EVALUATION OF DRY BLENDING FOR INFANT FORMULA MANUFACTURE

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ABSTRACT

Blending experiments and storage trials were carried out to assess the feasibility of manufacturing infant formula through dry blending of high fat whey powder (HFWP) with whole milk powder (WMP) or a base powder (BP) made from skim milk, sucrose and corn oil.

An indication of cohesiveness of the components of the blends was obtained by measuring compressibility using an Instron testing machine. Compressibility decreased in the following order: BP, WMP, HFWP, lactose and ascorbic acid. Particle size determination using a laser sizer indicated that the particle size increased in the above sequence.

Scanning electron microscopy revealed no evidence of an ordered mixture for either whey powder with milk powder or the powders mixed with ascorbic acid. The mixtures did not exhibit complete randomness and segregation. They are thus termed 'pseudorandom mixtures'.

HFWP was blended with WMP or BP to achieve a target ratio 50:50 in both an experimental ribbon blender and a pilot ribbon blender. Using Response Surface Methodology, load ratio and mixing time but not rotation speed were found to have significant effects on the homogeneity with the experimental ribbon blender. At load ratio 0.4, the time for reaching a certain homogeneity was shorter than that at load ratio 0.8. The cohesiveness of BP impaired its mixing.

A mixing index based on a satisfactory sample standard deviation has an acceptable value of 1. Both powder ratio scores and ascorbic acid level could be mixed below a MI of 1.5 but above 1. As to

protein, fat, carbohydrate, the mixtures reached the acceptable MI. The secondary nutritional requirements such as the ratio of whey protein to casein and the ratio of unsaturated fatty acid to saturated fatty acid were above 1 when the powder ratio MIs were higher than 1.

After mixing WMP and HFWP for 10 minutes differences of sensory quality could not be detected by the taste panelists even though the MI was still above 1.

Unblended and blended samples of WMP and HFWP were tested through a 180 day storage trial at 20°C, 30°C and 40°C. There was no significant difference between unblended and blended samples on the criteria of TBA, PV, HMF, oxidised flavour and caramel flavour at the 5% probability level.

Using the Arrhenius approach, at 20°C, the shelf lives of unblended and blended samples were estimated as 1628 days and 1090 days respectively, with an oxidised flavour limit of 3.5 out of 7 points. The shelf lives were 480 days and 466 days based on a PV limit of 2 milliequivalents O_2 per kg fat.

Dry blending is a feasible technique for manufacturing infant formula, with acceptable homogeneity of the main components of the blended samples and with normal storage stability. The cohesiveness of the components and the design of blender are important factors in improving homogeneity. Further trials are recommended in both experimental and commercial plants.

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CHAPTER 1 INTRODUCTION

Whey powder is being increasingly used in the food industry. Demineralized whey powder has been becoming an important ingredient in infant formula for its non-casein protein content and lactose content which leads to the humanization of infant formula. As an innovation, high fat whey powder has been made from incorporating cream into demineralized whey concentrate. Its fat content gives rise to possibility of dry blending high fat whey powder with a pre-dried mix of skim milk, vegetable oil and sucrose to make infant formula in contrast to the normal technique of wet blending, evaporation and drying.

This technique possesses the potential of saving energy and preventing whey protein denaturation in secondary thermal processing. It may offer the advantage of economic use of vitamin additives by avoiding the need to over-dose to cope with processing losses. In the countries where milk fat is in shortage, dry blending of high fat whey powder with skim milk mix will save local fresh milk fat that can be shifted to make more valuable products. However, there are few reports on dry blending milk products for making infant formula. Packard (1982) commented unfavorably on dry blending, stating that infant formula can be made from either dry blending ingredients or wet blending ingredients and drying, but from a microbiological point of view, the latter is preferred. This is a problem of hygienic production control. Delvalle (1981) reported that an infant formula was made from oat, soy and milk powder through a cooking/blending

process under strict hygienic control. The vital problem of blending may remain on the homogeneity of the ingredients through the mass and the effects of blending on nutrition value and sensory quality.

Blending is part of powder technology which is one of least understood (Earle, 1966) or gets modest attention in food processing (Peleg, 1982). To assess the feasibility of this technique, first of all, the powder properties related to blending must be determined. A system of sampling, testing, data analysis and evaluating the mixing operation is required to be established. This study was aimed at developing a methodology to understand the relationship between input variables such as the cohesiveness of the powders, the operation parameters of the blenders, and the output variables such as homogeneity of the mixture and the mixing rates. The methodology is hoped to be utilized in other blending equipments.

As to the effects of blending, Krasheninnin (1983) reported that fat destabilization occurred after blending whole milk powder with sucrose at 6.6 rev/sec for a maximum 10 minutes. The stability of the product would be the main concern in developing this technique. The oxidation of milk fat causes flavour deterioration in a storage. The effects of blending will not be apparent immediately after blending. A storage trial was to be carried out to determine the difference between blended and unblended products through chemical testing and sensory evaluation. Until the effects of blending are known, little can be done towards the practical application.

It is hoped that the basic understanding of the blending of milk powder and whey powder will be increased through this work.

CHAPTER 2 LITERATURE REVIEW

Powder mixing, one of the most widely applied operations in different industries, has come under detailed scrutiny since the 1950s (Clarke, 1971). Inorganic mixtures of relatively simple mixing characteristics have been examined and a statistic base for mixing and mixtures have been developed and reported on, especially in the pharmaceutical industry. Various food powders are mixed in numerous food industries such as soup powder making, drink powder making and nutrient fortification. However, the theory and practice of the mixing of food powder is rarely reported on. It is necessary to review the problems relating to food powder mixing under the following titles:

2.1 Cohesiveness of food powders

The physical properties of food powders have obvious implications in major or common food processing including mixing. Cohesiveness is one of these factors which closely affects the mixing of food powders. Cohesive powders, as defined by Peleg (1978), are those in which interparticle forces play a significant role in the powder beds mechanical behaviour. These factors can reduce flowability, form stable bridging between particles and cause agglomeration. The latter phenomenon is usually referred to as caking problem which can vary from soft lumps to the total solidification of the powder bed (Pietsch, 1969). Most food powders, if not protected, become cohesive especially due to moisture adsorption. The various kinds of mechanisms by which particles are attached or interlocked have been classified and discussed in the classical work by Rumpf (1961), and reviewed by

Pietsch (1969) Peleg (1977). The major mechanisms which are concerned in food powder operations are (1) liquid bridge, (2) molecular force, (3) mechanical interlocking.

The presence of a liquid bridge in itself is sometimes sufficient to obstruct flow (Peleg and Mannhein, 1973). Peleg (1982) showed that the cohesiveness of food powder increases with moisture or water activity. Takada (1974) reported that the cohesiveness of milk powder increases effectively with the increasing of fat content. On the contrary, Buma (1971) stated that the cohesion of whole milk powder is independent of the fat content in the range of 20-45% of fat. The free fat on the surface of the particles was considered directly linked with cohesiveness of the milk powder, but a small amount of fat is sufficient for the cohesiveness (Buma, 1971).

Molecular force can be considered as significant only at a short range. Van der Waals forces for example have an effective range of up to 100 Angstrom. It is obvious that the effect of such forces become significant in very fine powders or in compacted beds (Peleg, 1981). Indirect evidence of the contribution of such a short range force in foods can be found in the work of White et al (1967).

