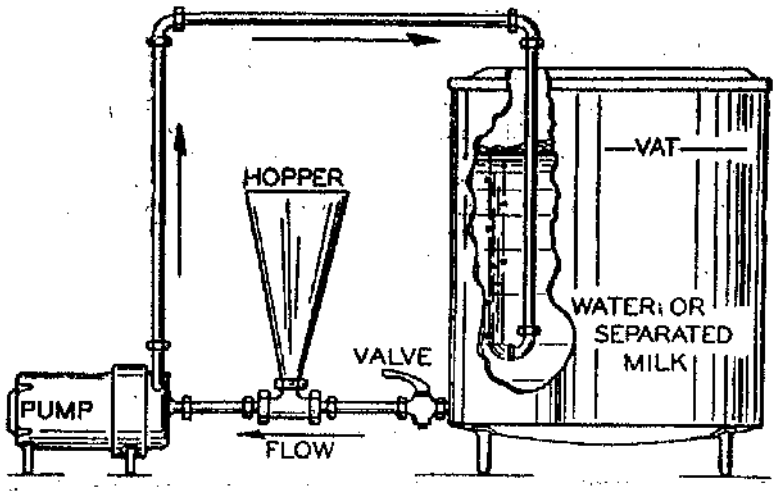


Figure 2: The suggested equipment for mixing dried skim-milk with water, Gibson (1952)

The "Milk-Made" machine (White,1953) for reconstitution of dry milks, is a modification of the standard milk pump fitted with a fine mesh screen on the discharge side and connected to a dry milk hopper and vibrator feeder and water supply. The feeder is electrically controlled to regulate the amount of powder and the volume of water is controlled by a manually operated valve. In the same article White (1953) mentioned the Homoloid equipment for reconstitution which is in effect a high speed hammer mill forcing the dry milk and water through a 300 mesh screen.

Lee, Binnington & Crossland (1953) reported that an agitator consisting of a shaft upon which is mounted a disc with tubes rigidly attached tangentially to its periphery could be used for reconstituting dried milk.

Mann (1964) reviewed the work carried out at the Dairy Research Institute at Hillerod in Denmark by Poulstch & Mondrof (1964) on a milk recombining process developed at the Institute. The process is started by reconstituting skim milk powder in a circulated stream of water at 8°C. The resultant milk is held for 4 hours to allow the hydration of the powder, and the fat is metered continuously into the reconstituted skim milk at 65°C by means of a flow regulated membrane-type pump. Using such a pump it has been found possible to control the milk fat content of the recombined milk to within ±0.05%. The recombined milk then passes via a fat distributing pump (where some breaking up of the fat globules takes place) to a normal homogenizer where it is homogenised at 60 to 65°C and a pressure of 150-200 atm. It is finally pasteurised in a plate heat exchanger and cooled to about 5°C.

Factors affecting the dispersion of skim milk powder have been studied by Koslov (1965). At 20°C product temperature and in mixtures of water with 9% dried milk, the degree of the dispersion of particles at a constant speed of the mixer (700 r.p.m.) increased with the treatment time. It also increased with increasing speed of the stirrer (up to 10,700 r.p.m.), and increasing concentration of the dried milk.

Lölliger & Schmied (1974) explained the method and the apparatus for continuously dissolving a powdered product in a liquid. The dried

milk was continuously dissolved in a liquid by spraying it with the finely divided liquid and allowing the resulting solution to flow in a thin layer down the inner wall of a chamber.

Experimental scale equipment suitable for producing 1500 litres reconstituted milk/hour (Ujhelyl & Szabo, 1974) contained a rotary-comb unit made up of a horizontal tube with four lines of teeth on the inner surface and a rotary axle with similar teeth to dissolve dried milk and water mixture. Insoluble particles are removed by applying a clarifier in the line.

Another continuous reconstitution process was used in two liquid milk dairies in Northern Norway to produce modified milk (fresh milk combined with 33% recombined milk) to avoid long-distance transport of fresh milk (Hadland & Solberg, 1974). Butter, skim milk powder and water was used in a continuous recombining process which involves the addition of unmelted butter and dried skim milk during circulation of about half the water required at 35°C followed by the addition of the remainaing water and mixing with fresh milk. The advantage of this process is the early introduction of fat and skim milk in a concentrated mixture providing rapid and efficient integration of the fat and solids-not-fat components and the water phase. The short time process contributed to the good bacteriological condition of the product.

A batch process which has lately been described by Newstead et al., (1979) is similar to the process described by Gibson (1952). To achieve a continuous operation they recommended the use of two recombining vats used in rotation. The water was heated up to 40-50°C before adding the powder and only 15 minutes was recommended for hydration. The temperature of the mixture was raised to 55 to 65°C to dissolve the fat. Clarification or filtration before or after the addition of the fat was found to be necessary. Two stage homogenisation was used, the second stage is intended to break up clusters of fat globules formed during the high pressure first stage. Pasteurisation at 72°C for 15 seconds was recommended for pasteurised milk production.

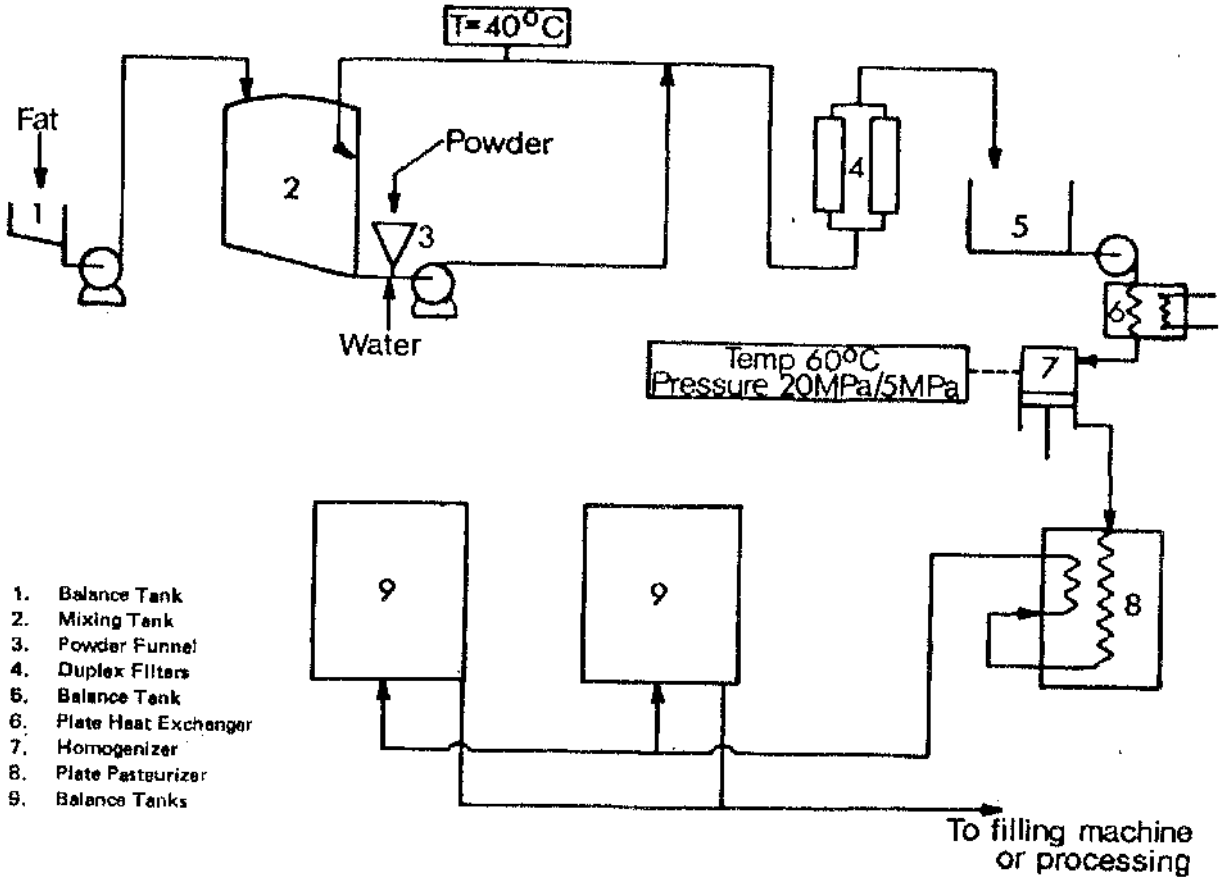


Figure 3: Recombined pasteurised milk, Newstead et al. (1979)

The Primodan continuous recombiner with a capacity of 5000 l/h has been described (Anon, 1981). In using a completely new powder-dosing system the milk powder is dosed under a vacuum down into the water in a continuous process with the advantage of reducing the amount of air sucked in the mixture. Melted fat is dosed into the recombined milk on a continuously operated basis. The pasteuriser and homogeniser are connected into the line with the CIP-cleaning system to make it a complete continuous compact system.

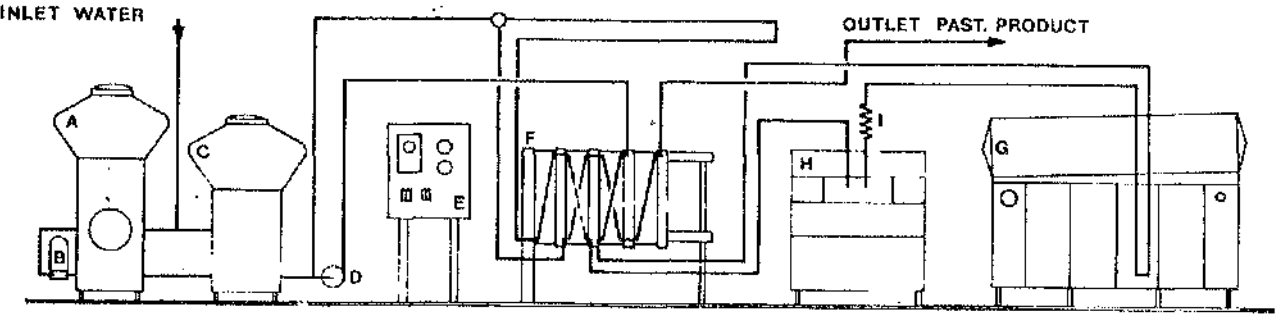


Figure 4: Flow diagram to a Primodan recombiner, Anon. (1981)

Two mixing stages as a modified system has been recommended for recombined evaporated milk with high total solids of 31% (Fotheringham & Choat, 1979). First water, fat and approximately 20% of the milk powder are mixed in the usual way, homogenised and then returned to be mixed with the rest of the powder. Another way of handling high total solid mixes is using Cowles type dissolver which also allows batches of powder to be reconstituted rapidly (Gilles & Lawrence, 1981).

3. Reconstituted Whole Milk Powder

Generally speaking the difficulties encountered with reconstituting whole milk powder in ordinary and cold water may be listed as follows:-

(1) rate of dispersion,

(2) loss of solubility with powder age,

(3) formation of foam or scum at the surface of the milk,

(4) adhesion of undispersed powder particles to the container wall,

(5) formation of a distinct layer at the top and bottom of the container after several hours in refrigerator,

(6) stale flavour of the reconstituted powder.

All these defects in appearance and flavour have materially affected the acceptability of reconstituted whole milk powder as a beverage. Many investigators have dealt with the problems of improving the keeping quality of whole milk powder and its reconstitutability.

The problems of undispersed particles clinging to the container wall, surface scum and distinct layer formation at the top and bottom of the container after several hours in a refrigerator were studied by Wilster, Schreiter & Tracy (1946). They found the film deposit and scum were reduced

(1) by manufacturing small particle-size powders,

(2) by spraying homogenised milk concentrate in which the fat globules were of small and uniform size,

(3) it was also important to ensure that the powder was not overheated in the drying chamber.

The completeness of the dispersion of the dry milk particles in the water was favourably influenced by the following procedure for reconstitution process:-

(1) reconstituting in water at 38.5°C (110°F) with a high speed agitation,

(2) heating the reconstituted milk to 65.5°C (150°F),

(3) homogenising the reconstituted milk at 23°C (75°F) and a pressure of 6.89 to 13.78 $M.N.m^{-2}$.

(4) clarifying the reconstituted milk,

(5) adding a small amount of citric acid (protein stabiliser) to the reconstitution water.

Ashworth & Bendixen (1947) found that complete dispersibility of whole milk powder could be achieved to a maximum concentration of 12.5 total solids, using an agitator with approximately three back and forth movements per second over a total amplitude of one foot during a period of 7 seconds. Unlike Wilster et al. (1946) Ashworth et al. (1947) implied that 20°C was the optimum temperature of the reconstitution water, although reference is made to an unspecified maximum temperature above which casein denaturation reduces the rate of dispersion of the milk powder. Dispersibility of the powder was improved by increasing the level of the total solids in the milk concentrate, from 20% to 40%. Preheating the milk in the range 71.5°C to 82°C for periods up to 10 minutes had no significant effect on powder dispersibility.

Litman et al. (1956) reported the evidence for the development of a fat-protein complex in whole milk powder which contributes to loss in solubility. In reconstituted milk powder, the formation of "scum", as insoluble material clinging to the walls of the container, occurred only with powders containing fat, and increased during storage at room temperature and above. The "scum" contained 48% fat, which had higher melting point (and lower iodine value) than the original fat, and 34% protein which was mostly casein plus denatured whey protein, the extent of which related to the preheat treatment of the original milk.

Storage of whole milk powder at room temperature results in deterioration in its solubility within a few days (Litman & Ashworth, 1957). Extensive "scum" development with decrease in free fat content was found on milk from powder stored at 29.4°C (85°F). There was little "scum" found when powders had been stored at 7.2°C (45°F). The free fat content of powder rapidly decreased at 29.4°C storage. Scum development was related to the initial free fat

content of the powder. The "scum" developed extensively with powder containing more than 26% fat which was stored at 29.4°C but little "scum" was found when the same powder was stored at 7.2°C. These authors related the reduction in free fat to the "scum" formation in which free fat combined with other milk components, especially protein to form insoluble fat-protein material. The chemical analysis showed that the "scum" contained a larger percentage of fat and protein together with a smaller percentage of ash and carbohydrate than the soluble fraction. The fat associated with the "scum" had a higher melting point and was more saturated than the remaining free fat. "Scum" protein consisted of 32.95% of casein plus denatured whey protein. The authors believed that calcium involved in the complex.

The insoluble material formed in reconstituted milk prepared from whole milk powder was examined by Julien & Baker (1957). Both foam fraction which did not pass through 100-mesh seive and sediment fraction which collected by centrifugation of the passing milk through the seive were studied. The foam fraction contained 98% fat and the sediment fraction 42% fat and 48% protein.

Baker, Bertok & Samuels (1959) prepared four kinds of agglomerated whole milk powder containing three different butter oil fractions (melting points of 19-21°C, 22-24°C, 28-30°C) and unfractionated butter oil. Their fat content was mostly the same around 24-25.5%. Wettability and dispersibility of these four powders plus commercial skim milk powder (instant and non-instant) and commercial whole milk powder (agglomerate and ordinary) were measured at different temperatures of water and powder. Samples of WMP were held at various temperatures (16-36°C) for 48 hours. Wettability and dispersibility in water at the same temperatures as the powders were then measured. Their results showed that the whole milk powder which contained the butter fat fraction melting at 19-21°C had a very good quality and the wettability and dispersibility were nearly the same as the commercial instant skim milk powder. As the melting point of the fat component increased, the dispersibility and wettability of the corresponding milk powders decreased. Finally their results showed that the temperature of the powder and of the water affected greatly the dispersibility of the powders containing

fat, but had little effect on the skim milk powder.

Large clusters and clumps showed under the microscope from the "scum" of reconstituted spray dried whole milk powder (King, 1960). The clusters consisted of a conglomeration of casein micelles and small fat globules. On the other hand the clumps consisted of intermingled free fat and protein with air bubbles and occasional fat globules. When fluorescence microscopy is used the free fat exhibited only a pale green fluorescence and it appeared semi-transparent, whereas the fat in globular form was intense opaque yellowish green. The protein fluoresced intensely red-orange. The low fluorescence intensity of the free fat may be ascribed to its rather high content of crystalline fat fraction which does not take up the fluorochrome. This gives support to the finding of Litman et al. (1957) that the scum-fat had lower iodine value and higher melting point than the remaining fat. Finally, King (1960) found that the number of undissolved powder particles increased appreciably with the age of the powder.

Samuels, Coffin, Julien & Baker (1960) repeated the examination of (a) scum from the foam fraction, and (b) scum from the sediment fraction. Their results indicated that the fat content of both fractions was much higher than the fat content of the original powder. The dried foam fraction contained about 50% more fat than the sediment fraction. The fat of the sediment contained a higher proportion of unsaturated fatty acids than the fat of the foam. Samuels et al. (1960) reported on electrophoretic analyses of the foam and sediment which indicated that the major protein constituent of the sediment was beta-lactoglobulin, and that of the foam was casein.

Reviewing the factors affecting the dispersibility of dried milk, King (1966) referred to the following as being important:

1. The chemical composition of dried milk as milk fat, milk protein, calcium, lactose and moisture.
2. Effect of manufacturing method and storage conditions.
3. Effect of reconstitution process.

The first two factors are closely connected with the physical

structure of the powder, which in turn is of importance in reconstitution.

The laboratory work by Pisecky and Westergaard (1972) had resulted in an industrial process and equipment for the production of cold water instant whole milk powder by overcoming the problem of the free fat layer which covered the whole milk powder particles and made them water repellent. Interfacial tension was used to facilitate the wetting of the particles. Lecithin has been chosen as a surface acting agent (to the surface free fat); it has an advantage in being a natural product of milk and also in being both lipophilic and hydrophilic in action. Wetting takes place immediately after suitable interfacial tension has been reached. Pisecky et al. (1972) showed that to obtain a fully reconstituted milk in a reasonably short time and with minimum effort, capillary penetration of water into the powder must be avoided. Capillary attraction depends on the structure of the powder, i.e. the size of the agglomerates, the amount of interstitial air and the specific surface area of the powder. The penetration of water into the powder is easily avoided when the powder consists of large agglomerates. Such a powder having a coating containing lecithin and a sufficiently high particle density will be readily wetted and dispersed when dropped onto an inert surface of water.

Baldwin & Sanderson (1972) briefly outlined the important factors in the production of an instant whole milk powder with the same properties as instant skim milk powder. Particle size ranged from 100-300 microns giving good dispersibility of instant whole milk powder, and for improving the wetting properties they recommended the use of lecithin at a level of 0.2 per cent of whole milk powder in the presence anhydrous milk fat as a non-aqueous solvent which appears to be the most suitable carrier. The mixture will play a role of surfactant coated on the surface of the powder after drying. It results in a powder with a dispersibility of 85.90% - equivalent to instant skim milk powder - whereas normal whole milk powder has a dispersibility of less than 20%.

Wetting properties of the powder was the most important key in improving the reconstitution properties of the powder in cold water

(Baldwin & Sanderson, 1973). However, only 35% of the fat in commercial milk fat has a melting point of less than 18°C which means that the softening point of this milk fat is higher than the temperature of the water to be used for reconstitution. Under this situation a surface active agent was required to improve wettability. Lecithin with enhanced hydrophilic properties was found to be a particularly effective and stable surfactant for whole milk powder. Since the initial stage of reconstitution involves surface wetting, the agent must be concentrated on the surface of the powder. Finally they conclude that to be effective the lecithin/fat mixture must coat all particle surfaces and the efficiency of coating is greatly affected by the method of application. A further important factor in obtaining an instant powder is the size distribution of the powder which is coated.

Tamsma & Kontson (1974) showed that a foam process spray-dried powder of whole milk type with good sinkability (100% sinkability after holding at 27°C) was prepared by combining three influential factors in the preparation procedure: (1) use of liquid milk fat which is prepared by holding liquid milk fat at 20°C during 3 to 5 days and removing the solid fraction with a basket centrifuge. This liquid milk fat was recombined with skim milk, (2) use of high pressure homogenisation at 367 atm, (3) foaming with CO_2 to bulk density.

During the concentrating and atomising process in the manufacture of whole milk powder, the milk fat globules are more or less split up into small ones. Consequently the fat globules in whole milk powder are covered at least partly by casein micelles, which have a detrimental effect on the solubility of the product (Mol, 1975). According to his work, milk powders standardised by 'casein-free creams' were superior to those standardised by 'casein creams' and the amount of dried sediment per 100 grams of each milk powder were 0.12 grams and 4.6 grams respectively. Starch gel electropheric studies of the sediment from reconstituted milk provide evidence that the main protein component of the sediment was casein. This observation is in contradiction to results obtained by Samuel, et al. (1960) who identified β-lactoglobulin as the major protein constituent in the sediment.

For the reconstitution of whole milk powder in warm water, such as for baby feeding, lecithin is not an essential additive (Lascelles & Baldwin, 1976). The best dispersibility was achieved for unlecithinated agglomerated whole milk powder when the fine particles (<90 mm in diameter) comprised less than 20% by weight of the product. At reconstitution temperatures below 45°C, irrespective of the percentage of fines, the dispersibility of the powder was poor because the powder wettability was low. This is believed to be due to the temperature of the water being too low to give rapid melting of the fat in the powder. Above 70°C, reconstitution of all powders was difficult because of the formation of a paste on the outside of the particle aggregates, giving rise to lumps. This viscous paste protects particles within the lumps from further penetration by water. A reconstitution temperature between 55°C and 65°C appears optimal.

From experimental work on flavour deterioration of instant whole milk powder stored at 22°C, Baldwin & Humphries (1976) reported that in general there were no significant differences detected up to 45 weeks storage. After storage for 61 weeks a significant difference between the powders* was detected by the taste panel but they were unable to agree on the flavour superiority of either one of the products.

Sanderson (1978) reviewed the manufacture of the instant whole milk powder and its reconstitution properties in addition to the effect of different storage conditions on its reconstitution properties and its flavour. The formation of large particles or agglomerates is the main factor in the manufacture of instant whole milk powder. But due to the hydrophobic nature of the milk fat of the powder a further treatment of coating the whole milk powder with a lecithin/ fat mixture makes it readily reconstituted in cold water.

The reconstituting properties for good instant powder mainly are wettability, penetrability, sinkability, dispersibility and solubility. Wettability of instant whole milk powder immediately after manufacture is normally very high due to the fact that the fat is still in the liquid state so the powder should be held for at least 48 to 72 h at ambient temperature before testing for its reconstitution properties. Sanderson (1978) reported that the

*conventional and instant whole milk powder

storage temperature is a very important factor in the deterioration of the wettability and dispersibility of whole milk powder. He mentioned that no change could be detected in the wettability and dispersibility of lecithin-treated whole milk powder after twelve months storage at 13°C whereas a significant deterioration in the wettability occurred at 30°C and was even greater when the lecithin was applied in the form of an emulsion with water rather than with milk fat. According to Sanderson (1978) this deterioration is not fully understood, although he explained either to the migration of the surface layer of lecithin into the powder particles or to the movement of free fat from within the particle to the surface. So the relative porosity of the individual powder particles in the agglomerate may therefore play an important role in the maintenance of good wettability. The other important property affected during storage is the stability of the particle agglomerates. Agglomerate breakdown not only results in the formation of fine particles which decreases the dispersibility of the powder but also adversely affects the wettability due to the exposure of powder surface which are not coated with the wetting agent.

The flavour stability of instant whole milk powder is mostly the same as normal whole milk powder. Cereal type off-flavour may occur if the lecithin used is not free of the soya oil from which it is normally extracted. The general flavour stability of instant whole milk powder is affected primarily by the availability of oxygen and the moisture and temperature conditions prevailing during storage.

Wetting properties of instant whole milk powder is only a starting step of a rather complicated reconstitution process (Pisecky, 1980). According to his views the reconstitution process consists of a number of phenomena requiring corresponding powder properties, as follows: wetting, penetrating, dispersing, sinking and dissolving the powder in the cold water. Bearing in mind there is no sharp line between these individual reconstitution steps, it is difficult to determine the effect of the individual properties independently of the others. Wettability and dispersibility tests proposed by the IDF (IDF, 1979) with other tests such as the solubility index by the ADMI (1971b) and a suggested modified solubility test for the determination of the rate of hydration have all been discussed by

Pisecky (1980) in relation to their contribution to the reconstruction properties of instant whole milk powder. Agglomerated powder provided with a wettable surface is the general requirement for achieving instant properties. The straight-through method for the production of agglomerated powder provided better properties for the final product than the rewet process (Pisecky, 1980). On the other hand lecithin dissolved in butter oil is the most acceptable surface active agent since it is a natural component of milk and it has superiority in functional performance.

4. Heat Stability

During many courses of manufacture, milk and milk products are subjected to high temperature heat treatment (as in the case of sterilisation process) which may result in an increased risk of milk coagulation when used. So the heat stability characteristic of milk powder is an important property in its use for the production of recombined sterilised milk or recombined condensed and evaporated milk.

Sargent, Biggs & Irvine (1959) studied the relation between the hard water which was used for the reconstitution and the heat stability of the reconstituted milk and according to their results the heat stability of reconstituted skim milk was adversely affected by high calcium and magnesium levels in the reconstitution water. Heat stability is affected by preheat treatment and pH adjustment prior to evaporation and drying of the skim milk powder during two dairying seasons (Newstead, Sanderson & Baucke, 1975). Better heat stability characteristics resulted from a preheat treatment of 120°C for 120 seconds than 120°C for 30 seconds. Adjustment of the pH was a great advantage in improving the heat stability specially during the earlier part of the season September to December when the optimum pH was usually between 0.06 and 0.12 units lower than the natural pH.

Seasonal variation in milk components is important in the processing characteristics but the combined effect of pH adjustment and manipulation of preheating conditions is a successful method of meeting the heat stability specification for producing powder suitable for use for recombined evaporated milk (Griffin, Hickey, Bailey & Feagan, 1976).

Heat stability characteristics of the powder play an important part in determining the stability of the recombined milk to ultra-high-temperature (UHT) treatment (100 to 150°C for 3 s) (Zadow & Hardham, 1978). Their results show that recombined UHT milk prepared from medium or high heat powder is more resistant to sediment formation than recombined UHT milk prepared from low heat powder. At the same time a slight reduction in the pH of recombined milk due to use of powder of high acidity or to the development of acidity in the

recombined product during storage may result in the formation of sediment in recombined UHT milk.

The effect on heat stability of recombined evaporated milk during one dairying season of solids-not-fat concentration, fat concentration and homogenisation pressure were highly significant (Newstead, Hughes & Baldwin, 1978). The increase of solids-not-fat concentration from 160 g/kg to220 g/kg reduced the average maximum heat stability from 57 to 21 minutes. Increasing fat concentration from 80 g/kg to 100 g/kg reduced the average heat stability from 49 to 32 minutes. An increase of first stage homogenisation pressure also reduced the heat stability of the product.

The manufacture of heat-stable milk powder is generally confined to mid-lactation milk, during which period the required heat-stable specification is more easily attained (Kelly, 1981). Seasonal variation of urea level in milk due to animal feeding could be a factor affecting the heat stability. Preheating of the milk to 120°C for 2 minutes together with the addition of urea brought the heat stability of powders produced from early lactation milk into line with those produced during mid-season without urea addition. Lactose, being a non-reducing sugar, does not increase heat stability due to lack of potential aldehyde or ketone function. Kelly (1981) found that when lactose is hydrolysed in milk using the enzyme β-galactosidase, its heat stability is increased due to protein-carbohydrate interactions.

Of all the variables that influence the heat stability of milk powders, only variations in the preheating of the raw milk is used as a routine in practice to establish the characteristics required (Kieseker, 1982a). He also mentioned that mineral level, especially calcium, pH changes due to seasonal variation at preheating, protein level, whey protein, lactose, and urea level in milk are all involved in determining the heat stability of milk.

5. Heat Treatment Classification

Heat treatment classification is not a grading requirement, but is of practical importance in indicating the suitability of spray process non fat dry milk for various uses. The whey protein nitrogen test which depends on measuring the turbidity of denatured whey proteins form the basis for the following heat classification according to the American Dry Milk Institute (ADMI) (1971a).

Heat Class	Undenatured whey protein nitrogen/g powder (WPN/g)
High heat	Not more than 1.5 mg/g
Low heat	Not less than 6.0 mg/g
Medium heat	1.51 - 5.99 mg/g

Baking quality of non fat dry milk solids was indicated by applying the method of estimating the whey protein (Harland & Ashworth, 1947). In a series of samples the upper limit for good baking quality was taken as 1.4 mg of whey protein nitrogen per gram of powder.

The method of Harland et al. (1947) has been studied and standardised by Kuramoto, Jenness, Coulter & Choi, 1959. A calibration curve was prepared from turbidemetric measurement on a mixture of filtrates from two lots of non fat dry milk, one containing a low, and the other a high, level of undenatured whey protein. The method was considered to be sufficiently accurate and reproducible for use for determining dried milk specification.

The method of Kuramoto et al. (1959) was modified by Leighton (1962) by means of giving a more precise definition of reconstitution procedure, and by using a saturated salt solution so buffered that when added to one ml filtrate, the final pH was always between 2.7 and 3.1 regardless of the type of powder under test. At this pH range the method has greater sensitivity and reproducibility.

The methods referred to above depend on the precipitation of the casein and denatured whey protein with sodium chloride, followed by measurement of the turbidity of the precipitated undenatured whey protein in the filtrate by reducing the pH.

Determination of undenatured whey protein nitrogen in skim milk

powder by dye binding was carried out by Sanderson (1970a). The filtrate produced by the method of Leighton (1962) was obtained and the amount of undenatured whey protein in the filtrate was determined by precipitation with an amido black solution in citric acid buffer and measuring the optical density of the supernatant liquid. Reproducibility of this method was better than by the usual turbidimetric method applied to the acidified filtrate, and the results were less affected by seasonal and compositional changes in the milk than in the turbidimetric method.

A series of standard curves prepared from low- and high-heat dried skim milk made at various times of the year using the turbidimetric method and the amido black method has been shown by Sanderson (1970b) The divergence of the standard curve prepared by the turbidimetric method is consistently greater than that of those obtained by using amido black. The standard curves prepared from the same set of milk powder made from the different season milk was considerably less varied by using the amido black than by using the turbidimetric method, due to the fact that whey protein from milk produced during the later stages of lactation developed less turbidity than those produced in the earlier stages. The seasonal change of the major individual whey protein components was more involved in measuring the whey protein by turbidimetric method due to the differences of turbidity between the equal concentration of β-lactoglobulin and α-lactalbumin solution, where the two portions showed very similar complexing ratio with the amido black.

Application of the technique of pro-milk difference (PMD) method introduced by McGann et al. (1972) to in-line process control of undenatured whey protein nitrogen in the manufacture of skim milk powder was demonstrated by Marschke & Houlihan (1980). Skim milk powder reconstituted, then at pH 4.8-4.7 casein and denatured whey protein were precipitated by adding acetic acid solution followed by sodium acetate. Duplicate reading of Pro-milk difference (PMD) were obtained on the undenatured filtrates at 20°C by using Pro-milk Mk II which is manufactured by A/S Foss Electric, Hillerod, Denmark. The results of the PMD showed a close linear relationship with the Kjeldhal nitrogen determination. The standard deviation for PMD repeatability was 0.011 PMD values which would be equivalent to the

ADMI heat treatment classification for skim milk powders. Three different preheat treatments of 73°C for 15 seconds, 80°C for 3 minutes and 88°C for 10 minutes were used to produce low, medium and high heat powders respectively in the pilot plant of their laboratory. The undenaturated whey protein nitrogen (UDWPN) was reduced by only 10% in the low heat powder compared with the raw skim milk values. UDWPN was reduced by 56% during preheating and some further reduction occurred during concentration in medium heat powder. All denaturation of whey protein (79%) occurred during preheating in the high heat powder. In all three products, denaturation during spray-drying was very slight. These results indicate that the rapid PMD technique has a useful application in process control during the manufacture of dried skim milk. The PMD method does not require calibration curves, although an occasional check with standard powder is recommended to confirm satisfactory operation of the Pro-milk.

A method for heat classification based on the determination of the heat number was proposed as a reference method by the International Dairy Federation (1982). An advantage of the method is that it enables the total nitrogen content of the dried milk to be found. The powder is classified according to the heat number which is the casein content plus heat denatured whey protein nitrogen content, expressed as a percentage of the total nitrogen content. Seasonal variation in casein number makes it impractical to use the heat number of dried milk to identify a dried milk that has received minimal heat treatment during manufacture which could be named as extra-low heat dried milk. According to this test the proposed heat classification scheme (IDF: 1982) is presented as below with the approximate correspondence of each heat class to the poly-acrylamide gel (PAG) electrophoresis pattern of the undenatured milk-serum protein (UMSP) and to the concentration of undenatured milk-serum protein nitrogen (UMSPN) obtained by the heat classification method of the ADMI.

Heat number	Heat class	PAG elcctrophoresis pattern of UMSP	UMSPN mg/g dried milk
80.0 or less	Low heat	All bands clearly visible or immunoglobulin band feint	⩾ 6.0
80.1 to 83.0	Medium Heat	Immunoglobulin band just visible or absent; blood serum albumin band feint	4.5 to 5.9
83.1 to 88.0	Medium high heat	Blood serum albumin and just visible or absent; β-lactoglobulin bands feint; α-lactalbumin band reduced	1.5 to 4.4
88.1 or more	High heat	β-lactoglobulin bands just visible or absent; α-lacalbumin band just visible or absent	⩽ 1.4

6. Anhydrous Milk Fat

Anhydrous milk fat (AMF) was originally produced by a special technique in Australia during World War II from melted butter which was purified by subsequent treatment in centrifugal separators to yield a milk fat product with an improved keeping quality compared to the original butter.

AMF made from fresh unsalted sweet cream butter has excellent keeping qualities and remains edible for months at room temperature. It has been widely used for standardisation of reconstituted milk in the Pacific area (White, 1953). This author also mentioned the United States military specification in 1950 for the AMF as a product with not less than 99.8% milk fat, not more than 0.1% moisture, not more than 0.25 ppm copper, a peroxide value of zero and free fatty acid value of not more than 0.5%.

Two methods of producing AMF from butter and cream were discussed by Fjaervoll (1970a). When butter was used as raw material, blocks were loaded into a cutter and mixer unit and the resulting paste pumped to a patented melting system which included a plate heat exchanger with automatic temperature control. The liquid butter was piped to a holding tank at a pre-set temperature and kept for a certain time to convert any remaining globular fat into free fat, allow escape of entrapped air and protein to agglomerate. The liquid was fed to a self-desludging separator and concentrated up to 99% or more. For highest purity, desludging was adjusted such that a small amount of fat left with the water phase, and was subsequently recovered. The small amount of water still present in the fat is flushed off in an apparatus consisting of a plate heat exchanger for heating and cooling and a vacuum chamber. The final moisture content of the fat was about 0.1%.

The other method (Fjaervoll, 1970a) of producing AMF was directly from cream. The cost is low compared to the first one, and there is a good recovery of all proteins and phospholipids from the cream in a protein fraction. Destruction of lipase by pasteuring the cream to 75°C and then regeneratively cooling to 55°C-58°C was recommended. Concentrated cream 30-40% to 70-75% fat was passed through an emulsion-breaking pump where most of the fat globule

membrane was destroyed. For higher fat concentration, a self-desludging machine was used to give a pure fat with a water content not exceeding 0.3-0.4%. The fat was then passed on to vacuum treatment for removal of water.

Separation of high-and-low melting fractions from AMF was described by Fjaervoll (1970b). A high-melting fraction with a melting point of 38°C shows in the composition of fatty acid, determined by gas chromatography, a high percentage of the most important saturated fatty acids as myristic (C_{14}), palmitic (C_{16}), stearic (C_{18}). Fatty acids with short carbon chains and unsaturated fatty acids were concentrated in the low-melting fraction with a melting point of 22.1°C. The original AMF had a melting point of 28.2°C with a complicated mixture of triglycerides composed of a large number of fatty acids of varying carbon chain length and degrees of saturation, dominated by palmitic (C_{16}) and oleic ($C_{18:1}$) acids.

Anhydrous milk fat is the highest quality of milk fat obtained from butter or cream with no change of the fat present in the original milk. No additives are used in its manufacture and the product retains its colour and vitamin content. Moisture content, peroxide value and free fatty acids content are the most important quality aspects of this product for routine quality assurance. AMF produced from fresh and good quality raw materials and then packed in oxygen-free conditions using nitrogen gas, will have a good stability to fat oxidation (Boersma, , 1982).

Further modification of AMF production from butter, using microwave treatment was reported by Entremont & Levardon (1981). Molten butter was fed to a U-shaped channel whilst being irradiated with microwaves. This caused the separation of the fat from water and protein with temperature rises of approximately 6°C. The degree of purification was 99.5% after 1 minute irradiation. The product may then be dried in a vacuum drier-degasser to 99.9% solids, and then pasteurised and packaged.

AMF has become the most common source of milk fat for producing recombined milk (Sanderson, 1982a). AMF does not require a refrigerated storage and even at elevated ambient temperatures of 30-40°C, for a period of 6-12 months it is claimed to keep its

quality. The use of high temperatures to melt the AMF should be avoided, to reduce the possibility of fat oxidation.

High quality AMF must be used for producing recombined butter with 75-80% milk fat. Recombined butter has the advantage of creating products of different standards according to the preference of the consumer. Equipment for table margarine is the most suitable type for producing recombined butter (Spieler, 1982).

AMF used as a source of milk fat for producing recombined evaporated milk should be stored in a cool position and a programme of testing initiated to select for immediate use batches which show high peroxide values. All fat must be checked organoleptically before use (Kieseker, 1982b).

Kirkpatrick (1982) supported the need for cool storage of AMF when reporting that AMF used in the manufacture of recombined concentrated milk products should not be stored for a long time at temperatures above 35-40°C.

The quality of AMF used in recombined milk, cream and butter must be very high, otherwise it will affect the acceptance of the final products (Gunnis, 1982a). He dealt with the requirement for cold storage and concluded that shipment of AMF under chill (5°C) or freezer (-10°C) conditions would be uneconomic. Gunnis (1982a) suggested that consideration should be given to the use of antioxidants as a means of controlling the auto-oxidation component of deterioration. His reasoning was based on the point of view that the addition of antioxidants to other edible oils was frequently permitted.

Milk fat in cheese is finely dispersed in a casein/water matrix so the physical properties of normal AMF have no effect on the body characteristics of recombined cheese (Gilles & Lawrence, 1982).

Review articles on recombination of milk by Jensen & Neilsen (1982) have pointed out that AMF remains as a main source of fat in the recombined process. The comprehensive review article by Jansen & Neilsen (1982) summarises the three methods of AMF manufactured, which have been outlined earlier in the work (pp 27).

7. Lipolysis

Milk fat is susceptible to many chemical and physical changes which sometimes result in the development of unpleasant flavours often described as soapy, bitter, nutty, butyric or rancid. Among these changes is lypolysis which has been defined by Jensen (1964) as the enzymatic hydrolysis of glycerides or fats. The enzymes responsible for this hydrolysis are called lipases.

The presence of lipase in milk was reported by Rice & Markley (1922). The effect of storage, temperature, cream separation, pH, stage of lactation and shaking on the activity of lypolysis has been studied by Roahen & Sommer (1940).

As a result of work during the 1950s and 1960s on the mechanism, activation and physico-chemical properties of the milk lipase system, it was concluded that there was more than one lipase present in milk. Tarassuk & Frankel (1957) concluded that all cows milk contains at least two different lipase systems. One is a naturally active lipase which is irreversibly adsorbed on the fat globule membrane when fresh milk is cooled and is associated with spontaneous lipolysis. The other is lipase of normal milk which remains in the plasma and is associated with the caseinate fraction.

However, in contrast to the theory of a dual lipase system, it has been found (Castberg, Egelrud, Solberg & Olivecrona, 1975) that lipo protein lipase and tributyrate hydrolysing activity were similarly distributed in the skim milk and cream fraction of separate whole milk. This also applied when the cream was washed and freed from lipid. It was concluded that in bovine milk there is only one major lipase, and that it is identical with lipo protein lipase. Previous theories about the existence of more than one lipase in milk probably reflect ranging conditions of assay and the tendency for the enzyme to aggregate with other milk components. Other workers, Olivercrona, Egelrud & Hernell (1975) and Downey (1975) have concluded that bovine milk contains only one lipase enzyme.

Microbial lypolysis

Many of the micro-organisms which contaminate dairy products produce lipase and can contribute to the development of rancid flavours.

Deeth & Fitz-Gerald (1976) in their review article on lypolysis in dairy products, reported that the most common sources of these lipase are the psychrotrophic bacteria, which grow at refrigeration temperatures. Unlike the milk-based enzyme, many of these bacterial lipases can attack the intact fat globules in milk. Many of these bacterial lipases differ from milk lipase in that they are not inactivated by pasteurisation even though the organisms which produced them are destroyed. They can therefore be carried through in an active form in to a manufactured product such as butter, causing fat breakdown during storage of the products.

The work of Nashif & Nelson (1953) showed that the presence of lipase produced by Pseudomonas fragi in cream and butter caused development of objectionable flavours, lowering the quality of these products considerably. The optimum condition for the lipase activity was in the pH range 5.7 to 6.6, using incubation at 36°C for 24 hours.

Butter containing residual lipase undergoes considerable fat degradation during storage, even at -10°C, developing pronounced rancid flavour at 5°C above.

The heat resistance of extra-cellular lipases from 60 strains of psychrotrophic bacteria isolated from farm tank raw milk was studied by Kishonti (1975). Kishonti reported that about 40% of the strains were found to produce enzymes which, after 90°C for 2 minutes or 63°C for 30 minutes, lost no more than 75% of their original activity. Heat treatment at 120°C for 5 minutes changed this percentage to 20%. The D value of extracellular lipase was 1.7 minutes at 150°C. Thus, UHT treatment at 150°C would seem to be necessary to destroy the enzyme.

Causes of hydrolytic rancidity in milk and milk products

Milk when freshly secreted from a healthy udder has about 0.5 μ mol FFA/ml (Fitz-Gerald, Deeth & Kitchen, 1981).

These acids result from incomplete synthesis rather than lypolysis. Under proper handling and storage conditions, only small increases in the FFA level should occur. In some cases, however, substantial increases are observed which result from either:

Induced lipolysis

or

Spontaneous lipolysis

These two forms of lipolysis have been distinguished by Tarassuk & Henderson (1942) and Tarassuk et al. (1957). The so-called 'induced lipolysis' which requires certain 'activation' treatment of raw milk such as homogenisation, temperature changes, shaking or agitation by air as in the pipe line of milking system. References to 'spontaneous lipolysis' refer to the reported lipase activity in the milk of individual cows, especially those in late lactation and on dry feed - without the milk being subjected to any apparent activation treatment other than ageing.

In their review article about lipolysis in dairy products Deeth & Fitz-Gerald (1976) pointed out that it is important to distinguish between the induced and spontaneous lipolysis, because the causes of the problems associated with them and the steps which have to be taken to eliminate them are quite different. Induced lipolysis is started when raw milk is activated by certain physical treatments during or after milking, such as agitation and foaming, certain temperature changes, freezing and thawing, and homogenisation. Whereas milk from a small percentage of cows in a herd undergoes spontaneous lipolysis, it could be described as 'naturally active', 'Susceptible', 'spontaneously lipolytic' or 'spontaneous' milk. The 'kind' of cows which produce 'spontaneous' milk depends on factors such as stage of lactation, feed nutrition, seasonal variations, breed and heredity, and mastitis.

The effects of lipolysis in milk and milk products

Rancid flavour in mastitis milk has been detected organoleptically by trained experts when the acid degree value level approaches 1.2 to 1.5 milliequivalent kOH/100 ml fat. The average consumer may detect rancidity when the level reaches 2.0 to 2.2 milliequivalent kOH/100 ml fat (Tallamy & Randolph, 1969).

Rancidity in cream was detected organoleptically in all samples when the acid degree value exceeded 2.1 milliequivalent kOH/100 ml fat (Dunkley, 1951). Thus the link between the appearance of rancid

flavour and the increase of free fatty acid has been established on numerous occasions and by many authors (Kuzdzal-Savoie, 1975) and (Kintner & Day, 1965).

Rancid milk does not always have the same odour (Herrington, 1956). Sometimes the odour of butyric acid is easily recognised but sometimes the odour is described as 'dirty' or 'goaty' which suggested to him that the C_6, C_8 and C_{10} carbon atoms were responsible.

It was demonstrated by Kolar & Mickle (1963) that fatty acids with very short chains (formic, acetic, propionic) do not play a significant role in determining the degree of flavour induced in herd milk. However, their finding may have been influenced by the low levels of C_1, C_2, C_3 acids in the milk as well as intra-experimental variations.

It was also shown by Scanlon, Sather & Day (1965) that fatty acid with 14:0 to 18:0 carbon atoms contribute little if anything to rancid flavour.

Al -Shabibi, Langner, Tobias & Tuckey (1964) concluded from an organoleptic evaluation of a wide range of fatty acids added in different concentration to milk that the rancid flavour of milk is caused by several fatty acids. The most prominent ones are capric (C_{10}) and lauric acids (C_{12}). Addition of butyric acid to milk was claimed to impart a flavour resembling butyric acid but lacking the sensation encountered in rancid milk. On the other hand, Scanlan et al. (1965) added a mixture of even numbered fatty acids from 4:0 to 18:0 carbon atoms to milk in the same ratio to one another but at a higher concentration than the FFA which Kintner & Day (1965) found in moderately rancid milk. Individual fatty acids and groups were omitted from the mixture and the milk prepared with the resulting mixtures were presented to a flavour panel. From their results, they concluded that the even numbered fatty acids from C_4 to C_{12} have an important role in contributing to the rancid flavour in milk, but not one of these acids singly exerts a predominating influence in its flavour contribution.

Lipolysis in butter

Many authors reported the problem of the flavour defects in butter caused by lipolysis, Fouts (1940); Nielsen (1972); Bell & Parsons (1977) and Jamotte (1970). Various faults in raw milk handling at dairy farms may give rise to a rancid flavour in fresh or stored butter (Nielsen, 1972). They are:

1. Excessive foaming of milk in pipelines, improper positioning of inlet cocks in the line and splashing of milk from improperly placed outlet in the cooling tank.

2. Excessive agitation of milk in the tank and partially submerged agitator.

3. Excessive temperature variation of the milk in farm cooling tank due to inadequate compressor capacity.

Bell & Parsons (1977) found that many factors influence lipase flavour in butter. The effect of storing cream for 24 hours at 4.4°C did not have a significant ($p > 0.05$) effect on the butter flavour scores. Butter made from cream pasteurised at 85°C and 93°C for 18 seconds had a better flavour score than using 72°C for 18 seconds in the beginning of the trial. However, all samples deteriorated during the 6 months storage, indicating that the higher heat treatments did not inactivate the lipase completely. Finally, they found very significant ($p < 0.01$) differences between the increases of free fatty acid for butter at -28.9°C and 4.4°C, respectively. They concluded that a good quality butter placed in storage at -28.9°C is stable for at least 6 months.

O'Connell, Cogan & Downey (1975) felt that it is very important to distinguish between high initial FFA values and high developed FFA values in butter. High initial FFA levels are due to the use of milk which has undergone hydrolytic rancidity development. In such butter many of the FFAs, especially the more obnoxious lower chain ones, will be released in the butter milk during churning and thus will not affect the flavour. In butter, in which rancidity is developing there is no release of fatty acids and consequently one obtained more pronounced taste for the same level of FFA. At the same time, they found that salted ripened cream butter is more

resistant to developed hydrolytic than unsalted sweet cream butter. O'Connel et al. (1975) also showed that this effect was almost certainly due to the salt level of 1.6% (w/w) which is equivalent to a concentration of 10% in the aqueous phase of the butter - more than enough to inhibit microbial growth. The low pH is an additional inhibition of microbial growth in the salted ripened cream. The same observation for the effect of salt in butter has been mentioned earlier by Fouts (1940).

The presence of psychrotrophic bacteria in milk becomes very important due to their production of heat resistant lipases which can survive the cream pasteurisation process, even though this destroys the bacteria themselves, Nashif et al. (1958); Kishonti (1975).

Lipase probably plays the major role of lipolysis in butter during storage. Deeth et al. (1979) found that heat stable microbial lipases originating from the milk or from post-manufacture microbial contamination were responsible for lipolysis during butter storage. Microbial lipases which release the short chain acids with or without the longer chain acids will cause the most noticeable effects on flavour. They also agreed with O'Connel et al. (1975), that in butter production when lipolysis occurs in the raw milk or cream fat, the short chain fatty acids which are responsible for flavour defects are lost in the skim milk, butter milk and butter washings during processing. Due to their solubility in water they cause less flavour defects than lipolysis which occurs after manufacture. In the latter case the entire profile of fatty acid released is retained in the butter. So the resulting flavour depends more on the kind of free fatty acids than the acid degree value. These workers point out that apart from the smell of the very short chain fatty acids, it is possible to detect the presence of free fatty acids in butter by 'a sharp or soapy sensation on the tongue when first tasted, followed by a strong after taste and burning sensation on the back of the palate.'

Woo & Lindsay (1980) indicate that the usual routine indexing methods of flavour scoring and acid degree values in butter have proved to be of little value in detecting and predicting lipase flavours developed during commercial handling of butter due to the lack of measuring the variation in individual FFA.

8. Milk Fat Oxidation

Oxidised flavour defects in dairy products such as rancid, fatty, oily, metalic, nutty, and fishy, which develop due to the auto-oxidation of milk fat play a very important role in limiting the shelf-life of these products. Generally speaking the unsaturated fatty acids undergo auto-oxidation and form odourless, tasteless and unstable hydroperoxides, which readily decompose to give carbonyl compounds which have an unpleasant flavour even at very low concentration.

Badings (1970) summarised the mechanism of auto-oxidation of unsaturated fatty acids from the findings of previous workers as follows:

1. The majority of auto-oxidations proceed through free radical chain reactions. Peroxides are usually the first formed products.

2. There are three elementary reactions:

   (a) Initiation, formation of free radicals which are necessary to start the propagation reaction (formation of $R^{\bullet}$ and $RO2^{\bullet}$)

   (b) Propagation, this is the free radical chain reaction. If RH is the symbol for an alefin or an unsaturated fatty acid molecule, H being a hydrogen atom of the methylene group adjacent to the double bond (α -methylene group), the propagation reaction proceeds as expressed below:

$$\longrightarrow R^{\bullet} + O2 \xrightarrow{kp1} RO2^{\bullet}$$

$$RO2^{\bullet} + RH \xrightarrow{kp2} ROOH + R^{\bullet}$$

   (c) Termination, interruption of the free radical chain reaction by the formation of stable, non-radical products from these free radicals:

$$\left.\begin{array}{l} 2R^{\bullet} \xrightarrow{kt1} \\ R^{\bullet} + RO2^{\bullet} \xrightarrow{kt2} \\ 2\,RO2^{\bullet} \xrightarrow{kt3} \end{array}\right\} \text{non-radical products}$$

Auto-oxidation reactions can be accelerated by light and radical-forming products, such as benzol peroxide, initiating new chains. The rate of reaction is also dependent on the energy required for the rupture of the α-methylene c-H bond. Auto-oxidation reaction may be inhibited by compounds (e.g. antioxidant) which react with free radicals to form non-radical product. Finally, the outcome of the Auto-oxidation is often complicated by numerous other reactions such as the formation of polyperoxides by addition of peroxy-radicals to olefins.

The degradation of the hydroperoxides of unsaturated fatty acids are of particular interest because these products (saturated and unsaturated aldehydes, unsaturated ketones, secondary alcohols etc.) contribute strongly to flavour defects in oxidised lipids.

Spontaneous oxidation

Spontaneous oxidised flavour in milk and its relation with natural copper concentration in cows milk was investigated by King & Dunkley (1959a). In seeking the answer to why some milks become oxidised, Shipe (1964) started with differences in oxidation stability between milks from different cows. He agreed with the existing classification of milk into three categories of oxidative stability as follows:

1. Spontaneous milk: milk capable of developing oxidised flavour without the presence of iron or copper as a contaminant.

2. Susceptible milk: milk which does not develop oxidised flavour spontaneously but is susceptible if copper or iron is added.

3. Non-susceptible milk: milk which does not even become oxidised in the presence of iron or copper.

Shipe (1964) concluded that heredeity and stage of lactation of the cow as well as feeding practices were factors affecting the differences in oxidative stability for milk from different cows.

In a three-year study of 1198 raw milk samples from 137 herds in Californian countries, Bruhn, Franke & Goble (1976) found that 18%

of the samples scored 2 (slight oxidised flavour) or more when examined organoleptically for oxidised flavour in 48 to 72 hours after collection on a 0 (none) to 4 (pronounced) scale.

Factors affecting milk fat oxidation

1. Metals

Contamination of dairy products with metals (particularly copper) has been recognised for a long time (Brown & Thurston, 1940) as a major factor influencing oxidative changes in the product.

Milk, as secreted, contains low but variable amounts of copper ranging from 0.2 ppm of natural copper at early lactation to about 0.02-0.04 ppm during the remainder of the lactation period (King et al., 1959a). As a result of their experimental work on the effect of feeding and drenching the cows with doses of copper sulphate, King et al. concluded that the natural copper in milk is an important catalyst of oxidised flavour. In a later study, radioactive isotope tracers of Cu^{64} and Fe^{59} were used to determine the distribution of added and natural copper and iron in milk by King, Luick, Litman, Jennings & Dunkley (1959b). They found that natural copper and iron concentrated at the fat globule surface but only 2-3% of the total added copper and none of the added iron associated with the fat globules. Added copper mostly was associated with the skim milk protein. Natural copper and iron were not dialysable and the same applied for the added copper associated with fat globules. However, added copper and iron in the skim milk was slightly dialysable. Finally they concluded that natural and added copper associated with the fat globule membrane and could have similar properties in the catalysis of oxidised flavour. The observation that no added iron associated with the fat globule membrane could partially explain the lower catalytic activity of added iron as compared to added copper.

The concentration of copper in milk has been increased significantly by feeding supplement of copper sulphate and copper ethylenediamine-tetraacetate (EDTA) and sodium EDTA to cows. While there was a small but statistically significant ($p < 0.01$) decrease in the concentration of the tecopherol in the milk the experiment did not

provide a clear effect on the oxidative stability of the milk. Dunkley, Franke, Robb, & Ronning (1968).

Downey (1969) summarised the effect of copper contamination by handling, processing and packaging of butter and other dairy products. He has shown the profound effects that copper can have on the oxidative stability of the milk fat. Examples of light-induced lipid oxidation of butter and cheese in illuminated display cabinets were also given.

Bruhn et al. (1976) confirmed that the tendency of milk to spontaneous oxidation is related directly to copper concentration and inversely related to tocopherol concentration.

Both treatment of milk with trypsin or the ageing of milk was found to affect the milk fat globule membrane (MFGM) and skim milk component. Gregory & Shipe (1975) found that the changes due to ageing were mainly qualitative resulting in a decrease in recoverable membrane material with few compositional changes. By contrast, trypsin treatment caused a decrease in recoverable membrane material and extensive hydrolysis of MFGM protein. It was thought that membrane material and hydrolysates released from the globule surface would significantly increase the chelating capacity for metals, particularly copper, and thus improve oxidative stability.

2. Light

Oxidised flavour in milk caused by exposure to sunlight, daylight and fluorescent light has been referred to by Barnard (1972). Using an appropriate lighting intensity for dairy containers, closed cases, vertical fluorescent light tubes and changing to yellow, pink or other coloured fluorescent tubes seemed to be ways of reducing the problem of light-induced oxidation.

The response to the light activated flavour in milk packaged in plastic jugs has been investigated by White & Bulthans (1982). Preference testing over all portions of the study indicated that 63% preferred the milk with no off-flavour, 27% preferred the light activated flavour and 10% had no stated preference. It was suggested that light activated off-flavour could result in a reduction of total milk sales.

Aurand, Boone & Giddings (1977) concluded that singlet oxygen ($^1O_2$) is the immediate source of the hydroperoxides that initiate fatty oxidation in milk. So they inhibited the milk fat oxidation by using singlet oxygen trapper (1,3-diphenylisobenzofuran) or a single oxygen quencher (1,4-diazabycyclo-(2-2-2)octane). At the same time they found that singletoxygen formation was catalysed by copper ions, enzymes and light.

3. Oxygen

Removal of dissolved oxygen from fluid milk or its replacement by inert gasses is expected to reduce the incidence and intensity of oxidised flavour. Schroder (1982) found that oxidised flavour developed through the catalytic effect of either copper or light and the development of copper-induced and light-induced oxidised flavour in milk differs in three respects:

a. The oxygen requirement for flavour formation.

b. The relationship between flavour formation and ascorbic acid oxidation.

c. The organoleptic properties of the flavour.

Light-induced oxidised flavour in milk can be controlled by limiting O_2 availability, but this is not so for Cu-induced oxidised flavour unless the milk has been de-aerated to very low oxygen level, probably less than 0.5 mg/l. Copper induced oxidised flavour was described as 'cardboardy' and light-induced oxidised flavour as 'painty'.

Korycka-Dahl & Richardson (1980) in their article about the initiation of oxidative changes in food, explained the formation of hydroperoxide as a direct reaction between an unsaturated fatty acid (RH) and oxygen. However, this reaction ($RH + O_2 \rightarrow ROOH$) is unlikely because it would require a change in total spin during the lifetime of the collision complex, i.e. RH and ROOH are in a singlet state, while O_2 is in the triplet state ($^3O_2$). Reactions such as this are forbidden because their spin is not conserved, so singlet oxygen ($^1O_2$), an electronically excited state of oxygen reacts readily with unsaturated singlet compounds to yield singlet peroxides which can

4. Heat Stability

During many courses of manufacture, milk and milk products are subjected to high temperature heat treatment (as in the case of sterilisation process) which may result in an increased risk of milk coagulation when used. So the heat stability characteristic of milk powder is an important property in its use for the production of recombined sterilised milk or recombined condensed and evaporated milk.

Sargent, Biggs & Irvine (1959) studied the relation between the hard water which was used for the reconstitution and the heat stability of the reconstituted milk and according to their results the heat stability of reconstituted skim milk was adversely affected by high calcium and magnesium levels in the reconstitution water. Heat stability is affected by preheat treatment and pH adjustment prior to evaporation and drying of the skim milk powder during two dairying seasons (Newstead, Sanderson & Baucke, 1975). Better heat stability characteristics resulted from a preheat treatment of 120°C for 120 seconds than 120°C for 30 seconds. Adjustment of the pH was a great advantage in improving the heat stability specially during the earlier part of the season September to December when the optimum pH was usually between 0.06 and 0.12 units lower than the natural pH.

Seasonal variation in milk components is important in the processing characteristics but the combined effect of pH adjustment and manipulation of preheating conditions is a successful method of meeting the heat stability specification for producing powder suitable for use for recombined evaporated milk (Griffin, Hickey, Bailey & Feagan, 1976).