Particles with irregular or fibrous shapes can be mechanically interlocked (Rumpf, 1961, Mohsein, 1978). By the aid of vibration or pressure, they may reach mutual orientations in which they are physically bound. In the processing of milk powder and whey powder, the powders undergo a number of mechanical operations and fine particles are likely to be bound as agglomerates.

2.2 Determination of cohesiveness

Currently three methods are used for assessing the cohesiveness of powders.

The shear strength method was developed by Jenike (1967) and extended by Williams and Irks (1967). The experimental procedure was based on compaction of a powder specimen under a determined consolidation load followed by a shear operation.

The tensile strength method provides a direct indication of the interparticle force's presence and magnitude. The experimental procedures are based on measurement of the force necessary to dissect a powder compact whether directly or indirectly in a vertical or horizontal split cell (Peleg, 1977).

An investigation of the cohesiveness of milk powder was conducted by Takada et al (1974) with a tensile strength tester. They found that the cohesiveness of milk powder increases with the increase of temperature, fat, moisture but with the decrease of particle size. The empirical equation of milk powder cohesiveness was given as following:

$$H = 1.70 \times 10^{-7} \times t^{1.1} \times d^{-1.5} \times \exp(0.06F + 0.3M)$$

Where: H = cohesiveness, gram
t = temperature, ^{o}C
d = particle size, micron
F = fat content, %
M = moisture content, %

The relationship between applied stress and powder density has been used for characterizing food powders in regard to their cohesiveness. The concept by definition describes the influence of pressure on bulk density. There is only weak or no interparticle force in noncohesive powders. The cohesive materials form an open structure, which gives a

high compressibility when pressure is applied. It was found that compressibility under small load is a sensitive index of powder cohesiveness (Carr, 1976), which has been observed in a large variety of food powders (Peleg and Mannhein, 1973, Peleg et al, 1973, Moreyra and Peleg, 1981). Moreyra and Peleg (1980) firstly reported that an Instron testing machine was used to determine the compressibility of a number of food powders. Food powder under a low pressure range generally exhibits the following relationship:

$$BD = a + b \log P$$

Where: BD = bulk density, g cm^{-3}
a = constant,
b = compressibility
P = pressure, g cm^{-2}

2.3 The types of mixtures

It is important to ensure that the ingredients are homogeneously distributed in the mixture through mixing, packing, transportation and storage. It is of interest to know the types of mixtures formed in mixing operations.

Weidebaum (1958) and Williams (1968) stated that a random mixture of free-flowing particles of equal size, shape and density has the characteristics that the possibility of finding a particle of A at any point in the mixture is a constant equal to the proportion of that kind of particle in the whole mixture. If the particles are not identical, then a partially randomized mixture will be formed (Barbosa-canovas, 1985).

Hersey (1975) described the concept of an ordered mixture of cohesive powders. In ordered mixtures, the fine particles are adhering on the surface of large particles and are not randomly distributed.

Ordered mixing requires particle interaction through adsorption, chemisorption surface tension, frictional, electrostatic or any other type of adhesion. If the ordered units are not equally sized, a segregation process will occur and a segregated ordered mixture will be obtained (Thiel et al, 1981).

If the fines are in excess of the sites of larger particles where they can adhere, they, if cohesive, will form agglomerates with their own kind; they will create a 'partial ordered random mixture'. (Hersey, et al, 1979).

Egermann and Orr (1983) have questioned the terms 'random' and 'ordered' in classifying powder mixtures. They suggested that the term 'interactive mixture' be used instead of ordered mixtures when adhesional phenomena are dominant. Noninteractive mixtures will replace random mixtures when all the ingredients are free-flowing.

Egermann (1980) suggested the term 'pseudorandom mixture' for the mixture with a random homogeneity from non free-flowing materials.

As to food powders, Barbosa-canovas, et al (1985) showed that the mixture types of a system depend on interparticle affinity, concentration and relative humidity. The bridges between the aggregated particles depend on their chemical species and the availability of surface moisture.

2.4 Assessment of homogeneity of mixture

There are more than 30 mixing indexes derived in efforts to obtain a universal index of mixing that is completely unambiguous but they are all subject to limitations (Fan, 1975).

The widely used method of assessing the homogeneity of a binary mixture is to compute the sample standard deviation as follows:

$$\sigma = \sqrt{\Sigma(x_i-x)^2/(n-1)}$$

Where: x_i = the content of ingredient tested in n_i spot sample
x = the mean content of the ingredient of n samples
n = sample number

Thiel (1981) suggested that when an ordered mixture is produced, the population mean content (u) for each batch in the mixer is known, the standard deviation be determined by the following formula:

$$\sigma = \sqrt{\Sigma(x_i-u)^2/n}$$

Where: x_i = the content of ingredient tested in n_i spot sample
u = the population mean content of the ingredient
n = sample number

Gao (1985) stated that this mixing index is suitable for industrial cases since the population mean of each batch is known before the operation.

The American standard has been used as basis for evaluating the homogeneity of a mixture in the pharmaceutical industry (Chowhan, 1981). Assuming the distribution of the samples to be normal and 99.7% confidence interval is required, the acceptable standard deviation is defined as:

$\sigma_{acceptable}$ = ±15% of mean/3

A number of investigators have indirectly used standard deviation as a measurement of the degree of mixing. σ/σ_a has been used by Hersey (1976) and Chowhan (1981) as a mixing index to assess a multicomponent system. It appears to provide a better comparison of the homogeneities of individual components in a mixing system than a standard deviation.

2.5 Mixing practice

According to Clump (1967), there are three types of mechanisms involved in mixing of solids.

(1) Convection bulk movement of groups of particles which is analogous to turbulence of fluids.

(2) Diffusion movement or scattering of separated particles which is analogous to diffusion.

(3) Shear movement of particles within a group of relatively slow particles which is analogous to laminar fluid motion.

Commercial mixers combine several of these types of particle motion to obtain mixing. Clarke (1971) provided a scheme of classification of mixers based on mechanical elements rather than on mechanisms.

In comparison with V-type, planetary, and conical mixers, the ribbon blender was considered a better machine to produce a satisfactory homogeneity (Miles, 1970). The ribbon blender is classified into a non-segregation or less-segregation blender. Although there is some segregation due to rolling down particles down an inclined surface and some shearing, the main action of mixing action is convection (Williams, 1968). It is able to handle segregating materials very much better than a tumbling mixer (Williams, 1973). Gao (1985) stated that it can give better mixing for less free-flowing materials.

Chowhan (1981) showed that the materials with similar properties are easier to be mixed homogeneously. The basic material properties of the mixing components which are cohesive limit the degree of homogeneity that can be attained. However, under vibrating conditions, cohesive powders give a more homogeneous mixture and show no

segregation.

The load ratio, rotating speed, and mixing time are important operating parameters. With increased load ratio, the mixing time necessary to obtain the required value of variation is also increased. (Jiri Thyn, 1981). As mixing proceeds, standard deviation falls to a minimum at which the mixing is normally stopped. Further mixing will either merely maintain this minimum or cause it increase again (Clarke, 1971).

Hersey (1975) stated that the rate of ordered mixing follows first order kinetics, since the rate of mixing will be in proportion to the number of fine particles remaining to adhere onto the larger particles. Such a mixing rate is also applicable to random mixing (Conlson, 1950). This was confirmed by numerous experiment results (Earle, 1966).

2.6 Storage stability of milk powder and whey powder

The storage stability of infant formula is a major concern of both the consumer and manufacturer. As there are no report on the storage stability of high fat whey powder or the mixture of sugar, vegetable oil and skim milk powder, which is termed 'base powder', the information of whole milk powder or other milk powders may provide some indications on the mixture of high fat whey powder and base powder.

The storage stability of dried milk products is influenced by the quality of milk, processing technology, packaging methods and storage conditions.

Oxidation of milk fat is the main process causing deterioration of flavour in whole milk powder. Walstra and Jenness (1984) state that

the deterioration of flavour due to oxidation is caused by carbonyl compounds. These compounds cause off-flavour in concentrations of 1 microgram/kg or less.

Ipsen and Hansen (1988) found that the quality of milk exerts an influence on the storage stability of whole milk powder. There was a good correlation between the number of coliforms in the initial milk and the flavour and 2-thiobarbituric acid (TBA) value of whole milk powder stored 50 weeks at 30°c. The extent of lipolysis in milk was also found to correlate with the flavour and oxidative deterioration in whole milk powder.

Temperature significantly influences the storage stability of dried milk and whey. Driscoll (1984) noted that non-fat dried milk held at 32°c for 6 months began to develop off-flavours and by 24 months was considered unacceptable by a trained sensory panel whereas, storage at 10°c resulted in minimal flavour changes in 52 months. Boon (1976) reported that significantly greater oxidative deterioration occurred at 37°c compared with 22°c in whole milk powder.

The moisture content of dried milk and whey products is a critical factor on the storage stability (Holm, 1927; Henry, 1948, Labuza, 1983). An increase of water content above the monolayer value acts as to dissolve and mobilize reactants for the Maillard reaction; a decrease of water content below monolayer value exposes the reaction sites of fat to oxidants. Labuza (1981) reported that the maximum rate of browning occurred at a_w 0.44. The gain or loss of moisture is highly dependent on the properties of the packaging materials.

High pre-heat treatment of milk increases natural antioxidant activity due to the formation of sulphydryl groups and so reduces the

rate of development of oxidized flavour (Harland, Cloulter, and Jennes, 1952). Boon (1976) reported that a heat treatment of skim milk of 120°c/12 sec combined with heating the cream at 93°c/10 sec followed by 120°c/4 sec resulted in the best stability. Poulson (1971) stated that a heat treatment above 95°c should be used for manufacture of whole milk powder with a good storage stability.

Buma (1971) reported that the free fat content on the surface of milk powder does not correlate with the oxidation extent. On the contrary, Westergaar (1983) stated that the free fat of whole milk powder has a direct influence on the shelf life of the powder but he did not mention the direct effect of free fat on oxidation.

The oxygen absorbance of milk powder was reported by Labuza (1971) as 4 to 5 ul O_2/gram/day based on peroxide values. Shipstead and Tarassuk (1953) showed that development of oxidized flavour is not always related to the amount of oxygen used. Powders with an oxidative stability used larger amount of oxygen before off-flavour occurred than less stable powders.

Using a kinetic approach, Hall (1985) found that the fat oxidation of whole milk powder stored in darkness at 25°c, has a linear phase which gradually changes to an exponential phase which can be seen from the development of the unfavorable oxidation product hexanal. The break point at which the oxidation changes from the first to second phase was found to be at 37 weeks.

Therefore, the stability of an infant formula depends on multiple factors. The effect of blending is one of them which will be assessed in the following chapters.

CHAPTER 3 MATERIALS AND METHODS FOR CHEMICAL ANALYSIS AND SENSORY EVALUATION

3.1 Introduction

The materials and the methods described in this chapter were those used for the determination of mixing index, chemical analysis and sensory evaluation of the samples. Physical testing is described in Chapter 4.

3.2 Materials

High fat whey powder (product code 585) was obtained from the Mangaturoto factory, Northland Coop. Dairy Company.

Base powder was obtained from the New Zealand Dairy Research Institute. Base powder was made from skim milk, corn oil, and sucrose.

Whole milk powder (product code 6075) was obtained from the Te Rapa factory, New Zealand Coop. Dairy Company.

All three powders were packed in multiwall bags and stored at ambient temperature.

The main chemical compositions of the three powders are shown in Table 3.1

Table 3.1 Main chemical compositions of high fat whey powder, whole milk powder, and base powder.

		High fat whey powder	Whole milk powder	Base powder
Protein	%	16.30	26.35*	20.69
Casein	%	4.15*	20.82*	16.76
Fat	%	25.50	26.23*	28.10
lactose	%	51.23	37.54*	26.80
Sucrose	%			20.75
Ash	%	1.99	6.03*	3.74
Moisture	%	4.44*	3.85*	3.82
Free fat	%	4.45*	2.60*	0.83*
Calcium	ppm	1650*	9650*	5640*
Ascorbic acid	mg/100g		32.16	

* Analyzed by the author, the other data was from the suppliers.

3.3 Methods

3.3.1 Determination of powder ratio score

3.3.1.1 Background

The powder ratio score in this study is expressed as the percentage of whole milk powder or base powder in the mixture. Table 3.1 shows the considerable difference of calcium content and the ratio of casein to whey protein between high fat whey powder and whole milk powder or base powder. This information can be used to provide an indication of the powder ratios of the mixture.

The calcium figure was used as an indicator of milk content in ice cream and bread (Pearson, 1976). Calcium complexometric analysis (EDTA) has been adopted as the standard method for testing calcium content in dried milk by NZMAF (1979). The only modification of this method is using reconstituted samples instead of dried powders.

Whey protein is bound by Amido Black after casein is precipitated. The excess dye can be detected spectrophotometerically. It is a fast method for determination of the protein in milk (Pearson, 1976).

These two methods were attempted and compared.

3.3.1.2 Preparation of samples

High fat whey powder and whole milk powder or base powder respectively were reconstituted with distilled water at temperature 50°c at the concentration of 1 g powder per 10 ml water. Unknown samples were treated in the same way. Milk and whey were mixed at the following ratios:

Table 3.2 The ratio of milk and whey in standard samples

Milk	0	20	40	60	80	100
Whey	100	80	60	40	20	0
Ratio score	0	20	40	60	80	100

3.3.1.3 Calcium testing method

3.3.1.3.1 Procedure

a. Reagents

Sodium hydroxide 8.0 M solution

Ethylenediaminetetracetic acid 0.01 M solution.

Magnesium sulphate 0.05 M solution.

Patton and Reede's Indicator (2-Hydrox-4-sulpho-1-naphthylazo-naphthoic acid).

b. Procedure

Deionized water 30 ml was added into 4 ml reconstituted sample. 20 ml 0.01 M EDTA was then added and followed by 1 ml 0.05 M magnesium sulphate solution and 2 ml 8.0 M sodium hydroxide. The solution was mixed and allowed to stand, shaken occasionally until it cleared.

Calcium chloride solution 0.01 M was added until pink coloration persisted on standing for 15 seconds.

The calcium content was calculated with the following equation:

Calcium (ppm) = (A-B) x 1000

Where: A = EDTA solution added, ml

B = calcium chloride solution added, ml

3.3.1.3.2 Regression

The consumed calcium chloride (ml) was regressed with ratio score. The results are shown in Table 3.4. The ratio score of unknown sample thus can be calculated with the equations. When a new batch of reagent was used, the equation needs recalibration.