Heat stability characteristics of the powder play an important part in determining the stability of the recombined milk to ultra-high-temperature (UHT) treatment (100 to 150°C for 3 s) (Zadow & Hardham, 1978). Their results show that recombined UHT milk prepared from medium or high heat powder is more resistant to sediment formation than recombined UHT milk prepared from low heat powder. At the same time a slight reduction in the pH of recombined milk due to use of powder of high acidity or to the development of acidity in the

recombined product during storage may result in the formation of sediment in recombined UHT milk.

The effect on heat stability of recombined evaporated milk during one dairying season of solids-not-fat concentration, fat concentration and homogenisation pressure were highly significant (Newstead, Hughes & Baldwin, 1978). The increase of solids-not-fat concentration from 160 g/kg to220 g/kg reduced the average maximum heat stability from 57 to 21 minutes. Increasing fat concentration from 80 g/kg to 100 g/kg reduced the average heat stability from 49 to 32 minutes. An increase of first stage homogenisation pressure also reduced the heat stability of the product.

The manufacture of heat-stable milk powder is generally confined to mid-lactation milk, during which period the required heat-stable specification is more easily attained (Kelly, 1981). Seasonal variation of urea level in milk due to animal feeding could be a factor affecting the heat stability. Preheating of the milk to 120°C for 2 minutes together with the addition of urea brought the heat stability of powders produced from early lactation milk into line with those produced during mid-season without urea addition. Lactose, being a non-reducing sugar, does not increase heat stability due to lack of potential aldehyde or ketone function. Kelly (1981) found that when lactose is hydrolysed in milk using the enzyme β-galactosidase, its heat stability is increased due to protein-carbohydrate interactions.

Of all the variables that influence the heat stability of milk powders, only variations in the preheating of the raw milk is used as a routine in practice to establish the characteristics required (Kieseker, 1982a). He also mentioned that mineral level, especially calcium, pH changes due to seasonal variation at preheating, protein level, whey protein, lactose, and urea level in milk are all involved in determining the heat stability of milk.

initiate oxidative chain reaction. This singlet oxygen can be generated chemically, enzymatically or by sensitized photoxidation. They also showed that there are other varieties of oxygen species important in oxidative stability can be generated in or near biological and food system as hydroxyl radical ($HO^{\bullet}$) which is one of the most reactive species known and the ozone (O_3). Hydrogen peroxide and superoxide anion residues serve as precursors for the most reactive species of oxygen.

Air polluted by ozone over cities and industrial areas can be a factor in oxidative stability of dairy products as spray dried whole milk where intake air may contain O_3 (Kurtz, Tamsma, Selman & Pallansch, 1969).

4. Milk fat globule membrane (MFGM):

King (1962) used thiobarbituric acid for investigating the rapid oxidation in model systems containing fat globule membrane material and ascorbic acid.

Various phospholipid classes fractioned from fat globule membrane of cows milk were studied by O'Mahony & Shipe (1970). There was lower concentration of phosphatidylethanolamine in milk classified as being less susceptible to copper-induced oxidation.

Badings (1970) has extended the possible sources of auto-oxidation deterioration of cold-stored butter. He has pointed out that the initiation of oxidative deterioration could start at the fat/serum interface. At the same time, oxidation of the butter fat could start as a result of the diffusion of hydroperoxides from interface into the fat. This interpretation was confirmed by experimental findings that the 'fishy' flavour developed even in artificial butter which did not contain fat globule membrane material or phospholipids. The 'fishy' flavour developed wihin a few months at -10°C, provided the serum phase had a high copper content.

Lipids in the MFGM such as the cephalin fraction of the phospholipids contain a large proportion of polyunsaturated fatty acids which are very susceptible to oxidation and probably play an important role in initiation reactions.

The natural copper content in the membrane is about 10 µg/100 g fat globules and is not very dependent on natural copper content of the milk. The copper content of the MFGM is influenced by various factors such as:

a. Cooling - natural copper migrates from the MFGM to the plasma.

b. Heating - copper (mainly added) migrates from the plasma to the MFGM in heated milk but the migration is mostly prevented in heated cream.

c. pH - approximatley 30 to 40% of added copper migrates to the MFGM at pH 4.6.

These factors have been used by Mulder & Walstra (1974) to explain the selection of processing conditions which give minimum fat oxidation in sour cream butter. Thus the raw milk should be kept for at least a few hours at about 5°C before separation, pasteurisation at 85°C for 18 seconds after separation.

Allen & Humphries (1977) found that the maximum oxidative capacity of bovine MFGM fractions, in a buffered linoleate system, was greatly enhanced by copper addition and association with phospholipid. They also found that xanthine oxidase was highly pro-oxidative, even in fractions devoid of phospholipid. Further work (Allen & Wrieden, 1982) with purified milk metallo proteins (lactoferrin, lactoperoxidase, superoxide dismutase and xanthine oxidase) showed that xanthine oxidase had little effect on lipid oxidation in the absence of added metals. However, a strong pro-oxidative effect was found in the presence of 10 µM Cu^{2+} and was still found, though more slowly, down to levels of 1 µM Cu^{2+}. Heat treatment of xanthine oxidase at 80°C/20s was more effective than at 72°C/20s in reducing the pro-oxidative effect in the presence of 10 µM Cu^{2+}. The pro-oxidative effect of xanthine oxidase catalysed by 10 µM Fe^{2+} was less pronounced than with copper at the same concentration.

A study into the variation in the enzyme superoxide dismutase and its effect on the oxidative stability of individual cows milk concluded that superoxide dismutase decreased the pro-oxidant effect of xanthine oxidase (Holbrook & Hicks, 1978).

Gregory, Babish & shipe (1976) examined the role of the heme proteins of the milk fat globule membrane in the oxidative degradation of milk lipids. They concluded that MFGM heme proteins are involved in lipid oxidation, but are not the only factors.

Milk fat globule membrane (MFGM) consists of a complex mixture of proteins, phospholipids, glycoproteins, triglycerides, cholestrol, enzymes and other major components and acts as a natural emulsifying agent enabling the fat to remain dispersed throughout the aqueous phase of milk.

In a review of the bovine milk fat globule membrane, McPherson & Kitchen (1983) have explained that the oxidation of unsaturated fatty acids of MFGM phospholipids can produce off-flavours. This oxidation can occur as a result of heating the milk which causes migration of copper from milk serum to the MFGM and this subsequent increase in MFGM-associated copper may increase the susceptibility of the membrane to oxidation. They also mention that heme proteins (mainly cytochromes) can be involved in initiating the oxidation reaction. Finally they indicate that superoxide (a product of the reaction catalysed by xanthine oxidase) is another reactive oxidative factor. It is also suggested that the relative abundance of xanthine oxidase in the MFGM could result in the enzyme being responsible for the initiation of oxidative rancidity.

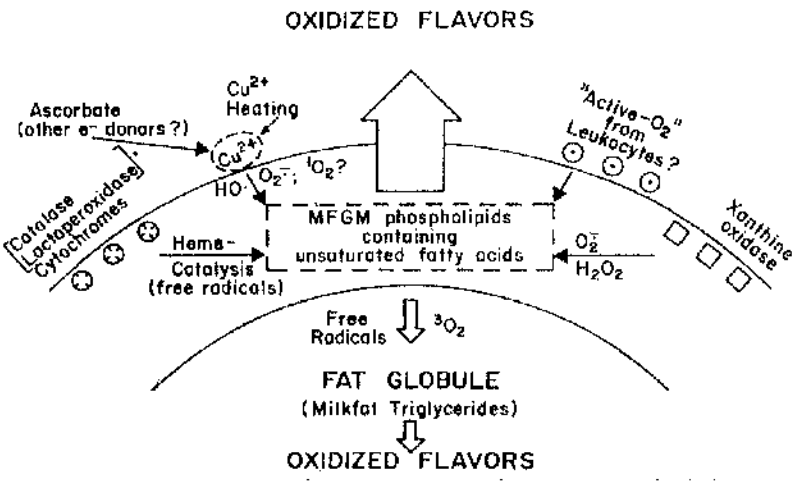


Figure 5: Factors associated with the fat globules membrane of milk that may favour the oxidation of milk lipids (from Richardson & Koryckn Dahil, 1983)

5. Tocopherol

The Tocopherol present in milk was found by Krukovsky (1952) to play an important part in resisting oxidised flavour development.

The direct addition of appropriately emulsified α-Tocopherol at a concentration of 25-100 µg/g milk fat was effective in controlling the spontaneous oxidised flavour in milk (King, 1968).

Attempts to overcome the problem of spontaneous oxidised flavour in raw milk by feeding vitamin E (DL-α-Tocopherol acetate) supplements to lactating dairy cows were reported in three experimental trials:

1. Clifton, Speck, Loewenstein & Martin (1971);
2. Dell, Loewenstein, Clifton, Vasavada, White & Speck (1973);

and 3. Loewenstein, Dell, Clifton & Fosgate (1974).

From these reports it is concluded that there is no significant correlations between Tocopherol intake, Tocopherol concentration in milk and spontaneous oxidation of milk.

Significant correlation (15.1% of variation) between copper and Tocopherol concentration and milks oxidised flavour score was found by Bruhn et al. (1976). However, attempts to control spontaneous oxidation in milk from cows on dry lot feeding by adding Tocopheryl acetate to the feed were unfavourable because only 2% of the added Tocopherol was transmitted in the milk.

Tocopherol is naturally occurring in many oils and fat. Under certain circumstances (probably high concentration) the Tocopherol can be a pro-oxidant but under the normal conditions existing in milk a pro-oxidant effect of Tocopherol is highly unlikely (Richardson & Korycka-Dahl, 1983).

6. Ascorbic acid

Early research indicated that ascorbic acid in association with copper is an important pro-oxidant in milk. Brown & Olson (1942) ascribed the mechanism as (a) reduction of cupric copper by ascorbic acid, which was oxidised to dehydroascorbic acid, (b) oxidation of the cuprous ion to the cupric ion by oxygen from the air with the

liberation of hydrogen peroxide which (c) oxidises phospholipids in the part fat globule membrane. As a result of their experimental work it was found that both ascorbic acid and dehydroascorbic acid were capable of producing an oxidised flavour in washed cream.

Smith & Dunkley (1961) concluded that copper and ascorbic acid are essential pro-oxidants in the spontaneous oxidation of milk.

Ascorbic acid can be a very effective antioxidant. Richardson et al. (1983) and also they explain that combinations of ascorbate and copper can be pro-oxidant or antioxidant depending upon their relative concentration.

$$AA + Cu^{2+} \rightleftharpoons AA\ Cu^{2+} + AA \rightleftharpoons AA\ Cu^{2+}\ AA$$

(I) (II)

unproductive complex

So from above hypothetical chelates between ascorbic and Cu^{2+} if one assumes that chelate (1) is pro-oxidant and that chelate (II) is antioxidant which means low concentration of ascorbic acid favour formation of pro-oxidant (I) where at high concentration of ascorbic acid favour formation of antioxidant (II). So they concluded from the numerous studies in this area that it was very difficult to define the complex role of ascorbate in the oxidative stability of milk.

7. Anti oxidants

Richardson et al. (1983) classified antioxidant into three major groups:

1. Inhibitors of free-radical chain reaction such as Tocopherol, butylated hydroxyanisale (BHA), butylated hydroxytoluene (BHT), ascorbic acid.

2. Peroxide decomposers such as the thioethers, methionine and thiodipropionic acid and its esters.

3. Ligands that de-activate metallic pro-oxidants.

At the same time any antioxidant intended for food use should possess the following properties.

1. It and its oxidation products, should be completely safe at the concentration used.

2. It should not react with any ingredients of the food or impart any odour, colour, or flavour to the products treated.

3. It should be effective at low concentrations.

4. It should remain active after the normal processing.

5. It should be soluble in the substances that it is to protect, so as to ensure uniform dispersion.

In our work we used some of these antioxidant in different levels of concentration to retard the oxidation of anhydrous milk fat and we are going to discuss these anti-oxidants more specifically in the course of this thesis.

CHAPTER ONE

MATERIALS AND METHODS

1.1 Materials

Medium-heat skim milk powder (SMP) and medium-heat lecithinated whole milk powder (WMP) were packed in 25 kg multi-wall bags with a polythene liner. The fat sources were anhydrous milk fat (AMF) and butter, packed in 25 kg polythene-lined cardboard boxes. The fats were supplied by the Aberdeen and District Milk Marketing Board and were of recent manufacture.

1.2 Reconstitution and Recombination

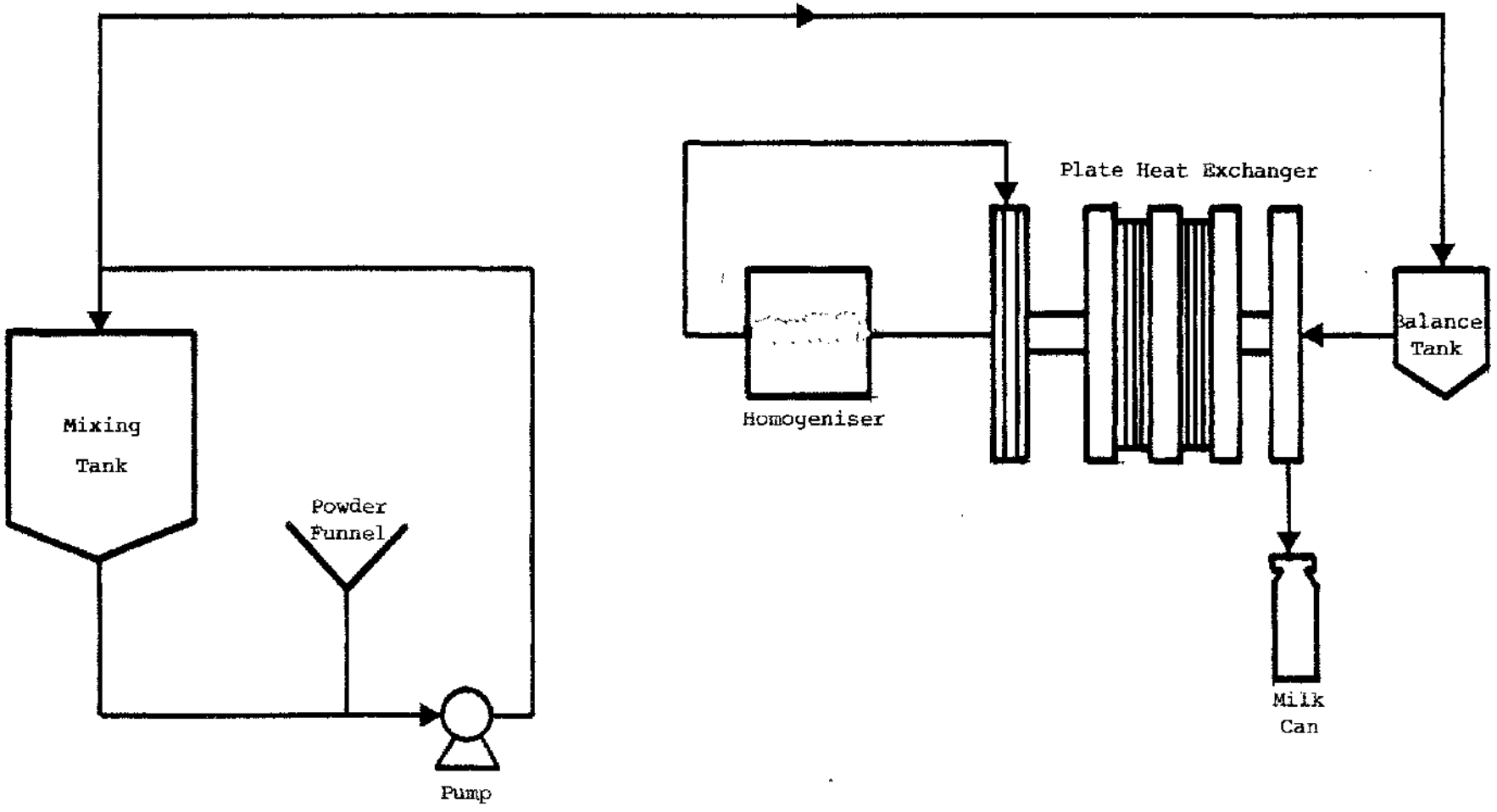
The SMP and WMP were each reconstituted in 100 litres of water at a temperature between 8 and 10°C (see Plates 1:1, and Figure 1:1). The powder was added through a funnel on the suction side of an APV Puma centrifugal pump (APV, Crewly, England). The flow of powder from the funnel was controlled manually by the setting of a butterfly valve. Dispersion of the powder in the water was achieved by circulation through the mixing tank during powder addition. When all the powder had been added the mix was allowed to stand for about half-an-hour to complete the reconstitution.

The temperature of the reconstituted SMP was raised to between 20 and 25°C prior to the addition of molten AMF or butter. Although higher temperatures have been used (IDF, 1979) it was found that fat separation and churning occurred at temperatures above and below the reported range.

The milk derived from either SMP or WMP was preheated in the first stage of an APV HX-Paraflow pasteuriser and then homogenised at a temperature of 50°C and a pressure of 20.7 M.N. m^{-2} in a Rannie LAB in-line homogeniser.

The pasteurisation was carried out for 15 seconds at a temperature slightly higher (approximately 1°C) than the normal (71.7°C) condition, in order to maintain the temperature during homogenisation. The milk was cooled to less than 6°C prior to collection in a sterilised milk can and overnight storage in a refrigerated cold store at 4°C.

Figure 1.1 Recombination Plant (present work)



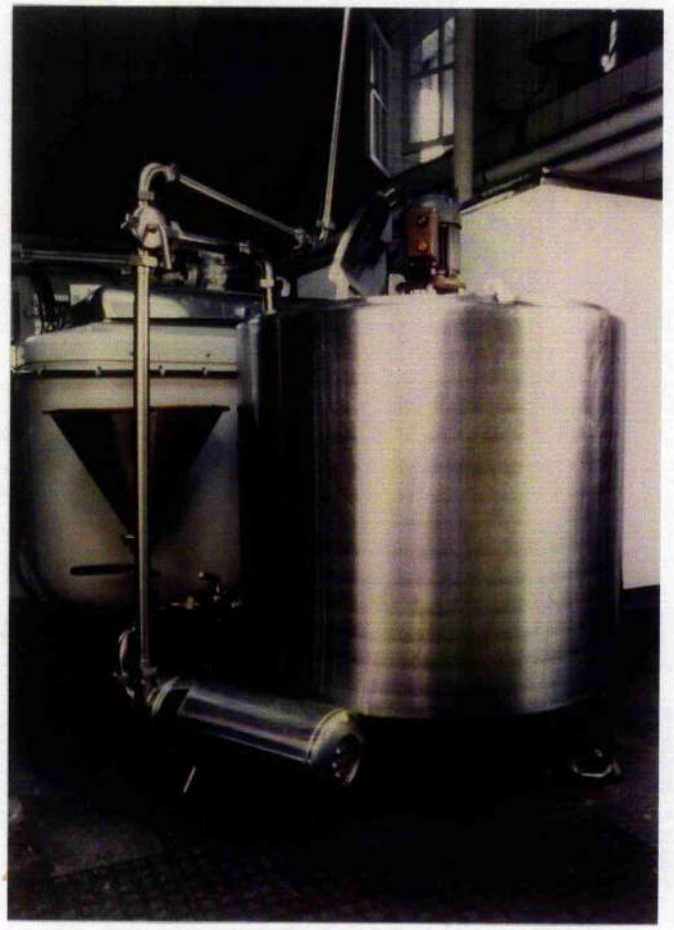


Plate 1:1 In foreground apparatus comprising steam heated mixing tank with funnel for addition of materials and pump and pipelines for circulation of water and ingredients during reconstitution or recombination

1.3 Taste Panel Evaluation

Samples of recombined or reconstituted milk mixed with the control freshly homogenised and pasteurised liquid milk at levels of 0%, 25%, 50%, 75% and 100% were presented to ten taste panelists. Two duplicates were included in the samples and the panelists were asked to score various acceptability qualities such as "flavour", "mouthfeel", "colour", "odour" and "overall acceptability" on an eight-point hedonic scale ranging from "Like Extremely" (8), to "Dislike Extremely" (1). The panelists were also asked to give detectability score rating for various characteristicssuch as "fattiness", "chalkiness", "acidity" and "rancidity" on a seven-point scale ranging from "Undetectable" (1), to "Very Pronounced" (7). *Each trial was carried out twice.*

The taste panel evaluation was used initially to see if there was any preference for a particular combination of raw materials. Subsequent taste panel trials were directed towards the effect of storage time and temperature on the raw materials and the acceptability of the recombined milk. In the latter case the trials were confined to the use of SMP and AMF with the freshly homogenised and pasteurised liquid milk as the control. For these trials nine panelists were used.

1.4 Raw Material Analysis

Samples of SMP, WMP and AMF were stored in approximately 2 kg lots using vacuum sealed nylon/polythene laminated pouches. The 2 kg lots were bulked together in 25 kg commercial skim powder bags using polythene liners and closure and sealing the outside of the bags with heavy cellotape. Each time a sample was required for testing, a 2 kg lot was removed from the bag, tested and then discarded.

A series of physical, chemical and microbiological tests were made on samples of SMP, WMP and AMF stored at temperatures of -18°C, 4.5°C, 11.5°C and 22.5°C for 20 months. The procedures used are as follows:-

1.4.1 Physical tests

1.4.1.1 Bulk density

The method of Sørensen, Kraag, Pisecky & Westergaard (1978a) based

on determining the volume of 50 g of powder after being tapped for 1150 times by using a Jolting Volumeter - JEL ST 2.

$$\text{Bulk density} = \frac{\text{Weight of Powder (g)}}{\text{Volume of Powder (cm}^3\text{)}}$$

1.4.1.2 Dispersibility

The IDF Standard Method 87 (IDF, 1979) was used to determine the dispersibility of both SMP and WMP. A test portion of 26 ±0.1 g of SMP or 34 ±0.1 g of WMP was evenly spread on the surface of 250 ±1 g of distilled water adjusted to 25 ±1°C in a glass beaker of 600 ml capacity. The mixture was stirred manually for 20 seconds and after standing, part of the mixture was filtered through a 150 µm sieve and the total solids content of the collected liquid was determined by drying.

The dispersibility was calculated by applying the following formula:

Skim Milk Powder

$$D = \frac{T \times 962}{100 - (W + T)}$$

Whole Milk Powder

$$D = \frac{T \times 735}{100 - (W + T)}$$

D is the dispersibility, in%;

T is the total solids content, in % (m/m), of the liquid;

W is the water content, in % (m/m) of the pre-treated test sample.

1.4.1.3 Wettability

The IDF Standard Method 87 Annex B (IDF, 1979) was used to determine the wettability of WMP. A test portion of 10 ±0.1 g of WMP was spread evenly on the surface of 250 ±1 g of distilled water and adjusted to 25 ±1°C in the same way for the dispersibility test. The time required for all the particles of the sample to become wetted (i.e. to sink below the surface of the water and any remaining on the surface to assume a typical "wet" appearance) was obtained.

1.4.1.4 Solubility index

The method of ADMI (1971b) was applied to measure the ability of the powder to dissolve in water. It is expressed as the volume of sediment in ml obtained by centrifugation of 10 g SMP or 13 g WMP reconstituted to 100 ml distilled water.

1.4.1.5 Scorched particles

The method of ADMI (1971c) was used to determine the scorched particles of the powder. Initially, 25 g of SMP or 32.5 g of WMP was dissolved in 250 ml of distilled water with 0.5 ml of anti-foam (Octan-1-01) in a Waring blender. The entire solution was filtered through a standard cotton disc, using a double-bulb bellows. The disc was dried at 30 to 40°C and compared with the ADMI scorched particle standards photoprint.

1.4.2 Chemical tests

1.4.2.1 Titratable acidity

The method of ADMI (1971d) was used to determine the titratable acidity of SMP and WMP. Initially, 10 g of SMP or 13 g of WMP was dissolved in 100 ml of distilled water using a Waring blender. Then 17.6 ml of mixture plus the same quantity of distilled water were titrated with 0.1 N sodium hydroxide solution using phenalphthalein as an indicator. The number of millilitres of 0.1 NaOH required for the titration, divided by 20, gives the percentage titratable acidity (in terms of lactic acid) of the reconstituted sample.

1.4.2.2 Water content

The water contents of SMP and WMP were determined by the IDF Standard Method 26 (IDF, 1964). The water content of the dried milk was expressed as the percentage loss in weight when the powder was heated in an oven (Townson and Mercer Limited, Croydon, England) at 102 ±2°C to constant weight.

1.4.2.3 Fat content

The fat content for SMP or WMP was determined by the Rose-Gottlieb method using the IDF Standard Method 22 (IDF, 1963). The fat content was determined gravimetrically by the addition of 1 ml of

25% (w/w) ammonia solution and 10 ml of 96% (v/v) alcohol to 10 g of powder. The fat was extracted into light petroleum/ethyl ether mixture and the solvents evaporated off in a vacuum oven at 80°C. The final weight of fat was determined by drying in an oven at 102°C ±2°C to constant weight.

1.4.2.4 Protein content

The improved micro-Kjeldhal method of the Association of Official Agricultural Chemists (1965) was used to determine the total nitrogen content of SMP and WMP (expressed as protein). Kjeldhal copper catalyst tablets (BDH Chemical Limited, England) were used instead of mercuric oxide.

Standard 0.02 N hydrochloric acid was used in the receiver for distillation of the ammonia. The excess of the acid was titrated with standard 0.02 N sodium hydroxide solution.

A blank sample was determined and the percentage of nitrogen (N) was determined from:

$$N = \frac{[\text{ml NaOH(blank)} - \text{ml NaOH(sample)}] \times \text{normality NaOH} \times 1400.7}{\text{mg sample}}$$

1.4.2.5 Surface free fat

The method of Sørensen *et al.* (1978b) was used to determine the free fat on the surface of the whole milk powder particles. Initially, 50 ml of carbon tetrachloride was added to 10 g of WMP. Extraction was carried out for 15 minutes by shaking in a flask with a Mk V Reciprocal Shaker. The mixture was then filtered. Carbon tetrachloride was evaporated from 25 ml of the filtrate in a vacuum oven at 80°C followed by drying in an oven at 102 ±2°C to constant weight.

The percentage surface free fat was calculated from:

$$\text{Percentage free fat in powder} = \frac{a \times 25 \times 2 \times 100}{\left(25 - \frac{a}{0.94}\right) \times b}$$

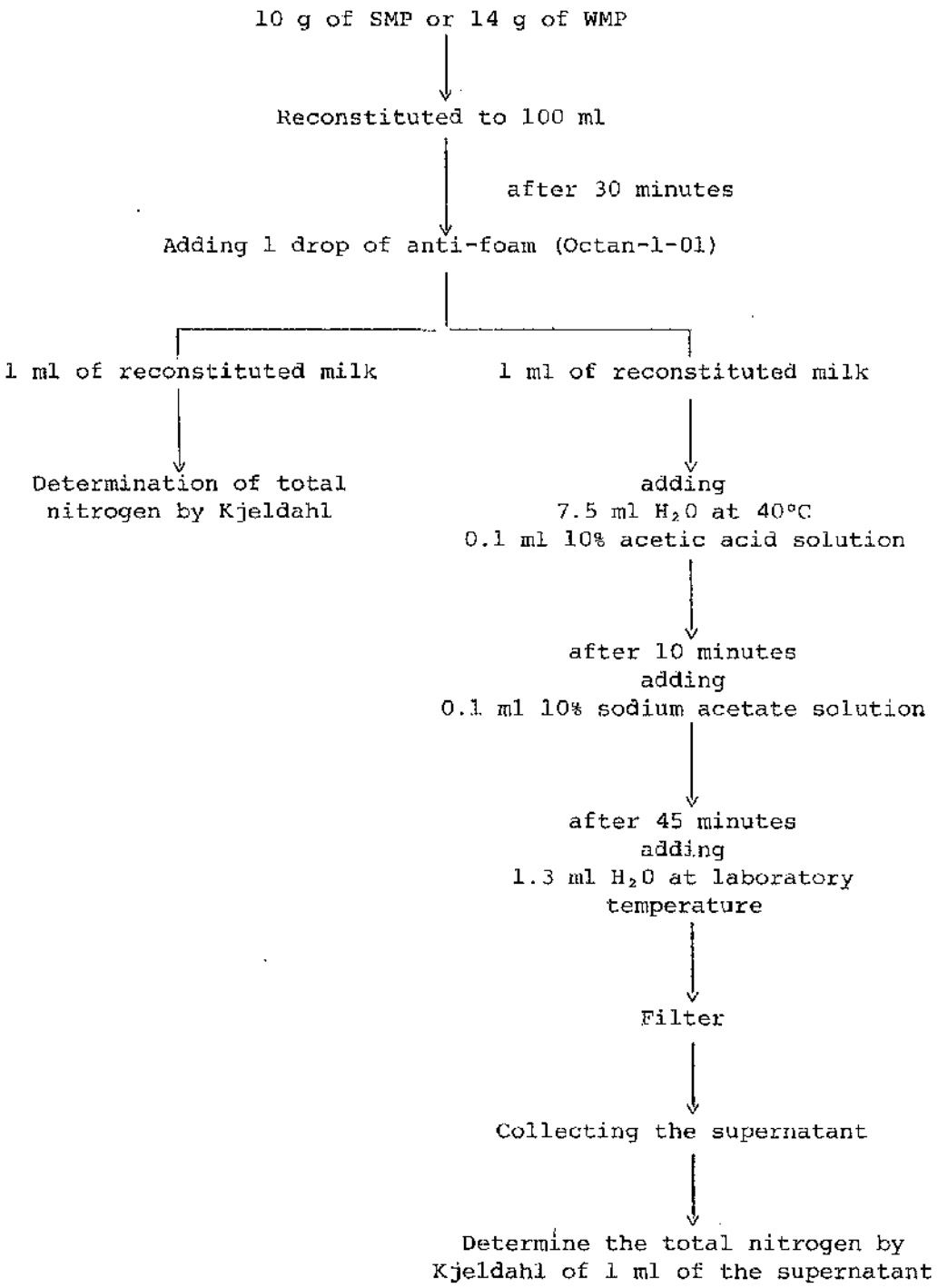
where,

a = evaporation residue from 25 ml of solvent

b = g of powder used.

1.4.2.6 Heat number

The determination of the heat number was based upon the IDF Standard Method 114 (IDF, 1982) according to the following scheme:



A blank sample was determined by Kjeldahl method.

$$H = 100 - \left[\frac{V_B - V_1}{V_B - V_2} \times 100\right]$$

H = heat number

V_B = the volume, in ml of the 0.02 N NaOH used in the Kjeldahl determination with the Blank

V_1 = the volume, in ml of the 0.02 N NaOH used in the Kjeldahl determination with the 1 ml supernatant multiplied by 10

V_2 = the volume, in ml of the 0.02 N NaOH used in the Kjeldahl determination with the 1 ml of reconstituted milk

1.4.2.7 Peroxide value

The determination of peroxide value of Anhydrous Milk Fat (AMF) was carried out by following the IDF Standard Method 74 (IDF, 1974).

a. Preparation of a Reference Curve

The reference curve was prepared according to the following scheme:

0.5 g iron powder was dissolved in about 50 ml of 10 N HCl

↓

add 1 to 2 ml of about 30% (m/m) H_2O_2

↓

remove the excess of H_2O_2 by boiling for 5 minutes

↓ <—— cool

dilute to 500 ml with H_2O

↓

1 ml of above solution transferred to measuring flask

↓

dilute with mixture of chloroform and methanol (4:1) to 100 ml

↓

transfer by means of burette to 10 cuvettes 0.1, 0.3, 0.5, 0.7, 0.9, 1, 1.25, 1.5, 1.75 and 2 ml respectively to obtain a series with 1,3, 5, 7, 9, 10, 12.5, 15, 17.5 and 20 µg of iron (III) ion

↓

add to these 10 cuvettes a 9.8, 9.6, 9.4, 9.2, 9, 8.9, 8.65, 8.4, 8.15 and 7.9 ml respectively the mixture of chloroform and methanol

↓

add to each cuvette 0.05 ml of ammonium thiocyanate solution

↓

add to each cuvette 0.05 ml of about 0.2 N HCl solution

↓ <—— wait 5 minutes

measure the extinctions at 500 mm against the mixture of chloroform and methanol

↓

plot the extinctions of the members of the series against the added amounts of iron (III) ion, expressed as µg Fe (III)

Figure 1:2 : Standard curve to measure the extinction of a series concentrations of µg Fe (III) by the spectrometer

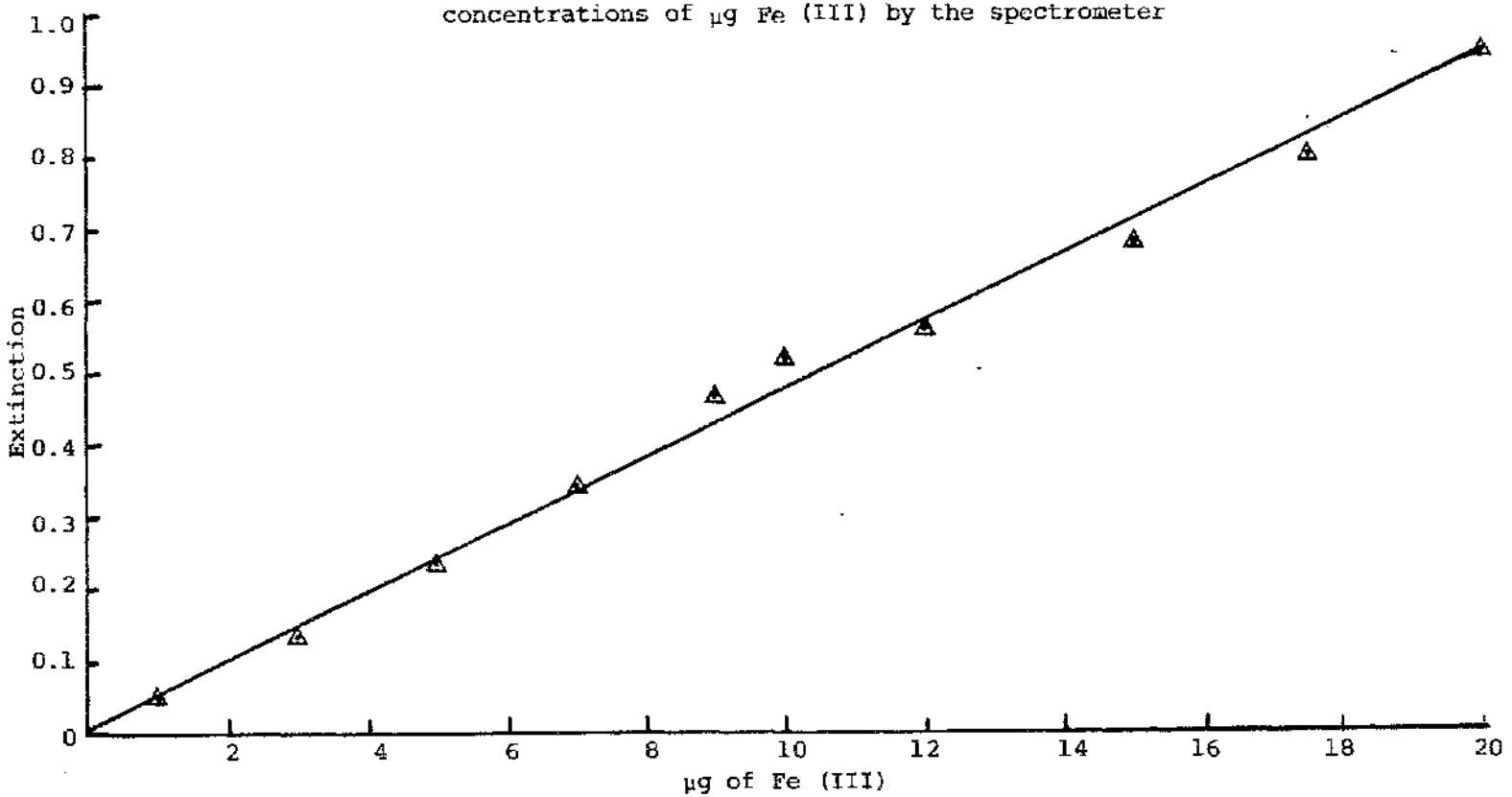


Plate 1:2 Milk processing equipment laboratory model homogeniser (left) connected in-line with H.T.S.T. pasteuriser (right)

µg of Fe (III)	1	3	5	7	9	10	12	15	17.5	20
Extinction	0.05	0.135	0.235	0.34	0.47	0.52	0.56	0.68	0.80	0.94
Factor	20	22.22	21.28	20.59	19.15	19.23	22.32	22.06	21.88	21.28

$$\text{Factor (F)} = \frac{\mu g \text{ Fe (III)}}{\text{Extinction}}$$

Mean value of the factor = 21.00 ± 0.86*

Standard deviation of factor values = 1.20

*At 95% confidence intervals for mean value

b. Peroxide value of the AMF

Firstly 0.3 g of AMF was dissolved in 9.6 ml mixture of chloroform and methanol, followed by the addition of 0.05 ml of the ammonium thiocyanate solution and 0.05 ml of ferrous chloride. After 5 minutes a photometric determination of the amount of ferric iron complex was carried out using the same procedure as for the reference curve.

$$\text{Peroxide value expressed as milligram equivalents/kg} = \frac{a}{55.84\ m}$$

a = the content of iron (III) in µg obtained from the sample

m = mass of test portion in grammes

The IDF method is only suitable for AMF having a peroxide value up to 1.0. For peroxide values in excess of 1.0, smaller weights of fat were used by dilution with the chloroform methanol mixture always ensuring that 9.9 ml were used for the extinction determination (Newstead & Headifer, 1981).

1.4.2.8 Acid value

The acid value of the AMF was determined according to the IDF Standard Method 6A (IDF, 1969). An 5 to 10 g sample of the AMF was dissolved in a 50 to 100 ml mixture of ethanol and diethyl ether and then titrated with 0.05 N alcoholic potassium hydroxide solution.

$$\text{Acid value} = \frac{vxt\ x\ 56.1}{a}$$

v = number of ml KOH solution

t = normality of KOH solution

a = mass in grams of test portion

1.4.2.9 Water content of AMF

The water content of the AMF was determined by the IDF Standard Method 23 (IDF, 1964), which depends on the Karl Fischer method. Water, either in the free or bound state in 10 g samples of AMF was converted into H_2SO_4 and HI by titration with a solution of SO_2 and I_2 in methanol and pyridine. The test was carried out by using the

Baird Tatlock AF 8 machine with accompanying calibration and calculation tables.

1.4.2.10 Milk solids-not-fat content of AMF

The MSNF content of the AMF was determined by the IDF Standard Method 11 (IDF, 1960). Initially 10 g of AMF were heated and then dissolved in 20-25 ml of light petroleum. The solution and the sediment were filtered and washed for five times by using a sintered glass filter crucible G.3 with a suction flask. Then the crucible was dried at 102 ±2°C for 2 hours to constant weight.

1.4.3 Microbiological tests

1.4.3.1 Colony count of dried milk

The IDF Standard Method 49 (IDF, 1970) was followed to determine the colony count of SMP and WMP. A series of dilutions of the sample (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) reconstituted at 47 ±2°C was made and 1 ml dilution mixed with Tryptone Glucose Yeast Agar (supplied by Oxoid) in petri dishes.
After incubation at 30 ±1°C for 72 hours, the colonies were counted.

1.4.3.2 Yeast and moulds count

The IDF Standard Method 94 (IDF, 1980) was used to enumerate the yeast and moulds of SMP and WMP. An agar medium was employed, in which organisms other than yeast and moulds was inhibited by using chloramphenicol. After incubation at 25°C, the colonies were counted.

1.4.3.3 Lipolytic count

The IDF Standard Method 41 (IDF, 1966) was used to determine the number of lipolytic organisms present in SMP and WMP. A sugar-free nutrient agar medium of pH 7.5, containing emulsified butter fat coloured with a small quantity of the fat soluble base of Victoria blue as an indicator, was used. The hydrolysis of butter fat yields free fatty acids and changes the base into the blue dye, so that colonies of lipolytic organisms were coloured blue. The colonies were counted after incubation at 30°C for 3 days.

1.4.3.4 Coliform count

The method of coliform count proposed by Harrigan & McCance (1976) was followed to determine the coliform count of SMP and WMP. The normal medium employed was violet red bile agar as a modified medium to give improved selectivity for, and definition of, coliform colonies. The colonies were counted after incubation at 35°C after 24 to 48 hours.

1.4.3.5 Thermoduric count

The British Standards Method B.S. 4285 (British Standards Institution, 1968) for the colony count of thermoduric organisms was used for SMP and WMP. A reconstituted sample of 10 g of powder in 90 ml of water was heated at 63.5 ±0.05°C in a water bath for 35 minutes, then cooled to 10°C. Dilutions (10^{-2}, 10^{-3}, 10^{-4}) were cultured on Tryptone Glucose Yeast Agar (supplied by Oxoid)
and the colonies counted after incubation at 30 ±1°C for 72 hours.

1.4.3.6 Psychrophilic count

The British Standards Method B.S. 4285 (British Standards Institution, 1968) for the colony count of psychrophilic organisms was used.
Tryptone Glucose Yeast Agar (supplied by Oxoid)
was employed as before but with incubation at 5 to 7°C for 7-10 days.

1.4.3.7 Proteolytic count

The proteolytic bacteria were determined in SMP and WMP by using an improved agar medium for the detection of proteolytic organisms proposed by Martley, Jayashankar & Lawrence (1970). This medium has been developed by adding sodium caseinate (1% w/v), sodium citrate (0.015 M) and Ca^{2+} (0.02 M) to Standard Method agar. Its greater sensitivity compared with existing milk agar media is related to its ability to detect the first stage of casein breakdown as shown by the formation of a white zone of precipitation.

1.5 Chemical and Microbiological Test of the Liquid Milk

A series of analyses for fresh, reconstituted and recombined milk were made before and after pasteurisation during each processing trial.

1.5.1 Total solids

The total solids content was determined by drying at 102 ±2°C according to the IDF Standard Method 21 (IDF, 1962).

1.5.2 Fat

The fat content was determined by Gerber method according to B.S. 696, Part 2 (British Standards Institution, 1969).

1.5.3 Phosphatase test

The phosphatase test for the pasteurised milk was determined according to the IDF Standard Method 82 (IDF, 1978). The liquid sample or the reconstituted sample was diluted with a buffer substrate at pH 10.2 and incubated at a temperature of 37°C for 2 hours. Any alkaline phosphatase present in the sample liberated, under these circumstances, p-nitrophenol from added disodium p-nitrophenyl phosphate. The p-nitrophenol liberated was measured by direct comparison with standard colour glasses in a simple comparator.

1.5.4 Total count

The total count for milk was determined according to the IDF Standard Method 3 (IDF, 1958).

1.5.5 Coliform count

The same method of 1.4.3.4 was followed.

1.5.6 Yeast and mould

The same method of 1.4.3.2 was followed.

1.6 Additional Tests for the Solubility Index of WMP and Scorched Particles of SMP

Further study was carried out on the solubility index sediment of WMP and the scorched particles of SMP to their increase through the storage time.

1.6.1 Solubility index sediment

Sediment from 16 centrifugal tubes each containing 50 ml of

reconstituted WMP were precipitated according to the method of ADMI (1971 b). These sediments were washed and centrifuged 5 times with distilled water and were then collected in a sample jar after having been suspended in distilled water. The suspension was examined for total solids according to the IDF Standard Method 21 (IDF, 1962); the fat content according to the IDF Standard Method 1A (IDF, 1969) which is based on the Rose-Gottlieb procedure and total nitrogen content according to the improved micro-Kjeldahl method of the Association of Official Agricultural Chemists (1965).

1.6.2 Scorched particles

Scorched particles form the SMP were collected on a standard cotton disc of known dry weight, according to the method of ADMI (1971 c). The particles were washed 5 times with distilled water then they were dried at 102 ±2°C in a hot air oven for 4 hours. Finally, the total nitrogen content was determined for the particles according to the improved micro-Kjeldahl method referred to above.

CHAPTER TWO

QUALITY CHANGES DURING THE STORAGE OF RAW MATERIALS USED FOR THE PRODUCTION OF RECOMBINED OR RECONSTITUTED MILK

2.1 Physical Changes

2.1.1 Moisture content

The moisture content of milk powder is defined by Woodhams & Murray (1974) as the part of the water contained by the solid which is in a form capable of taking part in deterioration of the powder.

Results: the moisture content of SMP and WMP showed a steady increase throughout the storage time, and these increases were more noticeable as the storage temperatures increased as shown in Figures 2:1 and 2:2 and Tables 2:1 and 2:2.

Discussion: the increases of moisture content of the powder through the storage time can probably be explained by increases in the permeability of the film packaging material and the increased diffusivity of the water vapour migrating through the powder as the storage temperature was increased (e.g. diffusivity of water vapour in air: $0°C = 21.8 \times 10^{-6}$ m^2/s; $40°C = 27.5 \times 10^{-6}$ m^2/s (Loncin & Merson, 1979).

This capability of the powder to gain moisture from the atmospheric conditions was confirmed by Woodhams *et al*. (1974) when they suggested producing powder from the drier at a lower moisture content than the specification so that the powder would remain within specification after some gain in moisture content during pneumatic conveying and blending, and to a lesser extent during storage.

Luquet, Mouillet, Boudier & Vincent (1982) reported that the moisture content of powder increased even at "very good" conditions of storage.

2.1.2 Bulk density

Bulk density is a measure of the mass of powder which occupies a fixed volume. The costs of packaging and shipping are both directly

TABLE 2:1

Variation of moisture content (expressed as a percentage) of SMP during storage at different temperatures

Storage temperature	Initial	Storage time/months			
		3	6	9	20
-18°C	2.61	2.62	2.65	2.69	3.19
4.5°C	2.61	2.72	2.91	2.99	3.57
11.5°C	2.61	2.91	3.08	3.10	4.01
22.5°C	2.61	3.07	3.18	3.33	4.17

Mean value from 2 trials

TABLE 2:2

Variation of moisture content (expressed as a percentage) of WMP during storage at different temperatures

Storage temperature	Initial	Storage time/months			
		3	6	9	20
-18°C	1.35	1.35	1.46	1.56	1.83
4.5°C	1.35	1.42	1.65	1.71	2.58
11.5°C	1.35	1.65	1.75	2.17	2.94
22.5°C	1.35	1.71	1.80	2.23	3.09

Mean value from 2 trials

Figure 2:1 Variation in the moisture content of SMP during storage at different temperatures

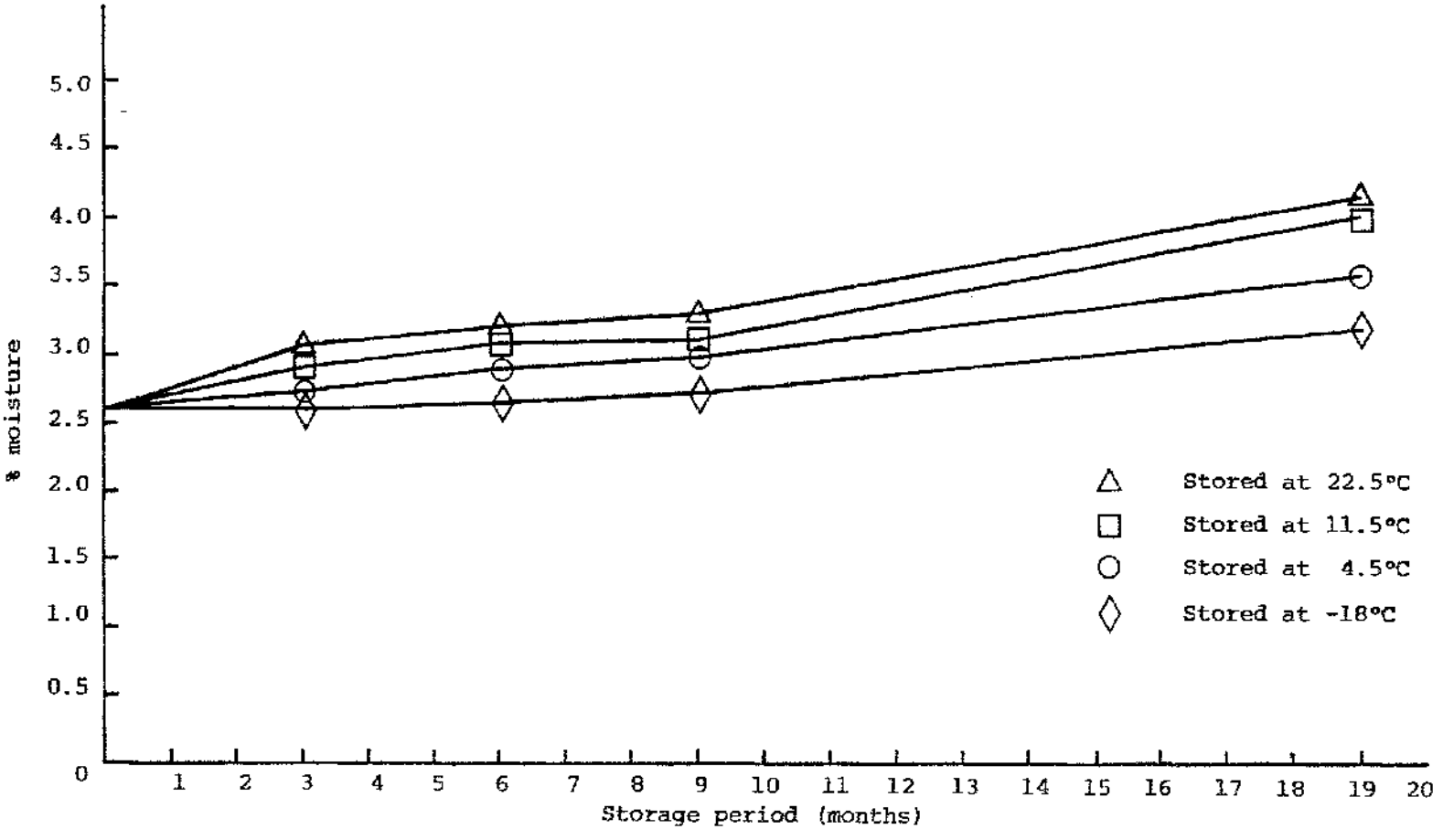
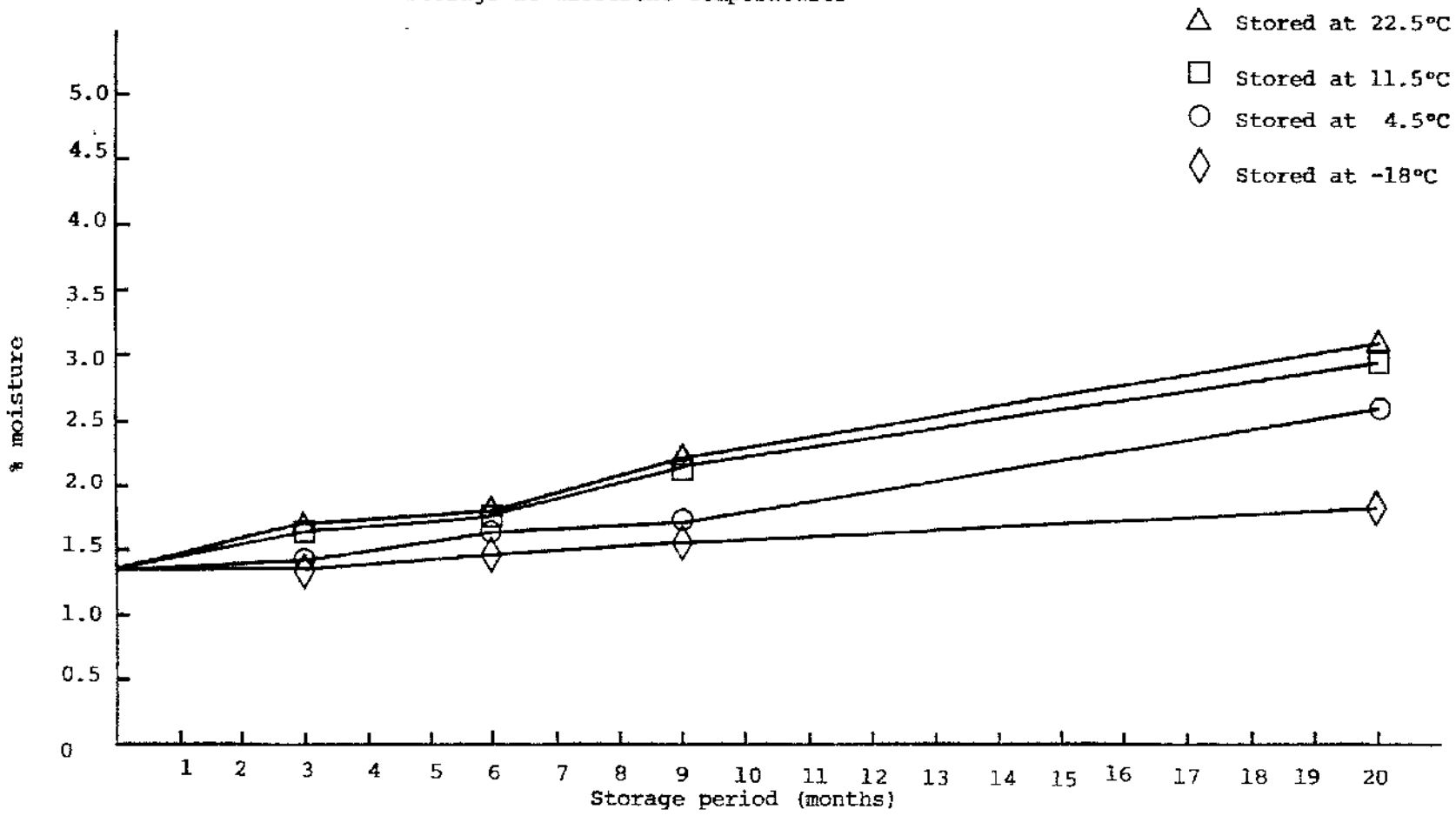


Figure 2:2 Variation in the moisture of WMP during storage at different temperatures



related to the bulk density. A powder with a low bulk density requires a larger bag to contain 25 kg and occupies more shipping space per tonne than a powder with a higher bulk density.

Results: the bulk density of the powder showed no change throughout the storage time at all temperatures and remained at a level of 0.69 g/ml for SMP and 0.54 g/ml for WMP.

Discussion: the bulk density of the WMP was always less than that of the SMP. These results were expected since the density of the fat in WMP is less than that of the SNF fraction in the SMP. The bulk density of powder also depends on the amount of air occluded within the particles and the way in which these particles are packed together. Sanderson (1978) reported that the bulk density of the instant product is relatively low due to the narrow size range of the prous particle agglomerates which contain a high proportion of vacuoles and capillaries. Crossley (1962) pointed out that the bulk density of normal spray powder may vary between 0.5 and 0.8 g/ml.

The absence of change in the bulk density figures can be explained by the relatively small amount of materials handling. In handling commercial powders, the formation of fine powder particles can increase the bulk density by decreasing the amount of free space not occupied by powder. Sanderson (1978) reported that the stability of the particles agglomorates were affected during storage. Agglomerate breakdown results in the formation of fine particles.

2.1.3 Dispersibility

Dispersibility is defined as a percentage by mass of the dry matter of the sample that can be dispersed in water (IDF, 1979).

Results: better dispersibility was found with the instant WMP at a level of 95% than the ordinary SMP which was at a level of 90%. The result showed decreases in the dispersibility of both WMP and SMP throughout the storage time and these decreases were more pronounced at higher storage tempertures as shown in Figures 2:3 and 2:4 and Tables 2:3 and 2:4.

Discussion: the results showed better dispersibility of instant WMP than the ordinary SMP, due to the fact that the water is more easily

TABLE 2:3

Variation of dispersibility (expressed as a percentage) of SMP during storage at different temperatures

Storage temperatures	Initial	Storage time/months			
		3	6	9	20
-18°C	90.0	86.7	80.9	75.7	60.3
4.5°C	90.0	85.5	80.2	75.5	58.0
11.5°C	90.0	82.1	76.7	72.3	49.0
22.5°C	90.0	79.3	75.6	71.7	44.9

Mean value from 2 trials

TABLE 2:4

Variation of dispersibility (expressed as a percentage) of WMP during storage of different temperatures

Storage temperature	Initial	Storage time/months			
		3	6	9	20
-18°C	95.0	92.3	90.5	88.7	70.6
4.5°C	95.0	91.5	89.4	87.1	65.8
11.5°C	95.0	90.0	87.7	85.9	64.3
22.5°C	95.0	89.2	85.6	81.5	61.0

Mean value from 2 trials

Figure 2:3 Variation of dispersibility of SMP during storage at different temperatures

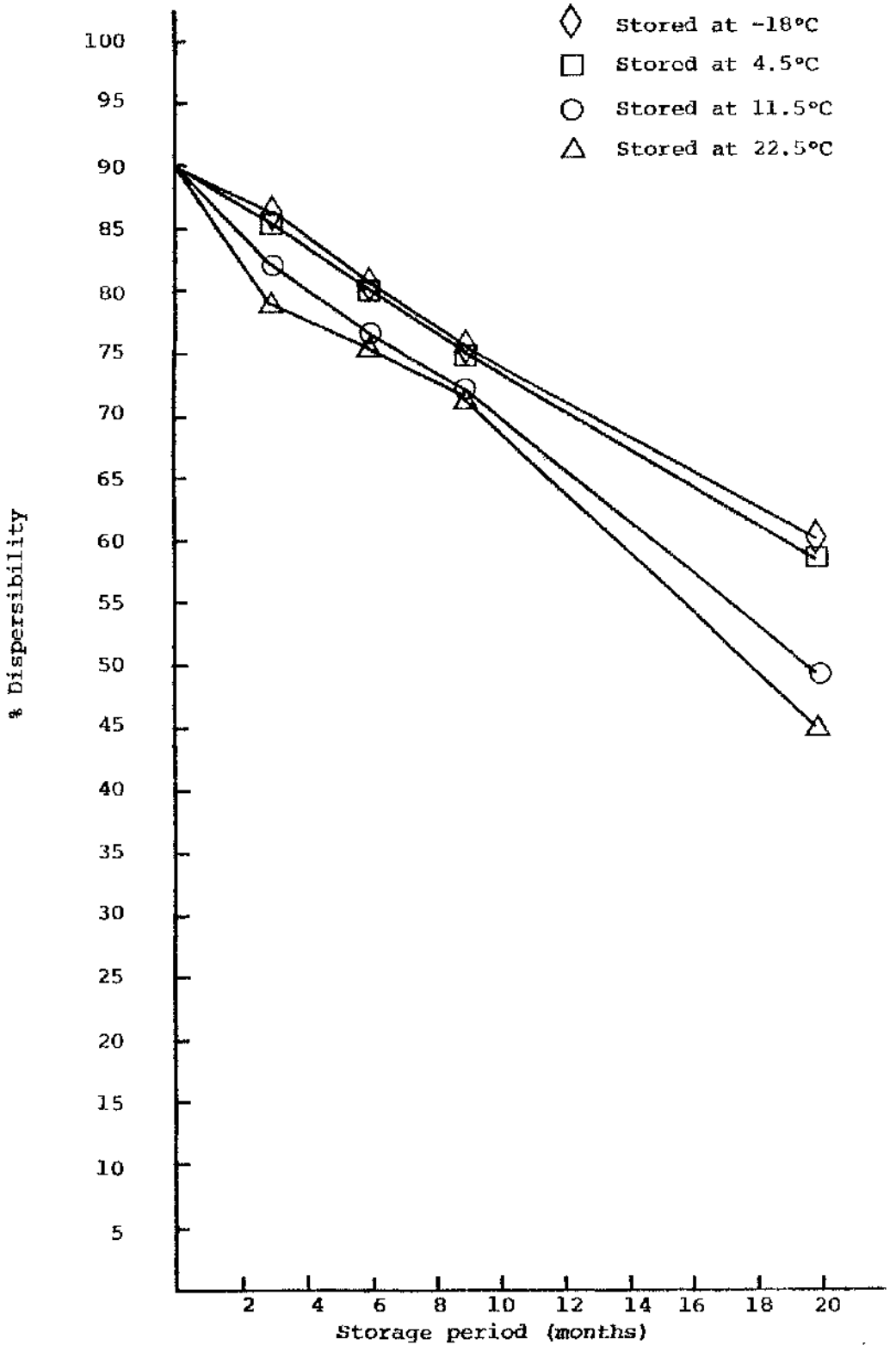
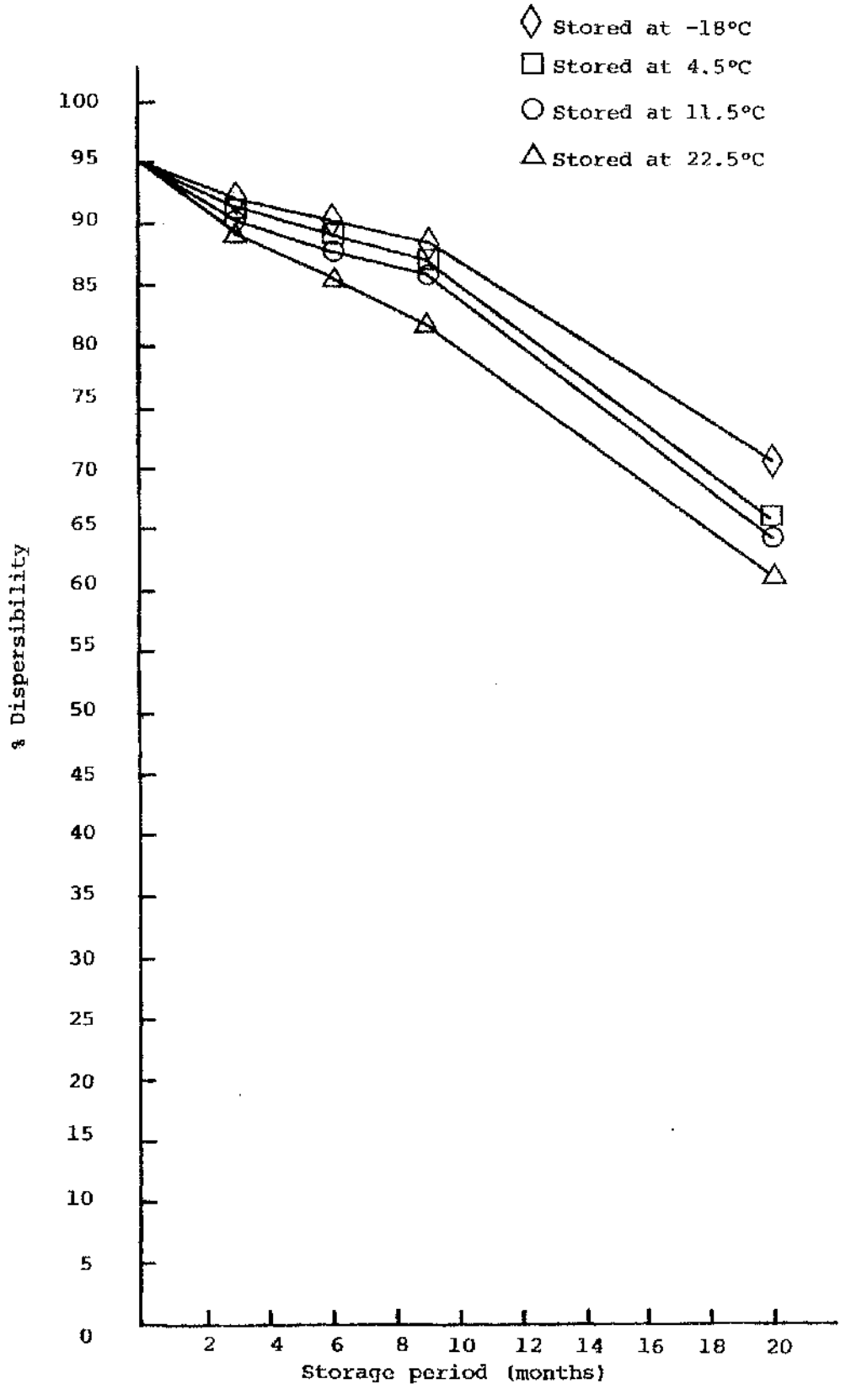


Figure 2:4 Variation of dispersibility of WMP during storage at different temperatures



able to penetrate the lump of large particles and to cause it to disperse as in the case of instant WMP which passes through an agglomeration process than the small particles which have the tendency to be cemented by a thick layer of wetted particles as in the ordinary powder which has not passed through an agglomeration process. Baldwin & Woodhams (1974) confirm that large particles in general are easier to disperse than small particles. Baldwin & Sanderson (1973; 1974) showed that increased particle size gave increased dispersibility. Lascelles et al. (1976) reported that dispersibility improved as the percentage of fine particles below 90 ml decreased.

The results also showed a decrease in dispersibility of powder throughout the storage time which can be explained by the formation of insoluble material which does not disperse in water as a result of a chemical reaction between the protein and the fat in case of WMP. This has been confirmed by many workers (Mol, 1975). In the case of SMP, the formation of these materials is most probably due to a reaction between the protein and lactose as has been confirmed by Crossley (1962) and Luquet et al. (1982). Chemical reaction rates are normally accelerated by the elevation of temperature, which would explain the greater decreases in dispersibility with higher storage temperatures.

Sanderson (1978) confirmed the decrease in dispersibility of instant powder due to the breakdown of agglomerate which results in the formation of fine particles. However, the lack of variation in the bulk density of both WMP and SMP during storage would seem to preclude this possibility in this work.

It should be pointed out that the manual operations involved in the IDF dispersibility method (IDF, 1979) lead to the possibility of variable results between test centres. Comparisons would be best made when the tests have been carried out by the same person.

2.1.4 Wettability

Is defined by the IDF Standard Method 87 (IDF, 1979) as the time, in seconds, required for all the particles of an instant dried milk to become wetted when placed on the surface of water at 25°C. Manufacturing instant WMP requires the agglomeration

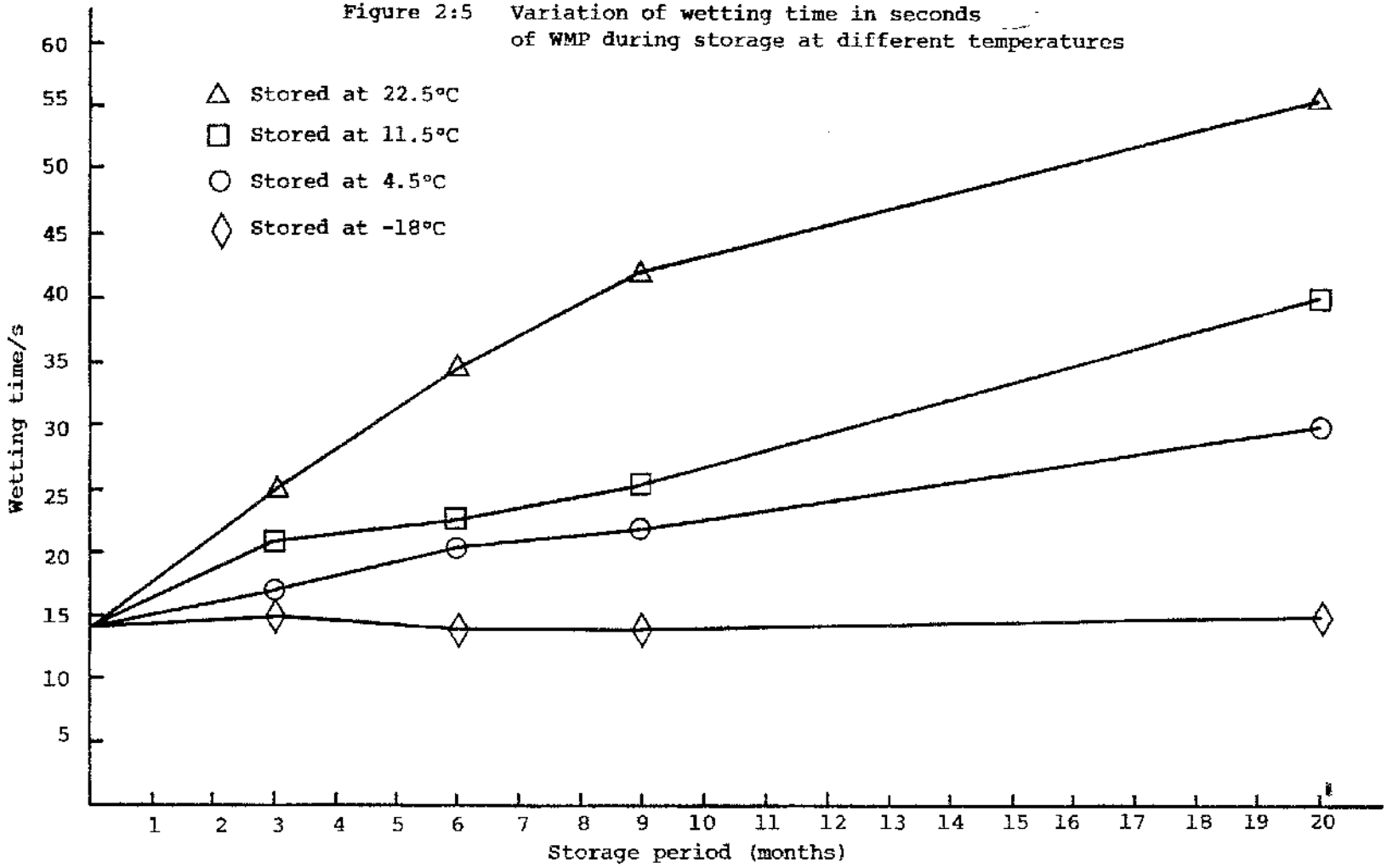
TABLE 2:5

Variation of wetting time in seconds of WMP during storage at different temperatures

Storage temperature	Initial	Storage time/months)			
		3	6	9	20
-18°C	14	15	14	14	15
4.5°C	14	17	20.5	22	30
11.5°C	14	21	22.5	25.5	40
22.5°C	14	25	34.5	42	55.5

Mean value from 2 trials

Figure 2:5 Variation of wetting time in seconds of WMP during storage at different temperatures



process to produce large particles followed by the coating of the surface of the powder with a lecithin-based wetting agent.

Results: the results of wetting time for the instant WMP showed an increase throughout the storage time at all storage temperatures except for the freezer storage. These increases were more noticeable at the higher storage temperature, as is shown in Figure 2:5 and Table 2:5.

Discussion: the increases of wetting time throughout the storage time could be explained by the low mechanical stability of the agglomerates which can be broken due to handling and transportation or some other physical treatments. However, the constant bulk density would not seem to support this explanation. The wettability can also be affected by the exposure of powder surfaces which are not coated with the wetting agent. Sanderson (1978) confirmed the deterioration in the wettability storage. This was not fully understood, although Sanderson (1978) mentioned the probability of either the migration of the surface layer of lecithin into the powder particles or the movement of free fat from within the particle to the surface.

2.1.5 Solubility index

The solubility index is the ability of the powder to dissolve in water. Pisecky (1980) reported that even the excellent solubility index (below 0.1 ml) does not represent any guarantee that the powder is sufficiently dissolved at the time of consumption.

Results: the solubility index of the WMP was constant at a level of less than 0.05 ml up to 9 months at all storage temperatures, whereas an increase in the solubility index of WMP was evident after 20 months storage time, as indicated below:-

Storage temperature	Solubility index
-18°C	0.05 ml
4.5°C	0.15 ml
11.5°C	0.25 ml
22.5°C	0.4 ml

At the same time the solubility index of SMP remained constant at a

level of less than 0.05 ml throughout the storage time at all storage temperatures. The results of studying the dry sediments obtained in solubility index test of WMP by means of chemical analysis showed a fat content of 52.3% and protein content of 34%.

Discussion: the formation of insoluble material in the WMP has been studied by many workers including Ashworth et al. (1957), Julien et al. (1957), Samuels et al. (1960) and Mol (1975). Their results showed the noticeable formation of insoluble materials during the storage of WMP for long times. These insolubles precipitate during the centrifugation of the reconstituted WMP in the solubility index test. The formation of this material was accelerated by raising the storage temperatures. The chemical analysis of these materials showed much similarity to the results of Mol (1975). The absence of these materials from the SMP throughout the storage time could be explained by the very low amount of fat in the SMP which is needed with the protein to form these insoluble materials.

2.1.6 Scorched particles

Scorched particles in milk powders are the over-heated or burnt particles which range from light brown to black in colour.

Results: the scorched particles in WMP remained constant at a level of grade A (ADMI, 1971) throughout the 20 months storage at all temperatures. At the same time an apparent increase in scorched particles was found in the SMP at all storage temperatures in a very short time (Plate 2:1). The scorched particles of SMP went from grade B to grade D after 9 months of storage at all temperatures. The chemical analysis of the scorched particles of SMP showed that about a third of the dry weight of these particles was protein.

Discussion: scorched particle formation may arise in a number of ways through the processing of the dried milk. The results showed an increase in these particles after post-processing storage of SMP. This could be explained by the formation of insoluble material resulting from the chemical combination of protein and lactose to form Maillard-type yellowis granules which cannot pass through the filter disc used in the scorched particles test. Luquet et al. (1982) confirmed that Maillard reactions progress with the age of the skim milk powder.

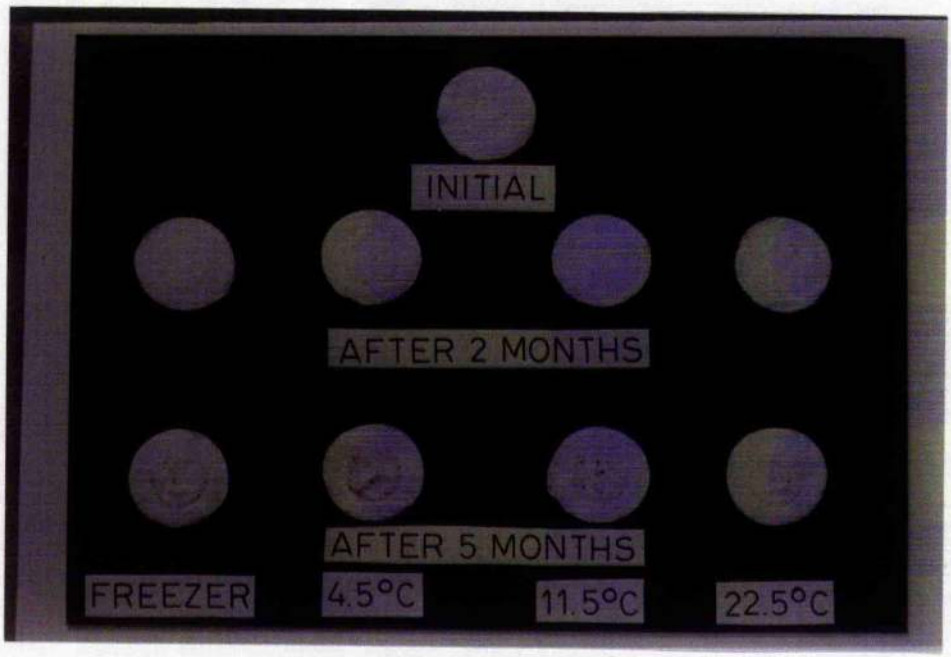


Plate 2:1 Scorched particle test on skimmed milk powder after storage for 2 and 5 months at various temperatures

The unavailability of the protein in the WMP probably results from its interaction with the fat, making the formation of protein-lactose material less likely during storage.

2.2 Chemical Changes

2.2.1 **Acidity** is related to the quantity of 0.1 N NaOH required to titrate a given amount of reconstituted milk with phenolphthalein as an indicator.

Results: the titratable acidity (expressed as a percentage of lactic acid) was not changed throughout the storage time at all temperatures for both SMP and WMP and remained at a level of 0.135%.

Discussion: the low level of water content of the dried milk is the main factor preventing any microbial development which might increase the acidity of the dried milk. Crossley (1962) pointed out that micro-organisms do not proliferate in dried milk, and storage defects are purely chemical in nature.

2.2.2 Total protein

It is based on the determination of total nitrogen in dried milk (expressed as protein).

Results: the total protein content of the powder was not changed throughout the storage time at all storage temperatures and remained at a level of 36.2% for SMP and 26.5% for WMP.

Discussion: the above results meet the specification for raw material for recombined products proposed by Jesen, Andersen & Nielsen (1983). The higher amount of protein in SMP is due to the low amount of fat in the SMP in comparison to the WMP. The protein content remained constant as was expected.

2.2.3 Fat content

The fat content of dried milk means the total content of fat and fatty substances expressed as a percentage by weight obtained by the Rose-Gottlieb method.

Results: ageing of the powder at all storage temperatures did not cause any change in the total amount of fat content of SMP (0.90%) and the WMP (28.7%).

Discussion: the above results meet the specification for raw material for recombined products proposed by Jensen et al. (1983). The low level of fat content in SMP has many advantages over the high level of fat in the WMP especially in relation to storage keeping quality, due to the flavour deterioration of WMP during the storage as a result of the fat oxidation and hydrolysis.

Coulter et al. (1948) showed that the general flavour stability of instant WMP and other powders containing fat is effected, primarily by the availability of oxygen and temperature conditions prevailing during storage. Crossley (1962) mentioned that oxidation of the fat leading to the production of flat, and finally marked tallowy flavours is a major storage defect of full-cream powder. Crossley (1962) also mentioned that true rancidity resulting from the hydrolysis of fat to free fatty acids such as butyric acid was formerly not uncommon.

2.2.4 Heat number is defined as the ratio of casein plus heat-denatured milk serum protein to total nitrogen multiplied by 100.

Results: the value of the heat number was unchanged throughout the storage time at all storage temperatures and was at a level of 83.5% for SMP and at 85.8% for WMP.

Discussion: according to the IDF Standard 114 (IDF, 1982) both the SMP and WMP would be classified as medium-high heat powders. The low water content of the dried milk probably prevented any enzymatic activity for protein proteolysis which kept the distribution of nitrogen constant throughout the storage time. Kieseker & Clark (1982) confirmed that no change occurred in the nitrogen distribution during storage of non-fat dried milk.

2.2.5 Fat on the surface of WMP particles

Free fat in dried milk is defined by Buma (1971a) as that part of the fat which can be extracted with organic solvents under standardised conditions. The value of the surface free fat is affected by the method of determination, such as the kind of solvent, the mixing ratio, duration of extraction and, above all, the extent of stirring the powder suspension after adding the solvent immediately, which can increase the free fat considerably.

Results: the free fat content of the WMP remained constant at a level of 1.25% throughout the storage time at all temperatures.

Discussion: The free fat content remained constant due to the absence of severe mechanical action on the powder during the storage causing an effect on the particle size. Buma (1971b) suggested that processing parameters such as particle size will influence the free-fat content of spray-dried WMP. Probably the storage temperatureswere never sufficiencly high for significant movement of the free fat from the surface. The previous findings that the wettability decreased with storage time and temperature, seem to indicate that it was the lecithin which diffused from zones of high concentration to regions of fat in the interior of the powder particle.

2.2.6 Peroxide value of AMF

The peroxide value is defined by the IDF Standard 74 (IDF, 1974) as the number of milligram-equivalents of oxygen per kilogram of AMF.

Results: there were no changes in the peroxide value of AMF stored at -18°C for 20 months. However, there were increases in the peroxide value at all other storage temperatures throughout the storage time and these increases were more noticeable at higher storage temperatures as is shown in Figure 2:6 and Table 2:6.

Discussion: The peroxide value is the parameter measuring the oxidation of the fat due to the chemical reaction of oxygen and unsaturated fatty acids. The oxidation of fat can be accelerated by increasing the temperature and exposure light. The result showed that no oxidation happened at a storage temperature of -18°C. At the same time fat oxidation occurred at all other storage temperatures and it was faster at the higher temperature. The peroxide value went higher than the recommended value of the (IDF, 1977) (maximum 0.2 mEq O_2 /kg) even at storage temperatures below the IDF recommended storage temperature of 10°C in IDF Standard 68A (IDF,1977). Badings (1970) found that oxidation of butter occurred at -10°C.

2.2.7 Acid value of AMF

The acid value is defined by IDF Standard 6A (IDF, 1969) as the number

TABLE 2:6

Variation of peroxide value (mEq O_2/kg fat) of AMF during storage at different temperatures

Storage temperature	Initial	Storage time/months			
		3	6	9	20
-18°C	0.104	0.107	0.105	0.103	0.104
4.5°C	0.104	0.112	0.113	0.115	0.63
11.5°C	0.104	0.194	0.253	0.361	1.16
22.5°C	0.104	0.243	0.357	0.612	5.11

Mean value from 2 trials

TABLE 2:7

Variation of acid value (mg of kOH/g fat) of AMF during storage at different temperatures

Storage temperature	Initial	Storage time/months			
		3	6	9	20
-18°C	0.37	0.37	0.37	0.37	0.37
4.5°C	0.37	0.37	0.37	0.37	0.37
11.5°C	0.37	0.37	0.37	0.38	0.39
22.5°C	0.37	0.40	0.44	0.46	0.55

Mean value from 2 trials

Figure 2:6 Variation of peroxide value (mEq O_2/kg fat) of AMF during storage at different temperatures

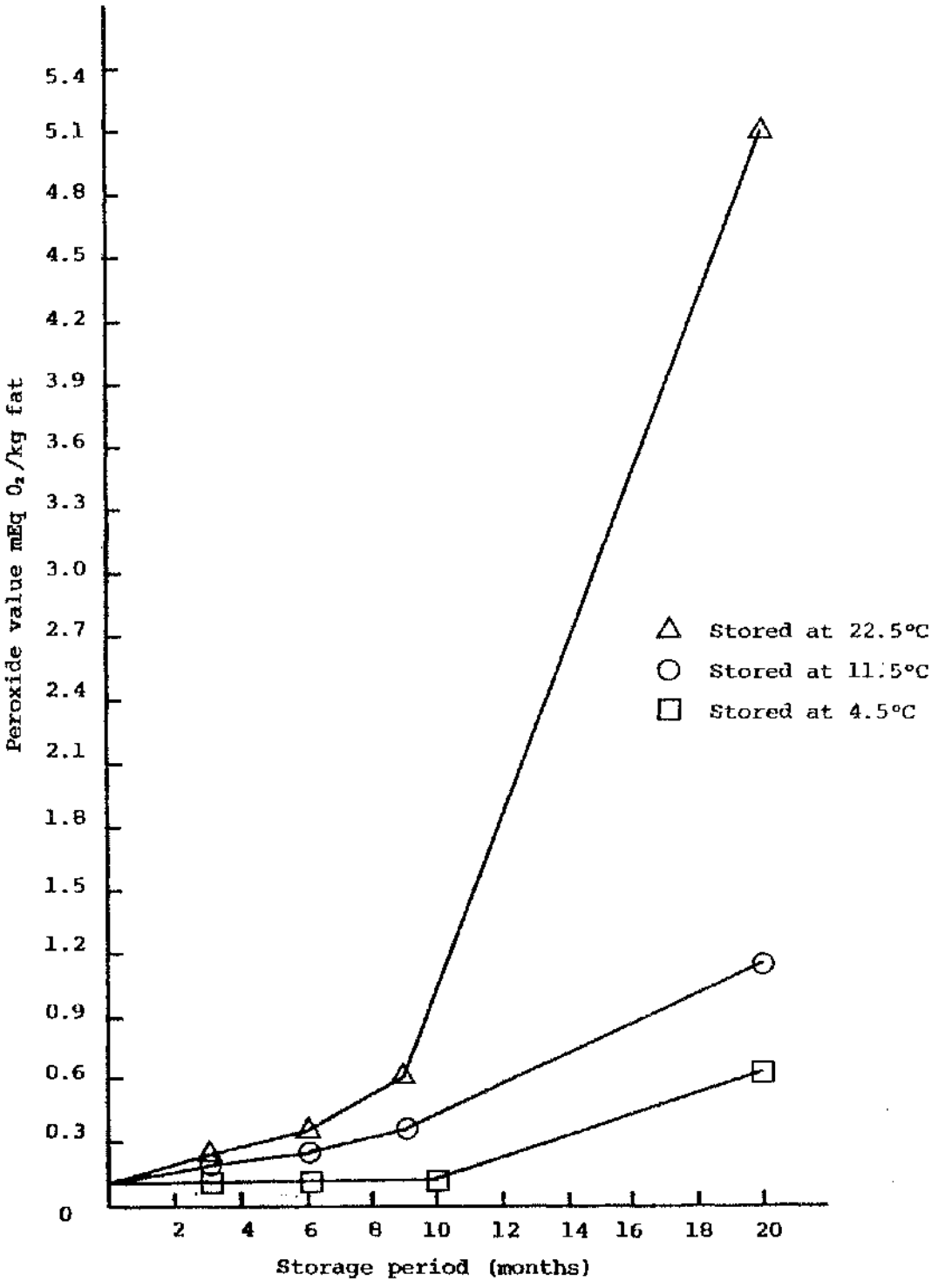
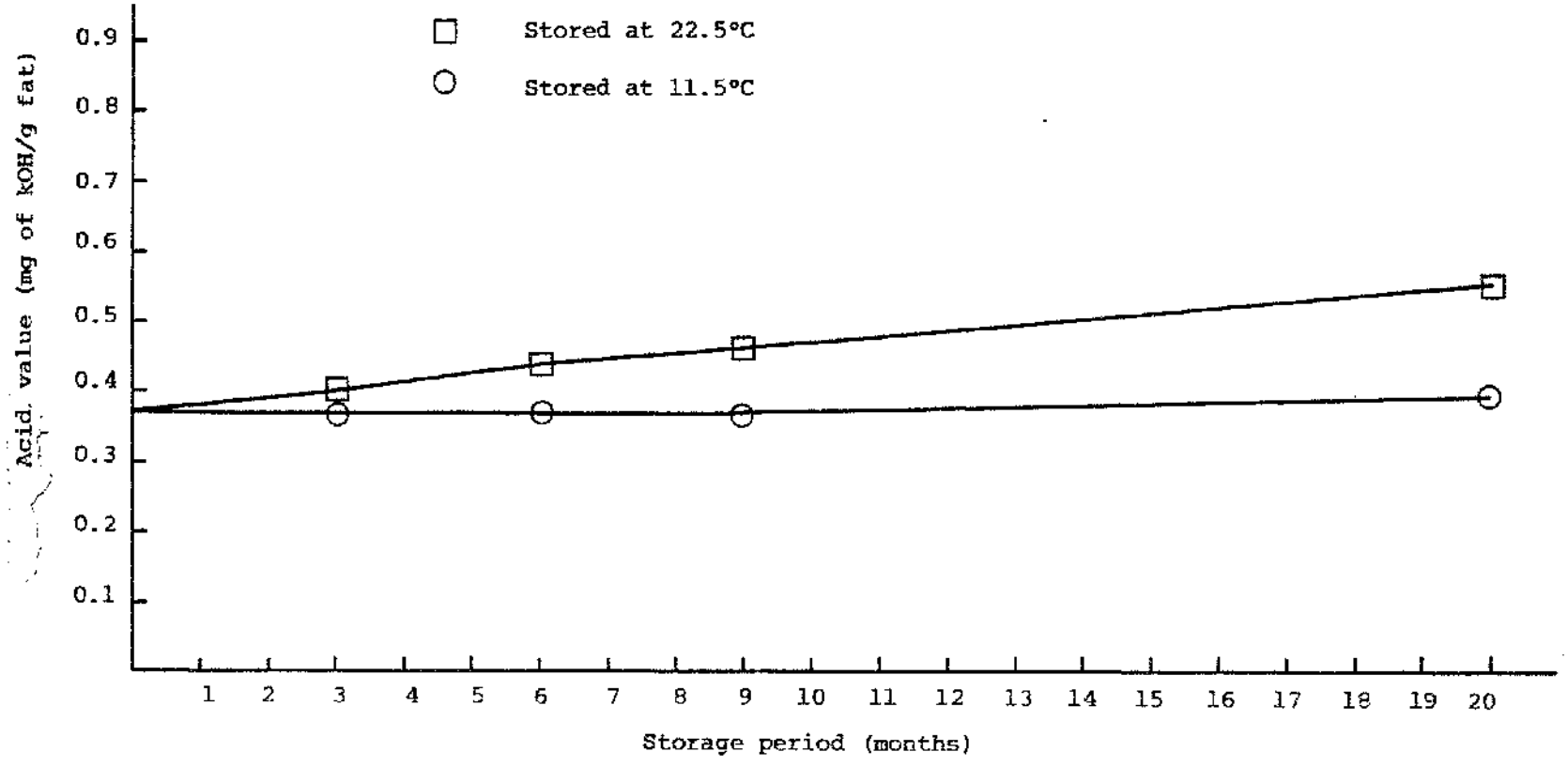


Figure 2:7 Variation of acid value (mg of kOH/g fat) of AMF during storage at different temperatures



of milligrammes of KOH required to neutralise one gram of the fat.

Results: the acid value of AMF kept constant at storage temperatures of -18°C and 4.5°C, with little increase at 11.5°C but a more noticeable increase at the storage temperature of 22.5°C, as shown in Figure 2:6 and Table 2:6.

Discussion: The acid value is the parameter measuring the amount of fat hydrolysis which occurs due to the activity of native lipase and microbial lipases. Many bacterial lipases differ from milk lipase in that they are not inactivated by pasteurisation, so they can be carried through in an active form into a manufactured product. This can cause fat breakdown during storage of the products (Deeth et al., 1976). Therefore, the lipolysis of the AMF is probably due to the activity of bacterial lipase. The results of 22.5°C, which means that the activity of these enzymes was stopped at a lower temperature and only started to be active at the higher temperature. O'Donnell (1975) confirmed that the majority of lipases exhibit maximum activity within a temperture range 30°C to 40°C. Connolly et al. (1980) reported that butter stored at +15°C showed increased FFA with time. In contrast butter stored at -18°C showed little, if any, change in FFA level.

2.2.8 Water content of AMF

The percentage by weight of water determined in the AMF by the IDF Standard Method 23 (IDF, 1964).

Result: There was no change in the water content of AMF which remained at a level of less than 0.1% throughout the storage time at all storage temperatures.

Discussion: the level of water content in the AMF was inside the IDF specification for AMF (0.1% maximum). The constant level of water content throughout the storage of AMF expresses the ability of this fat in preventing any gain in water during storage.

2.2.9 The solids-not-fat content of AMF is the residue of non-fat substances expressed as per cent by weight.

Results: there were no changes in the solids-not-fat content of AMF which remained at a level of less than 0.1% throughout the storage

time at all storage temperatures.

Discussion: the level of solids-not-fat in the AMF was inside the IDF specification. (IDF, 1977)

2.3 Microbiological Changes

2.3.1 Total count

The number of microbial colonies per gram of dried milk was obtained by the IDF Standard 49 (IDF, 1970).

Results: the total count was unchanged throughout the storage time at all storage temperatures for the SMP (at a level of about 1500/g) and for WMP (at a level of about 700/g).

Discussion: the level of total count was far below the maximum value of specification for both SMP and WMP (Jensen et al., 1983). This count depends on the quality of the milk which is used for the manufacture of the powder and the heat treatment of preheating the milk through the processing, besides the prevention of any contamination through the processing and after the processing. The SMP and WMP were classified as medium-high heat powder and that means they should have received a heat treatment sufficient to reduce the number of the total count. That the total count remained throughout the storage time was due to the low level of water content in the powder which prevented any bacterial growth. Lea et al. (1943) pointed out that at the low moisture content of the milk powder, microbial spoilage during storage will not occur unless water is absorbed by exposure to air. Luquet et al. (1982) confirmed that microbiological stabilisation was noted for SMP during storage.

2.3.2 Thermoduric count

The number of colonies per gram of powder obtained by the British Standards Method for thermoduric count B.S. 4285 (British Standards Institution, 1968).

Results: the thermoduric count was unchanged throughout the storage time at all storage temperatures for the SMP (at a level of 1200/g) and for WMP (at a level of 550/g).

Discussion: the thermoduric count was a high proportion of the total count, probably because the SMP and WMP were medium-high heat powders. The low level of water content in the powder prevented the growth of these bacteria throughout the storage time. Crossley (1962) pointed out that the flora of spray-dried milk is a specialised one and predominantly thermoduric.

2.3.3 Psychrotrophic count

The number of colonies per gram of powder was obtained by the British Standards Method for psychrophic bacteria B.S. 4285 (British Standards Institution, 1968).

Results: there was no change of psychrotrophic count at zero level for both SMP and WMP throughout the storage time at all storage temperatures.

Discussion: the heat treatment of the powder during manufacture was probably adequate to kill all the psychrotrophic bacteria during processing. The results showed no post-processing contamination by these bacteria. Deeth et al. (1976) pointed out that psychrotrophic organisms are destroyed by pasteurisation.

2.3.4 Lipolytic count

It is the number of colonies per gram of powder obtained by the IDF Standard 41 (IDF, 1966).

Results: The lipolytic count remained constant at a level of less than 6 lipolytic micro-organism per gram for both SMP and WMP throughout the storage time at all temperatures.

Discussion: the heat treatment kept the level of these bacteria very low and the low level of water content in the powder prevented any growth of all micro-organisms.

2.3.5 Proteolytic count

It is defined as the number of colonies per gram of powder obtained by the improved agar medium for the detection of proteolytic organisms in total bacterial count (Martley et al., 1970).

Results: the proteolytic count for SMP was constant at zero level.

For WMP, a constant level of proteolytic count of 2-4/g at a dilution of 10^{-2} was found up to nine months. There were no counts of these bacteria in powders after 20 months storage at all storage temperatures.

Discussion: the process heat treatment kept the level of these bacteria very low and the low level of water in the powder prevented any growth.

2.3.6 Coliform count of dried milk and AMF

The number of colonies per gram of powder as AMF obtained by the method of coliform count proposed by Harrigan & McCance (1976).

Results: the coliform count for SMP, WMP and AMF remained unchanged at zero level throughout the storage time at all storage temperatures.

Discussion: the heat treatment of the processing was adequate to kill all the coliform bacteria in SMP, WMP and AMF, and the results indicate that there was no contamination during the handling of these commodities or throughout the storage. Crossley (1962) pointed out that the pre-heating of the milk does ensure the destruction of any milk-borne pathogens which may be present in milk supply.

2.3.7 Yeast and mould

The number of colonies per gram of powder obtained by the IDF Standard 94 (IDF, 1980).

Results: the yeast and mould count remain constant at zero level for both SMP and WMP throughout the storage time at all storage temperatures.

Discussion: the heat treatment was adequate to kill all the yeasts and moulds in the milk. The results showed no sign of post-processing contamination. Nichols (1939) showed that the number of yeasts and moulds present in milk powder samples was found to be negligible.

CONCLUSIONS

1. The dispersibility of SMP and WMP decreased at all temperatures throughout the storage time and the decrease was more noticeable

in powders held at higher temperatures.

2. The wetting time of WMP increased at higher temperatures of storage.

3. The moisture content of SMP and WMP increased at all temperatures of storage and it was more pronounced at higher temperatures.

4. There was an increase in the level of scorched particles of SMP at all storage temperatures.

5. There was an increase in the solubility index of WMP and it was more noticeable at higher storage temperatures.

6. There were no changes in acidity, fat content, protein content, heat number for both SMP and WMP after storage at different temperatures.

7. There was no change in the microbiological condition of both SMP and WMP as a result of storage.

8. There were noticeable changes in the peroxide value of AMF at storage temperatures of 4.5, 11.5 and 22.5°C, but the change was more pronounced at higher temperatures.

9. There was a noticeable change in the acid value of AMF only at a storage temperature of 22.5°C.

10. No change in water content, solids-not-fat content, and coliform count of AMF occurred at all temperatures of storage.

11. There was no change in the peroxide and acid values of AMF stored at -18°C for 20 months.

CHAPTER THREE

THE EFFECT OF RAW MATERIALS ON THE ORGANOLEPTIC PROPERTIES OF RECOMBINED MILK

3.1 The Effect of Different Sources of Milk Fat on the Organoleptic Properties of Recombined and Reconstituted Milk

Pasteurised and homogenised recombined milk was prepared from SMP and three different sources of milk fat (AMF, unsalted butter, sweet cream). Reconstituted milk was prepared from WMP. A control batch was prepared on each occasion by the pasteurisation and homogenisation of raw fresh liquid milk under the same processing conditions.

The composition of recombined and reconstituted milk was in the range of 12 to 13% total solids and 3.5 to 3.7% fat. The control varied in composition from 11.6 to 12.5% total solids and 3.5 to 3.7% fat. All the milk showed a score of less than 6 for the phosphatase test after pasteurisation and they were free of coliform and yeast and mould with a total bacterial count of 200 to 400/ml for recombined milk and of 700 to 900/ml for the control milk.

The individual scores of each grader for each treatment in every 'method' were submitted to a statistical analysis of variance for each variate.

The term 'method' was used to differentiate between the four combinations of raw material (e.g. SMP + AMF, SMP + unsalted butter, SMP + cream and the WMP). The term 'treatment' was used to distinguish the five different levels (e.g. 0%, 25%, 50%, 75% and 100%) of fresh milk used for each organoleptic evaluation. The term 'variate' was used to differentiate between the five acceptability characteristics (i.e. mouthfeel, appearance, odour, flavour, and overall acceptability) and the four detectability characteristics (i.e. fattiness, chalkiness, acidity, and rancidity).

The statistical results of the organoleptic tests for the four different raw material combinations of recombined and reconstituted

TABLE 3:1:1

The mean value* of the scores for mouthfeel given by a Panel (10 graders) for (1) fresh pasteurised milk, (2) reconstituted WMP or recombined milk, (3) three blends of fresh milk + reconstituted WMP or recombined milk at different level. The recombined milk produced from SMP + 3 different sources of milk fat

% (v/v) of fresh milk in blend / Ingredients	100%	75%	50%	25%	0%
	5.99				
WMP		5.23	5.30	4.65	4.10
SMP + AMF		5.50	4.55	5.15	4.75
SMP + Butter		5.63	5.30	5.23	4.85
SMP + Cream		6.16	6.04	5.35	5.06

* mean value from 2 trials

	df	M.S	F
Method	3	14.7082	5.426*
Residual (Repeatability of expt)	4	2.7109	
Total	7	7.8526	
Grader	9	9.4691	9.424******
Treatment	4	22.3129	22.208******
Method x Grader	26	3.2566	3.241******
Method x Treatment	12	1.9237	1.915**
Grader x Treatment	36	1.3311	1.325*
Method x Grader x Treatment	104	0.7595	0.756
Residual (Grader error)	191	1.0047	
Total	382	1.5734	

* significant at 10% level
** " 5% "
*** " 2.5% "
**** " 1% "
***** " 0.1% "
****** " 0.01% "

Standard errors of differences of means

Table	Method	Grader	Treat.	Method Grader	Method Treat.	Grader Treat.	Method Grader Treatment
SED	0.1968	0.1894	0.1373	0.4098	0.3158	0.4340	0.8820

TABLE 3:1:2

The mean value* of the scores for appearance given by a Panel (10 graders) for (1) fresh pasteurised milk, (2) reconstituted WMP or recombined milk, (3) three blends of fresh milk + reconstituted WMP or recombined milk at different level. The recombined milk produced from SMP + 3 different sources of milk fat

% (v/v) of fresh milk in blend / Ingredients	100%	75%	50%	25%	0%
	6.6				
WMP		6.53	6.65	6.50	6.30
SMP + AMF		6.58	6.40	6.35	6.35
SMP + Butter		6.63	6.40	6.53	6.55
SMP + Cream		6.61	6.59	6.49	6.43

* mean value from 2 trials

	df	M.S	F
Method	3	0.36941	0.121
Residual (Repeatability of expt)	4	3.04662	
Total	7	1.89925	
Grader	9	25.87651	58.794******
Treatment	4	0.74764	1.699
Method x Grader	26	1.10731	2.516******
Method x Treatment	12	0.15380	0.349
Grader x Treatment	36	0.17645	0.401
Method x Grader x Treatment	104	0.11230	0.255
Residual (Grader error)	191	0.44012	
Total	282	0.96495	

* significant at 10% level
** " 5% "
*** " 2.5% "
**** " 1% "
***** " 0.1% "
****** " 0.01% "

Standard errors of differences of means

Table	Method	Grader	Treat.	Method Grader	Method Treat.	Grader Treat.	Method Grader Treatment
SED	0.2086	0.1254	0.0908	0.3164	0.2650	0.2873	0.6061

TABLE 3:1:3

The mean value* of the scores for odour given by a Panel (10 graders) for (1) fresh pasteurised milk, (2) reconstituted WMP or recombined milk, (3) three blends of fresh milk + reconstituted WMP or recombined milk at different level. The recombined milk produced from SMP + 3 different sources of milk fat

% (v/v) of fresh milk in blend / Ingredients	100%	75%	50%	25%	0%
	6.1				
WMP		5.73	5.70	5.20	4.70
SMP + AMF		5.58	5.50	5.20	5.20
SMP + Butter		5.90	5.50	5.45	5.20
SMP + Cream		6.21	6.13	5.82	5.57

* mean value from 2 trials

	df	M.S	F
Method	3	9.5944	2.711
Residual (Repeatability of expt)	4	3.5389	
Total	7	6.1342	
Grader	9	10.2018	11.609******
Treatment	4	12.4123	14.124******
Method x Grader	26	4.4744	5.092******
Method x Treatment	12	0.4667	0.531
Grader x Treatment	36	0.9442	1.074
Method x Grader x Treatment	104	0.5279	0.601
Residual (Grader error)	191	0.8788	1.735
Total	382	1.3616	

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

Standard errors of differences of means

Table	Method	Grader	Treat.	Method Grader	Method Treat.	Grader Treat.	Method Grader Treatment
SED	0.2248	0.1772	0.1284	0.4044	0.3224	0.4059	0.8349

TABLE 3:1:4

The mean value* of the scores for flavour given by a Panel (10 graders) for (1) fresh pasteurised milk, (2) reconstituted WMP or recombined milk, (3) three blends of fresh milk + reconstituted WMP or recombined milk at different level. The recombined milk produced from SMP + 3 different sources of milk fat

% (v/v) of fresh milk in blend / Ingredients	100%	75%	50%	25%	0%
	6.0				
WMP		5.08	5.10	4.33	3.50
SMP + AMF		5.48	4.70	4.58	3.90
SMP + Butter		5.50	5.05	4.60	3.70
SMP + Cream		5.98	5.71	5.18	4.81

* mean value from 2 trials

	df	M.S	F
Method	3	17.6833	1.773
Residual (Repeatability of expt)	4	9.9716	
Total	7	13.2766	
Grader	9	18.8830	12.171******
Treatment	4	56.1745	36.208******
Method x Grader	26	4.2302	2.727******
Method x Treatment	12	1.0900	0.703
Grader x Treatment	36	1.0867	0.700
Method x Grader x Treatment	104	0.9007	0.581
Residual (Grader error)	191	1.5514	
Total	382	2.4786	

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

Standard errors of differences of means

Table	Method	Grader	Treat.	Method Grader	Method Treat.	Grader Treat.	Method Grader Treatment
SE_D	0.3774	0.2354	0.1706	0.5847	0.4865	0.5393	1.1331

TABLE 3:1:5

The mean value* of the scores for overall acceptability given by a Panel (10 graders) for (1) fresh pasteurised milk, (2) reconstituted WMP or recombined milk, (3) three blends of fresh milk + reconstituted WMP or recombined milk at different level. The recombined milk produced from SMP + 3 different sources of milk fat

% (v/v) of fresh milk in blend / Ingredients	100%	75%	50%	25%	0%
	5.9				
WMP		5.23	5.15	4.50	3.70
SMP + AMF		5.23	4.65	4.78	4.25
SMP + Butter		5.55	5.05	4.65	3.95
SMP + Cream		5.85	5.69	5.03	4.80

* mean value from 2 trials

	df	M.S	F
Method	3	9.7157	0.694
Residual (Repeatability of expt)	4	14.0091	
Total	7	12.1691	
Grader	9	29.0199	21.999******
Treatment	4	43.5659	33.025******
Method x Grader	26	2.5628	1.943****
Method x Treatment	12	1.2233	0.927
Grader x Treatment	36	1.4027	1.063
Method x Grader x Treatment	104	0.8066	0.611
Residual (Grader error)	191	1.3192	
Total	382	2.3642	

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

Standard errors of differences of means

Table	Method	Grader	Treat.	Method Grader	Method Treat.	Grader Treat.	Method Grader Treatment
SE D	0.4474	0.2171	0.1573	0.6081	0.5294	0.4973	1.0820

TABLE 3:1:6

The mean value* of the scores for fattiness given by a Panel (10 graders) for (1) fresh pasteurised milk, (2) reconstituted WMP or recombined milk, (3) three blends of fresh milk + reconstituted WMP or recombined milk at different level. The recombined milk produced from SMP + 3 different sources of milk fat

% (v/v) of fresh milk in blend / Ingredients	100%	75%	50%	25%	0%
	2.14				
WMP		2.05	2.45	2.80	3.05
SMP + AMF		2.08	2.40	2.40	2.55
SMP + Butter		2.20	2.55	2.48	2.60
SMP + Cream		2.33	2.44	2.53	2.46

* mean value from 2 trials

	df	M.S	F
Method	3	0.7607	2.128
Residual (Repeatability of expt)	4	0.3574	
Total	7	0.5303	
Grader	9	20.4712	20.678******
Treatment	4	5.9453	6.005******
Method x Grader	26	8.2166	8.300******
Method x Treatment	12	0.7830	0.791
Grader x Treatment	36	2.1442	2.166******
Method x Grader x Treatment	104	0.5471	0.553
Residual (Grader error)	191	0.9900	
Total	382	1.9744	

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

Standard errors at differences of means

Table	Method	Grader	Treat.	Method Grader	Method Treat.	Grader Treat.	Method Grader Treatment
SE D	0.0715	0.1880	0.1362	0.3639	0.2554	0.4308	0.8564

TABLE 3:1:7

The mean value* of the scores for chalkiness given by a Panel (10 graders) for (1) fresh pasteurised milk, (2) reconstituted WMP or recombined milk, (3) three blends of fresh milk + reconstituted WMP or recombined milk at different level. The recombined milk produced from SMP + 3 different sources of milk fat

% (w/w) of fresh milk in blend / Ingredients	100%	75%	50%	25%	0%
	1.93				
WMP		2.25	2.25	2.45	2.95
SMP + AMF		2.03	2.25	2.30	2.55
SMP + Butter		1.98	2.30	2.53	2.40
SMP + Cream		1.83	1.90	2.09	2.11

* : mean value from 2 trials

	df	M.S	F
Method	3	3.5840	1.140
Residual (Repeatability of expt)	4	3.1427	
Total	7	3.3318	
Grader	9	20.4080	24.888******
Treatment	4	5.3723	6.552******
Method x Grader	26	3.2289	3.938******
Method x Treatment	12	0.7305	0.891
Grader x Treatment	36	1.3276	1.619***
Method x Grader x Treatment	104	0.8776	1.070
Residual (Grader error)	191	0.8200	
Total	382	1.5538	

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

Standard errors of differences of means

Table	Method	Grader	Treat.	Method Grader	Method Treat.	Grader Treat.	Method Grader Treatment
SED	0.2119	0.1711	0.1240	0.3877	0.3077	0.3921	0.8051

TABLE 3:1:8

The mean value* of the scores for acidity given by a Panel (10 graders) for (1) fresh pasteurised milk, (2) reconstituted WMP or recombined milk, (3) three blends of fresh milk + reconstituted WMP or recombined milk at different level. The recombined milk produced from SMP + 3 different sources of milk fat

% (w/w) of fresh milk in blend / Ingredients	100%	75%	50%	25%	0%
	1.23				
WMP		1.25	1.35	1.35	1.70
SMP + AMF		1.23	1.45	1.25	1.45
SMP + Butter		1.45	1.40	1.53	1.80
SMP + Cream		1.10	1.06	1.27	1.64

* mean value from 2 trials

	df	M.S	F
Method	3	1.98255	11.563***
Residual (Repeatability of expt)	4	0.17146	
Total	7	0.94764	
Grader	9	6.82555	33.371******
Treatment	4	2.43869	11.923******
Method x Grader	26	0.74312	3.633******
Method x Treatment	12	0.21767	1.046
Grader x Treatment	36	0.99153	4.848******
Method x Grader x Treatment	104	0.23037	1.126
Residual (Grader error)	191	0.20453	
Total	382	0.50219	

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

Standard errors of differences of means

Table	Method	Grader	Treat.	Method Grader	Method Treat.	Grader Treat.	Method Grader Treatment
SED	0.0495	0.0855	0.0619	0.1695	0.1219	0.1958	0.3911

TABLE 3:1:9

The mean value* of the scores for rancidity given by a Panel (10 graders) for (1) fresh pasteurised milk, (2) reconstituted WMP or recombined milk, (3) three blends of fresh milk + reconstituted WMP or recombined milk at different level. The recombined milk produced from SMP + 3 different sources of milk fat

% (w/w) of fresh milk in blend / Ingredients	100%	75%	50%	25%	0%
	1.15				
WMP		1.33	1.40	1.50	1.75
SMP + AMF		1.25	1.60	1.58	1.75
SMP + Butter		1.33	1.55	1.53	1.75
SMP + Cream		1.16	1.33	1.45	1.60

* mean value from 2 trials

	df	M.S	F
Method	3	0.7140	0.152
Residual (Repeatability of expt)	4	4.6930	
Total	7	2.9877	
Grader	9	12.0974	14.766******
Treatment	4	4.5756	5.585*****
Method x Grader	26	2.4485	2.989******
Method x Treatment	12	0.0885	0.108
Grader x Treatment	36	1.0755	1.313
Method x Grader x Treatment	104	0.3230	0.394
Residual (Grader error)	191	0.8193	
Total	382	1.1013	

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

Standard errors of differences of means

Table	Method	Grader	Treat.	Method Grader	Method Treat.	Grader Treat.	Method Grader Treatment
SE D	0.2589	0.1711	0.1239	0.4152	0.3417	0.3919	0.8184

milk and their different levels of fresh liquid milk are shown in Tables 3:1:1 to 3:1:9.

Results: the results of the effect of different sources of milk fat on the organoleptic properties of recombined and reconstituted milk showed:

1. There were no significant differences between the scores awarded by the panel to the four different combinations of raw materials except for the mouthfeel variate ($p < 0.1$).

2. There were very highly significant differences ($p < 0.0001$) between the scores awarded to the different level of fresh milk in the blends. Increasing the level of fresh milk resulted in higher overall acceptability preferences by the panel.

3. There were very highly significant differences ($p < 0.0001$) between the graders in their scoring of samples.

DISCUSSION

The results were used to analyse three major sources of variance i.e. Method, Treatment and Graders and their interaction for every variate. These results showed some significant differences ranging from ($p < 0.1$ to $p < 0.0001$). Each source of variance is discussed individually in the following sections.

1. The Method (different combination of raw materials): The small differences between the scores awarded to the different methods means that the taste panel was unable to detect big differences in the products and state preferences between the different combinations of raw materials. The level of significance ($p < 0.1$) for the mouthfeel responseto different methods was probably due to the higher preference for an SMP/cream combination as shown below:

Method	SMP + AMF	SMP + unsalted butter	SMP + cream	WMP
Mean values of the scores awarded	5.19	5.37	5.78	5.03

Mean value from 2 trials

The taste panelists found it very difficult to distinguish between the appearance of all the samples presented to them, including the fresh liquid milk. This was demonstrated very clearly by the close scores shown in Table 3:1:2.

The differences in the response to odour for the different combinations failed to be significant. However, there were higher scores for the combination of SMP plus cream than for the other combination as shown in Table 3:1:3. The differences between scores awarded to the flavour for different methods were even less than those for the odour as shown in Table 3:1:4.

The panel gave small differences between the acceptability scores for the different combinations. This gave rise to no significance of the results even at the level of $p < 0.1$ as shown in Table 3:1:5.

The detection of differences in fattiness between the different combinations failed to give a significant result and the scores were very close as is shown in Table 3:1:6. The detection of difference in chalkiness between the different methods was too small to show a significant result as well.

Moderately significant differences between the different methods resulted from the scores of the taste panel for the detectability of acidity. This was probably due to the low scores that were awarded to the combination of SMP plus cream, and to a lesser extent, by the SMP + AMF scores as shown in Table 3:1:8. Finally, the differences in rancidity levels between the different methods was very low. This was probably due to the fact that all raw materials were very fresh, with very low levels of milk fat oxidation. Even for milks based on 100% of recombined or reconstituted materials the rancidity was mostly unnoticeable.

2. **The Treatment:** The taste panel, in contrast to its inability to detect clear differences between the different methods (different combinations of ingredients), was very successful in detecting the differences between the treatments (different level of fresh milk) as shown in the results. It was very clear from the results that fresh milk gave consistent increases in the scores for all variates as its proportion in the mixtures increased. The exception was the

appearance variate, which showed no real differences between the different levels of fresh liquid milk as shown in Table 3:1:2.

Horner, Wallen & Caporaso (1980) reported that this type of organoleptic investigation can be strongly influenced by the extent to which the individual panel members were accustomed to consuming liquid milk. Thus, for example, responses to recombined and reconstituted milk could be expected to be totally different in European communities than in, say, South-East Asia.

The taste panel showed a positive response for increasing the replacement of recombined or reconstituted milk by the fresh liquid milk. At the same time, the taste panel showed a reasonable degree of acceptance for the recombined and reconstituted milk produced from recently manufactured raw materials despite the fact that all members of the taste panel were normally consumers of fresh pasteurised milk.

Sanderson (1968) carried out experimental work to study the possibility of replacing some of the town milk in New Zealand by the recombined or reconstituted milk in the dry seasons. He found that the following levels of recombined milk can be added to fresh milk without detection:

1. Reconstituted milk made from WMP - about 10-30% can be added, depending on the age of WMP.

2. Recombined milk prepared from low-heat SMP and unsalted butter - at least 60% can be added.

3. Recombined milk prepared from low-heat SMP and AMF - at least 50% can be added.

Whipple, Davidson & Sanders (1983) investigated consumer acceptability of recombined milk produced from a high grade low-heat, non-fat dry milk and raw cream. They estimated consumer demand response to change in price relationships between recombined and fresh fluid milk by a sensory evaluation study. Sensory panelists tested five samples, including 100% recombined milk, 100% fresh fluid milk and 75:25, 50:50 and 27:75 blends of each. They concluded that recombined milk can be an acceptable substitute for fresh milk.

Blending recombined milk with fresh milk seemed to be a way of introducing a substantial amount of recombined milk to the market.

Improvement of the acceptability of the recombined milk by blending with fresh liquid milk, as shown in the present work, agrees with the findings of Sanderson (1968) and Whipple el al. (1983). Like them, the view of the author is that this technique can be a key for solving the problem of many countries with insufficient production of fresh milk to meet the demands of their people throughout all the seasons. Al. Wendawi (1982) mentioned that the dairy industry in Iraq faces the problem of fresh milk shortage, mainly in the dry season during the months of September to March. In Egypt, where the production of milk was about 2.3 million tonnes in 1979, the pasteurised milk was often made by recombining imported butter oil and dried skim milk (with a clear declaration of origin on the label) to increase the production of this product (Hofer, 1981).

More experimental work in mixing the recombined milk with the existing fresh milk should be done to achieve a quality of final product more similar to the original fresh milk. In such countries as India, the milk has been made available to many millions of people by standardising reconstituted skim milk powder with buffalo milk, which has a fat content of over 7 per cent (Lucas Clements, 1981).

3. **Graders:** The differences between the graders influenced the results of the present study, especially for the different methods. This was shown from the results as a highly significant interaction between the graders and the methods obtained for most variates.

The differences between the individual taste panel members were very significant, due to the fact that the people who were chosen to carry out the organoleptic tests came from different backgrounds in testing the milk organoleptically, besides their personal preferences for milk in the diet. Nevertheless, it was thought that this panel would give more meaningful results than one formed of highly discriminating panelists with a bias towards milk.

Dietary habits can be affected by many factors such as climate, religion, class, age, sex, health condition, and many other factors.

3.2 The effect of different storage temperatures and time of SMP and AMF on the organoleptic propertiesof recombined milk

Taste panel evaluations were conducted parallel to the analytical studies. Mixtures of a control fresh milk with recombined milk produced from the bulk samples of SMP and AMF which has been stored at -18°C, 4.5°C, 11.5°C and 22.5°C for three, eight and twenty months were evaluated by a nine-member panel in the same manner as the initial study.

The individual scores awarded by each grader were submitted to a statistical analysis of variance for each of nine variates. The term 'control' was used for the initial tests (organoleptic test at zero months). The term 'storage' was used for the storage time. The term 'temperature' was used for different storage temperatures of ingredients and the term 'treatment' was used for different levels of fresh milk in the mixture.

The statistical results of the organoleptic tests for the five mixtures of fresh liquid milk and the recombined milk which was produced from SMP and AMF stored for 20 months at four different storage temperatures at four time intervals are shown in Tables 3:2:1 to 3:2:9.