Table 3.3 Coefficients of regression equations for ratio scores (calcium testing method)

	c	k (ml^{-1})	R^2 (%)
$HFWP_a/WP_b$	229.798	-12.544	99.9
$HFWP/BP_c$	454.757	-24.731	99.9

a HFWP = high fat whey powder
b WMP = whole milk powder
c BP = base powder

3.3.1.3.3 Reliability of the method

The method was used to determine the calcium content of whole milk powder and the reliability of the method was calculated. The results are shown in Table 3.4.

Table 3.4 Calcium content of whole milk powder.

Sample No.	Calcium content (ppm)
1	9650
2	9600
3	9650
4	9625
5	9670
6	9700
Mean ± SD	9649 ± 34.70
% precision	± 0.36%

3.3.1.4 Amido Black binding method

3.3.1.4.1 Procedure

a. Reagents

Acetic acid/sodium acetate buffer pH 4.6.

Amido Black 0.6175 g in 1 l 63 g citric acid solution.

b. Procedure

2 ml pH 4.6 buffer was added into 25 ml of standard sample or unknown sample to coagulate casein. The coagulum was filtered through Whatman #4 filter paper.

1 ml filtrate was added into 9 ml Amido Black dye solution in centrifuge tube in duplicate. The tubes were stoppered and mixed by inverting each tube 3 or 4 times. They were centrifuge at 2500 rpm for 5 minutes.

1 ml of the supertanant was diluted to 100 ml with distilled water and measure the absorbance at 615 nm using distilled water as a blank.

3.3.1.4.2 Regression

It was not necessary to find the exact whey protein content but the ratio of the two powders. The ratio scores of the standard samples were regressed with the absorbance readings. The results are shown in Table 3.5.

Table 3.5 Coefficients of regression equations for ratio scores (Amido Black method)

	c	k	R^2
$HFWP_a/WMP_b$	-200.506	699.412	99.6
$HFWP/BP_c$	-226.464	750.921	99.4

a HFWP = high fat whey powder
b WMP = whole milk powder
c BP = base powder

3.3.1.4.3 Reliability of the method

The method was used to determine the absorbance of reconstituted whole milk powder treated by Amido Black and the reliability of the method was calculated. The results are shown in Table 3.6.

Table 3.6 Aborbance of reconstituted whole milk powder tested by Amido Black.

Sample No.	Absorbance at 615 nm
1	0.428
2	0.433
3	0.423
4	0.428
5	0.440
6	0.439
Mean ± SD	0.432 ± 0.00673
% precision	± 1.6%

3.3.1.5 Comparison of calcium testing method with Amido black method

Fewer operation steps were involved in the calcium testing method. Calcium testing was more sensitive than Amido Black method as there was only 0.15 and 0.135 absorbance unit change from ratio score 0 to 100 whereas the calcium chloride consumed change were 4.06 ml, 7.9 ml for two mixtures, respectively. Therefore, the calcium testing method was used in this study. However, when calcium salt is added in the powder mix, the Amido Black method is of value of application as the calcium method can not be used.

3.3.2 Determination of ascorbic acid

3.3.2.1 Background

Ascorbic acid was extracted with metaphosphoric acid-acetic acid solution and titrated with 2,6-dichlorophenol-indophenol, which is

adopted as standard method for testing milk powder by NZMAF. The only modification was using reconstituted sample instead of powder.

3.3.2.2 Procedure

a. Reagents

Extracting solution: 30 g metaphosphoric acid was dissolved in 80 mi glacial acid and 600 ml water which was diluted to 1000 ml.

Standard ascorbic acid solution: Dry ascorbic acid was dissloved in extracting solution to the concentration of 1 mg/ml.

50 mg 2,6-dichlorophenol-indophenol was dissolved in 50 ml water to which 42 mg sodium bicarbonate had been added. The solution was shaken until dissolved and was diluted with 200 ml distilled water. It was filtered through Whatman #4 filter paper to amber glass bottle.

b. Procedure

10 ml of extraction solution was added to 20 ml reconstituted sample as in 3.3.1.1 (1). It was filtered through Whatman #4 filter paper.

10 ml filtrate was pipetted 50 ml erlenmeyer flask and titrated with 2,6-dichlorophenol-indophenol standard solution.

The ascorbic acid content in the sample is calculated based on the following equation:

$$A = (V_{smp} - V_o) \times s \times 3/2 \times 100$$

Where, A = Ascorbic acid, mg/100g
V_{smp} = Titration volume of the sample, ml
V_o = Titration volume of the blank (20 ml distilled water and 10 ml extraction solution), ml

3.3.2.3 Reliability of the method

The method was used to determine the mixture of whole milk powder and whey powder in which ascorbic acid was added at 60 mg per 100 g powder. The reliability of the method was calculated. The results are

shown in Table 3.7.

Table 3.7 Ascorbic acid content of the mixture of whloe milk powder and high fat whey powder.

Sample No.	Ascorbic acid mg/100g
1	90.45
2	89.05
3	88.75
4	90.05
5	87.25
6	86.45
Mean ± SD	88.67 ± 1.56
% precision	± 1.80%

3.3.3 Determination of 2-Thiobarbituric Acid (TBA) Value

3.3.3.1 Background

The oxidation products especially, malonaldehyde, react with 2 - thiobarbituric acid (TBA), the red colour of which gives an indication of the extent of lipid oxidation.

The use of TBA for estimating the degree of lipid oxidation in milk products has been described by many workers (Dunkley and Jennings, 1951; King, 1962; Mettler, 1973). Mettler (1973) postulated that reaction of steam distillates with TBA had been used as a routine procedure to assess deterioration of powdered milk products. The Mettler method has been used by a number of authors for its higher repeatability (Touhy, 1981).

3.3.3.2 Procedure

a. Reagents

2-Thiobarbuturic acid 0.67 g TBA was dissolved with warming (<50^{o}c) in 100 ml of 90 per cent v/v AR glacial acetic acid.

3 N hydrochloric acid AR.

b. Procedure

15 gram powder sample was added to glass distilled water by swirling in a 500 ml round bottom flask. 3 N hydrochloric acid 7.0 ml was then added. The distillation was carried at a rate controlled to collect 50 ml distillate in 10 - 15 minutes.

20 ml distillate was mixed with 2 ml of TBA reagent. The mixture was heated in boiling water bath for 35 minutes. A reagent blank was treated in the manner with 20 ml of distilled water replacing distillate. The cooled contents was read for absorbance at 530 nm.

3.3.3.3 Reliability of the method

The method was used to determine TBA values of whole milk powder. The reliability of the method was calculated. The results are shown in Table 3.8.

Table 3.8 TBA value of whole milk powder

Sample No.	TBA at 530 nm
1	0.016
2	0.017
3	0.015
4	0.018
5	0.015
6	0.016
Mean ± SD	0.016 ± 0.00117
% precision	± 7.3%

3.3.4 Determination of Peroxide Value

3.3.4.1 Background

The oxidation of ferrous to ferric iron and its estimation by the thiocyanate reaction has been the basis for a number of tests for determining peroxide in fats and oils. The ferric thiocyanate method

decribed by Loftus Hills and Thiel (1946) has been widely used for study of fat oxidation in dairy products (Hamm, 1969; Boon, 1976). It has been incorporated as a routine method for whole milk powder at the NZDRI (1979).