The results for each variate show significant and insignificant differences between the awarded scores as follows:

TABLE 3:2:1

The mean value* of the scores for mouthfeel given by a panel (9 graders) for blends of fresh pasteurised milk + recombined pasteurised milk produced from SMP and AMF stored at different temperatures for 20 months

Variate (1) Mouthfeel

Storage Temp. / Storage Time		Freezer (-18°C)	4.5°C	11.5°C	22.5°C
Initial	5.14				
3 months		5.23	5.06	5.01	5.05
8 months		5.41	5.25	5.36	4.98
20 months		5.26	5.12	5.22	5.02

* mean value from 2 trials

Treatment	100% Fresh milk	75% Fresh milk	50% Fresh milk	25% Fresh milk	0% Fresh milk
Means	5.87	5.54	5.17	4.13	4.47

	df	M.S	F
Control	1	0.024	0.007
Control x Storage	2	1.9876	0.592
Control x Temp	3	4.6733	1.392
Control x Storage x Temp	6	1.0374	0.309
Residual	11	3.3564	
Total	23	2.6593	
Grader	8	47.6941	36.464******
Treat.	4	88.4877	67.652******
Grader x Treat.	32	3.2486	2.484******
Residual	1012	1.3080	
Total	1056	2.0484	

Table	Control	Control Storage	Control Temp	Control Storage Temp	Grader	Treat.	Grader Treat.
SED	0.1705	0.1825	0.1885	0.2827	0.1248	0.0953	0.2859

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

TABLE 3:2:2

The mean value* of the scores for appearance given by a panel (9 graders) for blends of fresh pasteurised milk + recombined pasteurised milk produced from SMP and AMF stored at different temperatures for 20 months

Variate (2) Appearance

Storage Temp. / Storage Time		Freezer (-18°C)	4.5°C	11.5°C	22.5°C
Initial	6.49				
3 months		6.40	6.32	6.37	6.39
8 months		6.52	6.51	6.46	6.47
20 months		6.46	6.34	6.60	6.65

* mean value from 2 trials

Treatment	100% Fresh milk	75% Fresh milk	50% Fresh milk	25% Fresh milk	0% Fresh milk
Means	6.56	6.53	6.51	6.40	6.26

	df	M.S	F
Control	1	0.1819	0.116
Control x Storage	2	2.8634	1.821
Control x Temp	3	1.094	0.696
Control x Storage x Temp	6	0.8305	0.528
Residual	11	1.5729	
Total	23	1.3685	
Grader	8	92.7441	177.731******
Treat.	4	3.6866	7.065******
Grader x Treat.	32	0.3209	0.615
Residual	1012	0.5218	
Total	1056	1.2246	

Table	Control	Control Storage	Control Temp	Control Storage Temp	Grader	Treat.	Grader Treat.
SED	0.1167	0.12492	0.1290	0.19352	0.07882	0.06020	0.18059

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

TABLE 3:2:3

The mean value* of the scores for odour given by a panel (9 graders) for blends of fresh pasteurised milk + recombined pasteurised milk produced from SMP and AMF stored at different temperatures for 20 months

Variate (3) Odour

Storage Temp. / Storage Time		Freezer (-18°C)	4.5°C	11.5°C	22.5°C
Initial	5.42				
3 months		5.43	5.61	5.77	5.75
8 months		6.00	5.92	5.80	5.56
20 months		5.56	5.45	5.25	4.98

* mean value from 2 trials

Treatment	100% Fresh milk	75% Fresh milk	50% Fresh milk	25% Fresh milk	0% Fresh milk
Means	6.22	5.92	5.59	5.15	4.86

	df	M.S	F
Control	1	2.1251	0.807
Control x Storage	2	26.1160	9.914****
Control x Temp	3	3.7904	1.439
Control x Storage x Temp	6	5.4575	2.072
Residual	11	2.6342	
Total	23	5.5413	
Grader	8	67.5939	50.611******
Treat.	4	82.0768	61.455******
Grader x Treat.	32	3.120	2.336******
Residual	1012	1.3356	
Total	1056	2.1974	

Table	Control	Control Storage	Control Temp	Control Storage Temp	Grader	Treat.	Grader Treat.
SED	0.1510	0.1617	0.1670	0.2504	0.1261	0.0963	0.2889

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

TABLE 3:2:4

The mean value* of the scores for flavour given by a panel (9 graders) for blends of fresh pasteurised milk + recombined pasteurised milk produced from SMP and AMF stored at different temperatures for 20 months

Variate (4) Flavour

Storage Temp. / Storage Time		Freezer (-18°C)	4.5°C	11.5°C	22.5°C
Initial	4.87				
3 months		5.02	4.71	4.52	4.66
8 months		4.83	4.65	4.79	4.45
20 months		4.91	4.71	4.84	4.45

* mean value from 2 trials

Treatment	100% Fresh milk	75% Fresh milk	50% Fresh milk	25% Fresh milk	0% Fresh milk
Means	6.00	5.41	4.77	3.98	3.51

	df	M.S	F
Control	1	3.098	0.576
Control x Storage	2	0.665	0.124
Control x Temp	3	9.472	1.762
Control x Storage x Temp	6	2.163	0.402
Residual	11	5.375	
Total	23	4.563	
Grader	8	64.872	37.983******
Treat.	4	278.227	162.905******
Grader x Treat.	32	4.942	2.894******
Residual	1012	1.708	
Total	1056	3.32	

Table	Control	Control Storage	Control Temp	Control Storage Temp	Grader	Treat.	Grader Treat.
SED	0.2157	0.2309	0.2385	0.3577	0.1426	0.1089	0.3267

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

TABLE 3:2:5

The mean value* of the scores for overall acceptability given by a panel (9 graders) for blends of fresh pasteurised milk + recombined pasteurised milk produced from SMP and AMF stored at different temperatures for 20 months

Variate (5) Overall Acceptability

Storage Temp. / Storage Time		Freezer (-18°C)	4.5°C	11.5°C	22.5°C
Initial	4.97				
3 months		5.06	4.71	4.52	4.68
8 months		4.95	4.76	4.77	4.49
20 months		4.98	4.67	4.833	4.48

* mean value from 2 trials

Treatment	100% Fresh milk	75% Fresh milk	50% Fresh milk	25% Fresh milk	0% Fresh milk
Means	5.99	5.44	4.79	4.03	3.57

	df	M.S	F
Control	1	6.312	1.068
Control x Storage	2	0.210	0.036
Control x Temp	3	12.00	2.031
Control x Storage x Temp	6	1.820	0.308
Residual	11	5.908	
Total	23	5.158	
Grader	8	66.565	39.979******
Treat.	4	256.011	159.165******
Grader x Treat	32	5.603	3.365******
Residual	1012	1.665	
Total	1056	3.274	

Table	Control	Control Storage	Control Temp	Control Storage Temp	Grader	Treat.	Grader Treat.
SED	0.2262	0.2421	0.250	0.3750	0.1408	0.1075	0.3226

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

TABLE 3:2:6

The mean value* of the scores for fattiness given by a panel (9 graders) for blends of fresh pasteurised milk + recombined pasteurised milk produced from SMP and AMF stored at different temperatures for 20 months

Variate (6) Fattiness

Storage Temp. / Storage Time		Freezer (-18°C)	4.5°C	11.5°C	22.5°C
Initial	2.29				
3 months		2.41	2.61	2.68	2.56
8 months		2.40	2.35	2.33	2.45
20 months		2.67	2.79	2.58	2.69

* mean value from 2 trials

Treatment	100% Fresh milk	75% Fresh milk	50% Fresh milk	25% Fresh milk	0% Fresh milk
Means	2.27	2.41	2.56	2.67	2.68

	df	M.S	F
Control	1	8.0982	3.802
Control x Storage	2	9.6711	4.541**
Control x Temp	3	0.9182	0.431
Control x Storage x Temp	6	1.0891	0.511
Residual	11	2.1299	
Total	23	2.6156	
Grader	8	182.1613	193.518******
Treat.	4	10.7249	11.394******
Grader x Treat.	32	10.1105	10.741******
Residual	1012	0.9413	
Total	1056	2.6291	

Table	Control	Control Storage	Control Temp	Control Storage Temp	Grader	Treat.	Grader Treat.
SED	0.1358	0.1454	0.1501	0.2252	0.1059	0.0809	0.2426

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

TABLE 3:2:7

The mean value* of the scores for chalkiness given by a panel (9 graders) for blends of fresh pasteurised milk + recombined pasteurised milk produced from SMP and AMF stored at different temperatures for 20 months

Variate (7) Chalkiness

Storage Temp. / Storage Time		Freezer (-18°C)	4.5°C	11.5°C	22.5°C
Initial	2.21				
3 months		2.60	2.48	2.50	2.33
8 months		2.33	2.29	2.05	2.28
20 months		2.60	2.78	2.66	2.84

* . mean value from 2 trials

Treatment	100% Fresh milk	75% Fresh milk	50% Fresh milk	25% Fresh milk	0% Fresh milk
Means	1.90	2.18	2.52	2.79	2.93

	df	M.S	F
Control	1	8.9584	1.948
Control x Storage	2	28.0456	6.099***
Control x Temp	3	0.8138	0.177
Control x Storage x Temp	6	1.9455	0.423
Residual	11	4.5981	
Total	23	5.6410	
Grader	8	117.9321	113.488******
Treat.	4	49.2658	47.409******
Grader x Treat.	32	6.6158	6.366******
Residual	1012	1.0392	
Total	1056	2.2764	

Table	Control	Control Storage	Control Temp	Control Storage Temp	Grader	Treat.	Grader Treat.
SED	0.1995	0.2136	0.2206	0.3309	0.1111	0.0849	0.2548

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

TABLE 3:2:8

The mean value* of the scores for acidity given by a panel (9 graders) for blends of fresh pasteurised milk + recombined pasteurised milk produced from SMP and AMF stored at different temperatures for 20 months

Variate (8) Acidity

Storage Temp. / Storage Time		Freezer (-18°C)	4.5°C	11.5°C	22.5°C
Initial	1.27				
3 months		1.41	1.54	1.59	1.56
8 months		1.24	1.51	1.44	1.56
20 months		1.45	1.41	1.65	1.77

* mean value from 2 trials

Treatment	100% Fresh milk	75% Fresh milk	50% Fresh milk	25% Fresh milk	0% Fresh milk
Means	1.31	1.38	1.47	1.63	1.70

	df	M.S	F
Control	1	7.3653	17.39****
Control x Storage	2	1.3501	3.188*
Control x Temp	3	3.9432	9.311****
Control x Storage x Temp	6	0.9760	2.305
Residual	11	0.4235	
Total	23	1.4091	
Grader	8	31.1395	50.872******
Treat.	4	7.4368	12.149******
Grader x Treat.	32	1.5081	2.464******
Residual	1012	0.6121	
Total	1056	0.8964	

Table	Control	Control Storage	Control Temp	Control Storage Temp	Grader	Treat.	Grader Treat.
SED	0.0606	0.0648	0.0669	0.1004	0.0854	0.0652	0.1956

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

TABLE 3:2:9

The mean value* of the scores for rancidity given by a panel (9 graders) for blends of fresh pasteurised milk + recombined pasteurised milk produced from SMP and AMF stored at different temperatures for 20 months

Variate (9) Rancidity

Storage Temp. / Storage Time		Freezer (-18°C)	4.5°C	11.5°C	22.5°C
Initial	1.52				
3 months		1.30	1.52	1.56	1.76
8 months		1.60	1.56	1.38	1.43
20 months		1.75	1.62	1.83	2.03

* mean value from 2 trials

Treatment	100% Fresh milk	75% Fresh milk	50% Fresh milk	25% Fresh milk	0% Fresh milk
Means	1.19	1.30	1.51	1.89	2.15

	df	M.S	F
Control	1	1.1121	0.441
Control x Storage	2	15.3745	6.097***
Control x Temp	3	3.7325	1.48
Control x Storage x Temp	6	2.7332	1.084
Residual	11	2.5216	
Total	23	3.7911	
Grader	8	51.6752	63.889******
Treat.	4	44.0189	54.423******
Grader x Treat.	32	7.0425	8.707******
Residual	1012	0.8088	
Total	1056	1.5468	

Table	Control	Control Storage	Control Temp	Control Storage Temp	Grader	Treat.	Grader Treat.
SE D	0.1478	0.1582	0.1634	0.2450	0.0981	0.0749	0.2248

* significant at 10% level
** " 5%
*** " 2.5%
**** " 1%
***** " 0.1%
****** " 0.01%

RESULTS

The effect of storage of the SMP and AMF on the organoleptic characteristics of the recombined milk and its mixtures with fresh liquid milk, showed a degree of significant change for some of the parameters. The tables for each parameter are presented to show the changes of the mean values for the awarded scores given by the panel at each storage temperature for four storage time intervals following the initial value. The other part of these tables show the mean values at each level of fresh milk replacement by the recombined milk produced from ingredients stored at different storage temperatures for twenty months. The results showed

1. The acceptability parameters

1.1 The Mouthfeel: The taste panel showed very little differences in their scores for the recombined milk which was produced from raw materials stored at different temperatures for different periods of time as far as mouthfeel was concerned. At the same time the taste panel showed very clear preferences for the mixtures with higher percentages of fresh liquid milk, as shown in Table 3:2:1. The individual graders showed a very wide diversity in their scoring each sample due to their individual preferences.

1.2 The Appearance: The taste panel failed to find significant differences between the appearance of the samples as a result of different times and temperatures of storage of the ingredients. Thus, for all intents and purposes, the colour and structure of all the milk remained constant throughout the trial. The increasing level of fresh milk in the mixtures was reflected by small changes in preferences ranging from 6.56 as a mean score for 100% fresh liquid milk to 6.26 mean scores for 100% recombined milk. Nevertheless, this change was highly significant statistically ($p < 0.0001$), as shown in Table 3:2:2.

The graders acted very differently in their scoring of the appearance of the samples. Responses ranged from 5.07 as a lowest mean value of one grader, to 7.65 as a highest mean value of another panelist and the level of awards depends on many personal factors.

1.3 The Odour: The taste panel showed a clear response (Table 3:2:3) to the change in the odour of the samples throughout the storage time and between the samples which had been produced from the recombined milk raw materials stored at different temperatures. The change of odour was mostly due to the effect of changes in the quality of the AMF and especially at 22.5°C after 20 months storage. This effect could be due to the liberation of carbonyl compounds as a result of fat oxidation. An increase in the level of free fatty acids could also be responsible, particularly the short chain free fatty acids arising from lipolysis. Fat oxidation and lipolysis of the stored AMF are discussed in Chapter Two, and the results clearly show pronounced increases in the peroxide values and acid values of AMF, particularly when stored at 22.5°C.

The taste panel showed a wide degree of differences in scoring for the different treatments involving the blending of the recombined milk with the fresh liquid milk. The level of fresh liquid milk was probably an important factor in masking the changes of odour of the recombined milk, especially those produced from raw materials stored at high temperatures.

The diversity between the graders was very significant due to their different responses to the degree of the odour of the samaples.

1.4 The Flavour: The effect of the different storage temperatures and time of the SMP and AMF showed some degree of lowering of the scores from the taste panel. These ranged from 4.93 as a mean value for the freezer samples to 4.53 mean value for the samples stored at 22.5°C. However, these differences were not enough to give a significant result statistically.

Very broad differences were found between the different treatments. The effect of fresh liquid milk was very pronounced by its influence in raising the scores of the recombined milk with increased level in the blend. This is shown very clearly in Table 3:2:4.

The taste panel showed a high degree of diversity between its members as for previous variates.

1.5 The Overall Acceptability: There were large similarities between the results for overall acceptability and flavour, confirming that flavour has an important influence on acceptability. This is shown in Tables 3:2:4 and 3:2:5.

The samples which represent the raw material stored at freezer temperature were always more acceptable than the samples which represented raw materials stored at higher temperatures for a long time. The effect of blending the fresh milk at different levels improved the acceptability of the recombined milk. The fresh milk was probably very helpful in masking some of the defects of the recombined milk. This applied to the samples which represent the raw materials stored at 22.5°C for 20 months.

2. The detectability parameters

2.1 The Fattiness: The taste panel showed a low degree of significant differences in fattiness due to the effect of storage time and temperatures. The recombined milk usually showed a degree of fattiness due to the presence of the AMF. The results showed that deterioration of AMF due to storage will increase this degree of fattiness.

The results showed that blending the fresh liquid milk with the recombined milk would decrease the level of detectability of the fattiness in the recombined milk.

The graders varied in their ability to detect fattiness of the samples. Scores ranged from 1.14 as the lowest mean value of one scorer to 3.83 as the highest mean value of another scorer.

2.2 The Chalkiness: Chalkiness is usually the most detectable characteristic of recombined milk, probably due to the effects of the reconstitution properties of the milk powder and the homogenisation step in processing. The results showed that the storage of milk powder increased the detectability of chalkiness in the recombined milk. This effect could be related to the observed decrease in the dispersability of SMP during storage.

Adding the fresh milk to the recombined milk is the best way to decrease the detectability of chalkiness in recombined milk, as the

results showed highly significant differences between the treatments.

Once again individual panelists varied in their ability to detect chalkiness.

2.3 Acidity: The results showed a high degree of differences between the acidity responses of the control on the duplicate sample. Storage time and temperature showed statistically significant changes in the level of detected acidity.

The levels of fresh milk positively affected the acidity of recombined milk. The graders again differed in their level of scoring to the acidity.

2.4 The Rancidity: Increases in the detectability of rancidity in the samples were very pronounced as a result of the effect of storage time and temperature changes on the AMF.

The level of fresh milk in the blends showed a very positive influence in overcoming the problem of rancidity in recombined milk as shown in Table 3:2:9.

The graders varied in their ability to detect the rancidity of the samples (e.g. their mean values of scoring for the detectability of rancidity ranged from 1.0 to 2.46).

DISCUSSION

The growth in imports of milk powder (whole and skimmed) and milk fat into developing countries for the production of recombined milk and its products increases all the time. At the same time the increasing production of these raw materials by the developed countries of the world, opens a new commercial activity between the two parties.

Progress has been continued in producing better quality powder and introducing higher standards for the raw materials. Progress has been made in developing machinery for the recombination of the milk, to achieve a product with greater similarity to milk itself, Newstead et al. (1979); Anon (1981). The production of recombined milk in the developing countries should always be concerned with the storage conditions of the raw material for at least 3 months in advance of

production. This period represents a reasonable reserve of ingredients for production. Furthermore, high temperatures and humidities can be limiting factors in the methods of storage in countries manufacturing recombined milk.

The results of the effects of storage temperature and time on the SMP and AMF and their effect on the pasteurised recombined milk, showed that there were many chemical and physical changes which could occur to these raw materials.

Nichols (1939) examined bacteriologically over 400 samples of spray-dried milk powder from eight factories operating in England and Scotland. Individual plate counts varied widely, from 1400 to 149,000.000 per g. This variation appeared to be mainly associated with the technique of manufacture. The number of yeasts and moulds present in the milk powder samples was found to be negligible. About 10% of the samples tested in 1 ml quantities gave positive coliform tests, but some of the positive tests were due to anaerobic sporeformers. Mattick et al. (1945) showed that the factors which determined the plate count of dried milk appeared to be, in the order of their importance:

1. plant cleanliness and sterility;
2. pre-heating temperature;
3. bacteriological quality of the raw milk supply.

Crossley (1962) pointed out that spray-dried milks containing less than 1000 organisms per gram are now produced regularly in commercial practice, and reasonable maximum limits are 50,000 per gram for low-heat powder and 20,000 per gram for high-heat powder. Dried milk has an extremely good record of freedom from pathogenic bacteria. Crossley (1962) also mentioned that micro-organisms do not proliferate in dried milk, and storage defects are purely chemical in nature. This microbiological stabilisation of dried milk was also confirmed by Luquet et al. (1982).

The results of the present work showed a low plate total count of 1500 colony forming units (c f u)/g for SMP and 700 c f u /g for WMP. The thermoduric count was a high proportion of the total count. These results are in agreement with Crossley (1962) that good bacteriological spray-dried milks are produced regularly in

commercial practice. The results also confirm that thermoduric bacteria is the predominant flora of dried milk, Crossley (1962). The results of the present work are in agreement with Nichols (1939) that yeast and mould do not exist in dried milk to any extent. The results of the present work confirmed that micro-organisms do not proliferate in dried milk,and storage defects were purely chemical in nature.

Newstead (1979) reported that pasteurised recombined milk is the type that most nearly approximates to fresh milk when low or medium-heat milk powder is used the flavour is not readily distinguishable from that of fresh milk. The results of the present work confirm that pasteurised recombined milk is an acceptable product as a milk beverage. However, the results also showed that fresh milk has a higher degree of preference compared to the recombined milk. The degree of preference were increased by raising the level of fresh milk in the blend of both of them.

Kieseker (1981) reported that recombined UHT milk is generally less white than UHT fresh milk, due to the additional heating applied during powder preparation. It was claimed by Kieseker (1981) that recombined UHT milk has smaller differences in flavour when compared with the fresh product. Kieseker (1981) also mentioned that storage flavours arising from the AMF and the SMP are sometimes very evident and influence product acceptance. The results of the present work with pasteurised milk showed very little differences between the colour of the recombined milk and the fresh milk. The results showed a strong agreement with Keiseker (1981) that storage defects of AMF and SMP has a big influence on the acceptability of recombined milk.

Recombined fluid milk requires a specification for the SMP, particularly in relation to the solubility index (0.5 ml maximum); scorched particles with Disc A minimum (ADMI, 1971c); moisture content, 4% maximum and heat treatment prior to evaporation (low heat - WPNI not greater than 6.0 mg undernatured WPN/g) (Sanderson, 1979).

Nichols (1979) reported that damage to SMP during storage can result from moisture gain. The rise in moisture content should be slow

with proper packing which has not been damaged.

The results of the present work showed an increase in the moisture content of SMP and an increase in the amount of the scorched particles. Choat (1979) pointed out that SMP used for recombination process should be free from scorched particles and other extraneous matter which could be carried through to the final product. Through the production of the recombined milk in the present work these scorched particles were very pronounced in the mixing tank. However, these scorched particles diminished in the final product as a result of their removal in the filter during processing.

Kieseker (1981) reported that SMP for recombined UHT milk is normally of the medium-heat type. SMP is often produced very much on a seasonal basis, requiring to be stored for 6 to 8 months in some cases, at temperatures up to and in excess of 30°C. The moisture content of powder has a very marked influence on storage life, even powder with a generally acceptable level of 4% moisture can lose solubility, colour, flavour and nutritional properties, particularly if ambient temperatures are high.

The increases of moisture and scorched particles and the clear decreases of the dispersibility of SMP throughout the storage time in this work, showed a significant effect on the acceptability of the recombined milk. The results showed a significant increase in the detectability of chalkiness of the recombined milk produced from SMP stored for increasing lengths of time. Chalkinessis a very important characteristic of recombined milk which could affect its consumption level. Generally speaking, all recombined milk has a degree of chalkiness and this property was more pronounced in the recombined milk which was produced from SMP stored for 20 months, particularly at 22.5°C. In contrast, the detectability of chalkiness was decreased by increasing the level of fresh pasteurised milk in the mixtures of recombined and fresh milks. Fotheringham et al. (1979) pointed out that insufficient hydration timemay be lead to a 'chalky' defect in the recombined evaporated milk.

Kieseker (1981) reported the importance of AMF in influencing the flavour of recombined milk. Storage of AMF, particularly at high temperature will increase its oxidation rate and the time taken for

the AMF to deteriorate will fall rapidly. The effect of the AMF oxidation resulting in the formation of a distinctive and often objectionable off-flavour in the recombined milk was reported by many workers (Sanderson, 1982; Kirkpatrick, 1982; Gunnis, 1982).

Fotheringham et al. (1979) reported that only top quality AMF with a low level of copper and low peroxide value should be used for recombination. The quality of AMF should be checked for free fatty acids and for oxidation. AMF held in storage for any period longer than three months (particularly at high ambient temperatures) should be examined organoleptically, and regular checks performed on all fat as it is used.

The result of the present work showed that the peroxide value and the acid value (level of FFA) in the AMF were increased throughout the storage time. These increases were more pronounced at higher storage temperatures (22.5°C) particularly after 20 months storage time. These results would confirm the susceptability of AMF to deterioration as a result of the storage of AMF for long times at high temperatures, Nichols (1979). A storage temperature of 4°C for AMF was recommended by Sanderson (1979) and Nichols (1979). The results of this work showed that even at 4.5°C storage temperature for AMF there was an increase in its peroxide value. These results are in agreement with those of Badings (1970), which showed that oxidation in butter developed during cold storage (-10°C).

The organoleptic results showed that significant changes were found in the odour of the recombined milk. This change in odour was more pronounced in the recombined milk produced from AMF stored at high temperature (22.5°C) after 20 months. This probably can be as a result of the AMF deterioration which was mentioned earlier. The results also showed increases in the detectability of the rancidity and fattiness characteristics of the recombined milk. Once again, these characteristics were more pronounced in the recombined milk produced from AMF stored at 22.5°C for 20 months time. These results again probably can be explained by the increases of peroxide values and acid values of the AMF. The increases of the AMF oxidation would liberate carbonyl compound which can cause flavour defects in the AMF. On the other hand, increasing the level of the FFAs results in undesirable flavour and odour, particularly the short-chain FFAs.

These flavour and odour defects in the AMF probably can contribute to the recombined milk which is produced from this AMF. Sanderson (1982) pointed out that AMF can be expected to remain of good quality even at elevated ambient temperatures of 30 to 40°C, for 6-12 months. However, Kieseker (1982) mentioned that trials have shown that AMF stored at 30°C deteriorated to second quality within 6-8 weeks. The result of the present work showed that the peroxide value and the acid value of AMF stored at 22.5°C increased to a level which exceeded the IDF (IDF, 1977) recommendation maximum 0.2 mEq O_2/kg after 6 months. These results show that AMF is susceptible to deterioration during storage, particularly at elevated temperatures.

CONCLUSION

1. There were no clear preferences of either SMP + AMF, SMP + Butter, SMP + Cream or WMP as a raw material for recombined or reconstituted milk.

2. The level of fresh milk included in the blends of recombined milks had a very highly significant influence on the acceptability rankings.

3. There were very significant influences on overcoming the detectability of fattiness, chalkiness and rancidity for recombined or reconstituted milk by increasing the level of fresh pasteurised milk in the blends.

4. The recombined or reconstituted milk produced from high grade, recently manufactured raw materials can be very acceptable substitutes for fresh liquid milk, either by themselves or in blends with the fresh milk.

5. The long storage of SMP, especially at high temperatures, resulted in the development of chalkiness in the recombined milks.

6. Storage of AMF for a long time, especially at high temperatures had detrimental effects on the odour of recombined milk.

7. Fattiness and rancidity of recombined milk was more easily detected when milks were prepared using AMF stored for long periods, especially at high temperatures.

8. There were very wide diversities between the scoring of the taste panel members for all the parameters.

CHAPTER FOUR

Auto-oxidative stability of AMF with and without added antioxidants

4.1 Introduction

Fat stability is the resistance to auto-oxidation under prescribed conditions of ageing and is measured in units of time required for the product, (a) to achieve a state of oxidation which correlates with organoleptic detection of rancid flavour, or (b) to reach the end of the induction period if oxygen absorption measurements or peroxide analyses are used (Swern, 1964).

An antioxidant is any substance which retards the development of rancidity and increases shelf life of the fat. Such substances are radical acceptors which break the propagation sequence by forming new radicals which are insufficiently reactive to continue the propagation reaction. A single molecule of antioxidant prevents the oxidation of the hundred of thousands of olefin molecules which would have occurred in the absence of the chain-breaking antioxidant (Gunstone, 1967).

The stability of a fat is one of its most important requirements. Under normal conditions of storage, particularly when antioxidants are involved, the time would be too long for practical evaluation. Therefore, it is necessary to employ accelerated oxidation tests.

Thomson (1966) surveyed accelerated tests for determining the stability of oils and fat and he discussed three methods developed for this purpose:

1. The Oven Test,
2. Swift or Fat Stability Test,
3. The Quality Test.

The Oven Test correlates best with the actual shelf life or room temperature stability. It is a simple incubation of the fat at 64°C. Experience has shown that each day of Oven Test conditions is equivalent to somewhere in the range of 6 to 12 days at 21°C.

Each day of the Oven Test conditions is equivalent to somewhere in

the range of ¾ to 1¾ hours under the conditions of Fat Stability Test.

The Quality Test is very similar to the Fat Stability Test, from which it was developed. The main modification is the increase in the temperature of 98°C used in the Fat Stability Test to 130°C in the Quality Test, typically giving a result in 1-4 hours.

4.2 Materials

4.2.1 Anhydrous milk fat (AMF)

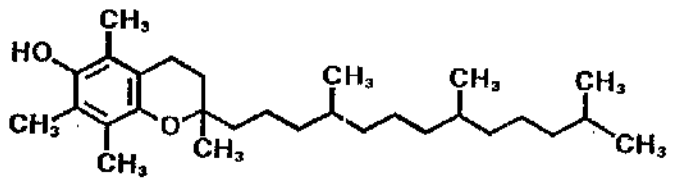
Supplied by the Aberdeen and District Milk Marketing Board and of recent manufacture, packed in 25 kg polythene-lined cardboard.

4.2.2 Antioxidants

Two types of antioxidants were used. One kind was based on naturally occurring compounds and the other on synthetic materials not found in fats and oils. All antioxidants used are on the permitted list of 'The Anti oxidants in Food Regulations', 1978, S.I. 1978 No. 105 (H.M.S.O., 1978).

4.2.2.1 Antioxidants based on naturally occurring compounds were supplied by ROCHE, 318 High Street North, Dunstable, Bedfordshire.

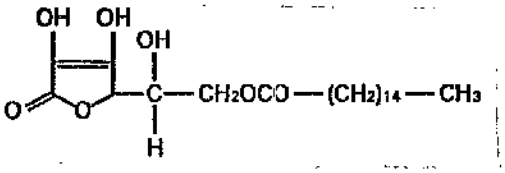
4.2.2.1.1 DL-α-Tocopherol (Vitamin E)



$C_{29}H_{50}O_2$ Molecular weight = 430.72

This is a yellow, clear, viscous oil, odourless, insoluble in water but soluble in alcohol.

4.3.3.1.2 Ascorbyl palmitate



$C_{22}H_{38}O_7$ Molecular weight = 414.54

This material is a white to slightly yellowish, crystalline powder, odourless, insoluble in water but soluble in alcohol.

4.2.2.1.3 Ronoxan A

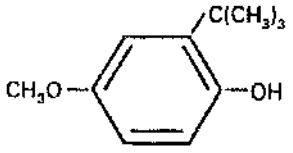
It consists of ascorbyl palmitate (min. 25%) with DL-α-Tocopherol (min. 5%) and lecithin. It is a brown to dark brown paste with a slight odour.

4.2.2.2

The synthetic antioxidants used were chemical compounds produced specifically for the food industry. They were supplied under the Embanox trade name by May & Baker Limited, Dagenham, Essex.

4.2.2.2.1 Embanox BHA

Butylated hydroxyanisole

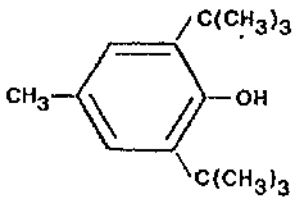


$C_{11}H_{16}O_2$ Molecular weight = 180.2

It is a white or slightly cream coarse crystalline powder, has a slightly phenolic odour, soluble in fats, oil, alcohol and ether, but insoluble in water.

4.2.2.2.2 Embanox BHT

Butylated hydroxytoluene



$C_{15}H_{24}O$ Molecular weight = 220.3

It is a fine, colourless or white crystal, soluble in alcohol or ether but insoluble in water.

4.2.2.2.3 Embanox 2

It has the following composition:

BHA	18% w/w
BHT	20% w/w
Vegetable oil	62% w/w

It is a pale yellow, oily liquid and is easily soluble in hot animal fats and vegetable oil. This formulation takes advantage of the marked synergistic action of mixtures of BHA and BHT, which results in improved stabilities for a given concentration of the mixture of antioxidants.

4.2.2.2.4 Embanox 7

It has the following composition:

BHA	67% w/w
Dodecyl gallate	33% w/w

It is a pale, slightly pinkish-brown powder, soluble in fats and oils alcohol and ether, but insoluble in water. Taking advantage of the marked synergistic action of mixtures of dodecyl gallate and BHA. The ratio of dodecyl gallate to BHA has been chosen to fit 1:2 ratio of the maximum incorporation rates permitted by the antioxidant regulations in the UK and some other countries.

4.3 Apparatus

4.3.1 A constant temperature heater (Tecam Dri-Block DB-4) which maintained all samples at a temperature of 97.8 ± 0.2°C, supplied by Techne (Cambridge) Limited, Duxford, Cambridge, England.

4.3.2 An air pump (Nimrod 3) for supplying low pressure, clean, oil-free air, supplied by Armitage Bros plc., Colwick, Nottingham, England.

4.3.3 The glasswear of air washing columns, water cooled condenser, manifold, the sample tubes (provided with a two-hole neoprene stopper) and aeration tubes were supplied by R & J Wood Ltd. Paisley (see Plate 4:1).

4.3.4 A rotameter-type air flow meter was supplied by Fischer

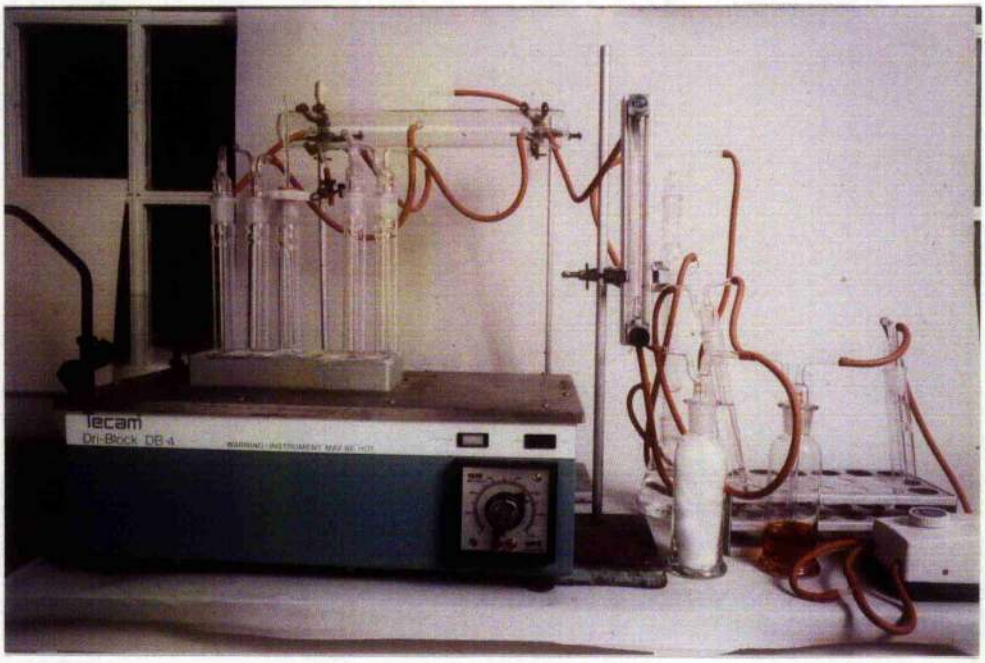


Plate 4:1 Fat stability test - apparatus

& Porter Limited, Workington, England.

4.4 Method

The Official Method Cd 12-57, Revised 1981 (American Oil Chemists Society) was used to measure the stability of AMF and AMF plus the different antioxidants mentioned in Section 4.2.2.

Melted samples of 20 g of AMF were poured into each tube. The aeration tubes were inserted into the sample tubes and adjusted so that the end of the air delivery tube was 5 cm below the surface of the sample. The tube and the samples were placed in a beaker of vigorously boiling water for a period of 5 minutes. At the end of this time the tubes were removed from the water, dried on the outside and then transferred to the constant temperature heater maintained at 97.8°C. The aeration tubes were connected to the capillary outlets from the manifold and the air flow rate was adjusted by using the rotameter to 2.3 ml per tube per second. The starting time was recorded and the peroxide values measured according to the IDF Standard Method 74 (IDF, 1974) for each duplicate sample. Peroxide value measurements were made initially on the untreated AMF sample. Measurements were made throughout the fat stability test at intervals of 4, 7, 12, 24, 28, 31, 36 and 48 hours.

4.5 Results

Duplicate results of the peroxide values obtained throughout the experimental time for the AMF itself and for the AMF plus the different kinds and levels of antioxidants are shown in Table 4:1. The means of duplicate test values given in Table 4:1 are shown in Table 4:2. The statistical analysis of variance for the different treatments throughout the experimental time were carried out and the results are shown in Table 4:3.

4.5.1 AMF

The development of the peroxide values for the AMF under the experimental conditions was very noticeable and it was accelerated with time. Peroxide values developed to in excess of 100 mEq O_2/kg at 48 hours (see Tables 4:1 and 4:2). The colour of the AMF changed from yellow to white and was accompanied by a very pronounced and obnoxious odour.

4.5.2 The effect of the antioxidants

4.5.2.1 Ascorbyl palmitate:

It was found that the crystals of ascorbyl palmitate were more difficult to disperse in the AMF than the other antioxidants tested. The effect of this antioxidants was very clear (see Fig. 4:3), especially at the early stages. For example, at a level of 200 ppm ascorbyl palmitate in the AMF, the initial peroxide value of 0.107 mEq O_2/kg was held for at least 7 hours (see Tables 4:1 and 4:2). A level of 200 ppm ascorbyl palmitate was more effective than 100 ppm in controlling the peroxide value. However, this difference only became marked after 36 hours aeration as shown in Fig. 4:3. In each case there was no change of colour, even at 48 hours aeration.

4.5.2.2 The DL-α-Tocopherol exhibited a pro-oxidant effect at both test levels of 100 and 200 ppm. The peroxide values of AMF containing this material at 100 ppm were greater than the corresponding values for untreated AMF for aeration times up to 36 hours. This pro-oxidant effect was even greater with AMF containing 200 ppm DL-α-Tocopherol. However, after 48 hours aeration the untreated AMF had entered the rapid stage of auto-oxidation with a peroxide value of 112 mEq O_2/kg. At this stage the DL-α-Tocopherol was no longer pro-oxidant, but the 100 ppm level of added material was still more effective than 200 ppm in holding the peroxide value to 17.1 as compared with 20.9 mEq O_2/kg. There was no change in colour for AMF with either 100 or 200 ppm levels of DL-α-Tocopherol, even after 48 hours.

4.5.2.3 Ronoxan A

This material dissolved very easily in the AMF even at addition levels of 2000 ppm. Its effect as an antioxidant under the experimental conditions was very good. It also exhibited the ability to reduce the initial AMF peroxide value of 0.107 mEq O_2/kg to zero for 7 hours at a level of 2000 ppm of Ronoxan A. A similar effect was also shown at 1000 ppm of Ronoxam A addition (see Fig. 4:1 and Tables 4:1 and 4:2). The overall antioxidant effect of Ronoxan A was most pronounced at the 2000 ppm level (Fig. 4:1). In this case, the peroxide value after 48 hours was only 0.5 mEq

O_2/kg. The antioxidant effect of Ronoxan A became progressively less effective as the dosage rate was lowered.

4.5.2.4 BHA

The upper permitted level SI 1978 No. 105 (H.M.S.O., 1978) of 200 ppm of BHA was effective in slowing down the development of the peroxide value of the AMF as shown in Tables 4:1 and 4:2. The peroxide value was only 5.6 mEq O_2/kg after 48 hours.

4.5.2.5 BHT

The upper permitted level SI 1978 No. 105 (H.M.S.O., 1978) of 200 ppm of BHT was always more effective than the same level of BHA in controlling the auto-oxidation. After 48 hours aeration, the AMF treated with 200 BHT had a peroxide value of only 3.4 mEq O_2/kg or 61% of the corresponding figure for BHA.

4.2.5.6 Embanox 2

The upper permitted level SI 1978 No. 105 (H.M.S.O., 1978) of 500 ppm of this antioxidant formulation showed a better effect in slowing down the development of the peroxide value of AMF compared with BHA and BHT as shown in Tables 4:1 and 4:2 and Fig. 4:3. The peroxide value was only 2.7 mEq O_2/kg after 48 hours aeration.

4.2.5.7 Embanox 7

The upper permitted level of 300 ppm of this antioxidant SI 1978 No. 105 (H.M.S.O., 1978) was more effective in slowing down the development of the peroxide values of the AMF than Embanox 2, BHT and BHA as shown in Tables 4:1 and 4:2 and in Fig. 4:3. Its effect as an antioxidant was greater than Ronoxan A at levels of 200 and 500 ppm, but was less effective than Ronoxan A at levels of 1000 and 2000 ppm as shown in Fig. 4:1.

4.5.2.8 Mixture of BHA and BHT

The upper limit SI 1978 No. 105 (H.M.S.O., 1978) of 100 ppm each of BHA and BHT showed a slight improvement in controlling the development of the peroxide value of AMF compared with individual levels of 200 ppm BHA or BHT as shown in Table 4:2. This slightly synergistic effect became most noticeable after 12 hours aeration.

TABLE 4:1

The development of the peroxide value (mEq O_2/kg fat) of AMF with and without different antioxidants after various times of the stability test

	Initial	4 h	7 h	12 h	24 h	28 h	31 h	36 h	48 h
AMF	0.107	0.460	0.604	1.013	2.74	4.79	5.14	5.71	108.11
	0.106	0.521	0.749	1.281	3.13	5.14	5.48	6.66	115.93
AMF + 100 ppm ascorbyl palmitate		0.144	0.181	0.371	1.207	1.357	2.335	3.138	7.172
		0.104	0.174	0.315	1.189	1.271	2.194	2.969	6.643
AMF + 200 ppm ascorbyl palmitate		0.072	0.092	0.250	1.007	1.185	1.936	2.960	5.370
		0.065	0.090	0.235	0.982	1.167	1.806	2.856	5.062
AMF + 100 ppm DL-α-Tochopherol		0.664	0.898	1.493	4.659	5.500	6.603	7.269	15.618
		0.709	0.965	1.665	4.795	5.747	7.043	7.452	18.618
AMF + 200 ppm DL-α-Tocopherol		1.326	2.355	2.938	9.898	12.943	13.560	15.042	21.646
		1.323	2.534	3.045	10.116	12.306	12.955	14.718	20.172
AMF + 200 ppm Ronoxan A		0.31	0.413	0.548	1.801	2.712	3.215	3.736	6.336
		0.29	0.440	0.593	1.942	2.963	3.516	3.962	6.725
AMF + 500 ppm Ronoxan A		0.069	0.154	0.244	0.577	0.667	0.693	0.833	1.81
		0.072	0.142	0.221	0.553	0.682	0.715	0.862	1.89

cont'd.....

(Table 4:1 cont'd.)

	Initial	4 h	7 h	12 h	24 h	28 h	31 h	36 h	48 h
AMF + 1000 ppm Ronoxan A		0.015	0.016	0.067	0.302	0.329	0.348	0.392	0.800
		0.005	0.011	0.071	0.316	0.321	0.389	0.428	0.834
AMF + 2000 ppm Ronoxan A		0.00	0.00	0.045	0.083	0.105	0.145	0.205	0.502
		0.00	0.00	0.042	0.087	0.102	0.141	0.211	0.495
AMF + 200 ppm BHA		0.338	0.426	0.699	2.742	0.899	3.138	4.029	5.903
		0.298	0.387	0.628	2.325	2.442	2.604	3.601	5.268
AMF + 200 ppm BHT		0.182	0.287	0.561	1.676	1.708	2.147	2.265	3.491
		0.189	0.322	0.573	1.678	1.712	2.291	2.314	3.369
AMF + 100 ppm BHA + 100 ppm BHT		0.442	0.529	0.710	1.328	1.591	1.659	2.019	2.887
		0.365	0.465	0.712	1.430	1.877	1.993	2.586	3.489
AMF + 500 ppm Embanox 2		0.278	0.367	0.459	1.331	1.677	2.151	2.631	2.818
		0.264	0.349	0.430	1.267	1.519	1.917	2.359	2.478
AMF + 300 ppm Embanox 7		0.244	0.291	0.360	0.772	0.818	0.904	1.057	1.326
		0.227	0.272	0.351	0.814	0.849	0.926	1.040	1.224

Each value represents a single test result on duplicate samples of AMF

TABLE 4:2

The means* of peroxide values (mEq O_2/kg fat) of AMF with and without added antioxidants through the time of the stability test

	Initial	4 h	7 h	12 h	24 h	28 h	31 h	36 h	48 h
AMF	0.107	0.491	0.677	1.147	2.936	4.965	5.309	6.183	112.02
AMF + 100 ppm ascorbyl palmitate		0.124	0.178	0.343	1.198	1.314	2.264	3.053	6.908
AMF + 200 ppm ascorbyl palmitate		0.069	0.091	0.243	1.03	1.176	1.871	2.908	5.216
AMF + 100 ppm DL-α-Tocopherol		0.687	0.932	1.579	4.727	5.623	6.823	7.36	17.12
AMF + 200 ppm DL-α-Tocopherol		1.325	2.444	2.991	10.007	12.624	13.257	14.88	20.91
AMF + 200 ppm Ronoxan A		0.30	0.427	0.571	1.871	2.837	3.365	3.849	6.53
AMF + 500 ppm Ronoxan A		0.071	0.148	0.233	0.565	0.675	0.704	0.847	1.850
AMF + 1000 ppm Ronoxan A		0.01	0.014	0.069	0.309	0.325	0.369	0.410	0.817
AMF + 2000 ppm Ronoxan A		0.00	0.00	0.043	0.085	0.104	0.143	0.208	0.499

cont'd.....

(Table 4:2 cont'd.)

	Initial	4 h	7 h	12 h	24 h	28 h	31 h	36 h	48 h
AMF + 200 ppm BHA		0.318	0.407	0.664	2.533	2.67	2.871	3.82	5.585
AMF + 200 ppm BHT		0.186	0.305	0.567	1.677	1.710	2.219	2.289	3.43
AMF + 100 ppm BHA + 100 ppm BHT		0.404	0.497	0.711	1.379	1.734	1.826	2.302	3.188
AMF + 500 ppm Embanox 2		0.271	0.358	0.444	1.299	1.598	2.034	2.495	2.648
AMF + 300 ppm Embanox 7		0.236	0.281	0.356	0.793	0.834	0.915	1.048	1.275

*Mean value from 2 trials

TABLE 4:3

Statistical analysis of variance of the results of the experiments which are shown in Tables 4:1 and 4:2

	df	M.S	F
Treatment	13	302.8477	973.341****
Storage	8	479.9194	1542.443****
Treatment x storage	104	183.6924	590.381****
Residual (Lab. error)	126	0.3111	
Total	251	107.2494	

Table	Treatment	Storage	Treatment x storage
SED	0.1859	0.1491	0.5578

* significant at 5% level
** " 1% "
*** " 0.1% "
**** " 0.01%

Figure 4:1 The development of peroxide values of AMF, AMF plus different levels of Ronoxan A and AMF plus 300 ppm Embanox 7 for 48 hours under the stability test condition

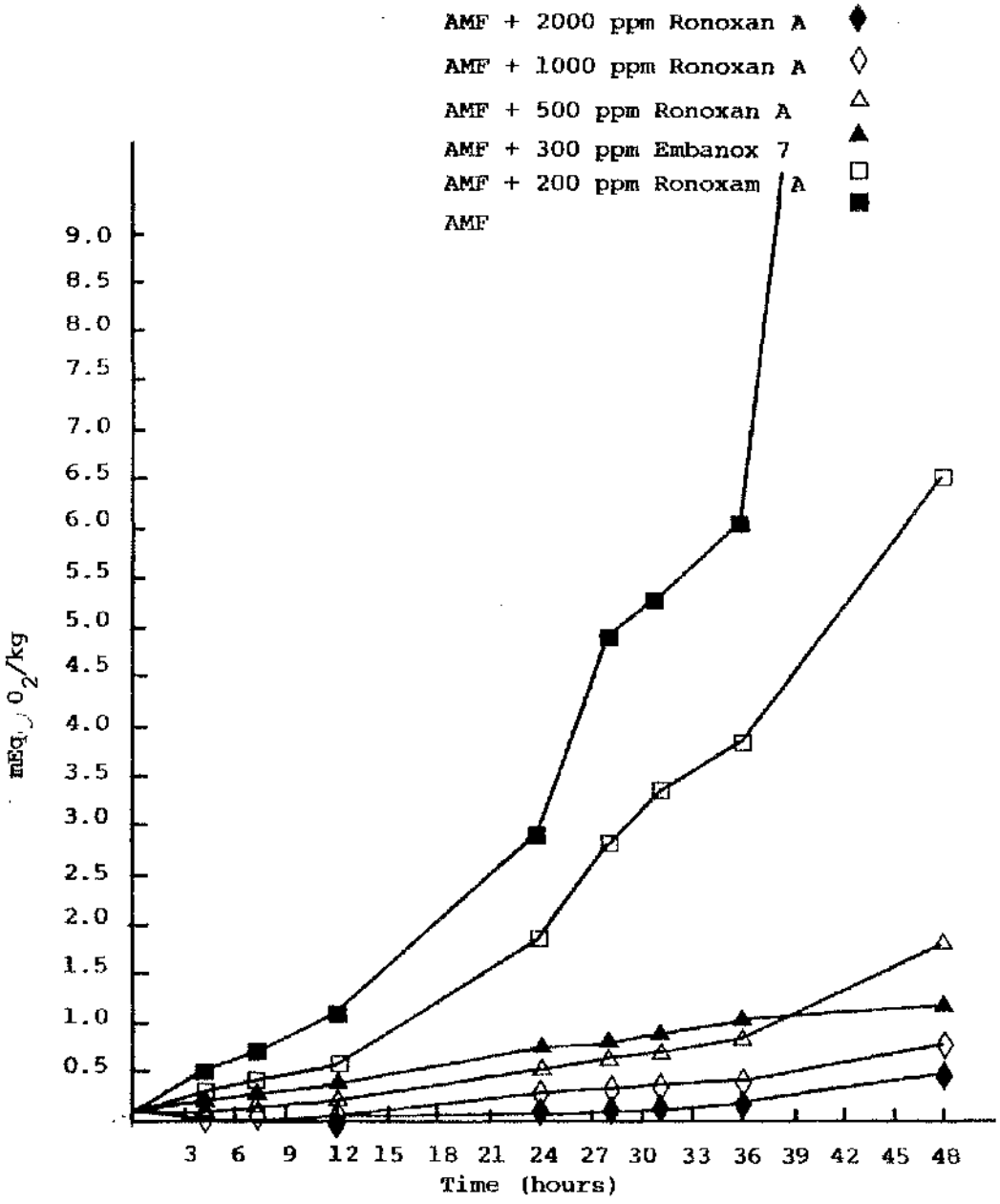


Figure 4:2 The development of peroxide values of AMF with antioxidants ascorbyl palmitate at levels of 100 and 200 ppm and DL- -Tocopherol at levels of 100 and 200 ppm for 48 hours under the condition of the stability test

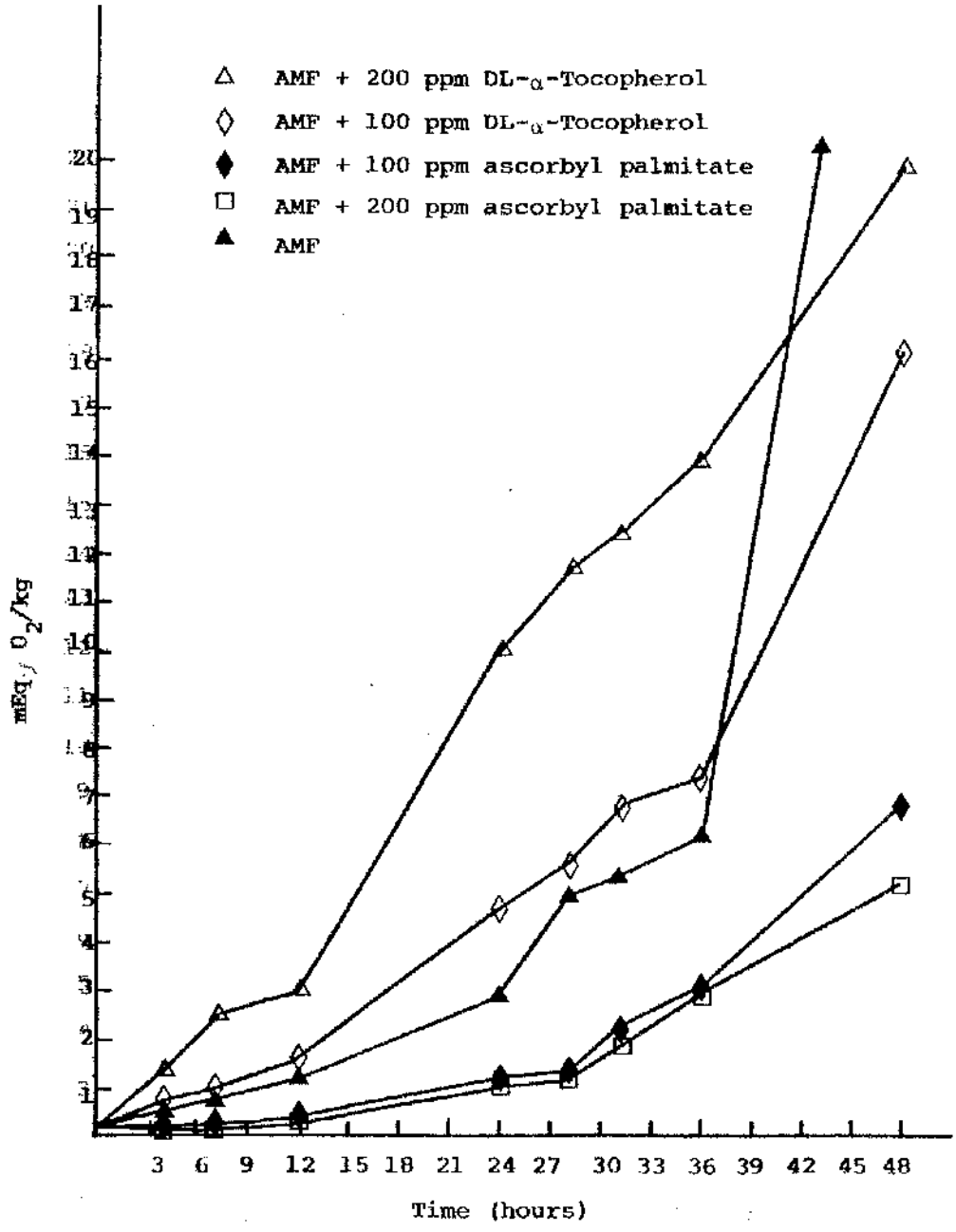
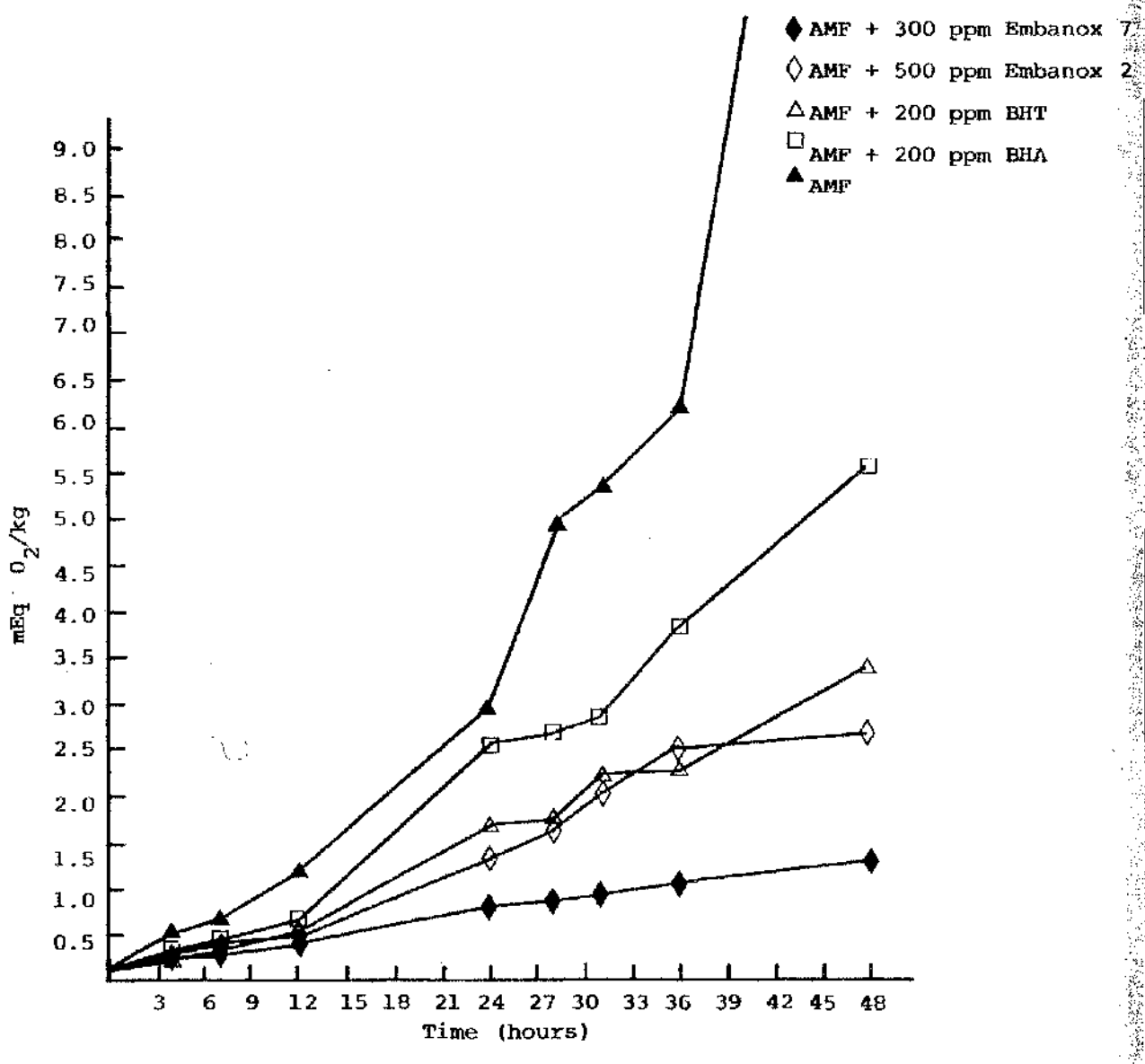


Figure 4:3 The development of peroxide values of AMF and AMF plus antioxidants during 48 hours under the stability test condition



4.5.3 Statistical Analysis

The statistical analysis of variance for the different treatments (e.g. AMF and various antioxidants and their interactions are shown for the complete experimental period in Table 4:3.

4.5.3.1 Treatment

The differences between the development of the peroxide value of the AMF and the AMF plus different kinds and levels of antioxidants showed very highly significant results ($P < 0.0001$).

4.5.3.2 Time

There were very highly significant differences ($P < 0.0001$) between the peroxide values throughout the experimental time.

4.5.3.3 There was a very highly significant interaction ($P < 0.0001$)between the treatments and the experimental time.

4.5.3.4 The laboratory errors which show the differences between the duplicate results for each treatment were very small.

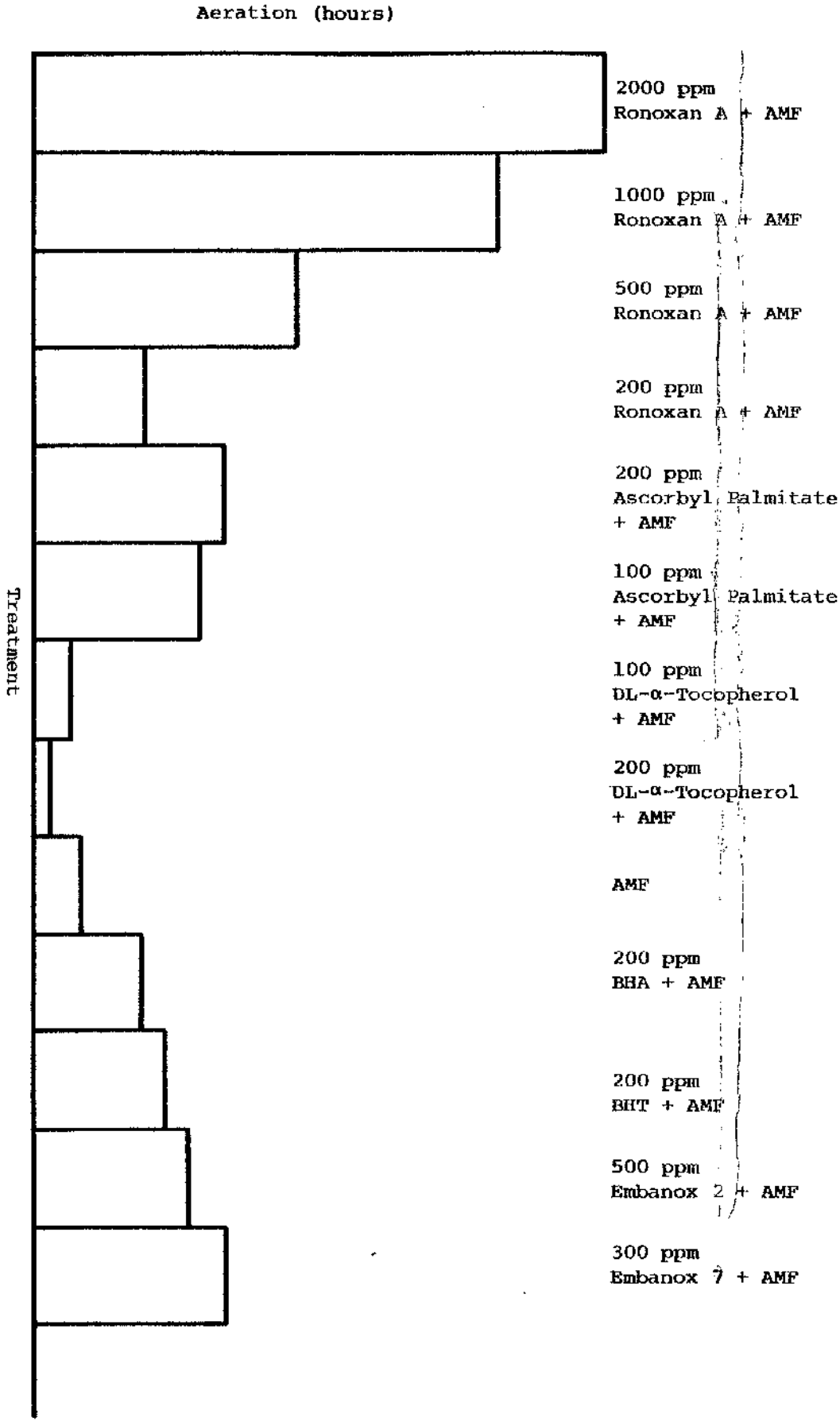
4.5.3.5 The experimental error was not estimable due to the lack of sufficient replication for each treatment to allow full statistical treatment of the results. The error term used in the analysis is laboratory errors (differences between duplicates) and is likely an underestimate of experimental error.

DISCUSSION

The fat stability experiments had three objectives. Firstly, to study the accelerated auto-oxidation of AMF. Secondly, to study the effect of various antioxidants in controlling the degree of auto-oxidation of AMF. Finally, to select the antioxidants and their levels to be used in the next experiments involving the storage of AMF at various temperatures for a period of one year.

It must be remembered, that the results of the accelerated fat stability test are limited by two main factors. In the first place the degradation reactions, which occur under the conditions of the test (i.e. aeration at 97.8°C) may not be the same as these which occur under typical storage conditions

Figure 4:4 Antioxidant effect as measured by the time for the peroxide value of AMF to reach 0.5 mEq O_2/kg (Fat Stability test - 97.8°C)



Secondly, the test depends only on the measurement of the peroxide value as a parameter for 'off-flavour' but it includes no measurement of changes in free fatty acid or other chemical compounds which may contribute to changes in flavour.

The inherent stability of food lipids against oxidation, depends upon the concentrations of natural antioxidant and pro-oxidant as well as such factors as the type and degree of saturation. The length of the induction period can be taken as a measure of the ability of the fat to resist oxidation. Thus, the effectiveness of any particular type and concentration of antioxidant may be measured by the increase in the length of the induction period. In practice the shape of an experimental fat stability curve is not always as simple as that predicted by the generalised theoretical model (Richardson et al., 1983). It is more useful to select the time to achieve a given peroxide value as the measure of auto-oxidative resistance during the fat stability test. The (IDF, 1977) have proposed that the peroxide value of AMF should not exceed 0.2 mEq O_2/kg prior to use in manufacture. However, for the purpose of comparing the performance of various antioxidants in the fat stability test, it was assumed that a peroxide value of 0.5 mEq O_2/kg would represent a maximum tolerable level in AMF that was to be recombined with SMP.

Ascorbyl palmitate: According to Fig. 4:4 ascorbyl palmitate has a clear antioxidative activity and the time for the AMF to reach a peroxide value of 0.5 mEq O_2/kg was extended from 4 hours to 14 hours when the ascorbyl palmitate was added at a level of 100 ppm. At 200 ppm this was increased to 16 hours. Richardson et al. (1983) described ascorbic acid as a very effective antioxidant and referred to it as one of the most efficient scavengers of alkoxy radicals, out proforming thiols and phenols. The present results confirm the highly effective antioxidative activity of ascorbyl palmitate. The peroxide values for the 200 ppm level are particularly significant at the beginning of the fat stability test because they show a decrease from the initial value for the AMF. This probably can be explained by the ability of this antioxidant to act as a peroxide decomposer. The results in Table 4:2 show that 200 ppm ascorbyl palmitate was better than 200 ppm BHA for the complete 48 hour test period. By contrast, 200 ppm ascorbyl palmitate out-performed

200 ppm BHT up to 31 hours but after this time the ascorbyl palmitate peroxide values increased more rapidly and exceeded those for BHT. Ascorbyl palmitate also can act as a synergist with DL-α-Tocopherol as will be shown in the discussion of the results for Ronoxan A. Cerutti (1956) showed that the addition of 0.02% ascorbyl palmitate to butter stored at 0°C for 150 days was more effect than the same amount of ascorbic acid, propyl gallate and butylhyroxyanisol.

DL- αTocopherol is commonly used as an antioxidant. It works as a radical scavenger and interrupts the propagation stage of the oxidation of lipids.

The results of the present work shows that DL-α-Tocopherol behaved as a pro-oxidant when it was added to the AMF at levels of 100 ppm and 200 ppm. This pro-oxidant activity was more pronounced at the level of 200 ppm.

Cillard et al. (1980a) reported that the pro-oxidant effect of α-Tocopherol has been described by many workers, in various oils such as grape seed, soyabean and cottonseed. Cillard et al. (1980a) also demonstrated the pro-oxidant effect of α-Tocopherol during linoleic acid auto-oxidation in an aqueous media, at pH 6.9. The addition of α-Tochopherol exhibited an important increase in the auto-oxidation of the linoleic especially during the first four days. Two oxidation products have been identified: α-Tocopherylquinone and a dimer of α-Tocopherol.

Cillard et al. (1980b) reported that the pro-oxidant effect of α-Tocopherol depends on the concentration of α-Tocopherol and the solvent.

Ronoxan A: Synergists are substances which, in combination with other substances, result in a mixture whose activity is greater than the sum of the activities of the individual components. Synergists may possess no activity of their own (e.g. citric acid) or may themselves be antioxidants (e.g. ascorbyl palmitate). Schuler (1980) demonstrated the synergism between DL-α-Tocopherol, ascorbyl palmitate and lecithin in lard. Various mixtures of the antioxidants were stored in open dishes at 65°C. The times taken to reach a peroxide value of 20 mEq O_2/kg were as follows:

Sample	Antioxidants & synergists mg/kg level			Duration days to reach 20 mEq O_2/kg
	Ascorbyl palmitate	DL-α-Tocopherol	Lecithin	
Control	0	0	0	1
1	250	0	0	4
2	0	50	0	4
3	0	0	700	1
4	250	50	0	17
5	250	50	700	30

The present results for Ronoxan A in AMF confirm strong synergistic interactions between DL-α-Tocopherol, ascorbyl palmitate and lecithin as follows:

Sample	Antioxidants & synergists mg/kg level			Hours to reach 0.5 mEq O_2/kg
	Ascorbyl palmitate	DL-α-Tocopherol	Lecithin	
AMF				4
AMF +	200			16
AMF +	100			14
AMF +		200		1.5
AMF +		100		3
AMF +	50	10	140	9.5
AMF +	125	25	350	22
AMF +	250	50	700	39
AMF +	500	100	1400	48

Ronoxan A also has the advantage that it is easier to dissolve in AMF than ascorbyl palmitate. The results also show the ability of Ronoxan A to initially reduce the peroxide value of the AMF to a level of zero, when it was used at levels of 1000 and 2000 ppm. Increasing the level to 2000 ppm improved its antioxidant effect giving the best results of all antioxidants used under the test conditions of this work.

BHA: According to Fig. 4:4 BHA has a clear antioxidant activity and the time for the AMF to reach a peroxide value of 0.5 mEq O_2/kg

was extended from 4 hours to 9 hours when the BHA was added to the AMF at a level of 200 ppm.

The other feature of this antioxidant was that, although the ascorbyl palmitate was a more effective antioxidant at the beginning, the BHA showed a more steady and stable activity with increasing time. Thus at the 200 ppm level of each antioxidant, the margin between their respective peroxide values had become very close after 48 hours aeration as shown in Table 4:2.

BHT: According to Fig. 4:4 BHT has a better antioxidant activity than BHA. The time for the AMF to reach a peroxide value of 0.5 mEq O_2/kg was extended from 4 hours to 11 hours when the BHT was added to the AMF at a level of 200 ppm. The BHT was less effective than ascorbyl palmitate at the beginning of the test when used at the same level, but BHT showed a better activity as antioxidant after the 31 hours of the test as shown in Table 4:2.

Embanox 2: The formation of this antioxidant gave the advantage of the marked synergistic action of BHA and BHT. According to Fig. 4:4 Embanox 2 has a better antioxidant activity than BHA and BHT. The time for the AMF to reach a peroxide value of 0.5 mEq O_2/kg was extended from 4 hours to 13 hours.

Embanox 7: The formulation of this antioxidant showed the advantage of the marked synergistic action of mixtures of dodecyl gallate and BHA. This antioxidant has a better antioxidant activity than Embanox 2, BHA and BHT (Fig. 4:4). The time for the AMF to reach a peroxide value of 0.5 mEq O_2/kg was extended from 4 hours to 16 hours.

CONCLUSION

1. The stability test is a reliable quick test for indicating the stability of AMF to oxidative rancidity and for testing the effect of different antioxidants in improving this stability.

2. The DL-α-Tocopherol acted as a pro-oxidant more than antioxidant when it was added to the AMF at levels of 100 to 200 ppm under the conditions of the experiment.

3. Ascorbyl palmitate has a good antioxidant activity and can act

as a synergist with DL-α-Tocopherol and lecithin as shown with the formulation of Ronoxan A.

4. Ronoxan A which has the advantage of synergistic action showed a very remarkable activity as antioxidant, especially when it was used at high levels (e.g. 1000 and 2000 ppm) with the AMF.

5. BHA and BHT each demonstrated a clear antioxidant activity at individual levels of 200 ppm. The synergistic effect, resulting from mixtures of BHA and BHT, was shown in the antioxidant activity of Embanox 2.

6. BHA and dodecyl gallate have a very good antioxidant activity when they are mixed together as a result of synergistic action.

7. No antioxidant could completely stop the development of the peroxide value in the AMF. Their individual effects were differentiated mainly by the ability to delay peroxide increase.

CHAPTER FIVE

The effect of storage of anhydrous milk fat on its quality and on the organoleptic properties of recombined milk produced from it

SECTION I - INTRODUCTION, MATERIALS, METHODS AND CALIBRATION OF GLC

5.1.1 Introduction

An experiment was designed to study the effect of storage conditions and antioxidants on the quality of AMF and the organoleptic characteristics of recombined milk made from it. Auto-oxidation and lipolysis are the major chemical changes which occur in AMF throughout storage. These changes influence the flavour, colour, odour and the appearance of the AMF.

The recommended maximum temperature for the transport and storage of AMF is 10°C. (IDF, 1971 and 1977). Earlier results (see Table 2:6) in the present work indicated that the peroxide value of AMF could increase from 0.104 to 0.115 mEq O_2/kg at 4.5°C over a 9 month storage period. Subsequent work showed that the peroxide value had increased to 0.63 mEq O_2/kg after 20 months at 4.5°C. The results at 9 months storage had also indicated that a temperature, somewhere between 4.5 and 11.5°C, would have a critical influence on the development of the peroxide value in AMF. For this reason, it was decided to investigate chemical and organoleptic changes at defined storage temperatures of 6, 8 and 10°C.

Parallel investigations were made at 32°C to represent non-refrigerated storage conditions in regions, such as the Middle East, which import and utilise AMF for recombined milk manufacture. A storage temperature of 55°C was also included in the study to represent a peak storage temperature condition and a condition of accelerated deterioration. The results and discussion of the experiments at 32 and 55°C are reported in Section 3 of this Chapter.

Two kinds of antioxidant were used at different levels to study their effect on selected chemical and organoleptic changes in the AMF during storage. Changes in the organoleptic quality of AMF were studied by recombining with SMP. Samples of the recombined milk were presented to a seven-member taste panel, with instructions to rank the samples, comment on their acceptability and indicate if certain parameters, such as rancidity, were detected.

5.1.2 Storage of the AMF at low temperature

Individual samples of approximately 1.4 kg of AMF were stored in Kilner jars at temperatures of 6, 8 and 10°C, in order to check the reliability of the IDF recommended maximum storage temperature of 10°C (IDF, 1977). At the same time, similar quantities of AMF containing 1000 ppm of the antioxidant Ronoxan A were stored at the same temperatures.

All the AMF used in the low temperature storage experiment was melted in a stainless steel tank with a steam-heated water jacket. Half of the melted AMF was run off into 1.4 kg capacity Kilner jars for storage and subsequent recombination. At the same time, 20 g samples of AMF were set aside in glass screw-top bottles, for storage and chemical analysis throughout the storage trials.

The AMF remaining in the melting tank was mixed gently with the measured amount of Ronoxan A, taking great care to avoid incorporation of air into the mixture. When the antioxidant had completely dissolved, 1.4 kg samples of the treated AMF were run into the Kilner jars and smaller samples taken for chemical analysis, as before.

5.1.3 Storage of AMF at high temperature

Individual samples of approximately 1.4 kg of AMF were stored in Kilner jars at temperatures of 32°C and 55°C.

All the AMF used in the storage temperatures of 32° and 55°C was melted in a stainless steel tank with a steam-heated water jacket. One quarter of the melted AMF was run off into the 1.4 kg capacity Kilner jar for storage and subsequent recombination. At the same time 20 g samples of AMF were set aside in glass screw-top bottles, for storage and chemical analysis throughout the trials.

Three quarters of the AMF remaining in the melting tank were subdivided into three parts and mixed separately with

- a measured amount of Ronoxan A to give a level of 1000 ppm of additive
- a measured amount of Ronoxan A to give a level of 2000 ppm of additive
- a measured amount of Embanox 7 to give a level of 300 ppm of additive.

Those samples of AMF with the antioxidants were run off into 1.4 kg capacity Kilner jars for storage and subsequent recombination. At the same time 20 g samples of AMF were set aside in glass screw-top bottles, for storage and chemical analysis throughout the storage time.

5.1.4 Chemical analysis

Three major chemical analysis were carried out to follow the changes of the AMF with and without the antioxidant at all storage temperatures:-

5.1.4.1 Peroxide value: The IDF Standard Method 74 (IDF, 1974) was used to determine the peroxide value of the AMF.

5.1.4.2 Acid value: The IDF Standard Method 6A (IDF, 1969) was used to determine the acid value of AMF.

5.1.4.3 Free fatty acis (FFA): The quantitative measurement of all the FFA in milk or milk products presents a difficult analytical problem. The acids range in solubility from water soluble (C_4) to almost completely water insoluble (C_{18}), and are accompanied by other water soluble acids, particularly lactic, which it is not desired to measure (Deeth & Fitz-Gerald, 1983). The methods which have been used to measure the FFA may be designated as follows:

1. Fat separation
2. Solvent extraction
3. Solid phase adsorption.

The short chain fatty acids, only represent about 5-6 per cent of the total fatty acids, and therefore are only a minor part of the total FFA and the final value of the acidity of the fat phase. However, since the short chain fatty acids have a strong influence on flavour defects in milk fat, Kuzdzal-Savoie (1980) reported that as the short chain fatty acids represent about 5 to 6 per cent of the total fatty acis, their presence or their absence has little influence on the intensity of the phenomenon and on the final value of the acidity of the fat phase, but their presence in the tested product may be related to an organoleptic defect. That is the reason why it is necessary to propose a method of FFA determination which takes the short chain acids into account.

Deeth, Fitz-Gerald & Wood (1975) described their proposed method for the measurement of FFA as the modified Dole extraction-titration method. This method has a better recovery of all the FFAs, (particularly the short chain acids) than the procedure of the Bureau of Dairy Industry (BDI) method; modification of the BDI method of Thomas, Neilsen & Olson (1955) was proposed by Driessen et al. (1977) for estimating fat acidity in raw milk. The wide variation in results between laboratories in the Netherlands was found to be much improved after the introduction of this modification.

Deeth et al. (1983) reported that the solvent extraction method estimated a higher proportion of the total FFA compared to the BDI method. This was due to the higher extraction of butyric acid and the higher probability of lactic acid and other acid components also being measured.

Fat separation and solvent extraction methods are usually employed for routine analyses of total acidity. According to the replies received by the International Dairy Federation (1982) in response to a questionnaire 282/A. By contrast, the quantitative determination of the individual FFAs is for more detailed. These determinations generally have two steps:

(a) Separation of FFA from the fat

(b) Gas chromatographic analysis for quantifying the acids.

Studying the FFAs by gas-chromatography can be done by isolating the ester derivatives of the individual FFAs, as in the method of Badings (1970) and Kisza et al. (1978). Alternatively, the free acids may be separated by themselves without derivatisation, as in the methods of Gray (1975), Woo et al. (1980) and Deeth, Fitz-Gerald & Snow (1982). Because of the risk of loss of short-chain acids during derivatisation, direct determination of the free acids is preferable.

5.1.4.4 The quantitative analysis of FFAs in the AMF

The quantitative method of FFA analysis by Gray (1975) was used to study quantitatively each fatty acid of the AMF from n-butyric acid (C_4) to oleic acid ($C_{18:1}$).

The FFAs were separated using a gas chromatography (GLC) Packard, Model 437 fitted with a programmed temperature controller, flame ionization detector and Packard Pen Recorder, Model 641. A glass column 2.5 m long x 3 mm (i.d.) packed with 7 per cent (w/w) stabilised diethylene glycol succinate (DEGS). The column was conditioned at 230°C for 48 hours prior to use. The nitrogen carrier gas was passed through a gas trap containing formic acid prior to entering the injection port at a rate of 20 ml/min. The formic acid was used to convert the potassium salts to the more volatile free fatty acids. The flow rates of hydrogen and air to the detector were 20 and 250 ml/min respectively. The injector port was held at 230°C. The chromatograph was programmed from 100°C to 210°C at a rate of 10°C/minute following injection.

5.1.4.4.1 Proportion of standards

Solutions of n-Butyric (C_4), n-Hexanoic (C_6), n-Octanoic (C_8) and n-Nonanoic (C_9) acids were prepared in distilled water:acetone (80/20 v/v). Five samples were converted to the potassium salts by titration with 0.05 N KOH to the phenolphthalein end point. The long chain acids n-Decanoic (C_{10}), Lauric (C_{12}), Myristic (C_{14}), Palmitic (C_{16}), Heptadecanoic (C_{17}), Stearic (C_{18}) and Oleic ($C_{18:1}$) were prepared in acetone:chloroform (1/1 v/v) and the potassium salts similarly prepared. The concentration of the acids were in the range of 0.06-0.27% (w/w). The response factors for C_4, C_6 and C_8 were measured using C_9 as standard FFA according to the equation of Woo et al. (1980).

$$\text{Response factor of FFA} = \frac{(\text{Wt of FFA}) \times (\text{Peak area of } C_9)}{(\text{Wt of } C_9) \times (\text{Peak area of FFA})}$$

Whereas C_{17} was used as a standard FFA for the long chain fatty acids (C_{10} to $C_{18:1}$) and the response factor was measured in the same manner.

5.1.4.4.2 Isolation of FFA from the AMF

Anhydrous milk fat (ca 20 g) was dissolved in 50 ml of diethyl ether ethanol (1/1, v/v) and titrated with 0.05 N-methanolic KOH to the phenolphthalein end point. Measured amounts of potassium salts of the standard acids (C_9 and C_{17}) were added. Removal of the diethyl ether was carried out by using a rotary vacuum evaporator

at a temperature of 30-35°C. Two to three ml of distilled water, 2 ml of methanol and 25 ml of hexane were added to the remaining mixture of the fat ethanol mixture before transferring to a 250 ml separating funnel. The aqueous layer, which contained the potassium salts, was collected after two more rinses with 25 ml hexane, then evaporated by the rotary vacuum evaporator at 30-35°C to 1 or 2 ml FFA mixture for GLC analysis.

5.1.4.4.3 Gas chromatographic analysis of the FFA concentrate

One to ten microlitres of sample were injected on - column with a Hamilton 10 µl syringe. The entire GC run required 45 minutes for the analysis form C_4 to $C_{18:1}$ FFA. Chromatographic peaks were identified by retention time, and peak areas were measured by triangulation. Individual FFA were quantified according to the equation of Woo et al. (1980).

$$\text{ppm of FFA} = \frac{(\text{response factor})(\text{Peak area of FFA})(\text{Wt of Standard FFA in } \mu g)}{(\text{Peak area of Standard FFA})(\text{Wt of Sample in g})}$$

5.1.5 Organoleptic tests

Low-heat skim milk powder (heat no. 77.6) packed in 25 kg multi-wall bags with a polythene liner was purchased from a commercial dairy factory. The AMF purchased from a commercial dairy factory was stored before recombination with the SMP for 6 and 12 months,

(a) at 6, 8 and 10°C

and (b) 32°C and 55°C.

At each storage temperature AMF was with and without antioxidants as specified in section 5.1.2 and 5.1.3 respectively.

Batches of AMF were withdrawn at 6 and 12 month intervals to be recombined with the low-heat SMP.

The recombination process was the same as that described in Chapter One with only the following one modification:

> the melted AMF was introduced into the reconstituted milk via the balance tank of the pasteuriser instead of introducing the melted fat in the mixing tank.

This modification eliminated all problems of fat separation.

Figure 5:1 The form used for the organoleptic tests of the recombined milk

Sample No.	Rank No.	Acceptability (Yes or No)	Detectability (Yes or No)							
			Chalky	Fatty	Powdery	Acid	Rancid	Metallic	Nutty	

Three groups of recombined milk were prepared:

1. Recombined milk prepared by using the low-heat skim milk and the AMF stored at low temperatures (6, 8, 10°C) with and without antioxidant (1000 ppm Ronoxan A).

2. Recombined milk prepared from low-heat skim milk powder and AMF stored at 32°C with and without the antioxidants (1000 ppm and 2000 ppm Ronoxan A and 300 ppm Embanox 7).

3. Recombined milk prepared from low-heat SMP and AMF stored at 55°C with and without the antioxidant (1000 ppm and 2000 ppm Ronoxan A and 300 ppm Embanox 7).

They were presented to seven taste panelists. A control sample was made from the low-heat SMP used in 1 to 3 above and AMF which had been kept in the freezer at -18°C. Each organoleptic evaluation included a control sample.

The instructions given to the panel are detailed in Fig. 5:1. Each organoleptic evaluation was carried out on single examples on two occasions.

5.1.6 Materials and equipments

- AMF of recent manufacture was supplied by the Aberdeen and District Milk Marketing Board, Twinspires, Aberdeen, Grampian Region, Scotland.

- SMP of recent manufacture was supplied by Dairy Crest, Lostwithiel, Cornwall, England.

- Antioxidants were supplied by (a) Roche, Dunstable, Bedfordshire, England, and (b) May and Baker Limited, Dagenham, Essex, England.

- Fatty acids were supplied by BDH Chemicals Limited, Poole, Dorset, England.

- Ravenhead Kilner Jars of about 1.4 kg capacity were used to store the AMF at all temperatures.

- Laboratory glassware was supplied by R & J Wood Limited, Paisley, Scotland.

- Incubators

(a) Low Temperatures

6°C ±0.5°C = an environmental cold room with chart recorder, regulated by thermostat

8 and 10°C ±0.5°C = Vindon Scientific Limited, Oldham, Lancashire, England.

(b) High Temperatures

32°C ±1°C = water jacketed incubator supplied by Baird and Tatlock, Essex, England

55°C ±2°C = anhydric incubator supplied by Astell and Hearson, London, England.

5.1.7 Results for calibration of GLC

5.1.7.1 Identification of the FFA: The retention times were measured in minutes for each FFA (i.e. C_4, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, $C_{18:1}$) by injecting them individually into the GLC as solutions of their potassium salts. Then a solution of low molecular weight salts of the FFAs (C_4, C_6, C_8) and a solution of high molecular weight salts of the FFAs (C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, $C_{18:1}$) were run separately through the GLC. Each group contained its relevant standard fatty acid (C_9 for the low molecular weight and C_{17} for the high molecular weight FFAs). The results as shown in Fig. 5:2.

5.1.7.2 Response factors: The flame ionisation detector responses of the individual acids were obtained experimentally by measuring the area under a chromatographic peak (Dickes & Nocholas, 1976) of a known amount of each fatty acid. Then, this peak area was directly related to a measured chromatographic peak area of a known amount of the standard (C_9 or C_{17}) by applying the equation of Woo et al. (1980) for calculating the response factor for each fatty acid. The mean and standard deviation of five response factors for each FFA are given in Table 5:1. These measurements were made at the beginning of the storage periods. Average response factors were calculated, in a similar way, prior to the GLC determinations of the FFAs that were carried out at approximately

Figure 5:2 The flame ionisation detector response for short chain even number (C_4, C_6, C_8) and long chain even-number (C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, $C_{18:1}$) free fatty acids with standard acids of n-nonanoic acid (C_9) for short chain fatty acids and heptadecanoic acid (C_{17}) for long chain fatty acids

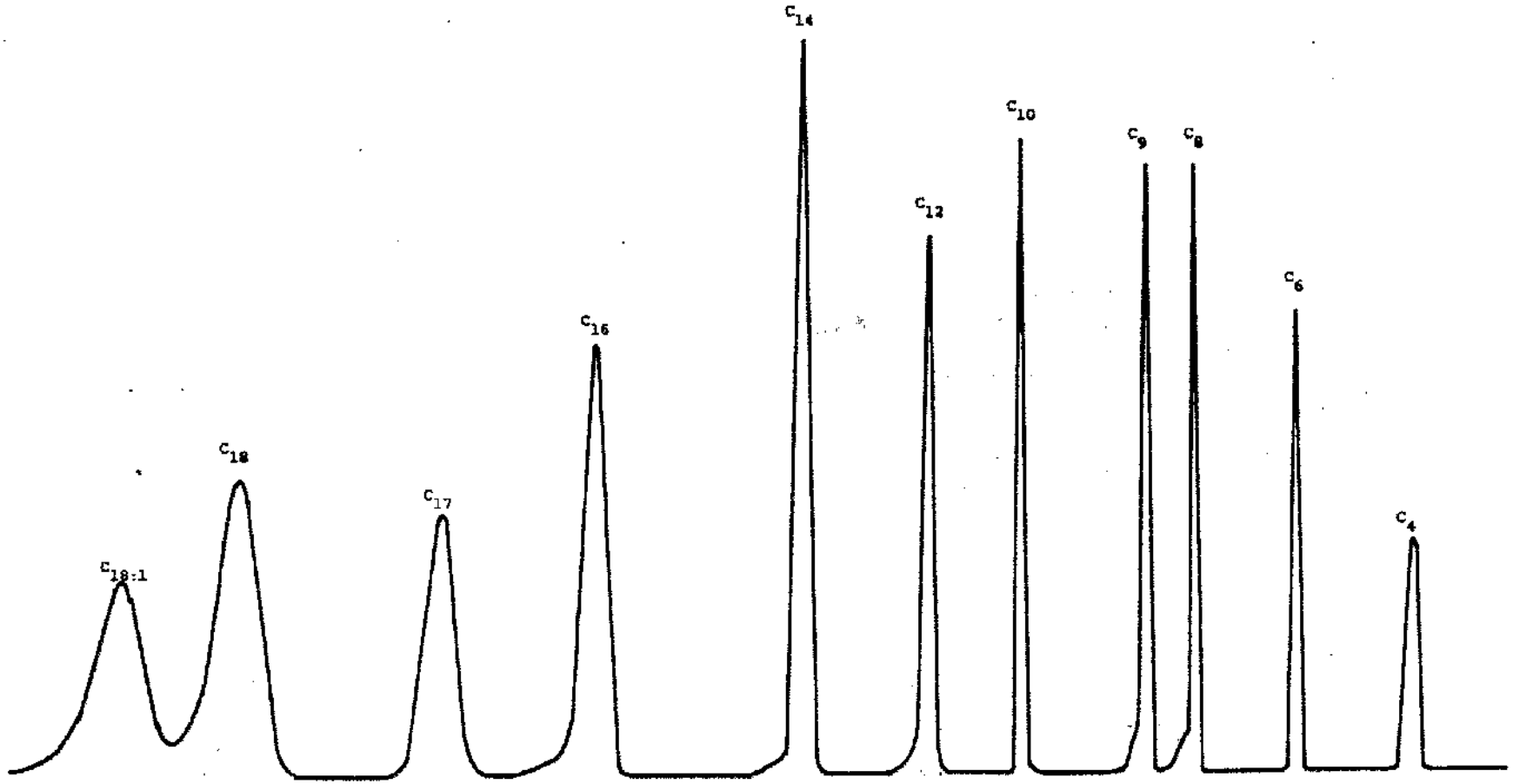


Figure 5:3 The flame ionisation detector response for the antioxidants BHA and BHT

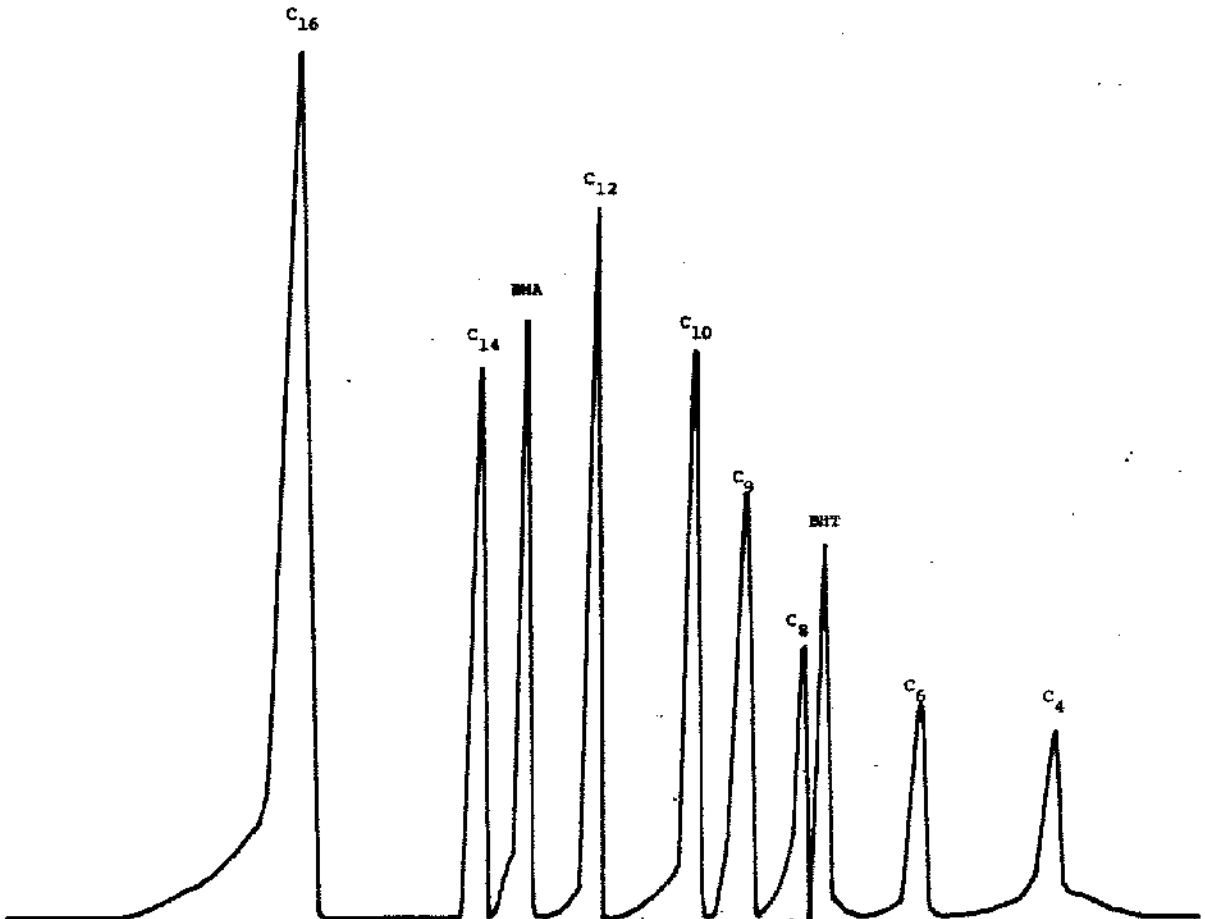


Figure 5:4 Gas chromatogram of FFAs isolated from recently manufactured AMF

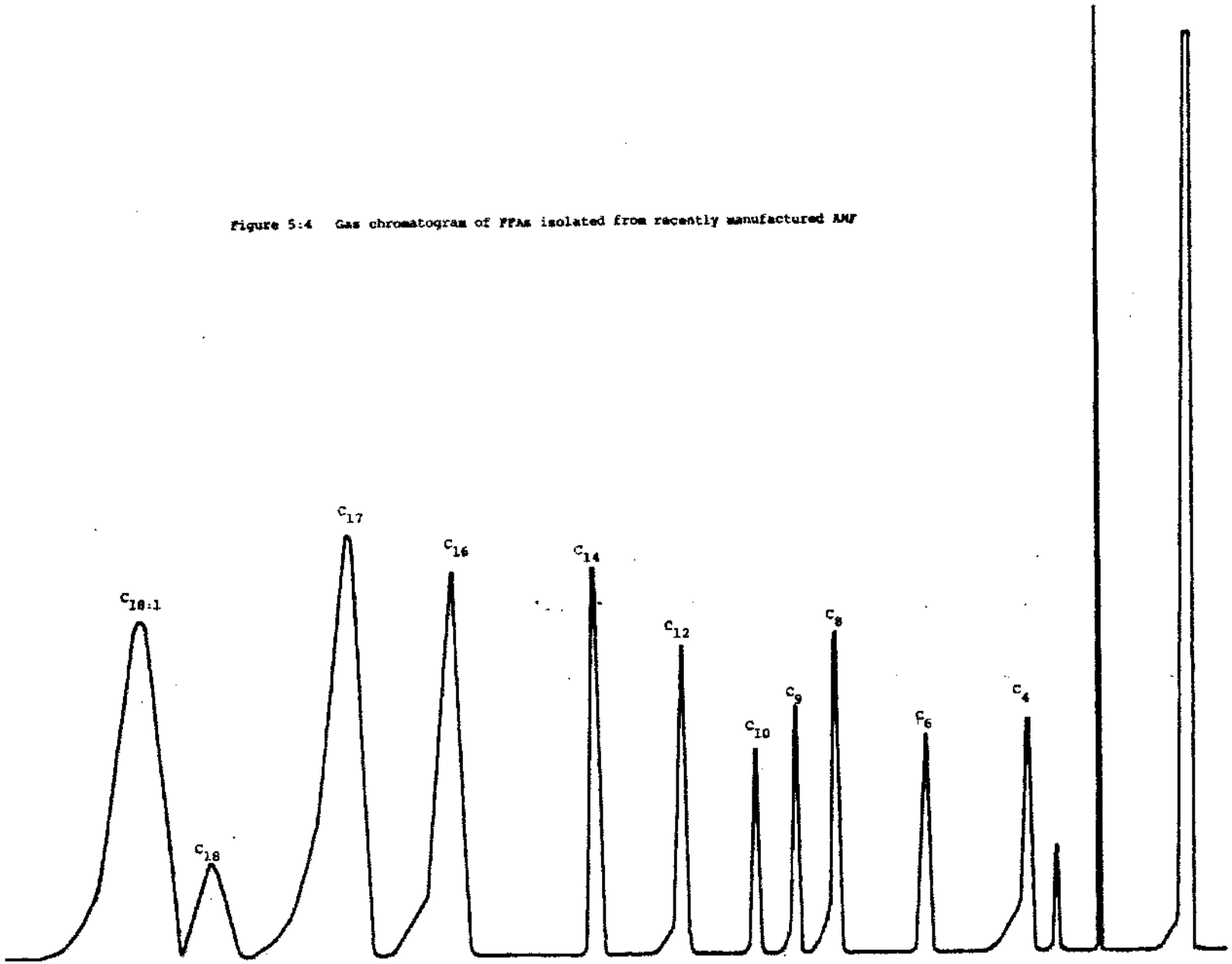
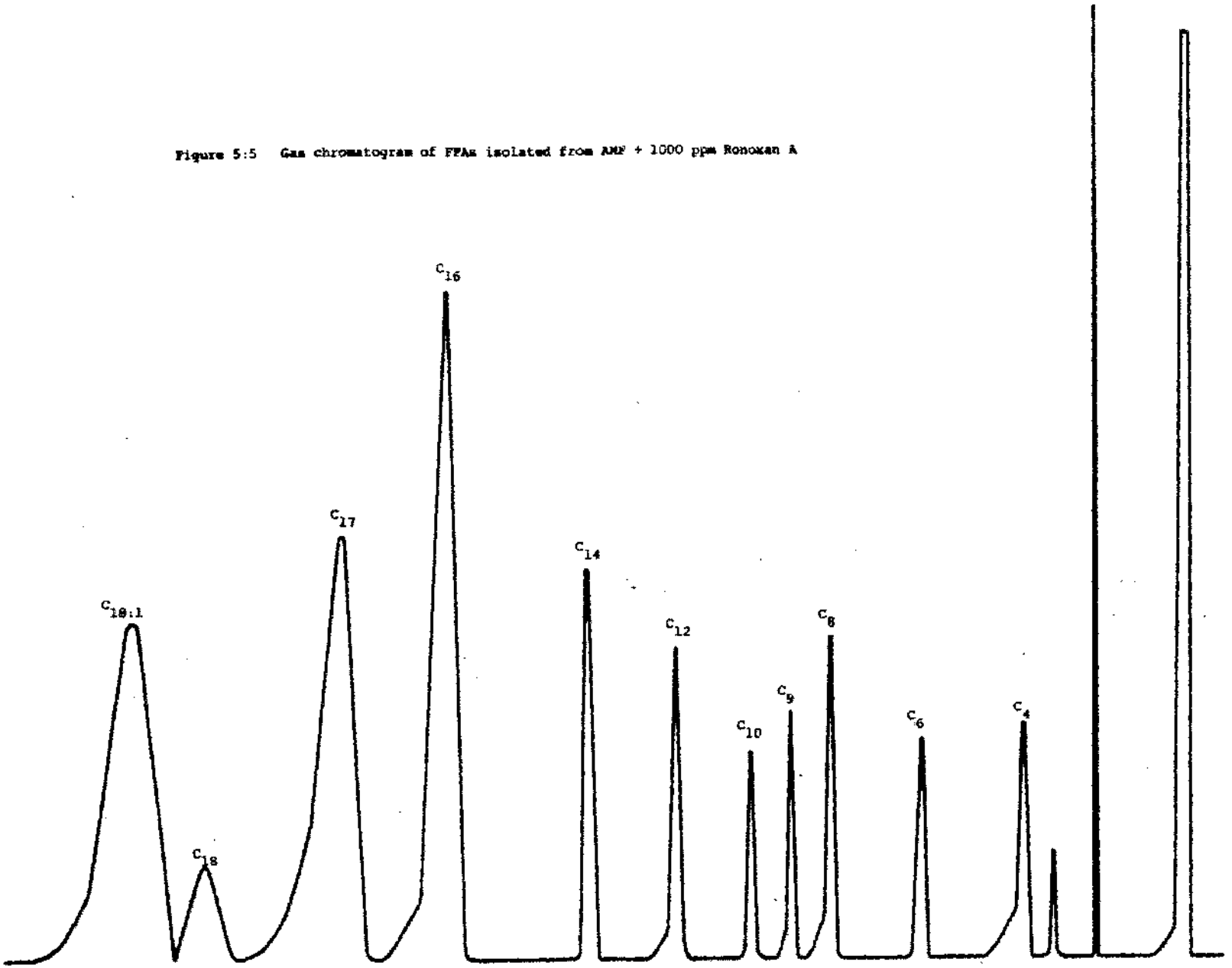


Figure 5:5 Gas chromatogram of FFAs isolated from AMF + 1000 ppm Ronoxan A



monthly intervals.

5.1.7.3. **Analysis of synthetic FFA mixtures:** Synthetic FFA mixtures (C_4 to $C_{18:1}$) were prepared in diethyl ether/ethanol (1:1, v/v) for analysis and were subjected to the entire procedure described for AMF (Section 5.1.4.4).

Recovery data were obtained for three concentrations of FFA and their mean values with standard deviation of these values from the mean are shown in Table 5:2. The data shows that nearly 100% recovery of FFA was achieved for each of the concentrations. A range of recovery from 95.7% to 115.2% was obtained for the different FFA. The results showed very low differences between the values obtained for each FFA.

5.1.7.4. **Recovery of FFA added to AMF:** It was essential to check for any influence that the AMF might have on the percentage recovery of free fatty acids formed from the AMF during the storage trials. To determine the influence of AMF on FFA recovery, four synthetic FFA mixtures (C_4 to $C_{18:1}$) were added to four AMF samples before analysis. All the added FFA with the original FFA of the AMF samples were recovered quantitatively as shown in Table 5:3.

TABLE 5:1

Flame ionisation detector response factors for even-number free fatty acids

	Response factor						
	1	2	3	4	5	Mean*	Standard deviation
C_4	1.24	1.28	1.29	1.23	1.29	$\pm^{1.266}_{0.037}$	0.029
C_6	0.89	0.90	0.92	0.91	0.88	$\pm^{0.900}_{0.020}$	0.016
C_8	0.89	0.90	0.90	0.89	0.88	$\pm^{0.892}_{0.010}$	0.008
C_{10}	1.12	1.12	1.14	1.10	1.13	$\pm^{1.122}_{0.0190}$	0.015
C_{12}	1.03	0.98	0.99	1.02	1.00	$\pm^{1.004}_{0.026}$	0.021
C_{14}	0.86	0.84	0.83	0.81	0.81	$\pm^{0.830}_{0.026}$	0.021
C_{16}	0.88	0.86	0.87	0.83	0.86	$\pm^{0.86}_{0.024}$	0.019
C_{18}	1.16	1.06	1.05	1.04	1.07	$\pm^{1.076}_{0.060}$	0.048
$C_{18:1}$	1.22	1.26	1.25	1.24	1.26	$\pm^{1.246}_{0.021}$	0.017

C_9 Standard FFA for short-chain FFA (C_4, C_6, C_8)

C_{17} Standard FFA for long-chain FFA (C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, $C_{18:1}$)

*t-test at 95% confidence intervals for mean

TABLE 5:2

Recovery of synthetic FFA mixtures

Fatty acids	mg added	Recover %	mg added	Recovery %	mg added	Recovery %	Mean*	Standard deviation
C_4	14.1	98	9.9	97.2	16	101	98.7 ±3.3	2.003
C_6	10.4	95	10.2	98	18.9	97	96.7 ±2.6	1.53
C_8	9.6	101	9.4	104.3	19.4	106.5	103.9 ±4.7	2.77
C_{10}	10.8	117	15.3	116.5	11.4	112	115.2 ±4.6	2.75
C_{12}	18.4	97.2	16.2	99	20.6	102	99.4 ±4.1	2.42
C_{14}	13.2	112.2	16.9	108	22.2	111	110.4 ±3.6	2.16
C_{16}	17.7	95.5	16.1	98	20.9	97	96.8 ±2.1	1.26
C_{18}	15.2	97	20.2	96	22.0	94	95.7 ±2.6	1.53
$C_{18:1}$	14.7	101	24.5	100.5	26.2	104.9	102.1 ±4.1	2.41

*at 90% confidence intervals for means

TABLE 5:3

Recovery of FFAs added to AMF

	ppm original	ppm added	Recovery %	ppm added	Recovery %	ppm added	Recovery %	ppm added	Recovery %	Mean* value of recovery	S.D
C_4	3.99	375.06	90.4	418.02	90.4	781.92	92.3	857.26	90.8	91.0 ±1.06	0.90
C_6	2.39	399.42	94.0	418.15	94.0	781.85	93	866.99	92.1	93.3 ±1.07	0.91
C_8	5.49	418.90	102.8	437.47	97.2	917.91	102.1	1042.35	98.4	100.1 ±3.2	2.74
C_{10}	40.4	569.90	122.9	680.50	120.6	1000.47	117.511	813.42	122.78	120.9 ±3	2.52
C_{12}	55.18	540.67	101.8	558.99	100.8	956.76	105.3	852.39	106.7	103.7 ±3.3	2.80
C_{14}	115.11	706.28	88.9	981.87	93.9	961.62	92.0	998.51	89.6	91.1 ±2.7	2.29
C_{16}	285.53	891.38	99.8	908.96	100.8	1199.59	100.7	940.06	102.3	100.9 ±1.2	1.04
C_{18}	138.86	769.61	94.8	539.54	97.5	835.35	95.3	1300.50	95.0	95.7 ±1.5	1.25
$C_{18:1}$	728.15	550.41	98.8	1011.03	98.50	951.91	97.0	1344.34	100.6	98.7 ±1.7	1.48

*t - test 90% confidence intervals for means

SECTION II - RESULTS OF LOW TEMPERATURE STUDY

5.2.1 Changes in the FFA profile of AMF stored for one year at 6, 8 and 10°C, with and without added antioxidant

Statistical analysis of variance was carried out for each variate (each FFA) as shown in Tables 5:6 to 5:16. The term 'Storage' is used for the storage time intervals. The term 'Treatment' was used to differentiate between the AMF and the AMF + 1000 ppm Ronoxan A, and term 'Temperature' was used for the different storage temperatures. The quantitative analysis values of each FFA are shown in duplicate in all the Tables.

The statistical results of these FFAs and their corresponding acid values showed:

1. Storage time: There were no significant differences between the amounts of all FFAs (C_4 to $C_{18:1}$) throughout the storage time. There were highly significant differences ($P < 0.0001$) between the acid values due to the effect of the length of storage time.

2. Treatments: Palmitic acid (C_{16}) showed very highly significant differences ($P < 0.0001$) between its values as a result of the antioxidant treatment. Myristic acid (C_{14}) showed a small significant difference ($P < 0.1$) as a result of the antioxidant treatment. There were no significant differences between the values of all the other FFAs due to the effect of the antioxidant treatment. Finally, there were very highly significant differences ($P < 0.0001$) between the acid values due to the effect of the antioxidant treatment.

3. Temperatures: There were no significant differences between the amounts of all the FFAs as a result of the different storage temperatures of the samples.

DISCUSSION

The method used to measure the entire range of FFA in AMF has two major advantages over those reported by previous workers (Iyer et al., 1967; Bills & Day, 1964; Ohren & Tuckey, 1969). Tedious

TABLE 5:6

The effect of various storage temperatures on the level (ppm) of n-butyric acid (C_4), over a one year period, in AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm

			Storage time				
			2 m	4 m	7 m	10 m	12 m
Initial		4.269					
AMF		4.271					
AMF	Stored at 6°C		4.144	4.262	4.635	4.422	4.120
			4.492	4.567	4.588	4.579	4.222
	Stored at 8°C		4.352	4.126	4.663	4.519	4.173
			4.307	4.144	4.209	4.477	4.210
	Stored at 10°C		4.328	4.123	4.290	4.429	4.213
			4.265	4.372	4.581	4.228	4.274
AMF + 1000 ppm Ronoxan A	Stored at 6°C		4.252	4.574	4.270	4.517	4.504
			4.300	4.575	4.279	4.245	4.472
	Stored at 8°C		4.319	4.112	4.151	4.539	4.643
			4.485	4.328	4.615	4.337	4.663
	Stored at 10°C		4.197	4.292	4.216	4.219	4.362
			4.487	4.147	4.574	4.577	4.322

	df	M.S	F
Storage	4	0.03747	1.573
Treatment	1	0.02642	1.535
Temperature	2	0.02921	1.697
Treatment x Temperature	2	0.01454	0.845
Treatment x Storage	4	0.07835	4.552*
Temperature x Storage	8	0.03028	1.759
Residual	8	0.01721	
Total	25	0.03229	

Table	Treatment	Temperature	Temperature x Treatment
SED	0.0339	0.0415	0.0587

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01% "

TABLE 5:7

The effect of various storage temperatures on the level (ppm) of n-hexanoic acid (C_6), over a one year period, in AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm

			Storage time				
			2 m	4 m	7 m	10 m	12 m
Initial		2.591					
AMF		2.697					
AMF	Stored at 6°C		2.597	2.475	2.422	2.580	2.581
			2.580	2.605	2.625	2.498	2.638
	Stored at 8°C		2.720	2.635	2.708	2.565	2.501
			2.617	2.761	2.402	2.764	2.490
	Stored at 10°C		2.750	2.574	2.413	2.644	2.617
			2.764	2.762	2.474	2.550	2.472
AMF + 1000 ppm Ronoxan A	Stored at 6°C		2.705	2.44	2.518	2.462	2.665
			2.534	2.546	2.681	2.799	2.401
	Stored at 8°C		2.606	2.774	2.586	2.469	2.602
			2.642	2.636	2.784	2.779	2.538
	Stored at 10°C		2.702	2.556	2.858	2.515	2.477
			2.632	2.722	2.656	2.420	2.557

	df	M.S	F
Storage	4	0.02018	1.521
Treatment	1	0.00381	0.402
Temperature	2	0.01919	2.026
Treatment x Temperature	2	0.00040	0.042
Treatment x Storage	4	0.02341	2.472
Temperature x Storage	8	0.01193	1.259
Residual	8	0.00947	
Total	25	0.01231	

Table	Treatment	Temperature	Temperature x Treatment
SED	0.0251	0.0308	0.0435

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01% "

TABLE 5:8

The effect of various storage temperatures on the level (ppm) of n-octanoic acid (C_8), over a one year period, in AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm

			Storage time				
			2 m	4 m	7 m	10 m	12 m
Initial		5.193					
AMF		5.221					
AMF	Stored at 6°C		5.173	5.174	5.255	5.159	5.273
			5.189	5.387	5.308	5.300	5.377
	Stored at 8°C		5.273	5.231	5.423	5.474	5.412
			5.149	5.279	5.373	5.207	5.162
	Stored at 10°C		5.131	5.139	5.426	5.475	5.366
			5.272	5.247	5.434	5.182	5.199
AMF + 1000 ppm Ronoxan A	Stored at 6°C		5.324	5.134	5.225	5.351	5.577
			5.147	5.100	5.284	5.403	5.265
	Stored at 8°C		5.210	5.233	5.160	5.467	5.187
			5.155	5.332	5.226	5.213	5.278
	Stored at 10°C		5.231	5.139	5.026	5.258	5.212
			5.213	5.189	5.153	5.281	5.097

	df	M.S	F
Storage	4	0.02596	2.445
Treatment	1	0.03169	3.201
Temperature	2	0.00951	0.960
Treatment x Temperature	2	0.02085	2.106
Treatment x Storage	4	0.02311	2.344
Temperature x Storage	8	0.00719	0.726
Residual	8	0.00990	
Total	25	0.01286	

Table	Treatment	Temperature	Temperature x Treatment
SED	0.0257	0.0315	0.0445

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01% "

TABLE 5:9

The effect of various storage temperatures on the level (ppm) of n-decanoic acid (C_{10}), over a one year period, in AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm

			Storage time				
			2 m	4 m	7 m	10 m	12 m
Initial AMF		40.059 / 40.023					
AMF	Stored at 6°C		40.260	40.180	41.926	40.892	40.315
			42.609	41.899	40.036	42.731	41.019
	Stored at 8°C		40.709	43.844	40.536	40.474	40.962
			40.617	43.880	41.260	40.664	40.895
	Stored at 10°C		40.234	42.927	43.687	41.792	40.185
			42.280	40.429	41.832	40.873	42.413
AMF + 1000 ppm Ronoxan A	Stored at 6°C		42.503	42.705	42.036	42.838	41.756
			42.097	44.793	41.812	42.352	40.862
	Stored at 8°C		41.424	42.750	42.594	41.267	41.165
			40.791	43.204	42.892	40.879	40.865
	Stored at 10°C		40.694	40.217	41.421	42.678	43.740
			40.302	41.813	41.285	40.092	40.257

	df	M.S	F
Storage	4	2.937	2.742
Treatment	1	2.291	1.983
Temperature	2	0.532	0.460
Treatment x Temperature	2	3.216	2.785
Treatment x Storage	4	0.044	0.038
Temperature x Storage	8	1.993	1.725
Residual	8	1.155	
Total	25	1.406	

Table	Treatment	Temperature	Temperature x Treatment
SED	0.277	0.340	0.481

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01% "

TABLE 5:10

The effect of various storage temperatures on the level (ppm) of lauric acid (C_{12}), over a one year period, in AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm

			Storage time				
			2 m	4 m	7 m	10 m	12 m
Initial		55.135					
AMF		55.085					
AMF	Stored at 6°C		55.095	56.383	56.985	53.756	54.835
			57.187	56.837	58.288	54.453	59.611
	Stored at 8°C		55.029	55.673	56.719	56.081	54.981
			56.144	54.629	54.692	58.222	56.111
	Stored at 10°C		55.483	55.160	58.671	56.099	54.438
			56.020	55.341	55.540	58.523	58.388
AMF + 1000 ppm Ronoxan A	Stored at 6°C		55.427	55.098	57.087	58.621	56.047
			57.884	54.216	55.287	58.907	54.846
	Stored at 8°C		56.272	55.892	58.297	55.849	54.416
			58.235	55.280	57.074	55.767	55.171
	Stored at 10°C		55.134	59.560	55.446	58.491	59.525
			57.567	54.750	57.089	55.252	54.818

	df	M.S	F
Storage	4	2.132	0.821
Treatment	1	1.049	0.216
Temperature	2	1.462	0.302
Treatment x Temperature	2	0.264	0.054
Treatment x Storage	4	1.353	0.279
Temperature x Storage	8	0.710	0.146
Residual	8	4.847	
Total	25	2.175	

Table	Treatment	Temperature	Temperature x Treatment
SED	0.568	0.696	0.985

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01% "

TABLE 5:12

The effect of various storage temperatures on the level (ppm) of myristic acid (C_{14}), over a one year period, in AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm

			Storage time				
			2 m	4 m	7 m	10 m	12 m
Initial		117.641					
AMF		116.573					
AMF	Stored at 6°C		117.731	117.677	119.025	117.010	114.541
			117.135	114.530	118.481	114.952	116.873
	Stored at 8°C		115.255	115.049	117.218	115.235	114.616
			117.177	115.345	114.800	116.683	115.543
	Stored at 10°C		115.517	117.755	118.838	118.673	114.739
			118.323	116.651	114.843	118.914	117.975
AMF + 1000 ppm Ronoxan A	Stored at 6°C		115.776	114.315	119.347	118.564	117.866
			119.203	114.454	119.155	115.740	117.603
	Stored at 8°C		115.656	119.059	117.519	118.211	117.370
			117.465	115.912	116.311	116.486	119.569
	Stored at 10°C		114.043	118.460	116.198	117.766	119.588
			116.344	116.175	119.891	117.920	118.403

	df	M.S	F
Storage	4	3.237	1.425
Treatment	1	9.021	7.916*
Temperature	2	3.444	3.022
Treatment x Temperature	2	2.987	2.621
Treatment x Storage	4	4.121	3.616
Temperature x Storage	8	3.600	3.159
Residual	8	1.140	
Total	25	3.051	

Table	Treatment	Temperature	Temperature x Treatment
SED	0.276	0.338	0.477

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01% "

TABLE 5:13

The effect of various storage temperatures on the level (ppm) of palmitic acid (C_{16}), over a one year period, in AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm

			Storage time				
			2 m	4 m	7 m	10 m	12 m
Initial		283.972					
AMF		291.463					
AMF	Stored at 6°C		293.29	293.88	296.04	292.42	285.55
			292.93	293.96	289.70	294.54	296.79
	Stored at 8°C		287.65	297.08	287.52	290.00	292.20
			286.58	289.83	295.96	288.98	294.99
	Stored at 10°C		294.88	291.85	293.02	290.52	296.14
			289.47	296.18	288.41	280.63	290.42
AMF + 1000 ppm Ronoxan A	Stored at 6°C		452.47	460.62	463.85	455.64	456.61
			443.92	460.27	460.59	454.61	454.67
	Stored at 8°C		456.02	458.38	453.41	454.10	459.48
			450.69	461.63	461.61	452.43	450.58
	Stored at 10°C		454.88	455.77	455.66	464.46	453.13
			446.95	459.89	460.59	449.02	459.35

	df	M.S	F
Storage	4	66.76	3.443
Treatment	1	405063.44	26057.941****
Temperature	2	8.27	0.532
Treatment x Temperature	2	3.14	0.202
Treatment x Storage	4	25.29	1.627
Temperature x Storage	8	5.41	0.348
Residual	8	15.54	
Total	25	16214.19	