3.3.4.2 Procedure

a. Reagents

The de-emulsification agent: tri-sodium citrate 50 g, sodium salicylate 50 g, N-butanol 86 ml were dissolved indistilled water and make up to 450 ml.

Chloroform/ methanol: 70/30 (v/v), redistilled reagents.

Ferrous chloride solution: Hydrated barium chloride 0.4 g was dissolved in 50 ml water and added slowly with stirring to 0.5 g of hydrated ferrous sulphate dissolved in 50 ml water. 2 ml of 10 N HCL was then added and mixed. The solution was centrifuged for 5 minutes at 100 rpm and the clear solution was used.

30% Ammonium Thiocyanate solution

b. Procedure

10 gram powder was mixed with 20 ml distilled water at 50^{o}c and transfered into 6"x1" test tube. After standing in 70^{o}c water bath for 3 minutes, 15 ml of de-emulsification reagent was added. The tubes were shaken vigorously and stood in water bath for another 5 minutes. The contents were transfered to the Babcock cream bottle. They were centrifuged for 3 minutes after standing in water bath for 1 minute. Distilled water was added at 60 ^{o}c to bring fat into the neck of the bottle. The bottles were centrifuged for 2 minutes then stood in 50^{o}c water bath until fat cleared.

9.7 ml of the chloroform/methanol reagent was added into the 6"x 1"

test tube. 0.2 ml fat, 0.05 ml of ammonium thiocyanate solution and 0.05 ml of ferrous chloride solution were added and mixed using a vortex mixer. The tubes were placed in the dark for 10 minutes. The absorbance of ferric thiocyanate was measured at 515 nm with Cecil 293 Spectrophotometer. The spectrophotometer was zeroed on the blank solution.

The peroxide value was calculated based on the following equation:

PV = ABS/ (F x 0.764 x 55.84 x 0.0325)

Where: PV = milliequivalents of O_2 per kg fat
ABS = absorbance at 515 nm
F = fat taken, ml
As 0.2 ml fat was taken, PV = ABS x 3.61

3.3.4.3 Reliability of the method

The method was used to determine PV of whole milk powder. The reliability of the method was calculated. The results are shown in Table 3.9.

Table 3.9 PV of whole milk powder

Sample No.	PV
1	0.88
2	0.93
3	0.84
4	0.81
5	0.92
6	0.86
Mean ± SD	0.87 ± 0.046
% precision	± 5.3%

3.3.5 Determination of 5-Hydroxymethylfurfral (HMF)

3.3.5.1 Background

It is a quantitive method for determining 5-hydroxymethylfurfral by spectrophotometeric measurement of 2-thiobarbuturic acid reaction

product. The result is a sensitive indication of the early stage of the Maillard reaction before visible color development. It is widely used for the study of the storage stability of dried milk product (Boon, 1976; Ipsen, 1988).

3.3.5.2 Procedure

a. Reagents

40% Trichloro acetic acid

0.05 M 2-thiobarbituric acid

b. Procedure

13 gram powder was reconstituted in 100 ml water using a solubility index mixer. 10 ml of sample and 5.0 ml 40% trichloric acetic acid were added into 50 ml stoppered test tube, mixed throughoutly, filtered through Whatman #42 filter paper. 4 ml of the filtrate was pipeted into test tube in which 1.0 ml 0.05 M TBA was added. The test tubes were placed in 40 ^{o}c water bath for 40 minutes, then cooled to room temperature.

The absorbance was measured in Varian 634 spectrophotometer at 443 nm. The blank was preparaed the same as the sample except substituting water for milk.

Free HMF was calaculated with the following equation:

Free HMF (micromoles per 100 g powder) = (ABS - 0.015)x 81

3.3.5.3 Reliability of the method

The method was used to determine HMF of whole milk powder. The reliability of the method was calculated. The results are shown in Table 3.10.

Table 3.10 HMF of whole milk powder

Sample No.	HMF (micromole/100 g powder)	
1	1.90	
2	1.91	
3	1.78	
4	1.75	
5	1.81	
6	1.82	
Mean ± SD	1.83	± 0.064
% precision		± 3.5%

3.3.6 Miscellanenous

The methods tabulated in Table 3.11 were those used for determining the composition only.

Table 3.11 Chemical methods for analysis of milk powder and high fat whey powder

Particulars	Methods	Principle of method
Moisture	NZDDM 4 2.9.2	Heating the sample at 108±0.5°c in an air oven.
Total nitrogen	Semi-micro[a] Kjeldhal	A weighed sample was catalytically digested with sulphuric acid converting the organic nitrogen into ammonia nitrogen. The ammonia nitrogen was released by the addition of sodium hydroxide, distilled and absorbed in boric acid and then titrated.
Casein-nitrogen	BS 1741[a]	Casein was precipitated with acetic acid-acetate buffer and filtered off. The nitrogen content of the filtered cake was determined.
Fat	NZDDM 4 2.6.1	Fat was extracted from an ammonia alcoholic solution of the sample with diethyl ether and petrolem ether, the solvents are evaporated, and the residue weighed.
Free fat	NZDDM 4 2.6.3	Free fat was the fat in whole milk powder extracted by organic solvents under standard conditions.
Ash	NZDDM 4 2.3.1	After all the organic material has been burt off the residue was weighed
Lactose	NZDDM 4 2.5.3	After deproteinisation of the reconstituted sample, the lactose content was determined indirectly by titrametric estimation of the amount of halogen reduced in the reaction between chloramine T/potassium iodide and lactose.

a From David Pearson, D. (1976). The others are from NZMAF.

3.3.7 Sensory Evaluation

3.3.7.1 Background

Descriptive analysis was used in this study for sensory evaluation. Descriptive analysis was considered as a methodology which provides a complete sensory description of the tested products and a basis for determining the sensory characteristics that are important to acceptance, as well as an aid in identifying underlying ingredient and process variables (Stone, 1985).

This method requires the use of a scale or score which corresponds to certain descriptors of a given product attribute. This involves the development of a linear scale word anchored at the ends describing varying intensities of a sensory stimulus (Anderson, 1970, Stone, 1974). A linear scale was used to describing varying intensities of milk and whey flavours and off flavours. The questionnaire for storage stability is shown in Appendix 1. The questionnaire for powder ratio variance was constructed in the same way as the one for storage stability. As to flavour, 1 point indicates the lowest intensity, and 7 points the highest intensity. It should be mention that evaluation was based on objective assessment of the characteristics of the sample rather than on preference.

The analysis of variance (ANOVA) is the most appropriate statistical procedure for analyzing responses, a twoway analysis of variance general linear model showing both subject and product effects (Stone, 1985). With one of the following: Duncan, Newman-Keul's, Tukey (a), Tukey (b), Scheffe, and Dunnet testing, the critical value of significant difference can be computed and the different samples are thus intentified.

3.3.7.2 Experimental conditions and procedure

3.3.7.2.1 Samples

The sample preparation method for powder ratio variance estimation and storage stability are detailed in Appendix 2.

3.3.7.2.2 Panelists

The panelists were selected from a group of employees of Food Technology Department and Food Research Centre, Massey University, who had proven ability to discriminate between milks with different sensory properties.

The panelists were trained to consistently recognise and quantify the descriptors including sweetness, mouthfeel, caramel flavour, oxidized flavour. In training sessions, the panelists were asked to score the reference sample. After completing the scoring, the panelists showed their scores on the board. In an open discussion, the panelists agreed on the reference score on each item on the questionnaire. Two training sessions were organised before testing started and another two for refreshing during the trials.

A total of nine panelists were used in each session for storage stability and seven panelists for identifying the powder ratio variance.

3.3.7.2.3 Panel facility

Sensory evaluation was conducted in a sensory room equipped with individual booths.

3.3.7.2.4 Time and temperature

All the samples were served at approximately 20^{o}C. Testing was done in the morning 10:00 am and 2:00 pm.

3.3.7.2.5 Procedure

All the samples were coded with 3-digit random numbers. 30 ml of sample was in a 50 ml PET cup. In a testing session, four samples plus reference were randomly placed in a tray with a glass of water. The panelists recorded their judgements on the linear scales.

3.3.7.2.6 Data analysis

Twoway ANOVA was performed to determine if significant difference existed among the samples and panelists for storage stability. The same analysis was done for powder ratio variance, followed by Tukey testing for the significant results.

CHAPTER 4 SOME PHYSICAL PROPERTIES OF HIGH FAT WHEY POWDER, WHOLE MILK POWDER AND BASE POWDER

4.1 Introduction

Blending of milk powder with high fat whey powder depends on the properties of the powders and the performance of the blender. The powder properties include surface properties, shape and particle size distribution. The common feature of food powders is the tendency to develop physical and chemical changes with dependency on the temperature-moisture history (Peleg, 1977), which in turn will affect the type and homogeneity of the mixture.

The work in this chapter was to establish fundamental knowledge of the compressibility, particle size, sorption isotherm, and the microscopy of the powders and their mixtures, to help understanding the characteristics of the mixing of high fat whey powder and milk powders.

4.2 Compressibilities of the powders

4.2.1 Introduction

The bulk density of food powders is increased with the application small load of pressure in handling and processing operations. The relationship between the bulk density and the load (stress) on the powder has been shown in 2.2. The slope of the equation is termed compressibility which provides an indication of other physical properties, notably internal cohesion, that may affect the powder's flowability and storage stability (Peleg and Mannheim, 1973). The compressibility of powder was determined by Peleg (1980) with an

Instron testing machine.

The compressibilities of base powder, whole milk powder, high fat whey powder, lactose and ascorbic acid were tested using an Instron food testing machine 1140 in order to obtain the indication of the cohesiveness of the powders.

4.2.2 Experimental

4.2.2.1 Material

The sources of the materials were as described in 3.1. Lactose are obtained from the Lactose Company NZ, Limited and ascorbic acid from Serva, Feinbiochemica. Each powder was used in its original form.

4.2.2.2 Procedure

The test cell was 30 mm deep with an internal diameter of 90 mm. The clearance between the cover plate and cell wall was about 1 mm. The clearance was proved to have an insignificant effect on the testing (Peleg, 1980).

The powders were poured into the test cell and their original bulk density determined by dividing the net weight of the powder by the known volume of the cell.

The cell was mounted on the base plate of Instron 1140 and the powder specimen compressed at a crosshead speed 50 mm per minute to a preset deform, one millimeter each time for milk powders and whey powder and 0.5 millimeter for lactose and ascorbic acid. The crosshead was stopped at this position and the stress was allowed to relax. The force range of the loading cell was 50, 100, 200, 500 Newton. The force-deform curves were recorded. Each test was replicated four times. Results were reported as average values.

The particle size distribution of three powders, lactose and

ascorbic acid were determined using a Malver Master Particle Sizer 2600c. Approximate 5 gram powder was added into holder and blown through the laser beam generated by the instrument. The light flux scattered by the particles was detected by the photo detector. Its output was fed to an analog digital convertor (APC). The APC produced a number of digital counts proportional to the input analog voltage. The microcomputer used these digital counts to compute the means and variance of the particle distribution.

4.2.2.3 Data analysis

The arithmetic average diameters of the powders were calculated with the following equation: (Stockham, 1977)

$$d_{av} = \Sigma nd/\Sigma n$$

Where: d_{av} = Arithmetic average diameter, um
d = Mid size of particle size interval, um
n = Frequency of occurrence

Since the initial weight of the powder and dimensions of the cell are known, the force deformation curve recorded could be transformed into bulk density-apparent stress relationships.

$$BD = W/V \qquad S = F/A$$

Where: BD = Bulk density, g cm^{-3}
W = Weight of the sample, g
V = Volume of the sample before and after deformation, cm^3
F = Force applied on the cell, Newton
A = Internal area of the cell, cm^2
S = Stress, Newton/cm^2

The empirical equation for the relationship between bulk density and the logarithm of stress were regressed using Mutab computer program (Boag, 1988) on an IBM PC-AT computer.

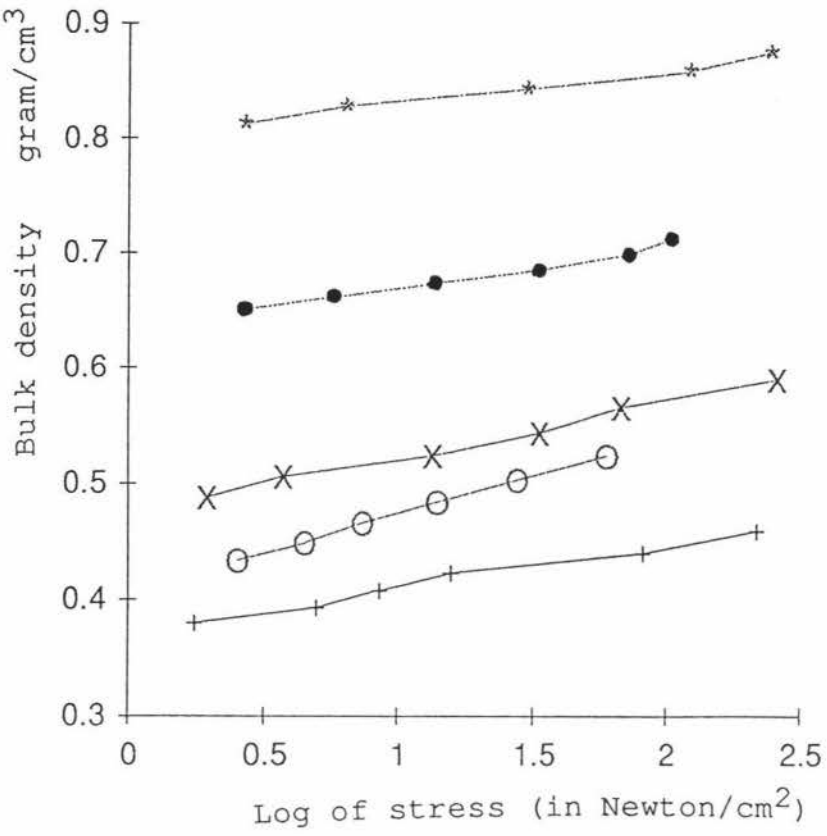

Fig. 4.1 Compressibilities of high fat whey powder (+), whole milk powder (x), base powder (o), lactose (●) and ascorbic acid (*).

4.2.2.4 Results and discussion

Loose bulk density, particle size and compressibilities of the five powders are given in Table 4.1. The compresibilities of five powders are also graphically presented in Fig.4.1.