Table	Treatment	Temperature	Temperature x Treatment
SED	1.018	1.247	1.763

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01% "

TABLE 5:14

The effect of various storage temperatures on the level (ppm) of stearic acid (C_{18}), over a one year period, in AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm

			Storage time				
			2 m	4 m	7 m	10 m	12 m
Initial		135.737					
AMF		138.723					
AMF	Stored at 6°C		135.64	132.29	132.09	137.10	134.54
			135.08	135.02	139.35	139.77	136.05
	Stored at 8°C		139.52	132.50	133.57	137.99	139.94
			133.04	135.10	136.91	134.88	137.82
	Stored at 10°C		137.00	133.27	138.23	135.62	139.47
			134.77	136.99	140.85	127.33	136.88
AMF + 1000 ppm Ronoxan A	Stored at 6°C		136.45	136.48	137.98	133.92	135.15
			133.39	135.62	140.74	134.41	137.15
	Stored at 8°C		137.42	139.71	140.27	127.83	138.17
			133.39	136.50	138.60	136.94	136.13
	Stored at 10°C		134.28	139.37	136.64	129.33	137.35
			136.47	134.79	137.78	137.38	133.16

	df	M.S	F
Storage	4	19.847	2.449
Treatment	1	0.517	0.070
Temperature	2	1.145	0.154
Treatment x Temperature	2	1.281	0.173
Treatment x Storage	4	13.406	1.809
Temperature x Storage	8	5.617	0.758
Residual	8	7.412	
Total	25	6.529	

Table	Treatment	Temperature	Temperature x Treatment
SED	0.703	0.861	1.218

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01% "

TABLE 5:15

The effect of various storage temperatures on the level (ppm) of oleic acid ($C_{18:1}$)), over a one year period, in AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm

			Storage time				
			2 m	4 m	7 m	10 m	12 m
Initial		729.39					
AMF		732.703					
AMF	Stored at 6°C		731.03	724.09	729.55	738.53	738.12
			733.07	727.17	736.76	730.37	739.20
	Stored at 8°C		734.74	729.79	731.98	732.05	731.78
			727.38	730.94	737.43	732.21	730.37
	Stored at 10°C		736.99	734.99	731.46	729.02	733.27
			725.99	722.64	738.62	735.99	734.90
AMF + 1000 ppm Ronoxan A	Stored at 6°C		736.25	733.34	726.48	727.53	732.65
			728.73	728.99	725.83	739.01	740.57
	Stored at 8°C		741.24	736.82	736.44	724.94	733.61
			733.08	740.05	729.57	736.09	737.84
	Stored at 10°C		729.76	730.53	732.74	733.88	736.11
			732.52	738.56	727.20	733.21	729.62

	df	M.S	F
Storage	4	19.57	0.918
Treatment	1	8.64	2.245
Temperature	2	7.16	1.861
Treatment x Temperature	2	21.54	5.596*
Treatment x Storage	4	48.39	12.574**
Temperature x Storage	8	21.40	5.560*
Residual	8	3.85	
Total	25	18.46	

Table	Treatment	Temperature	Temperature x Treatment
SED	0.507	0.620	0.877

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01% "

TABLE 5:16

The effect of various storage temperatures of a one year period on the acid value (mg KOH/g) of AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm

			Storage time				
			2 m	4 m	7 m	10 m	12 m
Initial		0.360					
AMF		0.360					
AMF	Stored at 6°C		0.352	0.360	0.369	0.367	0.366
			0.356	0.360	0.369	0.367	0.363
	Stored at 8°C		0.356	0.360	0.368	0.360	0.365
			0.356	0.362	0.362	0.367	0.365
	Stored at 10°C		0.356	0.362	0.367	0.365	0.365
			0.356	0.361	0.369	0.364	0.369
AMF + 1000 ppm Ronoxan A	Stored at 6°C		0.416	0.435	0.425	0.427	0.434
			0.406	0.439	0.429	0.423	0.431
	Stored at 8°C		0.429	0.437	0.425	0.428	0.436
			0.420	0.436	0.425	0.429	0.433
	Stored at 10°C		0.429	0.433	0.429	0.426	0.466
			0.429	0.432	0.424	0.429	0.466

	df	M.S	F
Storage	4	0.000419559	59.795****
Treatment	1	0.069564096	958.515****
Temperature	2	0.000145216	2.001
Treatment x Temperature	2	0.000108450	1.494
Treatment x Storage	4	0.000211775	2.918
Temperature x Storage	8	0.000095133	1.311
Residual	8	0.000072575	
Total	25	0.002890398	

Table	Treatment	Temperature	Temperature x Treatment
SED	0.0022	0.002694	0.003810

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01% "

TABLE 5:17

The mean* values (ppm) of each FFA in the AMF and AMF with 1000 ppm added antioxidant Ronoxan A stored at 6, 8 and 10°C for one year

FFA	AMF	AMF + 1000 ppm Ronoxan A
C_4	4.344	4.386
C_6	2.593	2.609
C_8	5.282	5.236
C_{10}	41.41	41.80
C_{12}	56.18	56.44
C_{14}	116.57	117.35
C_{16}	291.71	456.04
C_{18}	135.95	136.14
$C_{18:1}$	732.35	733.11

*Mean value from six time intervals (Initial, 2 months, 4 months, 7 months, 10 months and 12 months)

TABLE 5:18

The mean values (ppm) of each FFA in AMF and AMF with 1000 ppm added antioxidant Ronoxan A stored at 6, 8 and 10°C for one year

FFA	AMF			AMF + 1000 ppm Ronoxan A		
	Stored at 6°C	Stored at 8°C	Stored at 10°C	Stored at 6°C	Stored at 8°C	Stored at 10°C
C_4	4.403	4.318	4.310	4.399	4.419	4.339
C_6	2.560	2.616	2.602	2.575	2.642	2.609
C_8	5.259	5.298	5.287	5.281	5.246	5.180
C_{10}	41.19	41.38	41.67	42.38	41.78	41.25
C_{12}	56.34	55.83	56.37	56.34	56.23	56.76
C_{14}	116.80	115.69	117.22	117.20	117.36	117.48
C_{16}	292.91	291.08	291.15	456.32	455.83	455.97
C_{18}	135.69	136.13	136.04	136.27	136.50	135.65
$C_{18:1}$	732.79	731.87	732.39	731.94	734.97	732.41

Mean value from six time intervals (Initial, 2 months, 4 months, 7 months, 10 months and 12 months)

TABLE 5:19

The mean values (ppm)of each FFA in AMF and AMF plus 1000 ppm Ronoxan A (1RA) stored at 6, 8 and 10°C for one year at every time interval

	Initial	After 2 months		After 4 months		After 7 months		After 10 months		After 12 months	
	AMF	AMF	AMF + 1RA	AMF	AMF + 1RA	AMF	AMF + 1RA	AMF	AMF + 1RA	AMF	AMF + 1RA
C_4	4.27	4.315	4.340	4.266	4.338	4.494	4.351	4.441	4.406	4.202	4.494
C_6	2.65	2.671	2.637	2.635	2.612	2.507	2.680	2.60	2.574	2.55	2.54
C_8	5.21	5.198	5.213	5.243	5.188	5.370	5.179	5.299	5.329	5.298	5.269
C_{10}	40.1	41.12	41.30	42.19	42.58	41.55	42.01	41.24	41.68	40.96	41.44
C_{12}	55.12	55.83	56.75	55.67	55.80	56.82	56.71	56.19	57.15	56.39	55.80
C_{14}	117.1	116.86	116.41	116.17	116.40	117.20	118.07	116.91	117.45	115.71	118.40
C_{16}	287.78	290.8	450.8	293.8	459.4	291.8	459.3	289.5	455.0	292.7	455.6
C_{18}	137.2	135.8	135.5	134.2	137.1	136.8	138.7	135.5	133.3	137.5	136.2
$C_{18:1}$	731.1	731.5	733.6	728.3	734.7	734.3	729.7	733.0	732.4	734.6	735.1

TABLE 5:20

The mean* values of each FFA (expressed as a percentage of total FFA) of AMF stored at 6, 8 and 10°C for one year with and without added antioxidant Ronoxan A (1000 ppm)

	AMF	AMF + 1000 ppm Ronoxan A
C_4	0.3133	0.2824
C_6	0.18703	0.16797
C_8	0.38096	0.33712
C_{10}	2.9870	2.6915
C_{12}	4.052	3.634
C_{14}	8.408	7.556
C_{16}	21.041	29.363
C_{18}	9.806	8.765
$C_{18:1}$	52.824	47.203

*Mean value from six time intervals (Initial, 2 months, 4 months, 7 months, 10 months and 12 months)

TABLE 5:21

The means* of acid values (mg KOH/g) of AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm stored at 6, 8 and 10°C for one year

Treatments	Storage Temperature		
	6°C	8°C	10°C
AMF	0.363	0.362	0.363
AMF + 1000 ppm Ronoxan A	0.427	0.430	0.436

*Mean value from six time intervals (Initial, 2 months, 4 months, 7 months, 10 months and 12 months)

TABLE 5:22

The means* of acid values (mg KOH/g) of AMF with and without added antioxidant Ronoxan A at a level of 1000 ppm stored at 6, 8 and 10°C at six time intervals

Treatments	Storage time					
	Initial	2 m	4 m	7 m	10 m	12 m
AMF	0.360	0.355	0.361	0.367	0.365	0.366
AMF + 1000 ppm Ronoxan A		0.421	0.435	0.426	0.427	0.444

*Mean value from six time intervals (Initial, 2 months, 4 months, 7 months, 10 months and 12 months)

extraction procedures, often involving two silicic acid, column-chromatographic stages, are not required in the solvent extraction method. Conversion of the free fatty acids to volatile esters is not required, prior to injecting samples into the GLC.

The present method gave a better recovery of added stearic acid (C_{18}) than that obtained by Woo & Lindsay, 1980 and Woo & Lindsay, 1982. A better separation between stearic acid (C_{18}) and oleic acid ($C_{18:1}$) was also achieved.

The method showed a satisfactory separation of all the even FFAs (C_4 to $C_{18:1}$), with a very satisfactory separation of short-chain acids from the solvent front. Stearic acid was also satisfactorily resolved from oleic acid as shown in Fig. 5:2.

The use of n-nonanoic acid (C_9) as a standard for the short-chain FFAs and heptadecanoic acid (C_{17}) for the long-chain FFAs resulted in high and reliable recoveries of the FFAs. This was due to the obvious reason that the retention times of C_9 and C_{17} are similar to those of the short- and long-chain fatty acids, respectively. Gray (1975) reported that the use of formic acid in the nitrogen carrier gas would minimise peak tailing and completely suppress absorption of other acids on the column, in addition to acidifying the salts. Horwood & Lloyd (1980) also confirmed that formic acid could be used to liberate the FFA from the salts and to improve the shape of the fatty acid peaks in the GLC separation and this was recently confirmed by Deethet al. (1983).

The results obtained for the response factor (Table 5:1) of each FFA and the high recovery of both synthetic mixtures of FFA (Table 5:2) and the FFA added to the AMF (Table 5:3), show the high sensitivity of this method. The results also show that the method has good repeatability. A comparison between the present results for percentage recovery of (1) synthetic FFA mixtures, (2) FFAs added to AMF with those obtained by Woo et al. (1980) is shown in Tables 5:4 and 5:5.

The present method was also able to indicate the presence of the antioxidants, BHA and BHT, as shown in Fig. 5:3. In the case of Ronoxan A, the presence of ascorbyl palmitate in the formulation

caused an increase in the chromatographic peak of palmitic acid (C_{16}) as shown by comparison between Figs. 5:4 and 5:5.

Although the presence of BHA and BHT can be readily detected by this method, the presence of added ascorbyl palmitate can only be confirmed if the natural level of palmitic acid in the AMF is known. Alternatively, historical data on palmitic acid levels in AMF might provide a guideline. In any event, these facts might be difficult to unravel.

The method has been found satisfactory for measuring FFA in AMF. The relative proportions of FFA found in the recently manufactured AMF used in this study were similar to those reported by Deeth et al. (1983) for the butter as shown in Table 5:23.

TABLE 5:23

The level of individual FFAs expressed as a percentage of the total FFAs content of AMF (present work) and the level of individual FFAs expressed as a percentage of the total FFAs content of butter (Deeth et al. 1983)

FFA	The proportion of FFA found in recently manufacture AMF in the present work	The proportion of FFA found in a butter (Deeth et al. 1983)
C_4	0.31	0.40
C_6	0.19	0.37
C_8	0.38	0.50
C_{10}	2.9	2.9
C_{12}	4.0	5.0
C_{14}	8.5	8.4
C_{16}	20.8	22.7
C_{18}	9.9	12.5
$C_{18:1}$	52.9	47.4

The pattern of FFA was dominated by oleic acid ($C_{18:1}$) which represents more than 50% of the total FFA. This is followed by palmitic acid which represents about 20% of the total FFA. All the other FFAs combined, represent about 30% of the total FFA. The

amount of short chain FFAs (C_4, C_6, C_8) in the AMF was not very high at the beginning of the trial. This can be explained by the probable washing out of these relatively water-soluble short-chain FFAs during AMF manufacture. They may also be removed by the vacuum drying step in the manufacturing process. Fjaervol (1970b) showed that AMF had a complicated mixture of triglycerides composed of a large number of fatty acids of varying carbon chain length and degrees of saturation, dominated with oleic and palmitic acids. This supports the findings of the present work.

Downey (1980) has referred to the higher organoleptic preference for butter manufactured from lipolysed milk fat, compared with post-manufacture lipolysed butter with comparable FFA levels. He suggested that this might be due to the washing out of the strongly flavoured short-chain fatty acids during fat separation of the lipolysed milk, churning of the cream and washing of the butter granules. Thus, the ratio of short to long-chain fatty acids in butter may be significantly reduced. The loss of the short-chain FFAs during the butter production was also pointed out by O'Connell et al. (1975), and Deeth et al. (1979).

The level of FFA in the AMF showed no significant change during storage at 6, 8 and 10°C for one year (Tables 5.6 to 5.15). This would indicate that there was little if any enzymic activity for lipolysis at the above storage temperatures. The literature refers to the presence of two major kinds of lipases in milk (native lipase and bacterial lipases). The bacterial lipases differ from milk lipase in that they are not inactivated by pasteurisation, even though the organisms which produce them are destroyed. It has been confirmed recently by Cogan (1980) that natural milk lipase is completely inactivated by pasteurisation but many of the lipases produced by bacteria are not inactivated by pasteurisation. The majority of bacterial lipases exhibit maximum activity within the temperature range 30°C to 40°C (O'Donnell, 1975).

Hugo & Beveridge (1962) showed that the optimal temperatures for the lipases of many bacteria was in the range 32-37°C. Troller & Bozeman (1970) reported that the optimal temperature for Staphylococcal lipase was 32°C but considerable activity also appeared at 25°C.

Chorvath & Fried (1970) reported that the optimal temperature for Leptospiral lipase action on triglyceride emulsions was at 37°C. Finally, O'Donnel (1975) mentioned that certain lipases have the ability to hydrolyse fats at low temperatures but the rate of hydrolysis is directly related to the degree of unsaturation of the fat. He suggested that the substrate must be liquid for highest lipolytic activity.

The resistance of AMF to lipolytic activity at the lower storage temperatures (6, 8 and 10°C) for one year is probably due to the temperature having been significantly lower than that required for the optimum activity of the lipase. It is also likely that the levels of bacterial lipase was too low for significant activity to have occurred. Connolly et al. (1980) showed that no change in FFA levels occurred in butter stored at -18°C. In contrast butter stored at +15°C showed increased FFA with time. Thus the results of Connolly et al. (1980) and the present work indicate that lipolysis in milk fat might become significant between 10 and 15°C.

The presence of the antioxidant 'Ronoxan A' in the AMF influenced the pattern of FFA in AMF, due to its ability to hydrolyse to palmitic acid. The amount of palmitic acid increased very significantly as shown in Table 5:13. The extra amount of palmitic acid, derived from ascorbyl palmitate, also affected the percentage of each FFA by increasing the percentage of palmitic acid and decreasing the percentage of all other FFAs (Table 5:20).

The moderate statistical significance ($P < 0.05$) of the change (116.6 to 117.4 ppm) in myristic acid (C_{14}) levels (Tables 5:12 and 5:17) following treatment with Ronoxan A could be explained in two ways. Either there is a very low release of this acid from the lecithin in the antioxidant or, more probably, it is due to the experimental error.

The presence of a relatively high proportion (i.e. 53%) of oleic acid in the FFA profile of the AMF, suggests that AMF could be susceptible to deterioration, as the result of the availability of oleic and as a source of oxidisable unsaturated fatty acid. The high proportion of oleic acid in the FFA profile of the butter was also shown by Bills et al. (1969) and Deeth et al. (1983).

The acid value of AMF was also affected significantly by the presence of the antioxidant 'Ronoxan A'. The acid value increased significantly ($P < 0.0001$) in the AMF with added antioxidant due to the extra amount of palmitic acid released by the antioxidant.

The results indicated significant differences in the acid values throughout the storage time. However, the method is not very sensitive for several reasons. The titration can probably be read to ±1.5%. However, the end point was not always easily distinguished because of the colour of the fat. This problem was particularly marked in the case of samples of AMF with Ronoxan A. Thus, it is probably that high degree significant differences for the acid values is strongly influenced by the error of measurement. However, it would explain the definite tendency for the acid values to increase with time. Kuzdzal-Savoie (1980) pointed out that in parallel with the simple methods intended to obtain a global estimation of the FFA, it is necessary to have finer and more precise reference methods.

5.2.2 The effect of low storage temperatures (6°C, 8°C and 10°C) for one year on the peroxide value of AMF and AMF plus the antioxidant 'Ronoxan A'

The mean peroxide values of the AMF and AMF plus the antioxidant 'Ronoxan A' stored at 6, 8 and 10°C for one year were measured at approximately monthly intervals. These values and the initial values of AMF and AMF plus Ronoxan A (RA) are given in Table 5.24 and shown in Figs 5:6 and 5:7.

Statistical analysis of variance for the above data were carried out as shown in the bottom of Table 5:24. The term 'Storage' was used for the storage time, and the term 'Treatment' was used to differentiate between the AMF and AMF plus the antioxidant. Finally the term 'Temperature' was used to differentiate between the three different storage temperatures of 6, 8 and 10°C.

The statistical results of the peroxide values showed:

1. Storage time: the differences between the peroxide values of AMF with and without antioxidant were very highly significant ($P < 0.0001$) due to the effect of different storage time as shown in Table 5:24.

TABLE 5.24

The effect of low storage temperature (6°C, 8°C and 10°C) for one year on the peroxide value (mEq O_2^-/kg) of AMF and AMF + Ronoxan A (1000 ppm)

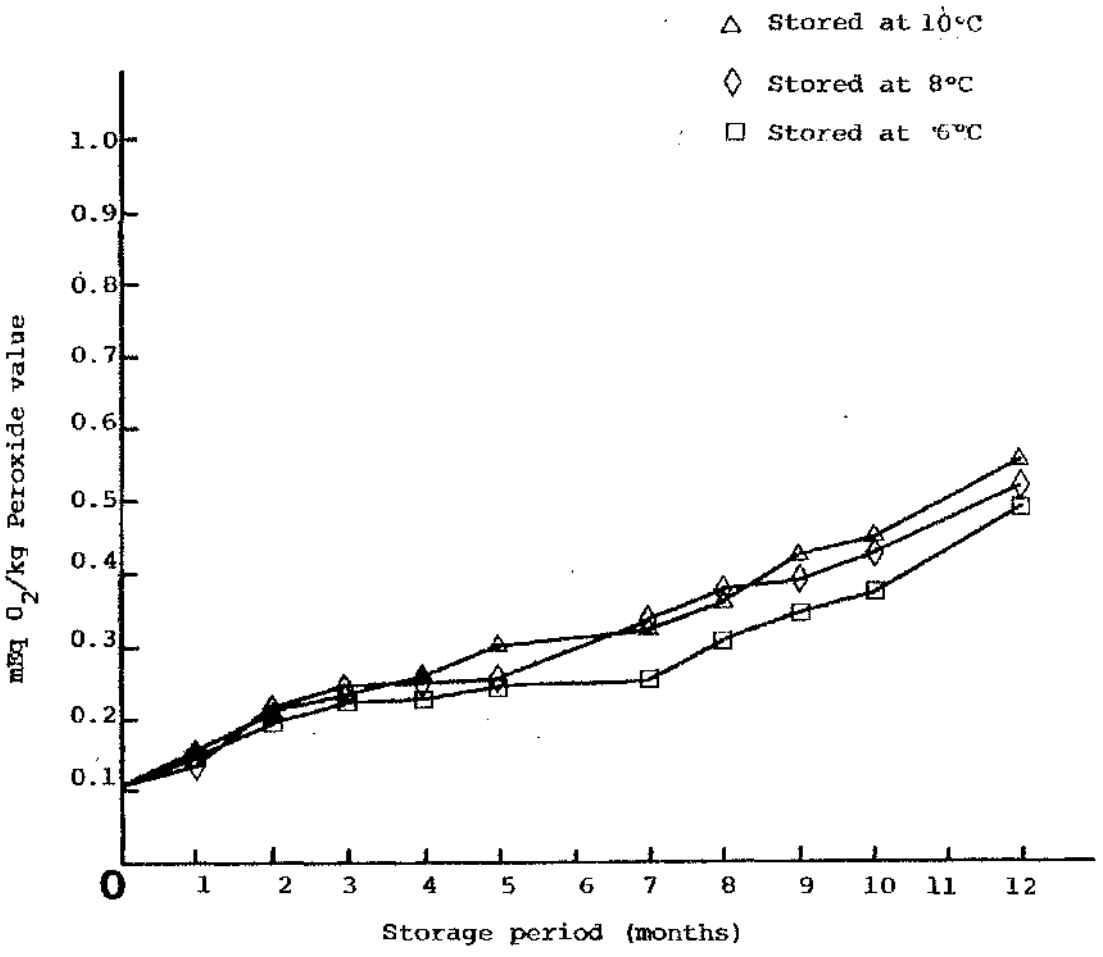
	Initial	After 1 month	After 2 months	After 3 months	After 4 months	After 5 months	After 7 months	After 8 months	After 9 months	After 10 months	After 12 months
Initial PV of AMF	0.106										
AMF stored at 6°C		0.146	0.199	0.227	0.229	0.245	0.25	0.31	0.349	0.377	0.498
AMF stored at 8°C		0.140	0.216	0.244	0.249	0.256	0.337	0.379	0.393	0.431	0.525
AMF stored at 10°C		0.154	0.206	0.231	0.253	0.303	0.321	0.360	0.429	0.449	0.560
Initial PV of AMF + RA	0.00										
AMF + RA stored at 6°C		0.014	0.023	0.029	0.039	0.040	0.053	0.053	0.055	0.060	0.064
AMF + RA stored at 8°C		0.012	0.024	0.029	0.031	0.052	0.055	0.056	0.059	0.060	0.068
AMF + RA stored at 10°C		0.012	0.030	0.044	0.046	0.047	0.054	0.056	0.059	0.062	0.073

	df	M.S	F
Storage	10	0.0621403	153.395***
Treatment	1	2.1077433	5202.785***
Temperature	2	0.0068896	17.007***
Treatment x Storage	10	0.0320774	79.180***
Treatment x Temperature	2	0.0045726	11.287**
Storage x Temperature	20	0.0004616	1.139
Residual	20	0.0004051	
Total	55	0.0448868	
Laboratory error	66	0.0001143	

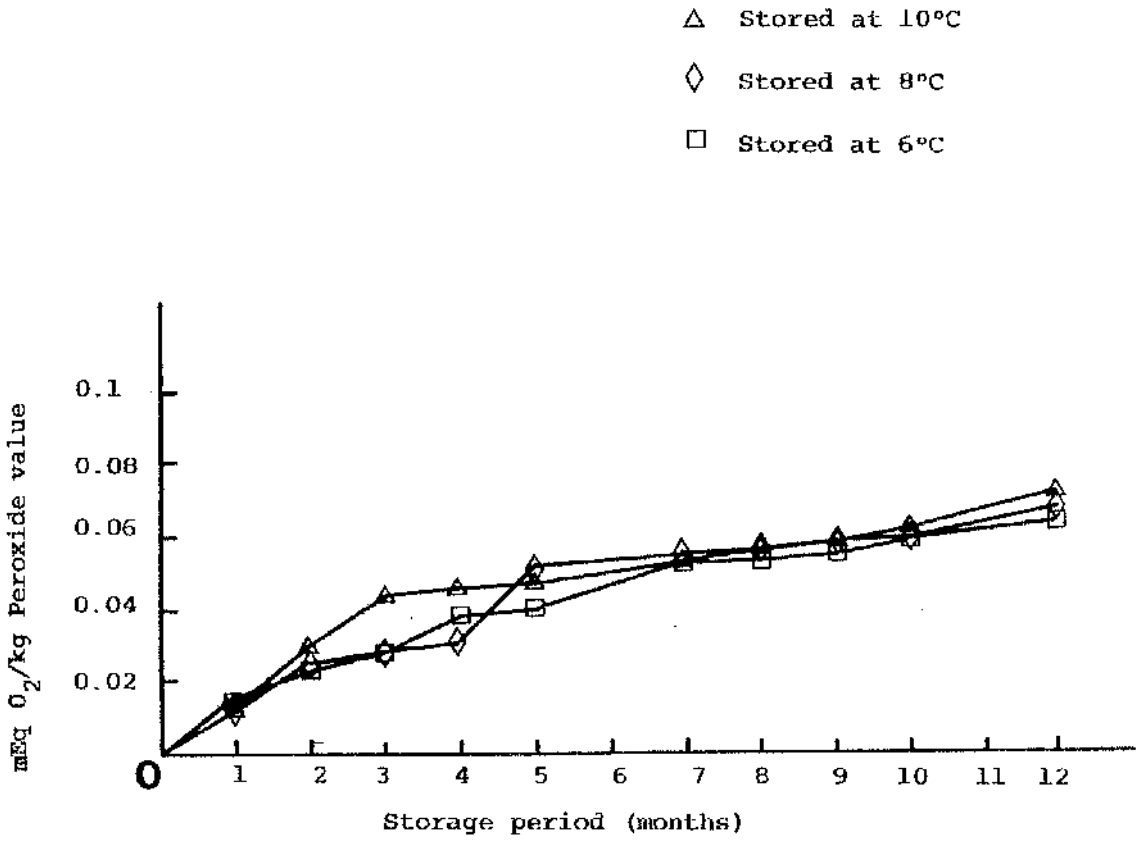
Table	Treatment	Temperature	Treatment x Temperature
SED	0.003504	0.004291	0.006069

** Significant at .1% level
*** " " .01% "

Figures 5.6 Variation in the peroxide value of the AMF during storage at low temperatures (6, 8 and 10°C)



Figures 5:7 Variation in the peroxide value of the AMF + 1000 ppm Ronoxan A during storage at low temperatures (6, 8 and 10°C)



2. Treatment: the differences between the peroxide values of the AMF and AMF + Ronoxan A were very highly significant ($P < 0.0001$).

3. Storage temperatures: the differences between the peroxide values of AMF and AMF plus Ronoxan A were very highly significant ($P < 0.0001$) due to the effect of different storage temperatures.

At the same time there were very highly significant interaction ($P < 0.0001$) between treatments and storage time. There were also a significant interaction ($P < 0.001$) between the treatments and different storage temperatures.

DISCUSSION

A major cause of flavour defects in cream and butter is the susceptibility of these products to oxidative deterioration (Badings & Neeter, 1980).

The results showed that very highly significant changes occurred in the peroxide values of AMF and AMF plus the antioxidant throughout the storage time. The increase in the peroxide value showed the presence of fat oxidation in AMF, even at a storage temperature equal to and below the IDF recommended maximum storage temperature of 10°C (IDF, 1977). This finding also confirms the early results of the present work, which showed an increase in the peroxide value of AMF at a storage temperature of 4.5°C. The results show that the storage time is an important factor which is responsible for a decrease in the quality of the AMF, as a result of oxidative deterioration.

The result also showed the highly significant differences ($P < 0.0001$) between the peroxide values of AMF and AMF with antioxidant (1000 ppm Ronoxan A). Ronoxan A exhibited the ability to lower the peroxide value of AMF initially to a level of zero. This finding confirms previous results (Chapter Four) of the present work. The results also showed that Ronoxan A effectively retards the oxidation processes. Koops (1964) found that the addition of 200 ppm (calculated on the fat) of ascorbyl palmitate to butter has a positive effect in retarding the oxidation during cold storage.

Even the small differences between the three storage temperatures used in the present work showed highly significant differences between the peroxide values of AMF. The development of the peroxide values were more pronounced as the higher temperature. Thus at 6°C the peroxide value of the untreated AMF increased approximately 4.07 times, compared with 4.95 at 8°C and 5.28 at 10°C, over the one year storage period. By contast, the AMF treated with 1000 ppm Ronoxan A exhibited a decrease by a factor of 0.60 at 6°C, 0.64 at 8°C and 0.69 at 10°C. Even though the addition of the antioxidant caused dramatic decreases in the rate of auto-oxidation, it was unable to completely stop this process. Additionally, temperature was still a factor in influencing the rate of peroxide development, even after the antioxidant had been added.

Cold storage defects in butter caused by oxidative degradation of lipid components were reported by Mulder & Kleikamp (1947). They also demonstrated that this defect does not occur when the natural surface layer of the fat globules is absent. They found that the addition of an antioxidant (hydroquinone) can prevent the cold storage defect in butter. Badings (1970) confirmed that there is no absolute proof that the off-flavours in cold-stored butter are, in fact, derived only from the oxidative degredation of the unsaturated fatty acids in the phospholipids. He showed that an artificial butter, which did not contain fat globule membrane material or phospholipids, exhibited a typical rancid flavour within a few months of storage at -10°C.

The result of the present work confirms the results of Koops (1964) and Badings (1970) that fat oxidation can occur in cold storage. Cerutti (1956) showed that the addition of ascorbyl palmitate at a level of 0.02% to butter stored at 0°C for 150 days prevented any change in peroxide value. The result of the present work confirms that 1000 ppm Ronoxan A (which contains 250 ppm ascorbyl palmitate) had the ability to keep the peroxide value of the AMF below its original value after one year storage at low temperatures.

The use of antioxidants in AMF was suggested by many workers including Kieseker (1981) and Keen (1982). The use of the antioxidant in the present work showed a significant effect in retarding the development of the peroxide values.

The result of the present work supports the view (Sanderson, 1979) that controlled storage of AMF at 4°C is preferable to the recommended storage temperature of 10°C maximum (IDF, 1977). Badings (1970) found that the TBA value of butter stored at -10°C increased from 0.034 to 0.212 after 5 months. Only at -18°C, in the present work, was there no change in the peroxide value of the AMF after 1 year. Thus, auto-oxidation changes of the AMF between 0 and -18°C require further investigation.

The present results show that fat oxidation is the main defect occurring in AMF under cold storage conditions. Various workers (Keiseker, 1981 and Sanderson, 1982[b]) have pointed out that it is possible to control the auto-oxidation of AMF by flushing the container with an inert gas such as nitrogen.

At least two factories in the UK use nitrogen flushing of 196 kg (net weight) drums of AMF.

5.2.3 Results of the organoleptic tests

Table 5:25 showed the superiority of the recombined milk produced from the control AMF at both storage times of 6 and 12 months. The mean values of the ranking scores obtained for this milk were 2.9 after 6 months storage time and 2.4 after 12 months storage time with a 100% acceptability. This is, every member of the panel graded all milk recombined from SMP and AMF stored in the freezer as being acceptable. The above values were the best of any recombined milk manufactured during this trial (see Tables 5:25, 5:26 and 5:27).

The mean values of the ranking and the acceptability of both AMF and AMF plus 1000 ppm Ronoxan A, stored at 6, 8 and 10°C for 1 year showed that:

1. Higher preference was given to the recombined milk produced from AMF stored at 6, 8 and 10°C than the recombined milk which was produced from AMF plus antioxidant stored at the same temperatures for the same time (Table 5:26).

2. There were only small differences between the scores awarded to the samples at the two storage times (6 and 12 months) as shown in Table 5:25.

TABLE 5:25

The organoleptic results of the control sample of recombined milk produced from AMF stored at -18°C and samples of recombined milk produced from AMF and AMF + 1000 ppm Ronoxan A stored at 6, 8 and 10°C

	6 months		12 months	
	Mean of all rank scores	% Acceptability	Mean of all rank scores	Acceptability
Control	2.9	100	2.4	100
AMF stored at 6°C	4.2	71	2.7	79
AMF stored at 8°C	4.1	85	4.7	71
AMF stored at 10°C	4.1	79	4.4	93
AMF +RA stored at 6°C	3.3	86	5.4	64
AMF +RA stored at 8°C	5.2	57	4.3	64
AMF +RA stored at 10°C	4.1	64	5.0	64

Mean value from 2 trials

TABLE 5:26

The mean values* of the organoleptic scores for the control sample of recombined milks produced from AMF stored at -18°C, and the recombined milk produced from AMF and AMF + 1000 ppm Ronoxan A stored at 6, 8 and 10°C

	Rank score	% acceptability
Control	2.7	100
AMF	3.9	80
AMF +ſRA	4.6	67

*Mean value of the control from 4 trials
*Mean values of AMF or AMF +ſRA from 12 trials

TABLE 5:27

The mean values* of the organoleptic scores for the control sample produced from AMF stored at -18°C and recombined milks produced from AMF and AMF + 1000 ppm Ronoxan A stored at 6, 8 and 10°C

	Rank scores	% acceptability
Control	2.7	100
6°C	3.9	75
8°C	4.6	70
10°C	4.2	75

*Mean value of the control from 4 trials
*Mean values of the AMF stored at 6, 8 or 10°C from 8 trials

TABLE 5:28

Statistical analysis of variance of the acceptability scores of the control sample of recombined milk produced from AMF stored at -18°C and samples of recombined milk produced from AMF and AMF plus 1000 ppm Ronoxan A stored at 6, 8 and 10°C

	df	M.S	F
Storage	1	0.0051	0.200
Residual	2	0.0255	
Grader	6	1.415	11.718*****
Storage x Grader	6	0.2551	2.113
Residual	12	0.1207	
Curest	1	1.7219	17.185******
Storage x Curest	1	0.0009	0.008
Grader x Curest	6	0.2358	2.354**
Treatment	1	0.7202	7.188****
Temperature	2	0.0536	0.535
Storage x Grader	6	0.0425	0.424
Storage x Treatment	1	0.0536	0.535
Grader x Treatment	6	0.3175	3.168****
Storage x Temperature	2	0.0774	0.772
Grader x Temperature	12	0.1091	1.089
Treatment x Temperature	2	0.1845	1.842
Storage x Grader x Treatment	6	0.2897	1.891*
Storage x Grader x Temperature	12	0.1190	1.188
Storage x Treatment x Temperature	2	0.2321	2.317
Grader x Treatment x Temperature	12	0.1567	1.564
Residual	96	0.1002	
Total	168	0.1378	

Table	Storage	Grader	Curest	Storage Grader	Storage Curest
SED	0.0228	0.0929	0.0646	0.1237	0.0826

Table	Grader Curest	Curest Treatment	Curest Temperature
SED	0.1752	0.0691	0.0733

* Significant at 10% level
** " 5% "
*** " 2.5% "
**** " 1% "
***** " 0.1% "
****** " 0.01% "

The score values awarded to the samples at the different storage temperatures did not show very much difference as shown in Table 5:27.

5.2.4 Statistical results for acceptability

A statistical analysis of variance was carried out for the acceptability of the samples. The term of 'Storage' was used for the storage time. The computer code of 'Curest' was used to differentiate between the control samples (sample which produced from AMF stored at freezer) and samples which produced from AMF or AMF plus antioxidant stored at 6, 8 and 10°C for the same time. The term 'Treatment' was used to differentiate between samples produced from AMF or AMF plus antioxidant, excluding the control. The term 'Temperature' was used to differentiate between the three different storage temperatures (6, 8 and 10°C) of the AMF, excluding the control sample.

The results of these statistical analysis shown in Table 5.28. These results showed that:

1. There were no significant differences in the acceptability scores of the recombined milks due to the effect of storage time or storage temperatures of the AMF stored at 6, 8 and 10°C.

2. There were very highly significant differences ($P < 0.0001$) between the degree of acceptability of the recombined milk produced from the control AMF and the other samples.

3. There were very highly significant differences ($P < 0.001$) between the graders in their degree of acceptability ranking of the samples.

4. There was no significant interaction between the graders and the storage time.

5. There was a low significant interaction ($P < 0.05$) between the curest and the graders.

6. There were highly significant differences ($P < 0.01$) between the treatments (e.g. samples produced from AMF or AMF plus antioxidant stored at 6, 8 and 10°C.

7. There was a highly significant interaction ($P < 0.01$) between the graders and the treatments.

8. There was a low significant interaction ($P < 0.1$) between the storage time, graders and treatments.

DISCUSSION

The control sample of recombined milk showed the best ranking scores with 100% acceptability. This result shows that the recombined milk can be an acceptable product. This acceptability depends very much on the quality of the raw materials (SMP and AMF). Low-heat manufactured SMP and an AMF free from any additive and of a quality which can meet the IDF specification (IDF, 1977) will lead to recombined processed with a high degree of consumer acceptability as shown in Table 5:25.

Adding the antioxidant, especially the natural one, as available in a commercial product such as Ronoxan A showed a very good effect in reducing the peroxide value of the AMF, but at the same time these antioxidants did not produce acceptability rating greater than those associated with antioxidant-free AMF. Firstly, adding 1000 ppm Ronoxan A resulted in a deepening of the colour of the AMF as shown in Plate 5:1. In most cases the AMF with the antioxidant was attributed the lowest preference by panelist ranking and the lowest percentage of acceptability. This was in spite of the fact that it had a better peroxide value than the control AMF and all of the untreated AMFs stored at 6, 8 and 10°C. Koops (1964) showed that high concentrations (0.05-0.1%) of ascorbyl palmitate in butter resulted in a slight but not unpleasant off-flavour.

The low storage temperatures of 6, 8 and 10°C for the AMF did not result in any change in the quantitative amount of the FFAs of these AMFs. At the same time these AMFs showed small increases in their peroxide values (ca P V = 0.5 mEq O_2/kg). The recombined milks produced from these AMFs showed less desirable ranking scores and lower percentage of acceptability than the recombined milk which was produced from the control AMF. Therefore, the superiority of the samples which were produced from the control AMF was either due to the absence of antioxidant from the AMF, the absence of auto-oxidation

1 2

Plate 5:1 The immediate effect of the addition of antioxidant Ronoxan A on the colour of anhydrous milk fat

1 - AMF with added antioxidants 2 - Control

of the AMF stored in the freezer or the absence of other deleterious chemical and physical changes not measured in this work.

There were small changes in the peroxide values of the AMF with storage temperatures of 6, 8 and 10°C over the storage time of 1 year. These small changes failed to give clear differences in the ranking of the recombined milk produced from their AMF and a significant result in the degree of their acceptability.

The off-flavour formed during oxidation of unsaturated fatty acids is actually composed of many flavours, often referred to as "oxidised flavour". This flavour appears in dairy products in different intensities and order during the progress of lipid oxidation (Badings, 1970).

The value of taste panels to study the development of off-flavour during the storage of dairy products, has been referred to by many workers, including Downey (1969) and Bruhn et al. (1976). Sidney et al. (1972) described a judging panel used to study the oxidised flavour in fluid milk. The results showed that shelf-life of milk can be limited due to the effect of the oxidised flavour in milk. Bruhn et al. (1976) showed that milk with an oxidised flavour was preferred less than the normal milk by organoleptic evaluation. The results of the present work indicate that oxidised flavour in milk can be a limiting factor to its acceptability.

The results of the present work support the views of Kieseker (1981); Kirkpatrick (1982); Keiscker (1982b) and Sanderson (1979) that AMF used for recombined diary products should be manufactured to the IDF specification (IDF, 1977). The AMF should be checked by a regular analytical programme supported by organoleptic assessment and only the best quality ingredients should be used for recombined milk.

The present work found that the organoleptic acceptability of AMF was usually decreased by adding antioxidant to the AMF at the same low temperatures, even though the development of the peroxide value was retarded. Thus the present work tends to agree with Keen (1982) that the addition of antioxidants to the AMF may alter what little advantage milk fat may have as a "natural product" over that of competitive vegetable oils and tallows.

SECTION III - RESULTS OF HIGH TEMPERATURE

5.3.1 The effect of high storage temperature (32 and 55°C) and different types and levels of antioxidant on the quantitative analysis of FFA in AMF stored for 1 year

The quantitative amounts of each FFA (C_4 to $C_{18:1}$), the corresponding acid values for AMF and AMF plus the different types and levels of antioxidant (1000 ppm Ronoxan A, 2000 ppm Ronoxan A and 300 ppm Embanox 7) stored at 32 and 55°C for one year were measured at regular intervals. The antioxidants used were:

(a) 1000 ppm Ronoxan A (1 RA);

(b) 2000 ppm Ronoxan A (2 RA);

(c) 300 ppm Embanox 7 (Emb 7).

The results for the experiment and the accompanying statistical analysis are shown in Tables 5:29 to 5:38 and Figs. 5:8 to 5:17.

Statistical analysis of variance was carried out for each variate (each FFA). The term of Storage time was used for the storage time intervals, the term Treatment was used to differentiate between the AMF, AMF plus 1000 ppm Ronoxan A, AMF plus 2000 ppm Ronoxan A and AMF plus 300 ppm Embanox 7. The term Temperature was used to differentiate between the two different storage temperatures (32 and 55°C). The quantitative analysis of each FFA are shown in duplicate in all the tables.

These statistical analysis showed the following results:

1. Storage time: There were very highly significant differences ($P < 0.0001$) between the amounts of all FFAs (C_4 to $C_{18:1}$) due to the effect of storage time. There were also very highly significant differences ($P < 0.0001$) between the acid values due to the effect of the storage time.

2. Treatments: There were very highly significant differences ($P < 0.0001$) between the amounts of all FFAs (C_4 to $C_{18:1}$) due to the effect of different treatments.

3. Storage temperatures: There were very highly significant differences ($P < 0.0001$) between the amounts of all FFAs (C_4 to

TABLE 5:29

The effect of storage temperature of 32 and 55°C for one year on the level of n-butyric acid (ppm) in AMF with and without added antioxidants

Variable (1) n-Butyric acid C_4

	Storage Treatment	Storage period (months)									
		0	2	3	4	5	7	8	9	10	12
32°C	AMF 1	4.269	10.48	14.33	17.13	20.62	23.02	27.80	29.24	34.18	38.24
	AMF 2	4.271	11.23	14.04	17.77	20.77	23.67	26.21	30.76	31.94	37.13
	AMF + 1000 ppm Ronoxan A		16.94	19.86	23.36	25.27	28.86	35.64	37.84	43.41	46.20
			17.04	18.58	22.77	26.29	29.07	36.12	37.97	42.93	46.11
	AMF + 2000 ppm Ronoxan A		22.73	26.05	28.89	34.96	37.90	44.39	49.01	54.58	63.42
			21.78	26.94	29.88	35.29	38.65	45.06	50.89	56.29	62.04
	AMF + 300 ppm Embanox 7		11.63	15.15	17.34	21.61	24.83	26.21	29.30	33.33	38.48
			12.39	15.63	17.21	21.89	24.21	27.68	30.76	32.36	39.01
55°C	AMF		99.14	122.34	139.94	158.83	N.D	168.10	193.96	211.92	228.03
			98.27	120.14	142.82	154.74	N.D	172.67	190.75	208.85	224.13
	AMF + 1000 ppm Ronoxan A		109.91	121.17	135.65	142.10	N.D	155.39	188.41	208.63	223.33
			110.94	124.47	133.64	145.76	N.D	153.76	185.68	207.19	228.03
	AMF + 2000 ppm Ronoxan A		120.04	131.70	142.19	150.56	N.D	166.58	210.31	216.86	234.81
			122.95	130.35	140.08	152.62	N.D	162.39	210.62	212.07	230.32
	AMF + 300 ppm Embanox 7		91.83	99.90	114.84	130.70	N.D	141.98	150.84	173.58	218.17
			89.72	99.26	111.88	126.34	N.D	140.40	151.56	170.97	214.58

N.D - Not done because of equipment breakdown

	df	M.S	F
Storage time	8	9792.87	4153.172****
Treatment	3	3424.435	61.195****
Temperature	1	598025.125	10686.707****
Treatment x Temp	3	1029.324	18.394****
Treatment x Storage	24	55.787	0.997
Temp x Storage	7	3805.144	67.998****
Residual	21	55.960	
Total	59	10856.547	

Table	Treatment	Temperature	Treatment Temperature
SED	1.763	1.247	2.494

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01%

TABLE 5:31

The effect of storage temperature of 32 and 55°C for one year on the level of n-octanoic acid (ppm) in AMF with and without added antioxidants

Variable (3) n-Octanoic acid C_8

	Storage Treatment	Storage period (months)									
		0	2	3	4	5	7	8	9	10	12
32°C	AMF 1	5.19	6.80	8.26	9.08	10.07	11.06	14.37	16.30	17.03	21.33
	AMF 2	5.22	6.61	8.34	9.20	10.01	12.18	14.11	16.47	18.12	22.04
	AMF + 1000 ppm Ronoxan A		6.61	8.02	10.19	11.65	14.12	15.94	17.77	20.99	29.25
			6.72	8.24	10.75	11.29	13.90	15.51	18.62	19.23	30.92
	AMF + 2000 ppm Ronoxan A		8.94	10.44	13.23	14.99	15.99	18.10	21.37	22.46	36.29
			8.41	10.23	12.24	14.66	16.19	19.36	21.97	22.41	34.37
	AMF + 300 ppm Embanox 7		6.76	6.92	8.90	9.62	11.49	13.99	16.09	17.18	24.90
			7.44	7.41	8.87	10.03	11.66	13.91	16.65	19.29	24.15
55°C	AMF		38.79	44.55	55.20	63.20	N.D	70.99	88.00	94.48	105.25
			37.95	45.29	59.70	64.95	N.D	71.06	86.30	91.70	102.54
	AMF + 1000 ppm Ronoxan A		35.37	41.33	51.53	55.78	N.D	63.01	79.79	80.98	87.22
			35.54	42.71	52.39	54.49	N.D	61.43	79.90	83.26	89.94
	AMF + 2000 ppm Ronoxan A		41.69	46.86	55.59	61.03	N.D	65.59	83.66	90.41	102.23
			40.70	44.51	55.55	58.96	N.D	64.91	82.51	87.65	102.10
	AMF + 300 ppm Embanox 7		32.43	34.78	46.73	50.19	N.D	59.59	67.88	73.97	89.85
			31.84	35.02	47.64	50.36	N.D	59.26	67.63	72.32	88.13

N.D - Not done because of equipment breakdown

	df	M.S	F
Storage time	8	2.681×10^3	3821.063****
Treatment	3	4.427×10^2	37.647****
Temperature	1	8.763×10^4	7451.336****
Treatment x Temp	3	3.033×10^2	25.786****
Treatment x Storage	24	7.236	0.615
Temp x Storage	7	8.163×10^2	69.410****
Residual	21	1.176×10	
Total	59	1.627×10^3	

Table	Treatment	Temperature	Treatment Temperature
SED	0.8083	0.5716	1.1431

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01%

TABLE 5:32

The effect of storage temperature of 32 and 55°C for one year on the level of n-decanoic acid (ppm) in AMF with and without added antioxidants

Variable (4) n-Decanoic acid C_{10}

	Storage Treatment	Storage period (months)									
		0	2	3	4	5	7	8	9	10	12
32°C	AMF 1	40.1	44.2	47.7	49.9	54.1	60.2	62.8	68.2	73.5	78.2
	AMF 2	40.0	43.7	47.4	50.8	53.3	60.4	63.8	66.2	73.4	77.0
	AMF + 1000 ppm Ronoxan A		48.1	56.5	64.7	67.3	72.7	76.3	81.3	87.2	92.8
			48.9	55.1	62.9	67.7	72.3	76.1	79.8	89.1	93.4
	AMF + 2000 ppm Ronoxan A		57.7	63.1	67.5	73.8	76.4	80.7	83.4	94.9	104.9
			55.9	64.8	67.9	73.0	76.3	81.6	84.9	93.1	102.7
	AMF + 300 ppm Embanox 7		43.9	46.8	48.5	53.5	60.2	62.5	69.6	74.3	77.7
			44.2	46.1	49.7	54.0	59.6	63.2	68.1	74.5	77.6
55°C	AMF		128.9	153.4	178.4	185.8	N.D	232.6	250.4	295.5	378.3
			127.2	152.6	180.7	188.9	N.D	236.6	245.9	297.5	383.4
	AMF + 1000 ppm Ronoxan A		145.2	163.2	169.7	173.8	N.D	196.3	210.3	281.6	309.2
			148.8	159.5	169.1	174.9	N.D	200.2	205.3	273.8	313.9
	AMF + 2000 ppm Ronoxan A		155.1	160.4	174.9	184.0	N.D	197.7	221.1	276.4	335.3
			151.6	158.4	171.6	186.7	N.D	194.4	215.1	274.0	329.5
	AMF + 300 ppm Embanox 7		106.9	113.1	135.7	142.4	N.D	162.2	182.2	202.2	308.0
			106.7	114.1	138.8	139.3	N.D	159.6	189.5	204.2	302.4

N.D - Not done because of equipment breakdown

	df	M.S	F
Storage time	8	22456.117	5881.531****
Treatment	3	7121.176	28.158****
Temperature	1	665037.125	2629.636****
Treatment x Temp	3	4890.645	19.338****
Treatment x Storage	24	169.109	0.669
Temp x Storage	7	11279.625	44.601****
Residual	21	252.901	
Total	59	13379.648	

Table	Treatment	Temperature	Treatment Temperature
SED	3.748	2.65	5.301

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01%

TABLE 5:33

The effect of storage temperature of 32 and 55°C for one year on the level of lauric acid (ppm) in AMF with and without added antioxidant

Variable (5) Lauric acid C_{12}

	Storage Treatment	Storage period (months)									
		0	2	3	4	5	7	8	9	10	12
32°C	AMF 1	55.14	63.2	65.9	69.8	72.5	75.5	80.6	88.7	96.7	99.8
	AMF 2	55.09	62.9	65.7	70.1	73.8	76.2	80.2	86.6	96.3	99.8
	AMF + 1000 ppm Ronoxan A		69.1	76.7	85.9	90.7	92.2	97.6	107.5	109.7	110.8
			70.3	78.2	86.4	89.2	92.5	97.8	105.6	110.5	112.1
	AMF + 2000 ppm Ronoxan A		73.0	82.9	94.8	96.5	101.4	104.8	109.3	112.3	129.0
			73.9	84.1	93.5	95.8	99.7	105.9	109.3	114.9	125.1
	AMF + 300 ppm Embanox 7		64.8	67.0	70.0	73.7	75.6	80.5	86.7	96.6	100.1
			64.3	67.3	69.8	72.6	76.4	81.1	87.9	96.3	100.9
55°C	AMF		174.3	192.3	225.2	235.9	N.D	286.6	328.0	372.0	405.5
			171.8	194.2	225.2	230.5	N.D	282.4	325.0	367.3	405.5
	AMF + 1000 ppm Ronoxan A		161.3	182.0	192.9	202.8	N.D	215.9	242.8	310.2	327.2
			162.4	182.4	190.1	204.0	N.D	218.0	232.1	303.7	333.2
	AMF + 2000 ppm Ronoxan A		174.0	189.4	194.3	217.8	N.D	227.9	256.4	313.8	352.6
			176.4	185.7	192.7	222.4	N.D	223.9	252.1	313.6	358.4
	AMF + 300 ppm Embanox 7		140.3	153.6	166.9	177.8	N.D	190.3	207.2	244.7	326.3
			143.2	151.0	167.6	178.5	N.D	187.7	207.2	248.4	320.1

N.D - Not done because of equipment breakdown

	df	M.S	F
Storage time	8	2.287×10^4	74.889****
Treatment	3	9.655×10^3	32.140****
Temperature	1	7.932×10^5	2640.389****
Treatment x Temp	3	1.002×10^4	33.367****
Treatment x Storage	24	2.344×10^2	0.780
Temp x Storage	7	1.081×10^4	35.998****
Residual	21	3.004×10^2	
Total	59	1.593×10^4	

Table	Treatment	Temperature	Treatment Temperature
SED	4.085	2.889	5.777

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01%

TABLE 5:34

The effect of storage temperature of 32 and 55°C for one year on the level of myristic acid (ppm) in AMF with and without added antioxidants

Variable (6) Myristic C_{14}

Storage Treatment	Storage period (months)									
	0	2	3	4	5	7	8	9	10	12
AMF 1	117.6	123.4	129.0	135.2	138.3	147.4	154.3	168.9	186.2	211.5
AMF 2	116.6	124.2	128.6	135.3	140.1	144.5	156.8	164.7	183.9	207.6
AMF + 1000 ppm Ronoxan A		133.5	148.9	157.4	164.1	169.3	176.0	188.5	198.7	208.0
		133.9	146.8	159.4	163.9	171.7	177.3	189.9	201.2	212.6
AMF + 2000 ppm Ronoxan A		142.6	154.0	169.8	182.1	190.4	207.4	218.5	231.3	253.2
		142.9	153.2	172.5	181.3	192.8	210.4	218.1	235.6	250.5
AMF + 300 ppm Embanox 7		123.3	129.8	137.3	142.8	146.2	154.7	164.3	188.9	208.4
		124.1	130.6	137.0	143.5	145.3	156.6	160.5	191.1	207.3
AMF		460.5	502.5	622.5	691.7	N.D	753.3	854.8	884.7	928.1
		464.7	506.0	615.3	697.9	N.D	754.7	852.2	879.3	941.8
AMF + 1000 ppm Ronoxan A		448.3	456.9	509.9	557.9	N.D	580.1	669.7	738.5	750.6
		449.0	457.8	510.1	558.5	N.D	582.3	668.8	739.3	762.9
AMF + 2000 ppm Ronoxan A		486.6	511.3	527.8	618.1	N.D	669.5	718.6	867.8	924.6
		490.7	507.5	533.9	608.2	N.D	661.0	713.6	861.7	917.1
AMF + 300 ppm Embanox 7		445.6	466.7	488.3	511.4	N.D	523.1	599.3	624.1	741.4
		449.9	464.3	489.7	511.6	N.D	520.6	608.4	630.4	732.1

N.D - Not done because of equipment breakdown

	df	M.S	F
Storage time	8	9.830×10^4	10420.313****
Treatment	3	5.506×10^4	27.085****
Temperature	1	7.598×10^6	3737.446****
Treatment x Temp	3	4.720×10^4	23.219****
Treatment x Storage	24	1.974×10^3	0.971
Temp x Storage	7	4.497×10^4	22.121****
Residual	21	2.033×10^3	
Total	59	1.408×10^5	

Table	Treatment	Temperature	Treatment Temperature
SED	10.627	7.515	15.029

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01%

TABLE 5:35

The effect of storage temperature of 32 and 55°C for one year on the level of palmitic acid (ppm) in AMF with and without added antioxidants

Variable (7) Palmitic acid C_{16}

	Storage Treatment	Storage period (months)									
		0	2	3	4	5	7	8	9	10	12
32°C	AMF 1	284.0	330.6	357.8	384.7	404.9	437.1	464.7	495.7	528.9	650.9
	AMF 2	291.5	332.7	360.3	388.6	410.4	441.9	457.4	497.0	520.1	643.3
	AMF + 1000 ppm Ronoxan A		506.8	540.1	577.1	597.1	631.7	696.2	719.6	743.8	816.2
			501.3	542.2	573.1	594.0	640.8	696.5	720.7	742.6	812.8
	AMF + 2000 ppm Ronoxan A		590.4	671.8	698.4	716.3	749.3	810.8	848.2	906.5	1060.1
			591.7	676.4	701.8	710.5	743.5	809.6	842.0	902.3	1037.4
	AMF + 300 ppm Embanox 7		333.9	363.4	399.1	419.7	435.9	462.1	470.7	486.3	647.3
			335.9	368.4	399.5	415.8	437.3	462.0	471.0	491.6	640.1
55°C	AMF		1059.7	1227.6	1404.1	1541.5	N.D	1733.6	2204.1	2348.6	2653.9
			1064.4	1219.6	1408.4	1550.6	N.D	1722.2	2213.0	2339.1	2637.4
	AMF + 1000 ppm Ronoxan A		1256.9	1306.2	1408.5	1545.8	N.D	1609.4	1976.9	2297.5	2418.9
			1268.4	1302.2	1404.3	1559.7	N.D	1597.5	1979.4	2298.3	2414.7
	AMF + 2000 ppm Ronoxan A		1377.6	1422.1	1467.7	1697.2	N.D	1833.3	2322.2	2413.0	2803.2
			1366.5	1428.2	1481.0	1685.2	N.D	1834.0	2329.0	2402.5	2807.9
	AMF + 300 ppm Embanox 7		1012.6	1155.0	1233.4	1324.5	N.D	1388.6	1542.1	1645.5	2121.8
			1023.7	1143.3	1237.8	1315.0	N.D	1391.8	1536.9	1639.1	2122.4

N.D - Not done because of equipment breakdown

	df	M.S	F
Storage time	8	1.180×10^6	44388.031****
Treatment	3	1.048×10^6	78.567****
Temperature	1	4.572×10^7	3425.983****
Treatment x Temp	3	1.828×10^5	13.696****
Treatment x Storage	24	1.726×10^4	1.293
Temp x Storage	7	5.184×10^5	38.850****
Residual	21	1.334×10^4	
Total	59	9.108×10^5	

Table	Treatment	Temperature	Treatment Temperature
SED	27.228	19.253	38.506

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01%

TABLE 5:36

The effect of storage temperature of 32 and 55°C for one year on the level of stearic acid (ppm) in AMF with and without added antioxidants

Variable (8) Stearic acid C_{18}

	Storage Treatment	Storage period (months)									
		0	2	3	4	5	7	8	9	10	12
	AMF 1	135.7	147.6	153.4	165.1	170.2	191.4	210.4	223.7	237.0	280.6
	AMF 2	138.7	150.8	154.7	167.3	172.5	193.3	209.0	222.9	236.6	274.4
	AMF + 1000 ppm Ronoxan A		163.6	179.4	195.2	202.5	211.0	228.1	234.9	246.7	287.7
			165.1	175.6	193.4	204.1	211.4	232.4	241.8	251.9	293.4
	AMF + 2000 ppm Ronoxan A		192.6	209.6	226.5	235.5	248.8	264.3	276.3	321.2	384.9
			194.5	210.1	226.6	239.7	251.3	262.8	279.7	329.9	380.9
	AMF + 300 ppm Embanox 7		147.3	153.7	166.2	173.5	194.4	205.9	214.8	216.4	271.3
			150.2	155.1	168.6	177.9	193.4	201.7	214.3	217.2	267.0
	AMF		511.4	553.9	706.8	829.9	N.D	914.2	995.7	1059.9	1185.2
			513.6	552.5	712.5	834.8	N.D	911.9	998.2	1056.6	1172.2
	AMF + 1000 ppm Ronoxan A		523.3	559.6	662.1	703.1	N.D	736.5	776.8	843.0	907.0
			525.0	564.3	655.5	705.1	N.D	733.8	789.0	836.1	918.3
	AMF + 2000 ppm Ronoxan A		546.9	596.6	678.5	727.4	N.D	795.6	841.6	871.3	1087.6
			542.0	599.2	674.9	728.2	N.D	788.2	835.8	871.0	1076.5
	AMF + 300 ppm Embanox 7		418.6	486.6	598.1	613.7	N.D	641.9	659.4	685.6	847.0
			420.8	477.6	594.5	610.3	N.D	635.9	664.0	690.0	847.6

N.D - Not done because of equipment breakdown

	df	M.S	F
Storage time	8	1.589×10^5	14116.523****
Treatment	3	1.012×10^5	33.399****
Temperature	1	9.624×10^6	3177.062****
Treatment x Temp	3	7.745×10^4	25.568****
Treatment x Storage	24	3.316×10^3	1.095
Temp x Storage	7	5.795×10^4	19.130****
Residual	21	3.029×10^3	
Total	59	1.815×10^5	

Table	Treatment	Temperature	Treatment Temperature
SED	12.973	9.173	18.346

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01%

TABLE 5:37

The effect of storage temperature of 32 and 55°C for one year on the level of oleic acid (ppm) in AMF with and without added antioxidants

Variable (9) Oleic acid ($C_{18:1}$)

Storage Treatment	Storage period (months)									
	0	2	3	4	5	7	8	9	10	12
AMF 1	729.4	759.6	769.6	782.8	792.0	833.6	877.0	898.3	1010.1	1152.9
AMF 2	732.7	758.7	771.3	786.7	797.3	827.3	885.0	900.9	1029.0	1148.9
AMF + 1000 ppm Ronoxan A		779.4	812.2	855.7	895.6	956.3	1013.9	1028.8	1070.6	1068.6
		782.7	820.1	848.9	884.2	946.4	1011.1	1035.4	1070.0	1080.9
AMF + 2000 ppm Ronoxan A		802.3	888.6	926.6	976.9	1021.5	1054.6	1166.6	1296.9	1435.1
		811.1	891.2	930.5	971.3	1039.9	1066.2	1169.2	1294.6	1410.7
AMF + 300 ppm Embanox 7		753.7	769.0	775.0	801.8	810.5	854.0	872.0	979.0	1019.8
		745.9	764.3	772.6	793.2	815.5	850.5	870.4	980.2	1028.7
AMF		1643.2	1877.6	2038.2	2195.3	N.D	2661.2	3144.6	3247.1	3633.9
		1660.7	1872.2	2051.2	2196.3	N.D	2664.1	3134.7	3237.1	3641.3
AMF + 1000 ppm Ronoxan A		1660.9	1830.7	1877.8	1925.5	N.D	2026.6	2492.1	2786.7	2857.1
		1645.9	1822.3	1879.0	1936.6	N.D	2028.3	2490.3	2780.4	2873.8
AMF + 2000 ppm Ronoxan A		1759.8	1862.8	1993.9	2023.8	N.D	2243.3	2740.8	3112.5	3248.5
		1758.6	1850.4	1981.3	2042.3	N.D	2263.8	2744.5	3110.2	3253.8
AMF + 300 ppm Embanox 7		1607.1	1730.8	1828.4	1928.9	N.D	2022.2	2356.2	2528.5	2716.4
		1601.7	1727.1	1819.5	1937.1	N.D	2027.1	2374.2	2537.8	2728.1

N.D - Not done because of equipment breakdown

	df	M.S	F
Storage time	8	1.618×10^6	35544.168****
Treatment	3	5.360×10^5	23.398****
Temperature	1	6.710×10^7	2929.45****
Treatment x Temp	3	3.903×10^5	17.038****
Treatment x Storage	24	2.868×10^4	1.252
Temp x Storage	7	6.450×10^5	28.159****
Residual	21	2.291×10^4	
Total	59	1.281×10^6	

Table	Treatment	Temperature	Treatment Temperature
SED	35.673	25.225	50.450

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01%

TABLE 5:38

The effect of storage temperatures of 32 and 55°C for one year on the acid values (mg of KOH/g) in AMF with and without added antioxidants

Variable (10) Acid value

Storage Treatment		Storage period (months)									
		0	2	3	4	5	7	8	9	10	12
AMF	1	0.360	0.403	0.426	0.433	0.484	0.574	0.607	0.654	0.702	0.763
	2	0.360	0.407	0.430	0.435	0.482	0.575	0.610	0.644	0.711	0.765
AMF + 1000 ppm Ronoxan A			0.482	0.494	0.529	0.570	0.709	0.731	0.767	0.840	0.899
			0.496	0.506	0.538	0.587	0.690	0.722	0.785	0.835	0.902
AMF + 2000 ppm Ronoxan A			0.530	0.551	0.575	0.632	0.786	0.807	0.849	0.891	0.962
			0.534	0.553	0.590	0.636	0.787	0.801	0.853	0.889	0.966
AMF + 300 ppm Embanox 7			0.432	0.448	0.456	0.484	0.602	0.614	0.668	0.720	0.765
			0.438	0.445	0.451	0.482	0.597	0.617	0.673	0.718	0.766
AMF			0.500	0.700	1.005	1.205		1.525	1.657	1.953	2.227
			0.500	0.705	1.007	1.210		1.526	1.691	1.984	2.222
AMF + 1000 ppm Ronoxan A			0.586	0.704	1.002	1.126		1.386	1.484	1.773	1.885
			0.589	0.703	1.001	1.056		1.397	1.492	1.786	1.913
AMF + 2000 ppm Ronoxan A			0.752	0.974	1.102	1.202		1.439	1.593	1.881	1.983
			0.752	0.969	1.105	1.201		1.433	1.601	1.873	1.975
AMF + 300 ppm Embanox 7			0.510	0.653	0.739	1.014		1.334	1.451	1.565	1.622
			0.512	0.671	0.721	0.967		1.352	1.466	1.558	1.599

N.D - Not done because of equipment breakdown

	df	M.S	F
Storage time	8	1.4188576	13114.395****
Treatment	3	0.2614692	23.357****
Temperature	1	14.5099325	1296.156****
Treatment x Temp	3	0.1142383	10.205***
Treatment x Storage	24	0.0079279	0.708
Temp x Storage	7	0.4599026	41.083****
Residual	21	0.0111946	
Total	59	0.3268086	

Table	Treatment	Temperature	Treatment Temperature
SED	0.024938	0.017634	0.035268

* Significant at 5% level
** " " 1% "
*** " " 0.1% "
**** " " 0.01%

TABLE 5:39

Individual FFAs as a percentage of the total FFA content of AMF and AMF with added antioxidants before and after 12 months storage at 32°C

	Initial %	Final percentages			
		AMF	AMF + 1 RA	AMF + 2 RA	AMF + Embanox 7
C_4	0.31	1.5	1.71	1.8	1.61
C_6	0.19	1.07	1.18	1.19	1.11
C_8	0.38	0.85	1.11	1.02	1.02
C_{10}	2.9	3.04	3.44	2.99	3.22
C_{12}	3.99	3.91	4.12	3.65	4.16
C_{14}	8.48	8.22	7.78	7.24	8.61
C_{16}	20.84	25.39	30.14	30.17	26.67
C_{18}	9.94	10.89	10.75	11.01	11.15
$C_{18:1}$	52.96	45.15	39.76	40.93	42.45

Mean value from 2 trials

TABLE 5:40

Individual FFAs as a percentage of the total FFA content of AMF and AMF with added antioxidants before and after 12 months storage at 55°C

	Initial %	Final percentages			
		AMF	AMF + 1 RA	AMF + 2 RA	AMF + Embanox 7
C_4	0.31	2.33	2.79	2.51	2.89
C_6	0.19	1.84	2.08	1.97	1.74
C_8	0.38	1.07	1.10	1.10	1.19
C_{10}	2.9	3.93	3.86	3.59	4.07
C_{12}	3.99	4.18	4.09	3.84	4.31
C_{14}	8.48	9.65	9.37	9.94	9.83
C_{16}	20.84	27.3	29.93	30.28	28.32
C_{18}	9.94	12.16	11.30	11.68	11.31
$C_{18:1}$	52.96	37.53	35.48	35.09	36.33

Mean value from 2 trials

TABLE 5:42

The effect of storage at 55°C for 12 months on the level of FFAs (expressed as a percentage of the total FFAs) in untreated AMF

	Initial	Storage period (months)							
		2	3	4	5	8	9	10	12
C_4	0.31	2.36	2.55	2.58	2.61	2.46	2.35	2.43	2.33
C_6	0.19	1.28	1.64	1.59	1.61	1.55	1.63	1.74	1.84
C_8	0.38	0.92	0.95	1.08	1.07	1.03	1.06	1.08	1.07
C_{10}	2.90	3.06	3.22	3.28	3.12	3.39	3.03	3.43	3.93
C_{12}	3.99	4.14	4.07	4.12	3.88	4.11	3.99	4.27	4.18
C_{14}	8.48	11.06	10.61	11.31	11.57	10.89	10.43	10.20	9.65
C_{16}	20.84	25.40	25.75	25.70	25.74	24.95	26.98	27.11	27.30
C_{18}	9.94	12.26	11.64	12.97	13.86	13.18	12.18	12.24	12.16
$C_{18:1}$	52.96	39.51	39.57	37.37	36.55	38.45	38.35	37.50	37.53

Mean value from 2 trials

TABLE 5:41

The effect of storage at 32°C for 12 months on the level of FFAs (expressed as a percentage of the total FFAs) in untreated AMF

	Initial	Storage period (months)								
		2	3	4	5	7	8	9	10	12
C_4	0.31	0.73	0.91	1.17	1.23	1.30	1.42	1.50	1.50	1.50
C_6	0.19	0.40	0.46	0.69	0.67	0.74	0.86	0.95	1.03	1.07
C_8	0.38	0.45	0.54	0.53	0.60	0.65	0.75	0.82	0.80	0.85
C_{10}	2.9	2.94	3.05	3.23	3.19	3.36	3.32	3.35	3.32	3.04
C_{12}	3.99	4.22	4.23	4.36	4.36	4.23	4.21	4.37	4.37	3.91
C_{14}	8.48	8.29	8.23	8.01	8.27	8.14	8.15	8.31	8.38	8.22
C_{16}	20.84	22.19	23.09	29.09	24.24	24.52	24.16	24.74	23.74	25.39
C_{18}	9.94	9.99	9.91	9.83	10.19	10.73	10.99	11.13	10.72	10.89
$C_{18:1}$	52.96	50.80	49.54	43.10	47.25	46.33	46.16	44.84	46.15	45.15

Mean value from 2 trials

TABLE 5:43

Free fatty acid (FFA) composition of a cream and several butters (Woo et al., 1980)

Sample	FFA concentration									Acid degree value	Flavour quality
	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	C_{18} congeners	Total		
	(ppm)										
Butter A	5	1	11	60	119	293	933	1571	2993	1.53	Bland, no criticism
Butter B	2	0	2	26	43	93	347	833	1346	.81	Bland, no criticism
Butter C	26	3	4	32	60	114	381	847	1467	.79	Full flavour no criticism
Butter D	29	12	16	73	141	282	900	1438	2891	1.72	Full flavour no criticism
Butter E	71	23	26	78	97	172	565	1494	2526	1.22	Distinctly rancid
Butter F	87	41	59	152	148	280	884	2481	4132	2.11	Distinctly rancid
Butter G	118	46	57	148	135	244	870	2373	3991	1.88	Strongly rancid
Butter H	58	14	11	122	311	878	2447	3446	7287	4.39	Soapy rancid
Fresh cream (35% fat)	13	11	9	20	29	60	264	515	921	1.08	Bland, no criticism

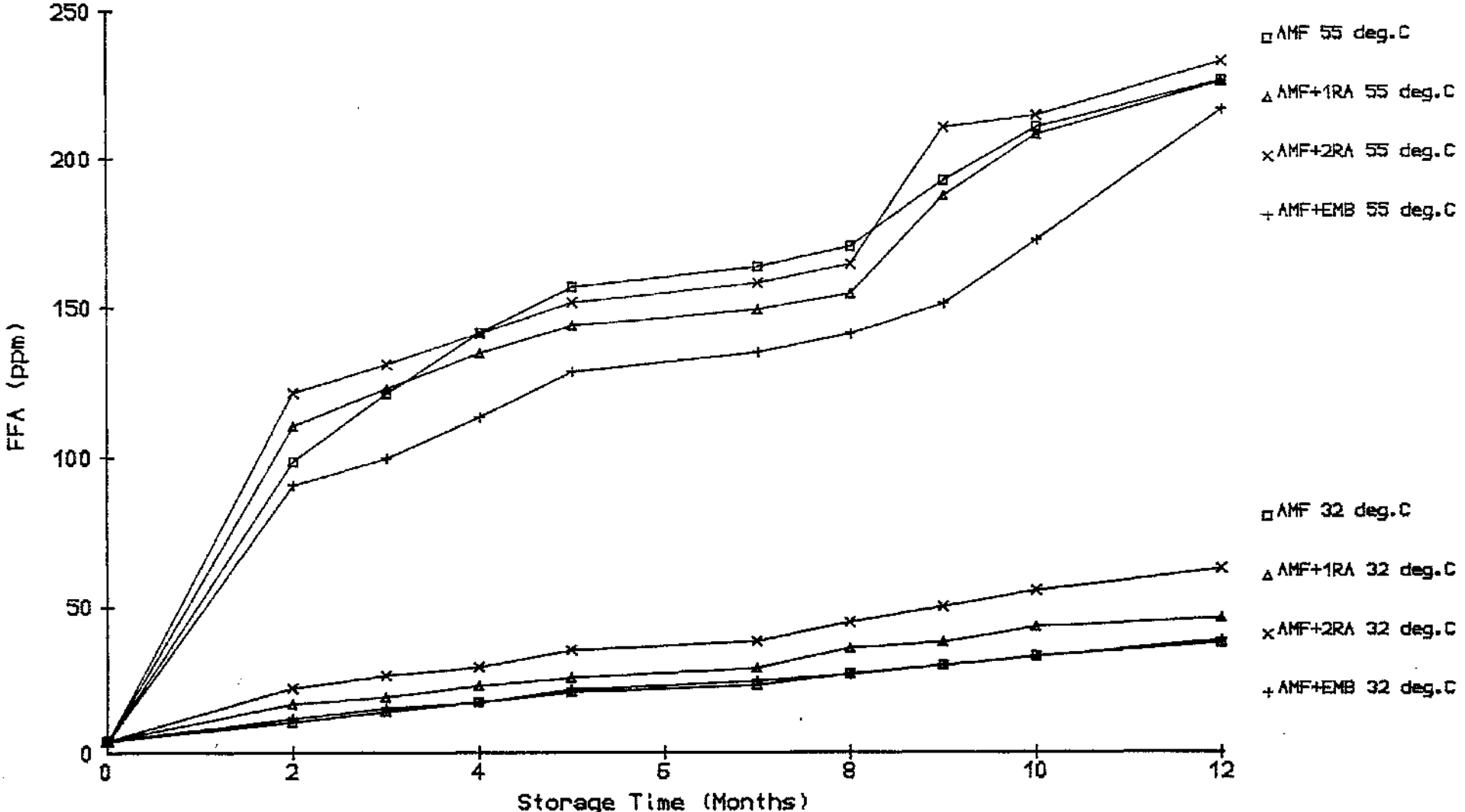

250
200
150
100
50
0
FFA (ppm)
0
2
4
6
8
10
12
Storage Time (Months)
AMF 55 deg.C
AMF+1RA 55 deg.C
AMF+2RA 55 deg.C
AMF+EMB 55 deg.C
AMF 32 deg.C
AMF+1RA 32 deg.C
AMF+2RA 32 deg.C
AMF+EMB 32 deg.C

Figure 5:9 Variation in the amount of n-hexanoic acid (ppm) in AMF and AMF + antioxidants during storage at high temperatures of 32 and 55°C

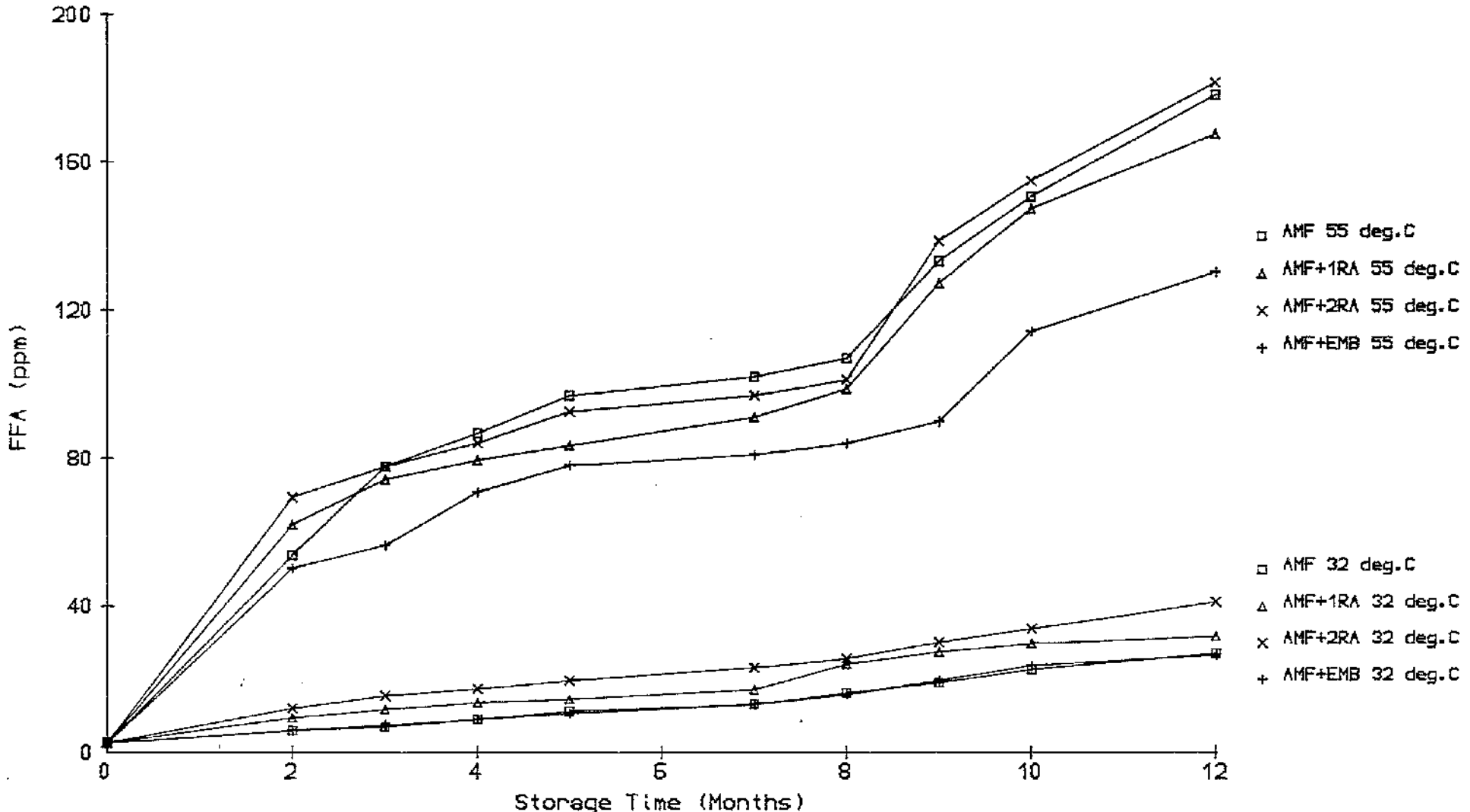


Figure 5:10 Variation in the amounts of n-octanoic acid (ppm) in AMF and AMF + antioxidants during storage at high temperatures of 32 and 55°C

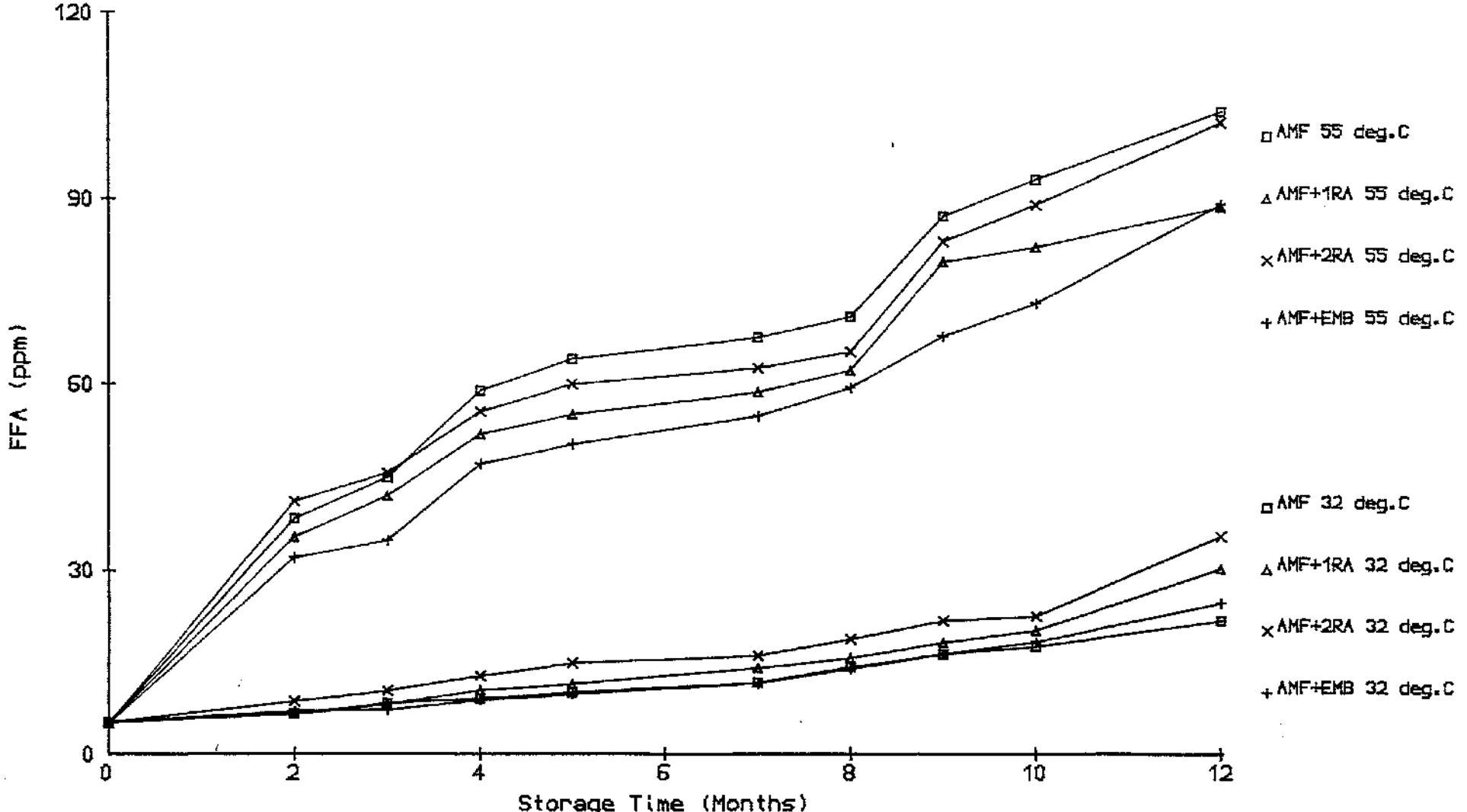


Figure 5:11 Variation in the amounts of n-decanoic acid (ppm) in AMF and AMF + antioxidants during storage at high temperatures of 32 and 55°C

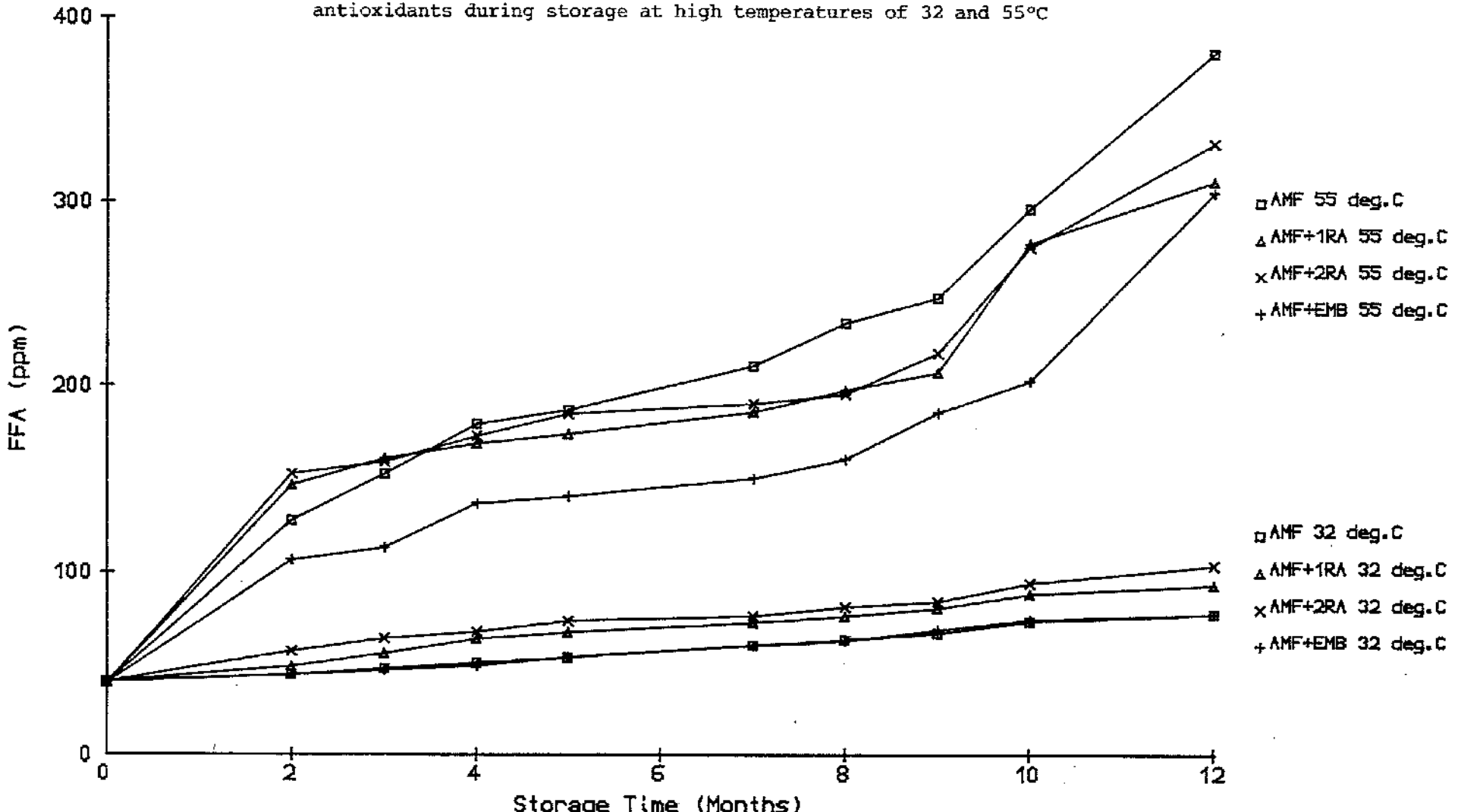


Figure 5:12 Variation in the amounts of lauric acid (ppm) in AMF and AMF + antioxidants during storage at high temperatures of 32 and 55°C

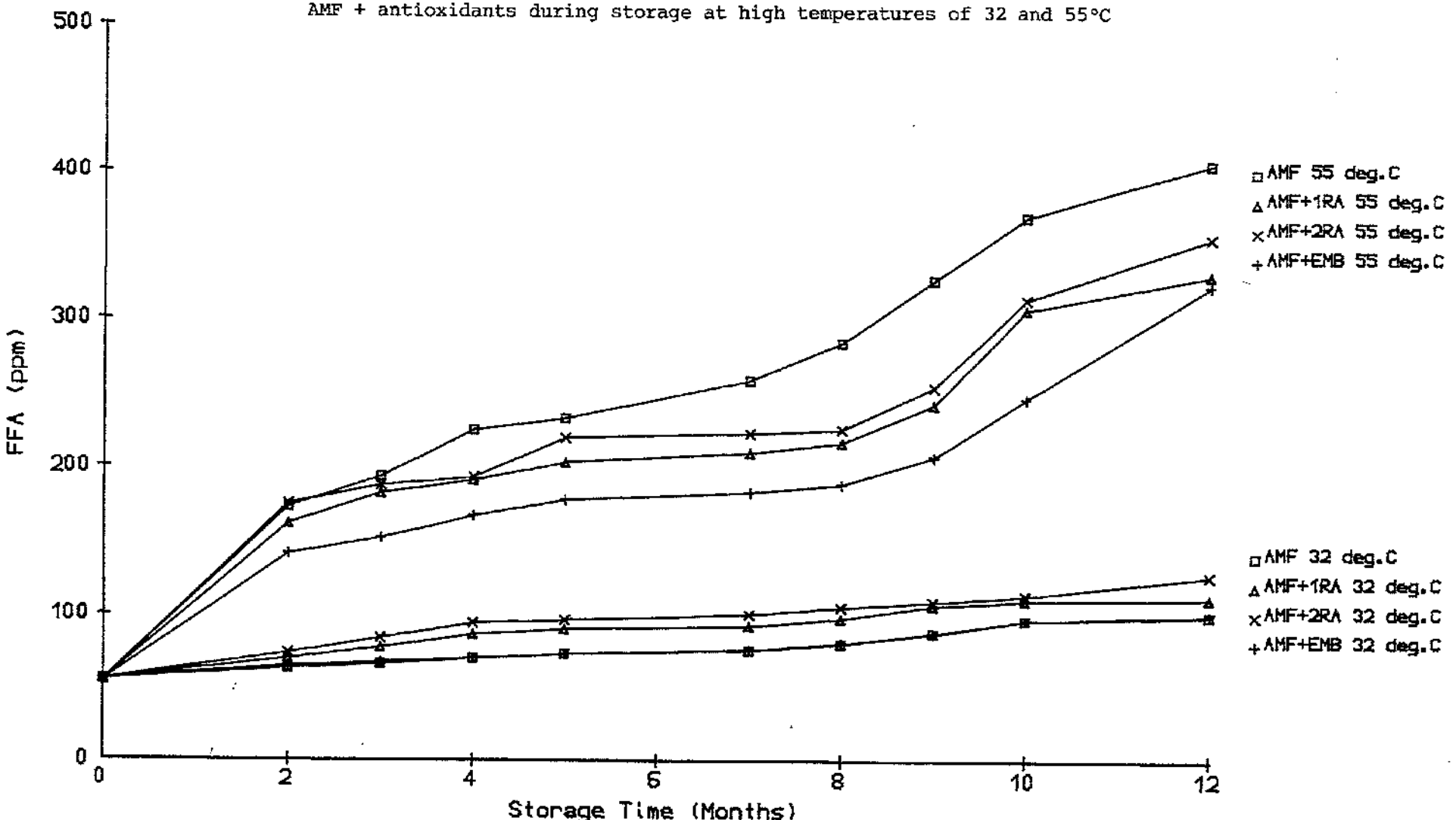


Figure 5:13 Variation in the amounts of myristic acid (ppm) in AMF and AMF + antioxidants during storage at high temperatures of 32 and 55°C

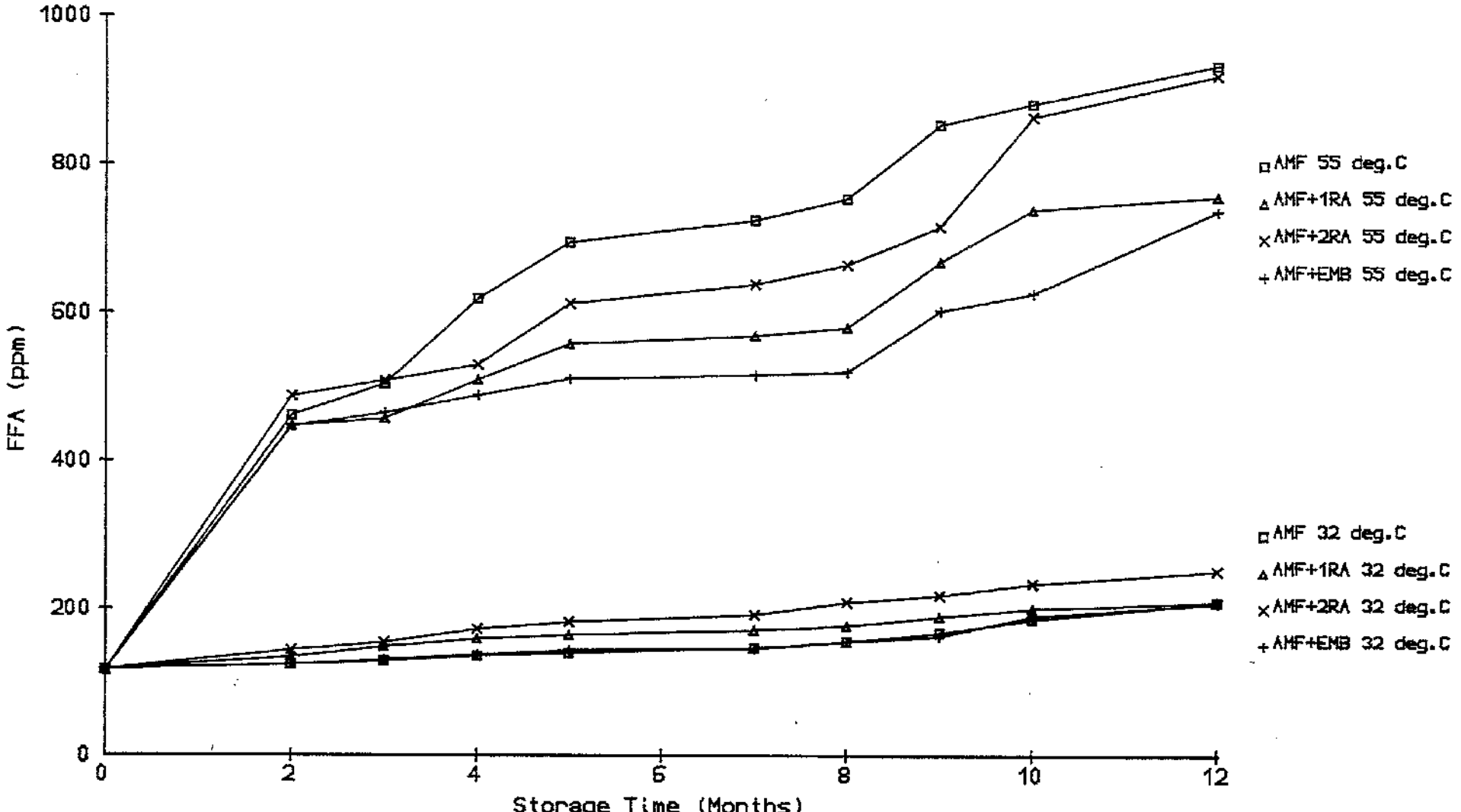
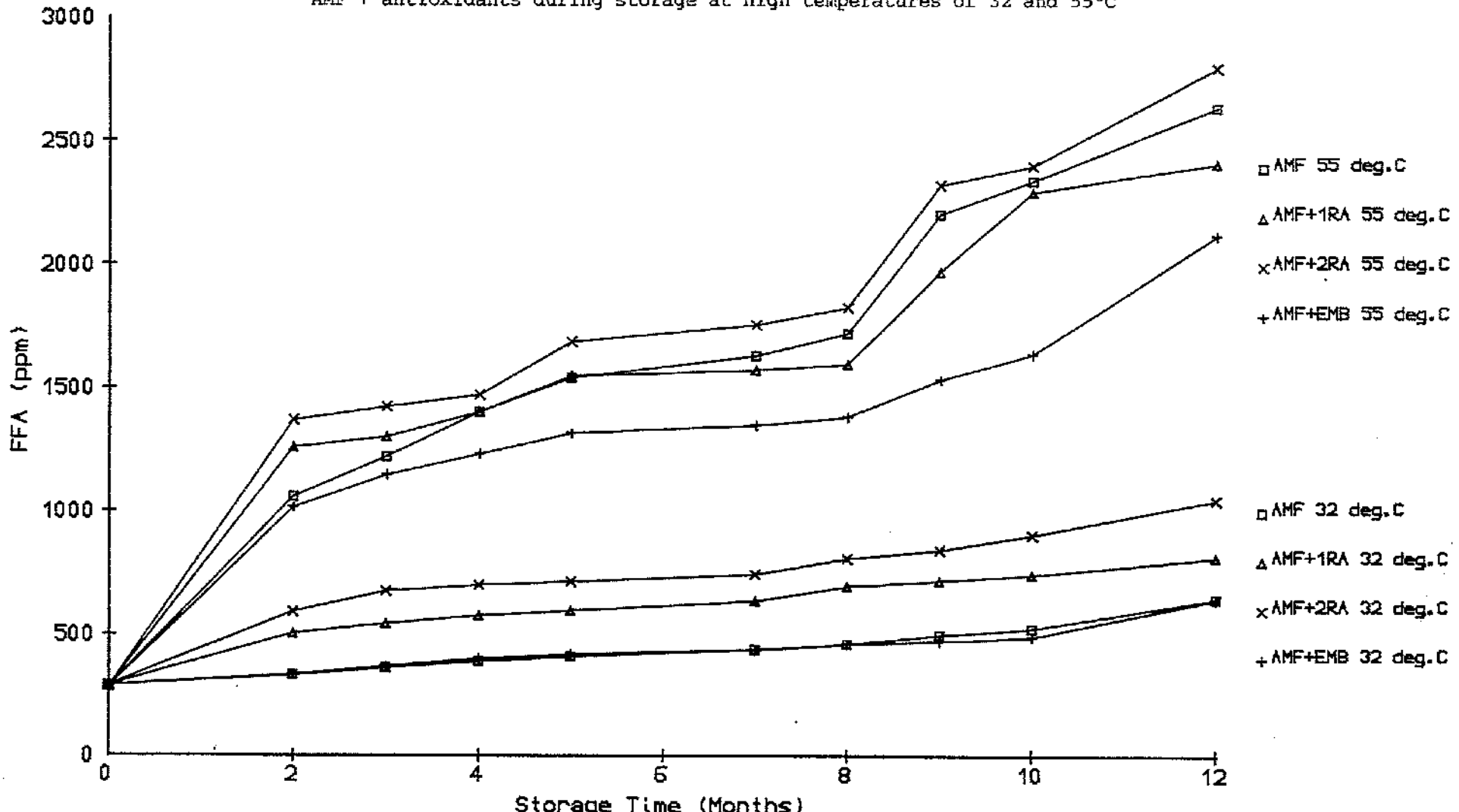


Figure 5:14 Variation in the amounts of palmitic acid (ppm) in AMF and AMF + antioxidants during storage at high temperatures of 32 and 55°C



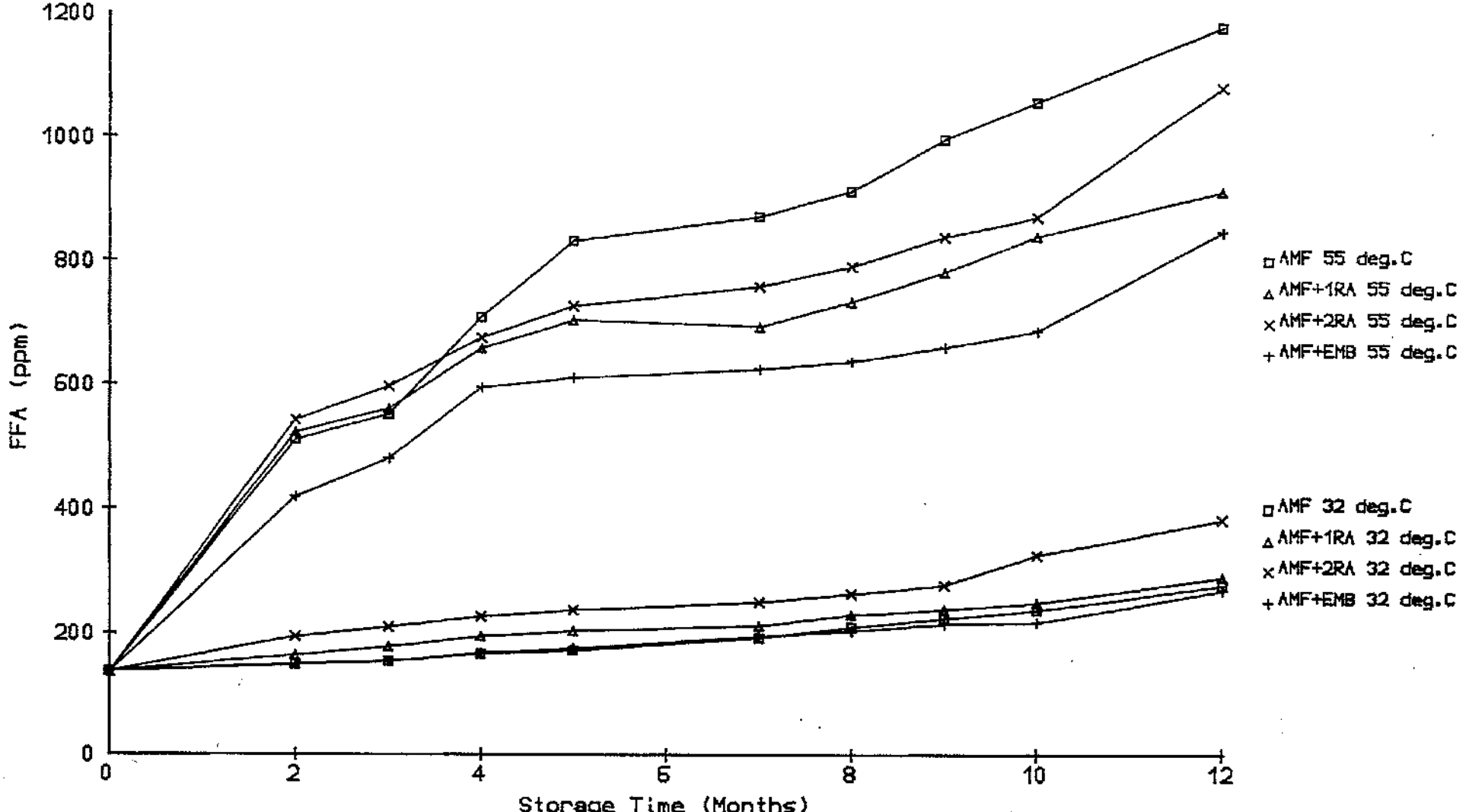

1200
1000
800
600
400
200
0
FFA (ppm)
0
2
4
6
8
10
12
Storage Time (Months)
AMF 55 deg.C
AMF+1RA 55 deg.C
AMF+2RA 55 deg.C
AMF+EMB 55 deg.C
AMF 32 deg.C
AMF+1RA 32 deg.C
AMF+2RA 32 deg.C
AMF+EMB 32 deg.C

Figure 5.16 Variation in the amounts of oleic acid (ppm) in AMF and AMF + antioxidants during storage at high temperatures of 32 and 55°C

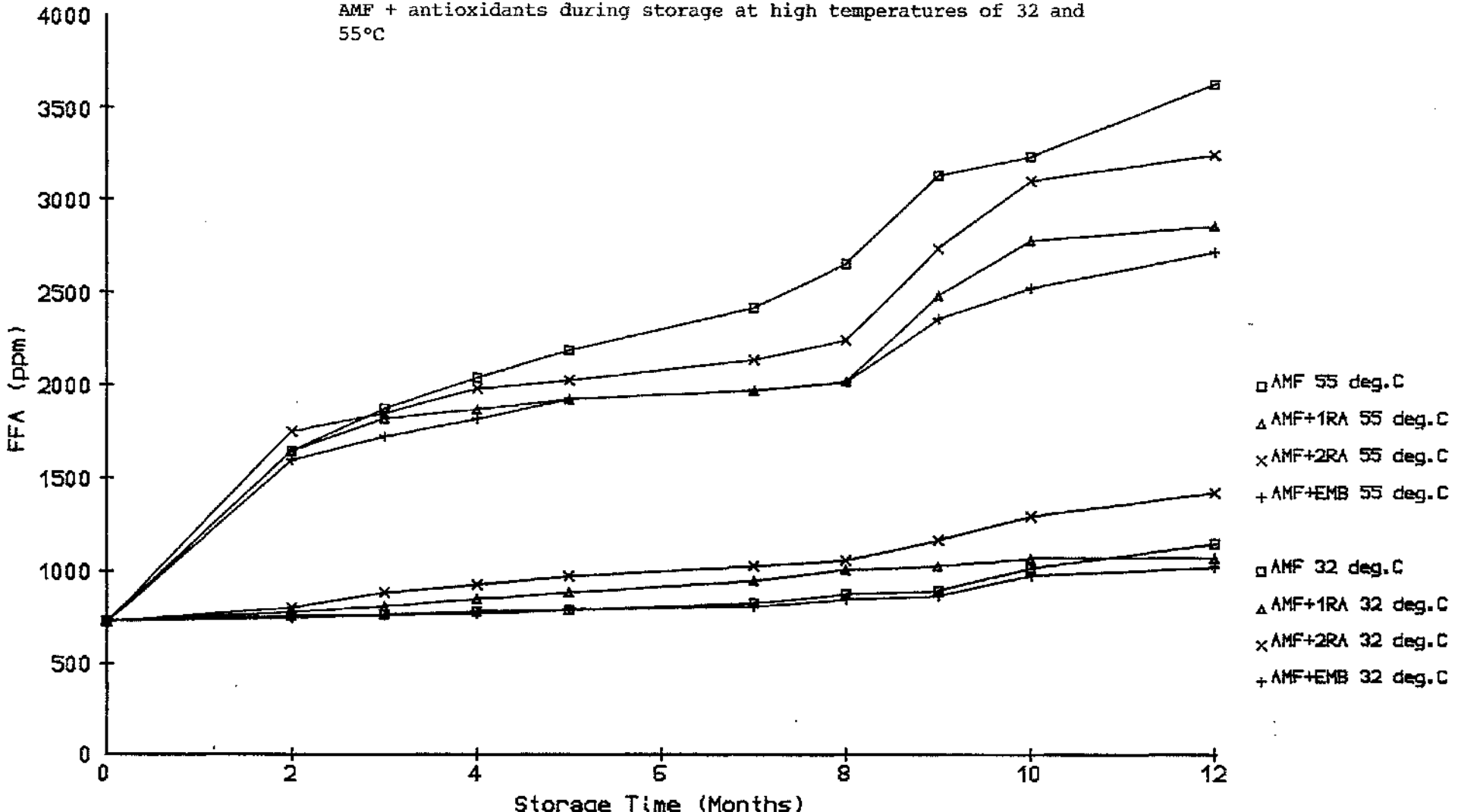
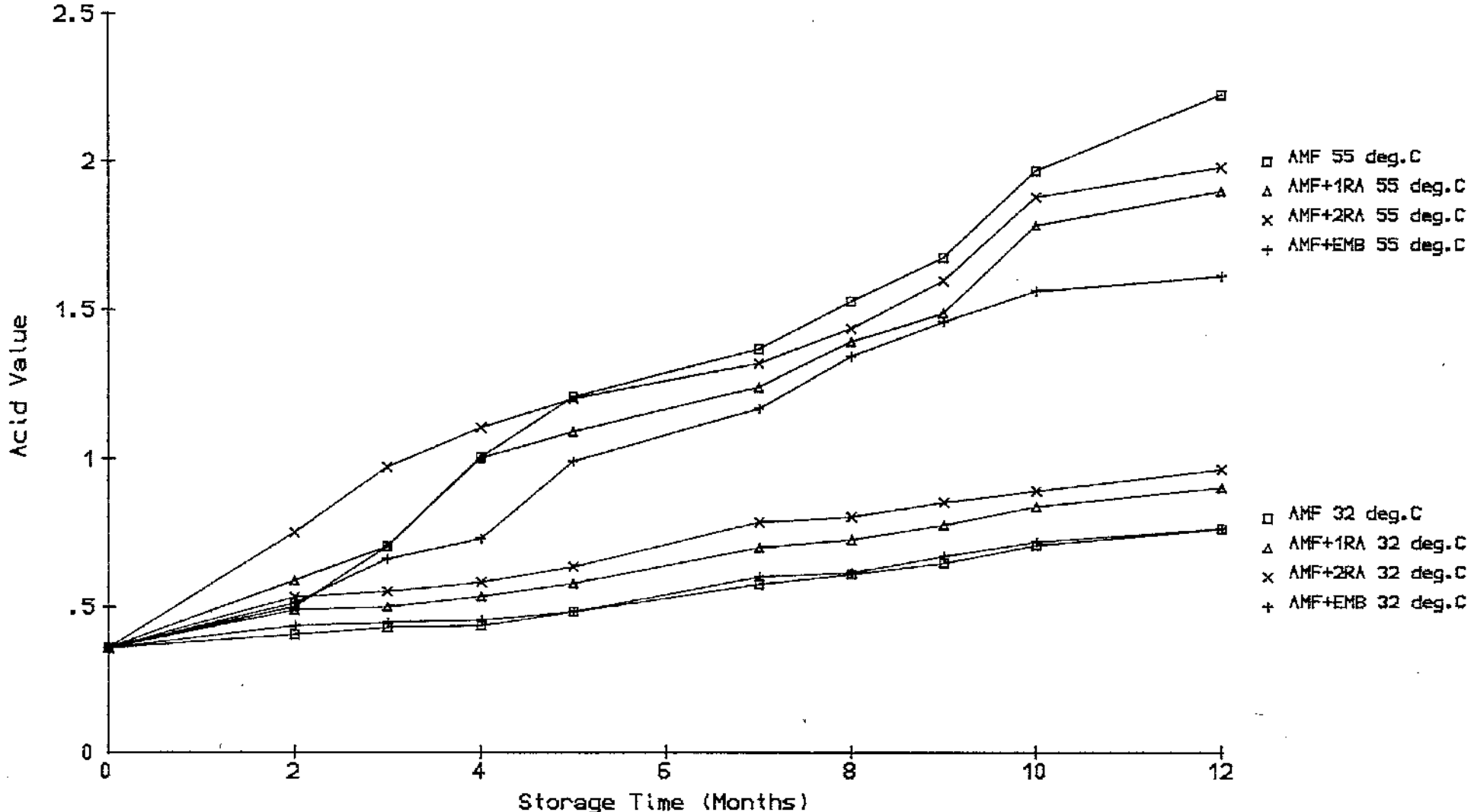


Figure 5:17 Variation in the acid degree values (mg KOH/g) in the AMF and AMF + antioxidants during storage at high temperatures of 32 and 55°C



$C_{18:1}$) due to the effect of different storage temperatures (32 and 55°C). The differences between the acid values were also very highly significant ($P < 0.0001$) due to the effect of different storage temperatures.

DISCUSSION

The storage temperatures of 32 and 55°C resulted in a completely different effect on AMF than the low storage temperatures discussed before. During storage at these temperatures AMF showed a considerable development of lipolysis with an increase in the amount of the FFAs. There was also a change on the ratio of each FFA within the mixture.

Lipases from a number of different species of psychrotrophic bacteria isolated from dairy products are resistant to heat treatment by pasteurisation. Shipe & Senyk (1981) concluded that processing at 76.7°C for 16 seconds should be adequate to protect most milks from lipolytic problems for 7 days after pasteurisation, but higher temperatures may be necessary for longer shelf-life of susceptible raw milk supplies. A further treatment combining more than one heat treatment for inactivation of bacterial lipases was examined by Griffiths et al. (1981). They found that there are many psychrotrophic bacteria which can produce extracellular enzymes which are extremly thermostable. The most important of these enzymes from the commercial viewpoint are the proteases and lipases. Even at very low concentrations, these enzymes are capable of breaking down the casein and fat in milk and milk products which are subject to long term storage. The use by Griffiths et al. (1981) of various combinations of heat treatments showed that no heat treatments can produce complete inactivation of the bacterial lipases as shown below.

Heat treatments	77°C/ 17s	77°C/17s +55°C/1h	140°C/ 5s	77°C/17s +140°C/5s	140°C/5s +55°C/1h	77°C/17s +55°C/1h +140°C/5s
activity of lipases remaining after heat treatment (% of original)	66	33	40	42	7	30

(Griffiths et al., 1981)

The other important feature of the bovine milk lipase and the bacterial lipases is their optimum temperature for maximum activity. Richter & Randolph (1971) demonstrated that maximum activity for bovine milk lipase occurred at 37°C. At 30 and 45°C the enzyme had 22% and 27% of the activity it had at 37°C. Hugo & Beveridge (1962) showed that the optimal temperature of lipolysis by the enzymes of many micro-organisms was in the range 32 to 37°C. However, it was noted that even at 20 and 42°C lipolytic activity persisted. Troller et al. (1970) reported that the optimal temperature for staphylococcal lipase was approximately 32°C, but considerable activity was also apparent at 25°C.

Reviewing the literature of the bacterial lipases can lead to the following general conclusions.

1. There is a very wide range of psychrotrophic bacteria in milk which can produce extra cellular lipases.

2. These lipases are extremely thermostable and there are no heat treatments that can completely inactivate them.

3. These lipases have a very wide range of temperatures through which they are active.

4. The thermostability and the activity of these enzymes depend on the media employed.

The results of the present work indicated the existence of the lipases in the AMF which had passed through a heat treatment of (75°C) during its production. The results also showed the activity of these enzymes through a wide range of temperatures. The storage temperatures of the AMF at 32 and 55°C showed a statistically significant lipolysis and this effect was more pronounced at 55°C.

The results of this study agree with the finding of O'Connel et al. (1975); Deeth et al. (1979) and Downey (1980) that it is important to distinguish between the pattern of the initial FFA and the developed FFA in butter. The initial feature of the FFA showed a very small amount of the short-chain FFA (C_4, C_6, C_8) (Tables 5.29, 5:30 and 5:31). These short-chain FFAs are water soluble and hence are mostly lost during the manufacture of the AMF. In order to show

the change in the pattern of the FFA development throughout the storage time, the percentage of each FFA was calculated on the following basis:

1. Initial percentages in AMF and final percentages in AMF and AMF plus the antioxidants stored at 32 and 55°C for one year as shown in Tables 5:39 and 5:40.

2. The change in all FFAs levels in AMF throughout the one year storage at 32 and 55°C as shown in Tables 5:41 and 5:42.

From the results in Tables 5:39 and 5:42 the following conclusions may be drawn:

1. There is very low percentage value of C_4, C_6, and C_8 initially in the AMF (0.19 to 0.38%).

2. There is a steady increase of FFAs throughout the storage time at both temperatures (e.g. C_4 increased by approximately five times at 32°C to end 1.50%. At 55°C, C_4 increased by about eight times to finish at 2.36%).

3. There were more pronounced increases of the percentage of C_4 and C_6 than C_8.

Other workers have confirmed that short-chain FFA contribute more to lipolysed flavour in butter, due to their lower flavour thresholds in fat, than do the longer-chain acids (Bills et al.,1969; Deeth & Fitz-Gerald, 1983). This characteristic is likely to be the same for the AMF because of its similarity to butter.

The results of this study confirm that lipolysis of the AMF has two distinct detrimental effects. Firstly, there is a quantitative increase of all FFA and secondly, there are more rapid increases of the strongly flavoured C_4 and C_6 components of the AMF than of other FFAs. The same result was obtained by Woo et al. (1980) for the FFA composition of different butters with different degree of hydrolysis as shown in Table 5:43.

The results showed that there were very highly significant differences in the quantitative amount of short-chain FFA as a result

of different storage temperatures (32 and 55°C). The amounts increased, both quantitatively and as a percentage, more rapidly at 55°C than the 32°C. This probably can be explained by the higher enzymatic activity, especially by enzymes with a high specificity for these short-chain FFAs at 55°C than the 32°C.

There were also very highly significant differences in the amounts of short-chain FFA in the various AMFs due to the effect of the antioxidants. These short-chain FFAs increased more significantly in quantity and as a percentage) in the AMF with the Ronoxan A, especially at level of 2000 ppm, than the untreated AMF and the AMF with the Embanox 7. This probably can be explained either by the presence of Ronoxan A favouring the activity of the lipolytic enzymes or by the release of these short-chain acids by the Ronoxan A which contains lecithin as a component.

The n-decanoic acid (C_{10}) and lauric acid (C_{12}) which contribute very significantly to the rancid flavour of milk (Al-Shabibi et al., 1964) showed significant quantitative changes. There were very highly significant increases ($P < 0.00.01$)at a temperature of 55°C, C_{10} increasing 9.43 x, and C_{12} increasing 7.36 x. The increases in FFAs were more pronounced at 55°C than at 32°C. Their percentage of the FFA mixture showed little change from the initial values as shown in Table 5.39 because their initial levels are relatively low. These results can once again indicate more lipolytic activity at 55°C than at 32°C. The effect of the antioxidant on the amount of C_{10} and C_{12} FFA produced was variable. At 32°C storage temperature, the amounts of these two FFAs were increased by the presence of Ronoxan A, which can probably be explained by the same reasoning as for the short-chain FFAs. At 55°C the AMF itself showed a higher release of these two FFAs, especially in the later stage of the storage time. Woo et al. (1980) also showed that these two FFAs (C_{10} and C_{12}) increased very much in quantity in rancid butter but their percentage of the whole FFA showed very little change (Table 5:43).

The amounts of palmitic acid (C_{16}) were very highly affected by the addition of Ronoxan A to the AMF (Table 5:35). There was an extra amount of this acid derived from the ascorbyl palmitate in the

Ronoxan A, which always increased in quantity and in its proportion of the mixture of the FFAs.

There were highly significant increases in the amount of oleic acid ($C_{18:1}$) (Table 5.37), but at the same time there were highly significant decreases in its percentage in the total mixture of FFA as shown in Table 5:40. These decreases in the proportion of the total FFAs made up by this acid were more pronounced at 55°C than 32°C as shown in Tables 5:41 and 5:42. These decreases in the percentage of oleic acid probably could be explained by the involvement of some amounts of this unsaturated FFA in fat oxidation. There is some evidence that free fatty acids oxidise more readily than esterified acids (Badings, 1970). Woo et al. (1980) showed that the percentage of the C_{18} congeners was decreased from 57.7% in butter C to 47.3% in butter H. Butter C was classified as a full flavour product with no abnormality whereas butter H was classified as having a soapy rancid flavour.