The compressibilities of milk powders and whey powder are smaller than 0.08 reported by Peleg(1981), which may be due to the different specimens and testing machines used.

The results indicate that in the case of non-cohesive powders like ascorbic acid, interparticle forces are of a weak nature only. Therefore, the powder occupies the volume of the container leaving

only random voids. The original structures were relatively dense; the pressure applied affected mostly the random voids and had a smaller effect on the bulk density resulting in lower values of compressibility.

In the case of cohesive powder like base powder and whole milk powder relatively strong interparticle forces enable the formation of an open structure. Pressure, when applied had a greater effect by causing the collapse of the open structure and higher values of compressibilities were obtained.

The compressibilities of high fat whey powder and lactose are very similar. The precrystallized lactose of whey powder forms random voids resulting in better flowability.

Although direct determination of cohesiveness was unable to be done the compressibilities of the powders provide indirect indications of the cohesiveness. It is clear that the cohesiveness sequence in decreasing order is: base powder, whole milk powder, high fat whey powder, lactose and ascorbic acid, referring to Table 4.1.

Table 4.1 Arithmetic average diameter, bulk density and compressibilities of base powder, whole milk powder, high fat whey powder, lactose and ascorbic acid.

	AAD_a um	BD_b g cm^{-3}	a_c g cm^{-3}	b_d	R^2 (%)
High fat whey powder	98	0.38	0.428	0.038	98.0
Base powder	77	0.43	0.507	0.066	99.9
Whole milk powder	96	0.49	0.545	0.048	99.4
Lactose	108	0.65	0.689	0.037	98.4
Ascorbic acid	208	0.81	0.845	0.028	98.4

a Arithmetic average diameter
b Bulk density
c Constant of regression equation
d Compresssibility

4.3 Sorption isotherms of high fat whey powder, whole milk powder and base powder

4.3.1 Introduction

Water activity has long been considered as one of the most influential factors in the safety and stability of foods. The relationships between water activity and microbial growth, the kinetics of deteriorative chemical reactions and other quality factors have been thoroughly investigated and reported (Karal, 1975, Schwimmer, 1980, Troller, 1980).

The physical aspects of water activity have also received considerable attention, especially in sugar containing foods that are renown for their hygroscopic nature and tendency to agglomerate and stick. The problem has been especially acute in milk powders when the water is liberated by lactose crystallization (Berlin et al, 1968), which is sufficient to cause severe caking problems. The capacity of amorphous sugar to absorb considerable moisture is probably one of the main reasons for the physical instabilities of a variety of food powders.

Since milk powder and high fat whey powders are fairly fine and bulk densities are relatively low, the bulk properties may be significantly affected by comparatively minor changes in surface moisture. Water activity is expected to be a more sensitive indication than moisture content in dealing with the bulk properties (Peleg, 1981). Furthermore, the water activities of the powders are related to the stability to oxidation and the Maillard reaction. Therefore, the sorption isotherms of high fat whey powder, whole milk powder and base powder were to be determined.

4.3.2 Experimental

4.3.2.1 Material

The materials used were the same as described in 3.1.

4.3.2.2 Procedure

Sorption devices as recommended by Lang (1981) were used for equilibration studies. The sorption containers contained a support for the weighing dish, which contained samples being exposed to the humid atmosphere in the cell. Saturated salt solutions corresponding to various water activities were chosen as recommended by Greenspan (1977). They were: lithium chloride (a_w of 0.113), potassium acetate (0.225), magnesium chloride (0.328), potassium carbonate (0.432), magnesium nitrate (0.529), sodium chloride (0.843), potassium nitrate (0.936).

Two gram samples of the three specimens were accurately weighed in the weighing dishes and placed in the equilibration cells, which were kept in the room maintained at 20°C. Samples were weighed every alternative day until apparent equilibrium was reached, i.e. until the sample showed no weight change for three successive weighings within 0.001 g. Three replicates of each isotherm were conducted.

Moisture contents of the samples were determined by the method described in Table 3.11.

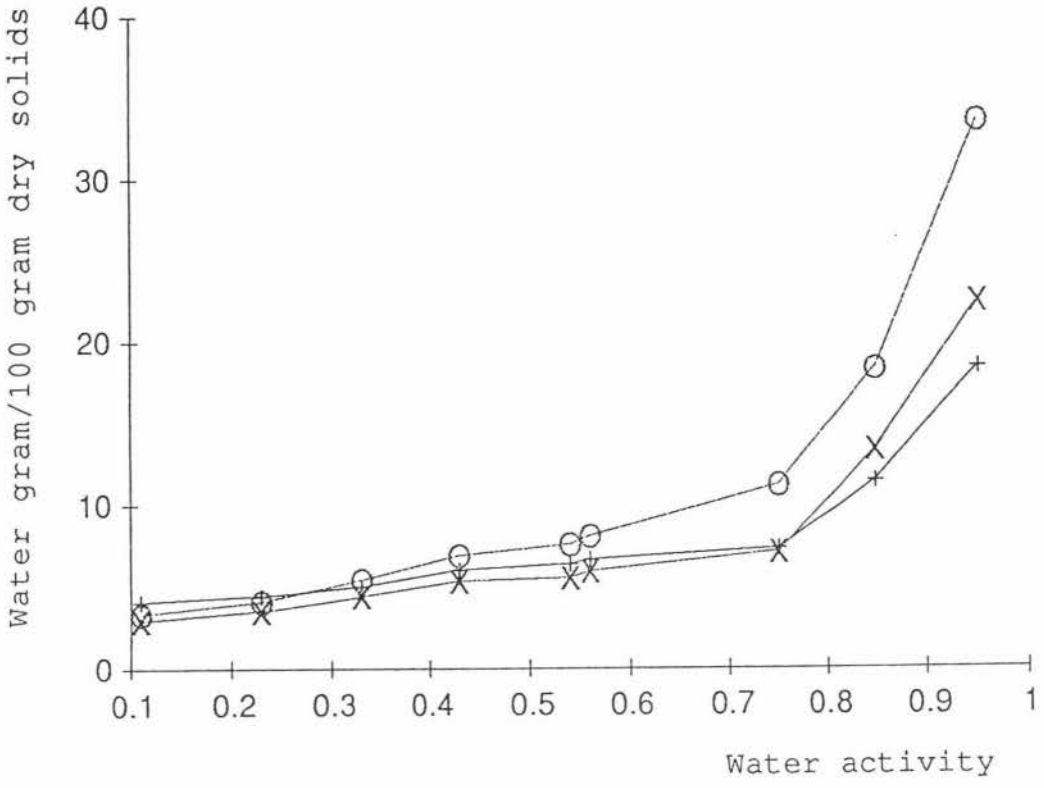

Fig. 4.2 Sorption isotherms of high fat whey powder (+), whole milk powder (x) and base powder at 20°C

4.3.3 Results and discussion

The sorption isotherm data were presented graphically in Fig.4.2. The data appear to have classic sigmoidal shape of type II isotherms according to B.E.T. classification (Labuza, 1968). At low water activities (0.11 - 0.43), a small increment of moisture would lead to significant change in water activity. There is a steep rise at a_w 0.75 in the isotherms of both milk powder and base powder and at 0.85 in the isotherms of two whey powders.