Generally speaking, all the FFAs (C_4, C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18} and $C_{18:1}$) showed very highly significant increases in quantity throughout the storage time at storage temperatures of 32 and 55°C. These increases were more pronounced at the 55°C storage temperature than the 32°C storage temperature. These results probably mean that enzymatic activity of lipases was very much higher at 55°C than the 32°C. These results also indicate that the occurrence of lipolysis in the AMF results in the liberation of all these FFAs without exception. This has been confirmed by many workers (Bills, et al., 1963; Woo, et al., 1980; Humbert, et al., 1969).

The antioxidant showed no positive effect in preventing the lipolysis of AMF. The 'natural' antioxidant (Ronoxan A) which contain ascorbyl palmitate, DL-α -Tocopherol and lecithin, had a negative effect by increasing the amount of the FFAs, especially palmitic acid, the extra amount being derived from the antioxidant itself.

There were also increases to a lesser extent, in the amounts of short-chain FFA in the AMF due to the presence of this antioxidant. These points should be considered, if there is any possibility of using such antioxidant.

The synthetic antioxidant (Embanox 7) did not have a great effect on the amounts of the FFA that would develop in AMF. This antioxidant however had a positive effect in delaying lipolysis, especially at 55°C (Tables 5:29 - 5:38). This effect can be considered as an important action in relation to the lipolysis defect.

The acid values showed significant increases throughout the storage time. This was obviously due to the increases of the amounts of FFAs present. The higher amounts of FFA liberated from the AMF at a 55°C storage temperature reflected the higher acid values at this storage temperature than the values of the AMF stored at 32°C. The effect of the antioxidants on the amounts of the FFAs in the AMF also was reflected in the acid values of the treated materials.

5.3.2 The effect of high storage temperature (32 and 55°C) for one year on the peroxide value of AMF and AMF plus the antioxidants

The mean values of the peroxide values of the AMF and AMF plus the antioxidants (1000 ppm Ronoxan A, 2000 ppm Ronoxan A and 300 ppm Embanox 7) stored at 32 and 55°C for one year measured at 10 timed intervals plus the initial values are shown in Table 5:44 and Figs. 5:18 and 5:19.

Statistical analysis of variance for the above data was carried out (Table 5:45). The term 'Storage' was used for the storage time, term of 'Treatment' was used to differentiate between the AMF, AMF plus 1000 ppm Ronoxan A, AMF plus 2000 ppm Ronoxan A and AMF plus 300 ppm Embanox 7. Finally, the term 'Temperature' was used to distinguish between the two storage temperatures (32 and 55°C).

These statistical analyses gave the following results:

1. Storage time: The differences between the peroxide values of each treatment throughout the storage time were very highly significant ($P < 0.0001$)(Table 5:44).

2. Treatment: The differences between the peroxide values of different treatments were very highly significant ($P < 0.0001$) (Table 5:44).

TABLE 5:44

The effect of storage temperatures of 32 and 55°C for one year on the level of the peroxide values as (mEq O_2/kg) in AMF and AMF plus the antioxidants

		Initial	After 1 month	After 2 months	After 3 months	After 4 months	After 5 months	After 7 months	After 8 months	After 9 months	After 10 months	After 12 months
AMF	Stored at 32°C	0.105	0.475	0.76	1.04	1.55	2.37	3.35	6.44	8.55	13.51	19.66
	Stored at 55°C	0.105	2.07	2.23	11.16	21.88	32.48	44.02	63.66	76.13	70.28	49.69
AMF + 1000 ppm Ronoxan A	Stored at 32°C	0.00	0.013	0.030	0.042	0.062	0.081	0.103	0.109	0.143	0.185	0.275
	Stored at 55°C	0.00	0.046	0.185	0.274	1.42	8.98	36.62	46.02	61.16	32.23	29.13
AMF + 2000 ppm Ronoxan A	Stored at 32°C	0.00	0.00	0.018	0.026	0.052	0.060	0.072	0.081	0.084	0.135	0.184
	Stored at 55°C	0.00	0.036	0.136	0.217	0.578	1.15	21.14	25.26	39.96	48.73	58.64
AMF + 300 ppm Embanox 7	Stored at 32°C	0.076	0.303	0.384	0.480	0.520	0.560	0.711	0.78	0.99	1.03	1.46
	Stored at 55°C	0.076	0.97	1.13	1.34	2.63	2.86	3.32	3.74	4.49	5.54	9.29

Mean value from 2 trials

cont'd.....

Statistical analysis of variance for the changes of the peroxide values in AMF and AMF plus the antioxidants stored for one year at 32 and 55°C

	df	M.S	F
Storage	10	1.495×10^3	8.974***
Treatment	3	2.321×10^3	13.933***
Temperature	1	1.298×10^4	77.935***
Treatment x Storage	30	2.122×10^2	1.268
Treatment x Temperature	3	1.298×10^3	7.794**
Storage x Temperature	10	1.097×10^3	6.58***
Residual	30	1.666×10^2	
Total	77	5.993×10^2	

Table	Treatment	Temperature	Treatment x temperature
SED	0.186512	0.131884	0.263767

** Significant at level 0.1%
*** " " " 0.01%

Figure 5:18 Variation in peroxide value of AMF and AMF + antioxidants during storage at an 'ambient' temperature of 32°C

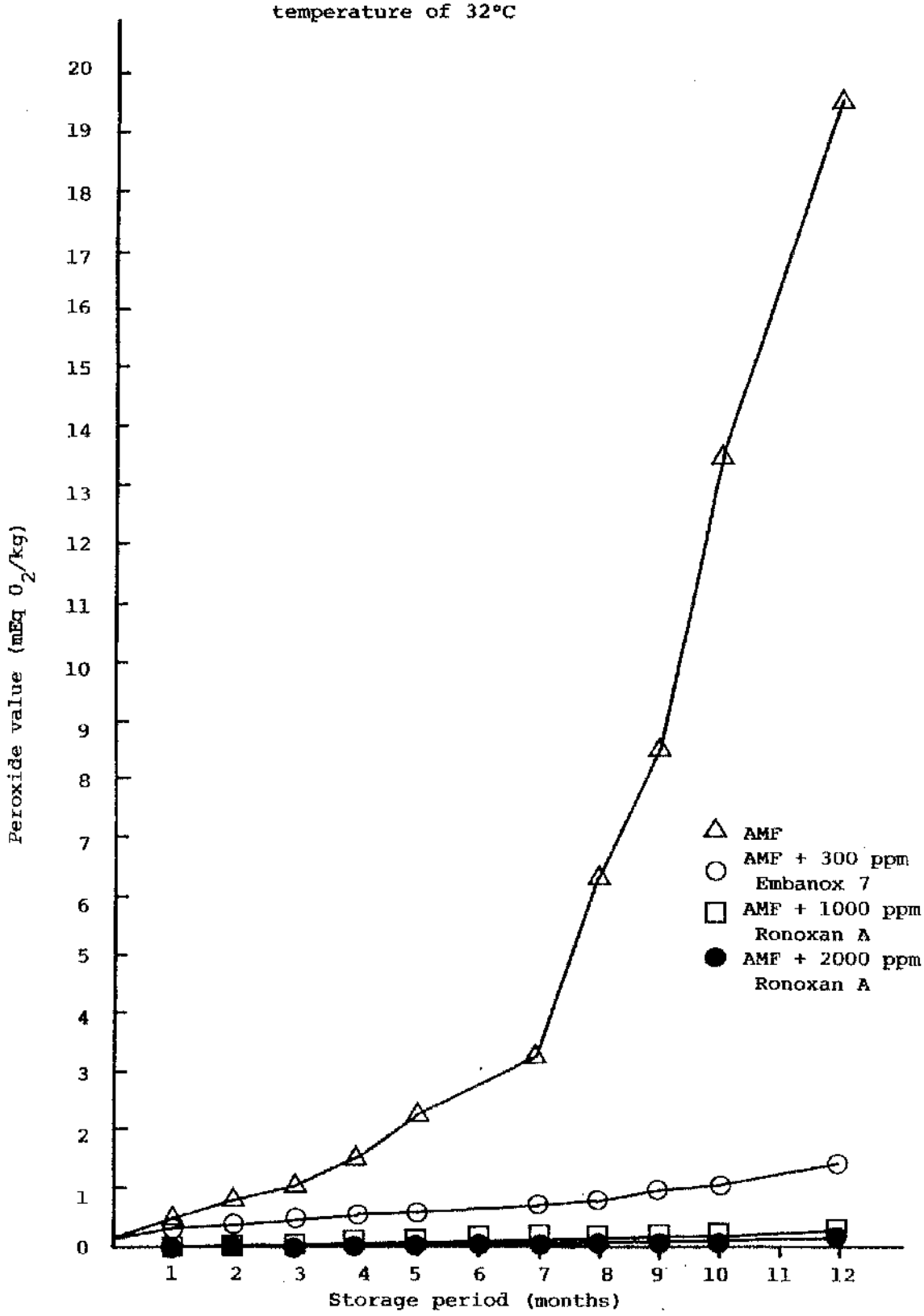
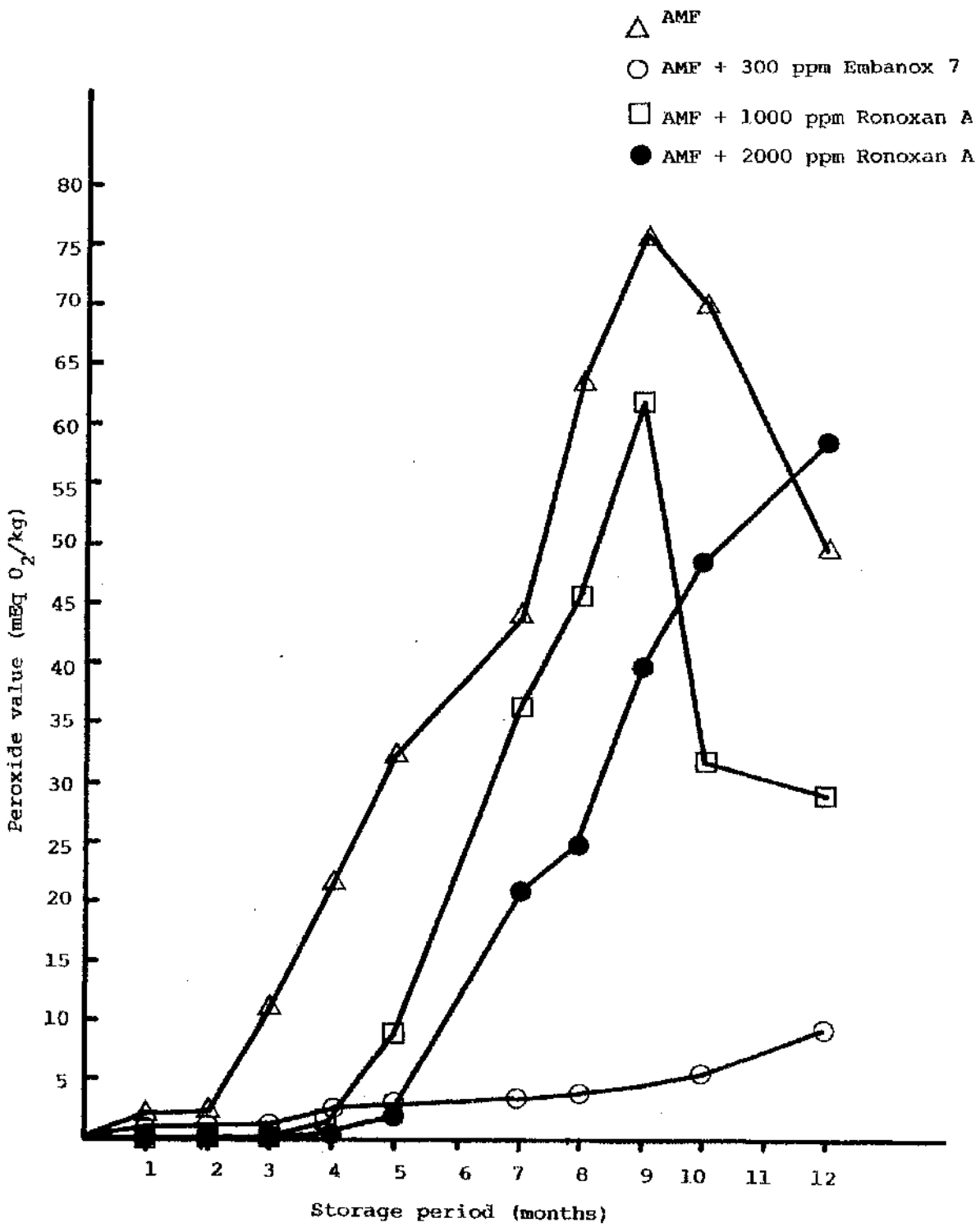


Figure 5:19 Variation in peroxide value of AMF and AMF + antioxidants during storage at 'high' temperature of 55°C



3. Temperature: The differences between the peroxide values of each treatment at different storage temperatures were very highly significant ($P < 0.0001$) (Table 5:4).

There was a highly significant interaction ($P < 0.001$) between the different treatments and different storage temperatures. Finally, there were very highly significant interactions ($P < 0.0001$) between the different storage temperatures and storage time.

DISCUSSION

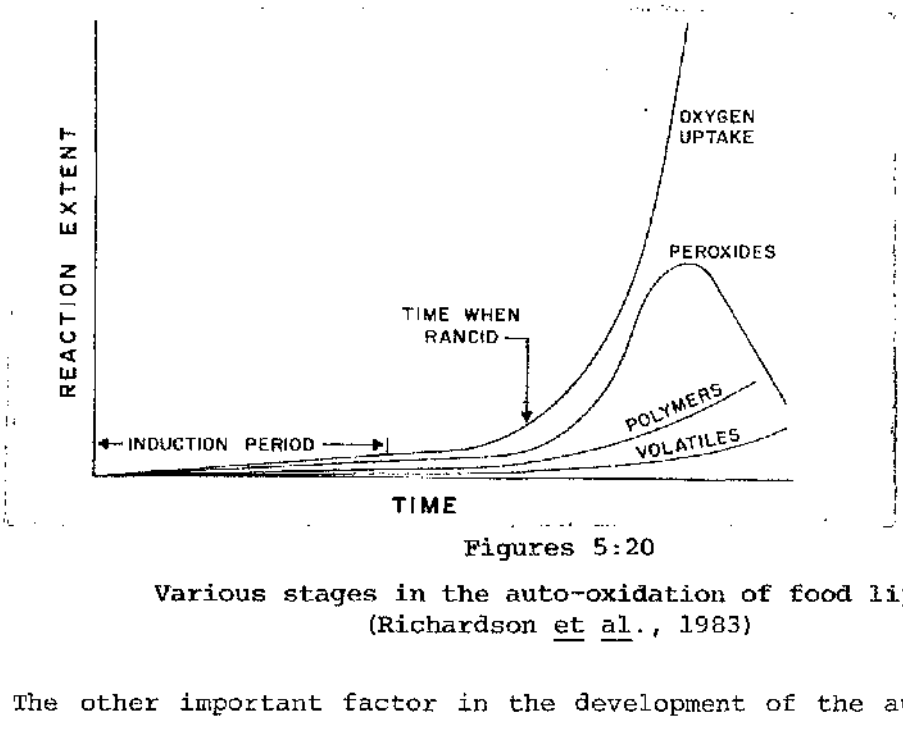
AMF sometimes deteriorates considerably in quality as a result of flavour defects which develop during storage. It is now well established that the auto-oxidation of unsaturated fatty acids is one of the main causes of these flavour defects. In the presence of oxygen and under certain storage conditions these unsaturated fatty acids undergo auto-oxidation to yield organoleptically unacceptable flavour in the AMF. Pont et al. (1960) and Badings (1970), showed that there are certain compounds or groups of compounds responsible for the major types of off-flavour resulting from fat oxidation.

The low storage temperatures showed very small increases in the peroxide values throughout the one year storage time. In contrast the high storage temperature (32 and 55°C) resulted in very high increases in the peroxide values of the AMF. The initial peroxide value increased from 0.107 mEq O_2/kg to 0.56 at 10°C and to 19.6 at 32°C for the untreated AMF.

The effect of the storage temperature is one of the most important factors in controlling the oxidation of the AMF. The results showed very highly significant differences between the peroxide values of the AMF when stored at 32 or 55°C. The storage temperature of 55°C gave a very marked increase in the peroxide value of the AMF compared with the effect of the 32°C as shown in the results. Comparing the results of the untreated AMF stored at 32 and 55°C shows that after a 3 month storage time at 32°C the peroxide value was 1.04 mEq O_2/kg. However, double this value was reached with AMF stored at 55°C after only one month. Changes in peroxide value of AMF increased rapidly after the third month of storage at 55°C. Thompson (1966) showed that fat oxidation could be accelerated by

increasing the temperature during the fat stability tests.

The untreated AMF stored at 55°C showed three distinct periods during the development of its peroxide values. The induction period lasts for two months and showed resistance to auto-oxidation. The rapid phase of change occurred between two and nine months and showed a highly accelerated development of the peroxide values. The third period, which started after nine months, showed decreases in the peroxide values, with presumably more peroxide being destroyed than formed. Richardson et al. (1983) showed the various stages in the auto-oxidation of food lipids as below.



Figures 5:20

Various stages in the auto-oxidation of food lipids (Richardson et al., 1983)

The other important factor in the development of the auto-oxidation was the storage time. The results showed that the auto-oxidation increased with time. The AMF showed a degree of resistance to auto-oxidation in the early stages of the storage time, even at 55°C and this was clearer in the presence of the antioxidants. This period, which is the so-called 'induction period', is the most important period in the auto-oxidation of any fat. The fat will start to become rancid and the peroxide values will start to increase dramatically after this period. Therefore, it is very important to consider the storage time as a limiting factor on the quality of the AMF at different storage temperatures. These stages of fat oxidation are shown very clearly in Fig. 5:20 reproduced from

Richardson et al. (1983) and based on the work of Labuza (1971).

The presence of the antioxidant in the AMF resulted in highly significant differences in the peroxide values of the AMF. The effect of the Ronoxan A at both test levels was very clear, especially in the early stages of storage. For example, this antioxidant brought down the peroxide level to zero mEq O_2/kg in the initial reading. These results confirm the early results (see Chapter 4). However, the presence of this antioxidant at a level of 1000 ppm held the peroxide value of the AMF below its initial value for at least 7 months at 32°C storage temperature, and for at least one month at 55°C. The level of 2000 ppm of Ronoxan A held the peroxide value of the AMF below its initial peroxide value for at least 9 months at 32°C storage temperature, and for at least one month at 55°C storage temperature. The level of 2000 ppm Ronoxan A was more effective than the 1000 ppm Ronoxan A in controlling the peroxide values of AMF at both storage temperatures. This confirms the early results. The presence of this antioxidant, at a level of 1000 ppm, kept the peroxide value to less than 0.3 mEq O_2/kg at 32°C where the peroxide value kept less than 0.2 mEq O_2/kg when the level of 2000 ppm of Ronoxan A was used at the same temperature. At a storage temperature of 55°C the presence of 1000 ppm Ronoxan A was effective in controlling the development of auto-oxidation up to the fourht month but after that the deterioration in AMF had entered a rapid phase lasting up to nine months. After this time it was very noticeable that the decomposition of the hydroperoxides was even faster with Ronoxan A than with the untreated AMF after the nine months storage time at 55°C. The level of 2000 ppm of Ronoxan A was effective in controlling the oxidation of the AMF at storage temperature of 55°C up to the five months storage time. However, the AMF after that time had entered the rapid phase of auto-oxidation which lasted until the end of one year of storage.

The presence of the Embanox 7 showed a completely different effect from the Ronoxan A. This antioxidant was less effective in the early stages of storage than the Ronoxan A at both storage temperatures. It was more effective in controlling the development of the peroxide values of the AMF at 32°C than the 55°C storage temperatures. Its presence in the AMF kept the peroxide value of

the AMF well below the peroxide value of the untreated AMF at 32 and 55°C storage temperatures all the time. However, the presence of the Embanox 7 was less effective in controlling the peroxide value of the AMF than both levels of Ronoxan A at 32°C. In the case of AMF held at 55°C Embanox 7 was more able to control the development of auto-oxidation of the AMF than the Ronoxan A, especially in the later stages of storage. However, the presence of Embanox 7 prevented the rapid stage of auto-oxidation of the AMF and held peroxide values at less than 10 mEq O_2/kg after one year storage time at 55°C.

Kieseker (1982b) pointed out that AMF stored in air at 30°C deteriorated to second quality within 6 to 8 weeks. The result of the present work showed that AMF stored at 32°C increased its peroxide value from 0.108 to 0.76 mEq O_2/kg in two months. This result strongly supports Kieseker's (1982b) work. However, this result does not support Sanderson (1982a) that AMF can be expected to keep, even at elevated temperatures of 30 to 40°C, for 6 to 12 months. This also was confirmed by Kirkpatrick that storage of AMF for long periods at temperatures above 35 to 40°C must be avoided.

Kieseker (1982b) suggested that it was time to find some international body to study the role of antioxidants and their use in milk fat - so illustrating the possible extension of use of those additives to secure improved quality of recombined dairy products.

The results of the present work showed a better result concerning the resistance to fat oxidation by using the antioxidants. This advantage of the antioxidant was more pronounced at 32°C. However, the antioxidant controlled only the fat oxidation and showed no positive resistance against lipolysis. Controlling the storage time and temperature is probably a better way of minimising fat deterioration while maintaining product acceptability. Sanderson (1982b) thought that many manufacturers of recombined dairy products would very much oppose the addition of antioxidants into the product called AMF.

5.3.3 The results of the organoleptic tests

Table 5:45 showed the results of the organoleptic tests of the

recombined milk which was produced from recently manufactured low-heat SMP and stored AMF. The control sample was produced from AMF stored at -18°C. The other four samples were produced from AMF and antioxidants treated AMF stored at 32°C. The results showed:

1. The superiority of the control sample which was produced from the AMF stored at -18°C. This sample showed the lowest ranking scores at both time intervals (6 and 12 months). Its acceptability was 93% at 6 months time interval and 100% at the 12 month interval.

2. The sample which was produced from the AMF plus 2000 ppm Ronoxan A stored at 32°C showed better results than the other three samples. The other three samples represent the recombined milk which was produced from AMF, AMF plus 1000 ppm Ronoxan A and AMF plus 300 ppm Embanox 7.

3. The sample which was produced from untreated AMF stored at 32°C showed the worst results, especially at 12 months time interval.

4. The results showed the high decrease in the degree of acceptability (except the control sample) due to the effect of storage time. The percentage of acceptability of the sample produced from untreated AMF dropped from 71% at 6 month intervals to 0% at 12 month intervals. The drop was from 79% to 43% for the sample produced from AMF plus 1000 ppm Ronoxan A. The samples which were produced from AMF plus 2000 ppm Ronoxan A and AMF plus 300 ppm Embanox 7 dropped from 86 to 57 and 71 to 36 respectively.

Table 5:46 showed the results of the organoleptic test of the recombined milk which was produced from recently manufactured SMP and stored AMF. The control sample was produced from AMF stored at -18°C. The other four samples produced from AMF and AMF treated with antioxidants were stored at 55°C. The results showed:

1. The superiority of the control sample produced from AMF stored at -18°C. This sample showed a ranking score of 1.0 (like best) and an acceptability percentage of 100% at the 6 months time interval.

2. All the other four samples showed a degree of acceptability between 0 to 14% at the 6 months time interval. The samples prepared from the AMF and AMF plus 1000 ppm Ronoxan A were completely rejected by all the members of the taste panel.

3. The scores showed that recombined milk produced from AMF plust 300 ppm Embanox 7 had the best ranking other than the control sample. The recombined milk produced from untreated AMF showed the poorest ranking score.

5.3.4 The statistical results for the acceptability

Statistical analysis of variance was carried out for the acceptability of the samples as shown in Table 5:47. The term 'Storage' was used for time and term 'Treatment' was used to differentiate between the samples produced from untreated AMF and AMF treated with different antioxidants. These results represent both storage temperatures of 32 and 55°C.

The results showed very highly significant differences ($P < 0.01$) due to the effect of storage time. There were very highly significant differences ($P < 0.01$) between the graders. There were also very highly significant differences ($P < 0.0001$) due to the effect of different treatments.

The results also showed very highly significant interaction ($P < 0.01$) between the graders and the storage time. There was very highly significant interaction ($P < 0.0001$) also between the storage time and different treatments. Finally, there was a low significant interaction ($P < 0.1$) between the storage time, graders and different treatments.

DISCUSSION

The results of the present work showed the important role of the AMF in affecting the acceptability of recombined milk. These acceptabilities changed from 100% for recombined milk produced from AMF with the recommended specification of the IDF (IDF, 1977) to complete rejection for recombined milk produced from AMF with a high degree of auto-oxidation and lipolysis. The unique, pleasant flavour inherent in AMF properly manufactured from good grade cream

TABLE 5.45

The organoleptic results for the samples of the control recombined milk produced from AMF stored at -18°C and samples of recombined milk produced from AMF with and without the antioxidants stored at 32°C

	6 months		12 months	
	Ranking scores	% Acceptability	Ranking scores	% Acceptability
Recombined milk produced from control AMF	1.3	93	1.0	100
Recombined milk produced from AMF stored at 32°C	3.7	71	4.8	0.0
Recombined milk produced from AMF + 1000 ppm Ronoxan A stored at 32°C	3.7	79	2.7	43
Recombined milk produced from AMF + 2000 ppm Ronoxan A stored at 32°C	2.9	86	2.9	57
Recombined milk produced from AMF + 300 ppm Embanox 7 stored at 32°C	3.4	71	3.6	36

Mean value from 2 trials

TABLE 5:46

The organoleptic results for the samples of the control recombined milk produced from AMF stored at -18°C and samples of recombined milk produced from AMF with and without the antioxidants stored at 55°C

Samples	After 6 months	
	Ranking scores	% Acceptability
Recombined milk produced from control AMF	1.0	100
Recombined milk produced from AMF stored at 55°C	4.5	0.0
Recombined milk produced from AMF + 1000 ppm Ronoxan A stored at 55°C	3.8	0.0
Recombined milk produced from AMF + 2000 ppm Ronoxan A stored at 55°C	3.2	14
Recombined milk produced from AMF + 300 ppm Embanox 7 stored at 55°C	2.5	7

Mean value from 2 trials

TABLE 5:47

Statistical analysis of variance for the acceptability of control samaples of recombined milk produced from AMF stored at -18°C and samples of recombined milk produced from AMF with and without added antioxidants stored at 32 and 55°C before use

	df	M.S	F
Storage	2	5.49047	115.3****
Residual	3	0.04762	
Total	5	2.22476	
Graders	6	0.37143	6.324****
Grader x Storage	12	0.27381	4.662****
Residual	18	0.05873	
Treatment	4	3.350	36.077******
Storage x treatment	8	0.58571	6.308******
Grader x treatment	24	0.10556	1.137
Storage x grader x treatment	48	0.13294	1.432*
Residual	84	0.09286	
Total	168	0.20714	

Table	Storage	Grader	Treatment	Storage Grader	Storage Treatment
SED	0.0369	0.0626	0.0665	0.1069	0.1094

Table	Grader Treatment	Storage Grader Treatment
SED	0.1693	0.2928

*	Significant at level 10%
**	" " 5%
***	" " 2.5%
****	" " 1%
*****	" " 0.1%
******	" " 0.01%

or butter constitutes a fundamental asset in creating a demand. Freedom from off-flavours is a deciding factor in consumer acceptance (Keen, 1982).

These off-flavours created from the unsuitable storage condition were derived from two different chemical processes. However, the off-flavour formed during unsaturated lipid auto-oxidation is actually composed of many flavours often referred to as 'oxidised flavour'. The second off-flavour formed as a result of fat hydrolysis to produce FFAs, some of which are responsible for this off-flavour, is described as rancid, butyric and soapy.

The chemical study of the AMF showed that lipolysis of milk fat and the auto-oxidation of this fat can be considerably extended at both storage temperatures (32 and 55°C). Although the addition of antioxidants may be proposed to improve the flavour stability of AMF, generally speaking, the antioxidants used in these experiments did not markedly improve AMF flavour stability.

The organoleptic results at the 6 months time interval showed very clearly the effect of storage temperature on AMF flavour stability. The recombined milk which was produced from AMF stored at 32°C showed a 71% of acceptability. At the same time, the recombined milk which was produced from AMF stored at 55°C showed zero acceptability. The same was true for the recombined milk produced from antioxidant-treated AMF as shown in Tables 5:45 and 5:46.

The effect of the antioxidants on AMF flavour stability was more clear at storage temperatures of 32°C than the 55°C. The recombined milk produced from treated AMF with 2000 ppm Ronoxan A showed a second preferred sample after the control sample with 86% acceptability. This was after 6 months storage time of AMF at 32°C. At the same storage time and temperature ofAMF, the recombined milk produced from treated AMF with 1000 ppm Ronoxan A scored 79% acceptability. The treated AMF with 300 ppm of Embanox 7 and the untreated AMF scored 71% acceptability. These results probably can be explained by the effect of these antioxidants, especially Ronoxan A in delaying the development of the auto-oxidation of AMF as shown in Table 5:44.

The twelve months storage time interval at 32°C, again showed the same pattern in preferences but, with a lower degree of acceptability. The recombined milk produced from treated AMF with 2000 ppm Ronoxan A showed 57% acceptability. This was followed by the recombined milk produced from treated AMF with 1000 ppm Ronoxan A which showed 43% acceptability. The recombined milk produced from treated AMF with 300 ppm Embanox 7 showed a 36% acceptability. Finally, the recombined milk produced from untreated AMF had a zero acceptability. These results show the effect of these antioxidants on AMF flavour stability by holding down auto-oxidation.

It must be stressed that even when a partial control of oxidative deterioration of milk fat can be achieved, off-flavours are only partially prevented. The result showed that these antioxidants have no positive effect on milk fat lipolysis and even some of them have a negative effect, as shown with Ronoxan A. The other important point about these antioxidants is that they cannot achieve a complete prevention of milk fat auto-oxidation. The results showed at ambient temperatures, the liberation of FFAs and the development of the peroxide value of the antioxidant-treated AMF was still significant. These, as a consequence, affected the acceptability of the recombined milk.

The storage time was a very significant factor in AMF stability. The storage temperature of 32°C resulted in a reduction in the acceptability due to the time factor as shown in Table 5:45. The amount of FFAs and the peroxide values of the treated and untreated AMF were considerably increased by the storage time at 32°C. These changes were reflected in less desirable organoleptic properties of the recoambined milks prepared from AMFs held over longer periods of time.

The recombined milk produced from AMF or antioxidant-treated AMF stored at 55°C for six months was of very poor quality. For this reason, organoleptic tests were not undertaken with milks prepared from AMFs stored at 55°C for longer periods of time. Obviously, 55°C is completely outside any temperature limit which might be considered for even short time storage of AMF.

Sanderson (1982b) pointed out that there are differences of opinion with regard to the effectiveness or suitability of fat stability tests such as the peroxide value and the Swift test. However, Sanderson (1982b) also commented that these tests do give at least a guide to the quality, though flavour acceptability was the more important criterion. Slamet (1982) mentioned a very strong cheesy flavour in AMF with a peroxide value not much different from the normal. In contrast he found that AMF with a peroxide value of up to 0.6 mEq O_2/kg still had a normal flavour. The results of the present work are in agreement with Sanderson (1982b) and Slamet (1982) that organoleptic grading is the best indicator for the flavour acceptability for human consumption. Unfortunately the grading results do not always agree with the chemical results because of the presence of numerous other flavours unrelated to the chemical analysis performed. The results of the present work are also in agreement with Deeth et al. (1976) that fat oxidation is not the only way in which the fat in dairy products can decompose. It can also undergo lipolysis through breakdown of the milk fat to produce free fatty acids with unpleasant flavours. Anderson et al. (1984) showed that increasing levels of FFAs are likely to contribute to an overall deterioration of cream flavour. Aalbersberg (1982) pointed out that auto-oxidation is only one aspect of fat deterioration. Fatty acid content is the other aspect and both have to be considered at the same time.

Several workers, Kiesoker (1981; 1982b) Gunnis (1982a & b) suggested the use of antioxidants to stabilise the AMF. The result of the present work showed an improvement in the quality of the recombined milk produced from stored AMF treated with antioxidant. This was mostly with storage temperature of 32°C compared with recombined milk produced from untreated AMF stored at the same temperature. The results also showed that the best organoleptic results were obtained in the recombined milk produced from untreated AMF stored at -18°C. The results of the present work probably can support the suggestion of adding the antioxidants only in places where cold stores are not available for a certain time. The time before use of the AMF is important because the results showed that these antioxidants can only retard fat oxidation but cannot stop it completely. Concerning the time of storage, the results of the present work support the

declaration of production dates on the containers of AMF suggested by Aneja (1982) and Fokkema (1982).

The other important change in the AMF due to the storage at 32 and 55°C was in the colour. The colour was changed from yellow to white as shown in Plates 5:2 and 5:3. It may be possible for colour comparisons of AMF to be used to give some indication of product quality.

CONCLUSION

1. The results of the present work showed that there were no changes in the levels of all the FFAs or in the peroxide values of the AMF stored at -18°C.

2. The results of the present work showed the superiority of the recombined milk produced from AMF stored at -18°C which was preferred to the recombined milks produced from AMF and AMF plus Ronoxan A stored at 6, 8 and 10°C.

3. The results obtained in this study showed that the level of the FFAs (C_4 to $C_{18:1}$) remains constant at low storage temperatures of 6, 8 and 10°C for one year.

4. The results of the present work showed that there were significant increases in the amounts of palmitic acid in the AMF as a result of adding the antioxidant Ronoxan A. Palmitic acid was derived from the ascorbyl palmitate which represents about 25% of the commercial product Ronoxan A.

5. The results of the present work showed that there were significant increases in the degree of auto-oxidation in AMF at low storage temperature of 6, 8 and 10°C throughout the storage time.

6. It is concluded that the addition of the commercial antioxidant Ronoxan A to the AMF had a significant effect in retarding the auto-oxidation of AMF. The results showed that this antioxidant had the ability to keep the peroxide value of the AMF below its original value after one year of storage at low temperatures (6, 8 and 10°C).

1 2 3

Plate 5:2 A comparison of the colour of molten anhydrous milk fat stored with and without added antioxidants

1 - Control AMF After 1 year at 32°C

2 - AMF with added Ronoxan A (1000 ppm) after 1 year at 32°C

3 - Control AMF after 1 year at -18°C

1 2 3 4 5

Plate 5:3 A comparison of the colour of molten anhydrous milk fat stored with and without added antioxidants

1 - Control AMF after 1 year at 55°C

2 - AMF with added antioxidant (1000 ppm Ronoxan A) after 1 year at 55°C

3 - AMF with added antioxidant (2000 ppm Ronoxan A) after 1 year at 55°C

4 - AMF with added antioxidant (300 ppm Embanox 7) after 1 year at 55°C

5 - Control AMF after 1 year at -18°C

7. It is concluded that the addition of Ronoxan A at level of 1000 ppm a negative organoleptic results by deepening the colour of the AMF. In most cases the recombined milk produced from the AMF plus the antioxidant was less preferable than the samples which do not contain the antioxidant.

8. The present work showed that there was a very highly significant increase in the amounts of all FFAs (C_4 - $C_{18:1}$) in AMF at storage temperatures of 32 and 55°C. These increases were more pronounced at 55°C.

9. Storage temperatures of 55 and 32°C resulted in very highly significant increases in auto-oxidation of AMF. These increases were more pronounced at 55°C than 32°C.

10. It is concluded that the antioxidants were useful in retarding the auto-oxidation of the AMF particularly at storage temperature of 32°C.

11. Recombined milk produced from deteriorated AMF cannot be classified as an acceptable product.

12. The results of the present work showed that the combination of storage time and temperature are the main factors in controlling fat deterioration.

CHAPTER SIX

The relationship between the level of free fatty acids in anhydrous milk fat and the acceptability of the recombined milk made from it

6.1 INTRODUCTION

The previous results of the present work showed the relationship between the flavour of recombined milk and the degree of lipolysis and auto-oxidation of AMF. At the same time, the results showed that lipolysis and auto-oxidation developed in a parallel way at certain storage conditions. Therefore, it was very difficult to study the effect of each of the parameters individually. The availability of food-grade pure fatty acid was an opportunity to study the effect of increasing the level of FFA in AMF and study its effect on the flavour of recombined milk.

There have been many workers who have studied the relationship between the FFA content and the flavour of milk. Considerable variation between their studies has been observed (Krukovsky et al., 1942; Tallamy & Randolph, 1969; Hunter et al., 1968; Gould, 1944; Magnusson, 1974; Pillay et al., 1980; Kuzdal-Savoie, 1975 and Kintner et al., 1965; Shipe et al., 1980).

Some workers (Al. Shabibi et al., 1964 and Scanlan et al., 1965) showed that certain FFAs, particularly C_4 to C_{12} mainly caused the rancid flavour of milk. Although, the results of the present work showed some change in the ratio of the FFAs, during lipolysis, all FFAs (C_4 - $C_{18:1}$) increased with storage time. Therefore, it was thought to be more useful to study the effect of adding all these fatty acids (C_4 - $C_{18:1}$) to the AMF, rather than adding some of them.

6.2 Materials and Methods

6.2.1 The fatty acids

6.2.1.1 The short chain fatty acids C_4 and C_6 were supplied by BDH Chemicals Limited, Poole, England.

n-Butyric acid (C_4) $CH_3(CH_2)_2COOH$

Molecular weight 88.11

n-Hexanoic acid (C_6) $CH_3(CH_2)_4COOH$

Molecular weight 116.16

6.2.1.2 The fatty acids of C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18} and $C_{18:1}$ were supplied by Croda Universal Limited, North Humberside, England.

Caprylic acid-n-octanoic acid (C_8) $CH3(CH_2)_6COOH$

molecular weight 144.24

contains 99% caprylic with 1% of capric acid (C_{10})

Capric acid-n-decanoic acid $CH_3(CH_2)_8COOH$

molecular weight 172.27

contains 98.5% capric with 0.5% caprylic (C_8) and 1% lauric acid (C_{12})

Lauric acid-dodecanoic acid $CH_3(CH_2)_{10}COOH$

molecular weight 200.32

contains 99% lauric acid with 0.2% capric acid (C_{10}) and 0.8% myristic acid (C_{14})

Myristic acid-tetradecanoic acid (C_{14}) $CH_3(CH_2)_{12}COOH$

molecular weight 228.38

contains 96.5% myristic acid with 1% lauric acid and 2.5% palmitic acid (C_{16})

Palmitic acid-hexadecanoic acid $CH_3(CH_2)_{14}COOH$

molecular weight 256.43

contains 96.2% palmitic acid with 1% myristic acid and 2.8% stearic acid (C_{18})

Stearic acid-octadecanoic acid (C_{18}) $CH_3(CH_2)COOH$

molecular weight 284.49

contains 93% stearic acid with 5.1% palmitic acid and 1.9% arachidonic acid (C_{20})

Oleic acid-Cis-9-Octadecenoic acid ($C_{18:1}$) $CH_3(CH_2)_7 CH = CH(CH_2)_7COOH$

molecular weight 282.47

contains 72-77% oleic acid with 8-12% linoleic acid ($C_{18:2}$) and linoleoic acid ($C_{18:3}$) and 7-12% saturated fatty acids

6.2.2 Adding the fatty acids

Four levels of fatty acids (C_4 - $C_{18:1}$) as follows:

	mg of FFAs added to 1 kg of AMF									
	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	$C_{18:1}$	Total
First level	30.2	30.8	32.5	41.3	43.3	67.1	114.5	65.5	177.7	602.9
Second level	65.7	56.3	60.9	75.8	87.7	130.6	248.9	125.8	362.8	1214.5
Third level	123.5	88.5	100.6	173.7	176.4	271.3	426.5	257.4	557.4	2175.3
Fourth level	272.2	230.5	234.7	418.3	447.9	558.8	811.9	452.8	1013.5	4440.6

These levels of FFA were dissolved in four small portions of AMF (about ½ kg) at a temperature of 60°C. Each of these portions were added individually to the bulk of four amounts of melted AMF at about 40°C. Four samples from these four levels of fatty acid-treated were analysed to measure the recovery of each added FFA. A control was run with untreated AMF. The measurement of the FFA was carried out by the GLC method described in Chapter 5.

6.2.3 The production of recombined milk

The production of ten batches of recombined milk was carried out by using recently manufactured low-heat SMP with AMF. Two batches of the recombined milk were produced from untreated AMF, which met the IDF specification (IDF, 1977). The other eight batches were made from the four levels of fatty acid-treated AMF. These ten batches were produced on two different days (five batches each day). These five batches represent the untreated AMF with the four levels of fatty acid-treated AMF. The recombination process was carried out using the method already described in Chapter 5.

6.2.4 The organoleptic tests

Five samples of recombined milk representing the untreated AMF and the four levels of fatty acid-treated AMF were presented to a taste panel. The taste panel was asked to assess the samples in the same way as is described in Chapter 5. The assessment of the samples was carried out twice by presenting five samples on two separate days. Six graders were used for the organoleptic evaluation.

TABLE 6:1

The gas chromatographic estimation of (a) the initial level of the FFAs in AMF, (b) the added levels of fatty acids and (c) their recoveries expressed as a percentage of initial addition. Mean values for recovery at different levels are given along with standard deviation

	Initial value	Added amount at first level	% of Recovery	Added amounts of second level	% of Recovery	Added amounts at third level	% of Recovery	Added amounts of fourth level	% of Recovery	Mean value* of the recovery percentage	S.D
C_4	4.10	30.2	98.5	65.7	96.8	123.5	99.7	272.2	98.8	98.5 ± 1.4	1.21
C_6	1.99	30.8	95.2	56.3	94.9	88.5	94.0	230.5	96.6	95.2 ± 1.27	1.08
C_8	4.85	32.5	98.8	60.9	98.0	100.58	95.3	234.7	97.3	97.4 ± 1.8	1.5
C_{10}	40.46	41.3	98.2	75.76	96.1	173.7	96.2	418.3	96.6	96.8 ± 1.14	0.97
C_{12}	58.39	43.3	95.1	87.66	95.1	176.40	95.1	447.88	95.4	95.0 ± 0.5	0.38
C_{14}	117.4	67.1	94.5	130.6	95.4	271.3	96.6	558.8	97.1	95.9 ± 1.4	1.17
C_{16}	300.5	114.5	95.0	248.9	96.4	426.5	95.9	811.9	95.9	95.8 ± 0.7	0.58
C_{18}	136.2	65.5	95.2	125.8	92.7	257.4	92.9	452.8	95.5	94.1 ± 1.8	1.5
$C_{18:1}$	784.3	177.7	94.7	362.8	90.6	557.4	92.8	1013.5	94.7	93.2 ± 2.3	1.95

*t - test 90% confidence intervals for means

TABLE 6:2

The acid values measured by the IDF Standard Method 6A (IDF, 1969) for the untreated AMF and the AMF plus the added levels of the fatty acids

	Acid value (mg of KOH/g fat)	Acid value (mEq KOH/100 g fat)
Untreated AMF	0.34	0.61
AMF + First level of added FFA	0.48	0.86
AMF + Second level of added FFA	0.61	1.09
AMF + Third level of added FFA	0.85	1.52
AMF + Fourth level of added FFA	1.29	2.3

The results obtained are expressed in two ways so that comparison may be made with published work and IDF Specification.

6.3 Results

6.3.1 The recovery of the added fatty acids

The initial level of each FFA (C_4 to $C_{18:1}$) and the added amounts of each of them at the four levels are shown in Table 6:1. The table also shows the recovered amounts of each FFA (which were measured quantitatively using GLC) as percentages of the recovered FFA and their mean values and standard deviations.

The percentages of the recovered FFAs range from 93 to 98%. The acid values for the five samples of the AMF are shown in Table 6:2.

6.3.2 The organoleptic results

6.3.2.1 Ranking results: The results of the ranking scores for the five samples given by six panelists were as follows:

TABLE 6:3

Sample of recombined milk made from SMP with AMF at different levels of FFA	Mean values of the ranking scores
Untreated AMF	1.0
AMF + first level of added fatty acids	2.58
AMF + second level of added fatty acids	3.25
AMF + third level of added fatty acids	3.33
AMF + fourth level of added fatty acids	4.83

Mean values from 2 trials

6.3.2.2 Acceptability results: The results of the acceptability percentage for the five samples by the six taste panel members were as follows:

TABLE 6:4

Samples of recombined milk made from SMP with AMF at different levels of FFA	Mean value* of the acceptability percentage
Untreated AMF	100
AMF + first level of added fatty acids	92
AMF + second level of added fatty acids	50
AMF + third level of added fatty acids	50
AMF + fourth level of added fatty acids	17

*Mean values from 2 trials

6.3.2.3 **Statistical results:** The statistical analysis of the acceptability rankings of the samples are shown as follows:

TABLE 6:5

	df	M.S	F
Graders	5	0.5367	4.879*
Residual	5	0.1100	
Treatments (different levels of FFAs in AMF)	4	1.4000	12.000***
Graders x Treatments	20	0.1200	1.029
Residual	24	0.1167	

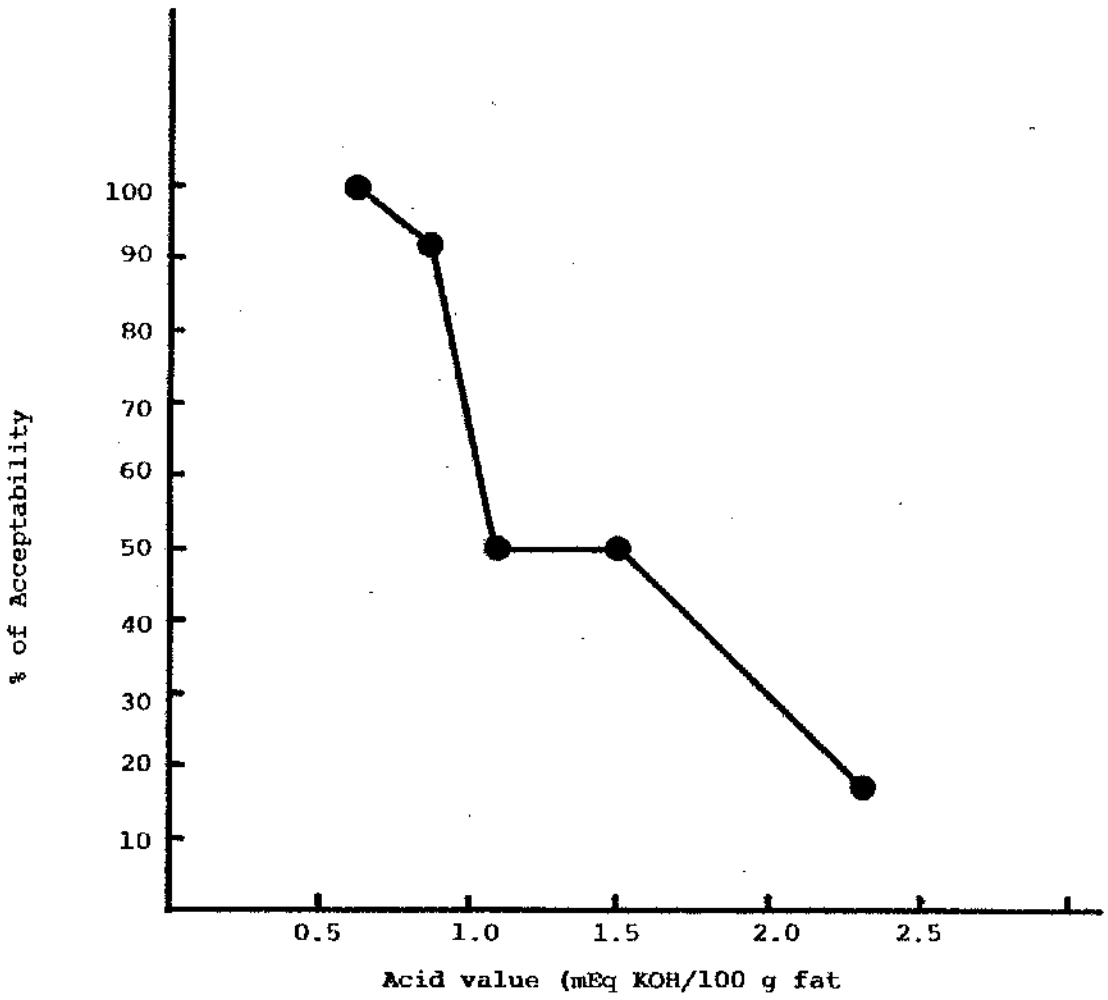
* Significant at level 10%

** " " 1%

*** " " 0.01%

The results represent the scores of 2 trials

Figure 6:1 Variation in the acceptability of the recombined milk produced from untreated AMF and treated AMF with four different levels of added FFAs.



DISCUSSION

Lipolysis is not the only way in which the fat in dairy products can decompose. It can also undergo oxidation by reaction with oxygen in the air entrained and dissolved in the products. This gives rise to oxidative rancidity and flavours described variously as 'oily', 'tallowy' or 'oxidised'. These flavours are different from thos resulting from lipolysis and should not be confused with them (Deeth et al., 1976). The results of the present work (Chapter 5) were in agreement with Deeth et al. (1976), that there was more than one way in which the milk fat could decompose. The present storage trials at 32 and 55°C clearly showed that lipolysis and auto-oxidation developed in parallel in the AMF.

Deeth et al. (1979) described the flavour of lipolysed butters as a rather subtle one which can sometimes be overlooked by experienced graders, if grading is done quickly. This is particularly so when the short-chain fatty acids are in low concentration. Although the very short-chain acids are detected first by smell, FFAs are usually detected by a sharp or soapy sensation on the tongue when first tasted. This sensation is followed by a strong after-taste and a burning sensation on the back of the palate. Deeth et al. (1979) also mentioned that the untrained palate may not recognise this latter sensation until the FFA level is quite high.

The results of the present work are in general agreement with those of Deeth et al. (1979) that the taste panelists differ in their recognition and response to lipolysed flavours in milk fats. The present results showed that 50% of the panel rejected the product

which was produced from AMF plus Third level of added fatty acids (acid value = 1.52 mEq KOH/100 g fat). This rejection level was increased to 83% when AMF plus Fourth level of added fatty acids (acid value = 2.3 mEq KOH/100 g fat) was used in the recombined milk. This establishes that lipolysed flavour is only rejected by some people when the level of FFAs is quite high. The unpleasant smell and the soapy sensation on the tongue were informally reported by many members of the panel during the organoleptic evaluation of the recombined milk produced from the AMF with added levels of FFAs. The panelists were supplied with slices of apples to overcome the after taste and the burning sensation on the back of the palate.

In view of the present results and from the remarks of Deeth et al. (1979) it is very difficult to nominate an acid value to be considered as the upper limit for flavour acceptability. Many workers have attempted to do this in the past and it is not surprising that values suggested vary considerably.

More than forty years ago Krukovsky & Herrington (1942) examined the relationship between acid degrees of raw milk and its flavour as scored by the judges. Their results indicated a threshold value for the recognition of rancidity at acid degrees near 0.8 mEq NaOH/ 100 g fat. Their results also showed that an increase of acid degrees corresponded to a decrease in the flavour score. The results of the present work show that increases in the level of the FFAs tend to result in a decreased acceptability of the recombined milk. A sharp drop in acceptability from 92% to 50% was found when the FFA level was changed from First level (acid value = 0.86 mEq KOH/100 g fat) to Second level (acid value = 1.09 mEq KOH/100 g fat). Gould (1944) showed that rancid flavour was generally detectable when the acid degree was within the range of 1.5 to 2 mEq NaOH/100 g fat. Gould (1944) also confirmed the direct relationship between the development of rancidity in milk and the increase in acid degree of the milk fat. Finally, Gould (1944) mentioned that the fat recovered from the milk by churning does not necessarily represent all the FFAs in milk. This was due to the release of at least a

portion of the lower fatty acids during the churning. It has already been suggested, in this work (see Chapter 5), that the low initial level of short chain FFAs found in the AMF was due to losses of these fractions during AMF manufacture.

Tallamy & Randolph (1969) showed that the rancid flavour in milk may be detected organoleptically by trained experts when the acid degree value level approaches 1.2 to 1.5 mEq KOH/100 ml fat. The average consumer may detect rancidity when this level reaches 2.0 to 2.2 mEq KOH/100 ml fat. Hunter et al. (1968) considered that milk samples with an acid degree value of 1.5 mEq KOH/100 ml fat or more were organoleptically unacceptable. Magnusson (1974) found that when the content of FFAs exceeds 1.8 mEq KOH/100 g fat, some deleterious effects were noted which may be attributed to hydrolytic rancidity.

A comparison between the BDI method (fat separation and titration) and the extraction titration procedure was carried out by Pillay et al. (1980). The relationship between the above two methods and the lipolysied flavour in milk was also studied in the same work. Pillay et al. (1980) found that greater amounts of free fatty acids were extracted by the extraction titration procedure than the BDI method. Pillay et al. (1980) indicated that the threshold for lipolysied flavour lies within the range of acid degree value 4.1 to 4.5 mEq KOH/100 g for the extraction titration method and 1.85 to 2.05 mEq KOH/100 g for the corresponding BDI values.

In view of the remarks reported above, it would seem that the relationship between the level of FFA and lipolysed flavour is influenced by two main variables as follows:

1. The variation between the FFAs determinations due to the effect of different methods. Johnson & Gould (1949) found that the solvent extraction of fat from rancid cream gives a greater recovery of fatty acid than does churning. Frankel & Tarassuk (1955) demonstrated that their proposed extraction-titration method makes possible a 95 to 100% recovery of added high molecular weight fatty acid, and 52 to 58% of added low molecular weight fatty acids. Frankel & Tarassuk (1955) estimated that about 90% of the total fatty acids released from

milk fat at any given degree of hydrolysis can be determined by the extraction-titration method. Similar calculations applied to data obtained from churned fat yield a value of approximately 65% for recovery of the fatty acids from milk fat. Perrin & Perrin (1958) showed that the recovery of FFAs added to milk by using the BDI method (separation-titration) was not quantitative. They quoted losses of 98%, 93% and 16% of added butyric, caproic and stearic acids respectively by using the BDI method. Deeth & Fitz-Gerald (1983) pointed out that solven extraction methods (extraction-titration) estimate a high proportion of the total FFAs. However, those samples in which the higher amounts of butyric acid are extracted and measured are the ones in which there is the greatest probability of lactic acid and other acidic components also being measured.

2. Variation in methods used to obtain the thresholds for the perception of lipolytic flavours. Individual people differ considerably in their ability to detect rancid flavour according to their natural flavour perceptions and their degree of training in taste and flavour perception.

The results of the present work confirm that individual people differ considerably in their ability to detect lipolysed flavour. Only 50% of the taste panel in the present work did not accept the lipolysed flavour in the recombined milk produced from AMF plus Second level and Third level of added FFAs (acid value 1.09 to 1.52 mEq KOH/100 g fat).

The results of the present work show the advantage of using a gas chromatographic method for analysing the FFAs in AMF. The quantitative determination of the amounts of individual FFAs could be the key to overcoming the variation of the acid values between different methods. The gas chromatographic method used in the present work showed its high recovery and reliability for estimating the FFAs in AMF. This method is suitable for the routine analysis of FFAs because of its simplicity.

Table 6:6 shows the ADV levels for the detection of rancid flavour in milk obtained by different workers. Some results of the present work were also included in the table in order to compare them with those of other workers.

Al-Shabibi et al. (1964) showed that the rancid flavour of milk is caused mainly by capric (C_{10}) and lauric (C_{12}) acids. On the other hand, Scanlan et al. (1965) found that the even numbered fatty acids from C_4 to C_{12} have an important role in contributing to the rancid flavour in milk.

Individual inspection of the pure fatty acids used in this work, showed that the short-chain fatty acids (particularly butyric acid) have a very sharp, unpleasant smell. The long-chain fatty acids, especially stearic acid, gave a very pronounced soapy taste. Several factors needed to be considered when planning the experiment to investigate the influence of free fatty acids on lipolysed flavour:

1. The differences between the conclusion of Al-Shabibi et al. (1964) and Scanlan et al. (1965) on which group of FFAs predominate in the rancid flavour of milk.

2. The inspection of the fatty acids, individually in the present work.

3. The lipolysis of the AMF resulting in the liberation of all FFA without exception as shown in the results of the present work (Chapter 5).

A conclusion can be drawn that some fatty acids have a more important role in contributing to the rancid flavour but, in general, all the FFAs can contribute to the rancid flavour to a certain degree.

TABLE 6:6

The ADV levels for the detection of rancid flavour in milk as measured by many workers and in the present work

Acid value mEq KOH or NaOH/100 g fat	Reference
0.8	Krukovsky et al (1942)
1.5 - 2	Gould (1944)
1.3 - 1.6	Thomas et al. (1955)
1.5	Olson et al. (1956)
2.74	Kintner et al. (1965)
1.5	Hunter et al. (1968)
1.2 - 1.5	Tallamy et al. (1969) (detected by trained panel)
2.0 - 2.2	Tallamy et al. (1969) (detected by average consumer)
1.8	Magnusson (1974)
2.0	Kuzdzal-Savoie (1975)
1.3	Shipe et al. (1980)
4.1 - 4.5	Pillay et al. (1980) ADV measured by the extraction-titration method
1.85 - 2.05	Pillay et al. (1980) ADV measured by the separation-titration method
1.1 - 1.5	50% of the panel of the present work did not accept the recombined milk
2.3	Nearly all the panel of the present work did not accept the recombined milk

CONCLUSIONS

1. The results of the present work showed the very highly significant relationship between the rancidity flavour of the recombined milk and the level of FFAs in AMF.

2. The present work showed that 50% of the panel did not accept the recombined milk produced from AMF with acid values of 1.1 to 1.5 mEq KOH/100 g fat. Most of the panel (83%) did not accept the recombined milk produced from AMF with acid value of 2.3 mEq KOH/ 100 g fat.

3. It is concluded that a gas chromatograpahic method for quantitative analysis of FFA in AMF has the advantage over the measurement of the acid value. The gas chromatographic method performed very effectively in estimating the individual FFAs and in determing the added fatty acids in AMF.

4. The results of the present work showed that the off-flavours of the recombined milk resulted from the increased level of FFAs in AMF are different from the oxidised flavour. The oxidised flavour derived from the auto-oxidation of the milk fat. The flavours derived from increasing the level of FFA are detected firstly by unpleasant smell and a sharp or soapy sensation on the tongue.

5. The results of the present work showed a variation between the individual people in their ability to detect the rancidity flavour.

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