Several mathematical models and treatments have been proposed to correlate sorption data (Labuza, 1968). The most widely employed model is Brumauer-Emment-Teller B.E.T. equation which assumes localized sorption of strongly-bound water followed by formation of multilayers of liquid-like water on the surface (Brunauer, 1938). The B.E.T. models has been applied in a numerous cases (Iglesias, 1976). The data between a_w value of 0.11 and 0.43 were fitted to the B.E.T. equation in the following form:

$$\frac{a_w}{(1-a_w)X} = \frac{(C-1)a_w}{X_m C} + \frac{1}{X_m C} \qquad (4.3)$$

Where: a_w = water activity
X = equilibrium moisture, g water/100 g solids
X_m = monolayer moisture content, g water/100 g solids
C = constant

The regression coefficients were used to determine the value of C and X_m. The R^2 indicated a good fit over the range of data used. The results are tabulated in Table 4.2.

Table 4.2 B.E.T. parameters for high fat whey powder, whole milk powder and base powder.

	X_{ma}	C	R^2 (%)
High fat whey powder	3.378	-148.00	99.9
Whole milk powder	3.145	28.82	99.3
Base powder	4.184	14.94	98.3

a g water/100 g solids

The B.E.T. model, however, best fits sorption isotherm data over a relatively narrow range of relative humidity (5-50%). A detailed collaborative study of water activity of foods, and food components, was recently undertaken by the European Economic Community in a project titled COST 90 (Spiess, 1983). The best analytical expression

was the Guggenheim-Anderson-de-Boer (G.A.B.) model (Bizot, 1983) which was applicable over a wider range than B.E.T. equation and usually gave a better fit to the data up to a water activity 0.9. It may also provide a better evaluation of the amount of water tightly bound by primary adsorption sites.

With the three samples, data up to a water activity 0.9 were fitted to G.A.B. expression as following:

$$\frac{a_w}{W} = \frac{k(1 - C)a_w^2}{W_m} + \frac{(C - 2)a_w}{W_m C} + \frac{1}{W_m Ck} \qquad (4.4)$$

Where: W = moisture, g water/100 g solids
W_m = moisture content corresponding to saturation of all primary adsorption sites by one water molecule (equivalent to the monolayer in BET theory), g water/100 g solids
C = Guggenheim constant
k = factor correcting properties of monolayer molecules with respect to bulk liquid

The G.A.B. coefficients are given in Table 2.4. The comparatively low values of the relative root mean square (%, R.M.S.) indicate a fairly good fit of data to G.A.B. model. An example of calculation of R.M.S. is given in Appendix 3.

Table 4.3 G.A.B. parameters for high fat whey powder, whole milk powder, and base powder.

	W_{ma}	C	k	RMS_b (%)
High fat whey powder	3.815	-620.991	1.157	7.50
Whole milk powder	3.089	66.202	1.223	10.45
Base powder	3.966	12.556	1.545	5.36

a W_m = g water/100 g solids.
b RMS = Root Mean Square.

The Wm and B.E.T. values of base powder are higher than those of

whole milk powder and high fat whey powder, which is due to the 20% sucrose content. The monolayer value of a whole milk product is 4.122 g water/100 g solids at 15% added sugar and is 4.480 g water /100 g solids at 30% added sugar, whereas it is 3.089 g water /100 g solids when no sugar is added (Sawhuey, 1988). Guilbot and Prapron (1969) give moisture sorption isotherms of amorphous and crystalline sugars. The crystalline sugar first begins to sorb after attaining a minimum water activity, while the amorphous sugar sorbs at any water activity.

Moreyra and Peleg (1981) reported that the bulk density of cohesive powder is less affected by water activity level. This was expected since the open bed structure could not be much further expanded. Of the other parameters, compressibility was strongly affected by the water activity level. It clearly indicated that moisture adsorption in these powders transformed the solid material progressively into a more plastic and softer mass.

However, it is obvious that in industrial practice, moisture adsorption equilibrium is not always reached at lower relative humidity and it would be a very unlikely event that the powder will be deliberately exposed to high relative humidity for an extended period of time. It should be mentioned that the data derived at equilibrium conditions do not provide sufficient information regarding the physical property changes of the powders. Peleg (1977) reported that the most decisive physical characteristics, cohesion, bulk density and compact mechanical stability are mainly attributed to the particle surface properties. This means that caking or flowability loss, for example, starts even when the particle interior is relatively dry. Therefore, moisture related flowability problems are to be expected at

times that are much shorter than necessary to reach equilibrium.

4.4 Electronic scanning microscopy on the powders

4.4.1 Introduction

Scanning Electronic Microscopy (SEM) has become a convenient and useful tool in microstructural analysis of non-food and food powders and mixtures since it offers the possibility of high resolution and large depth of field (Bhatttacharyya, 1969; Barbosa-canovas, et al, 1985).

The objective of this work was to observe the shapes and size of the powder particles and the microstructures of the mixtures of high fat whey powder with base powder, and with whole milk powder.

4.4.2 Experimental

4.4.2.1 Material

High fat whey powder, whole milk powder, base powder and ascorbic acid and lactose were used as the same materials described in 4.2.2.1.

4.4.2.2 Mixture preparation

The mixtures of high fat whey powder with whole milk powder and with base powder were made at the conditions of 60 rpm, 12.5 minutes and load ratio 0.4 in the experimental blender, which is detailed in 5.3.1.

4.4.2.3 SEM sample preparation and observation

Samples were fixed to double-sided tape on aluminum stubs. The sputter were coated with approximately 20 nm of gold and examined with a Cambridge Stereoscan 250MK3 scanning electron microscope using 20 kV accelerating voltage. Images were recorded on Ilford FP4 film. The components of the mixture were recognized from the micrographs of the pure ingredients and then the type of mixture was evaluated.

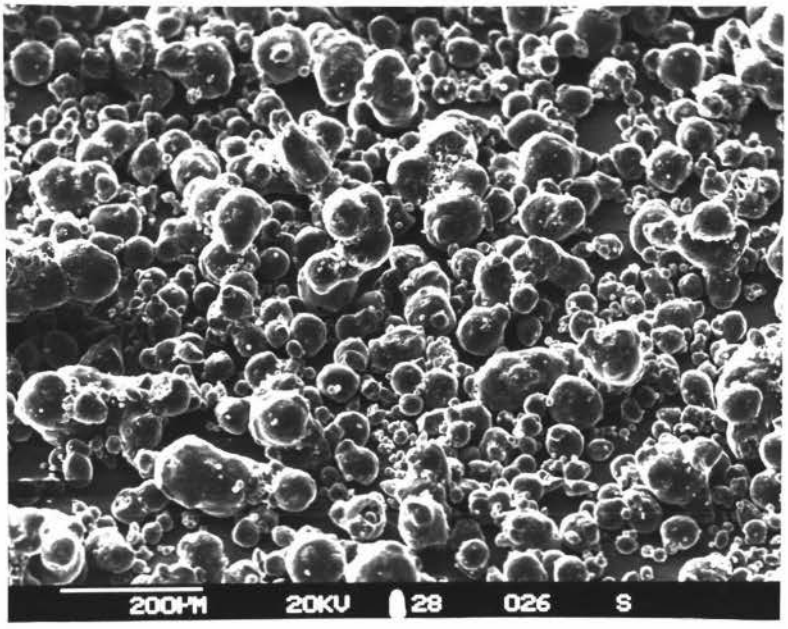

Plate 1 Scanning electron micrograph of high fat whey powder 100x

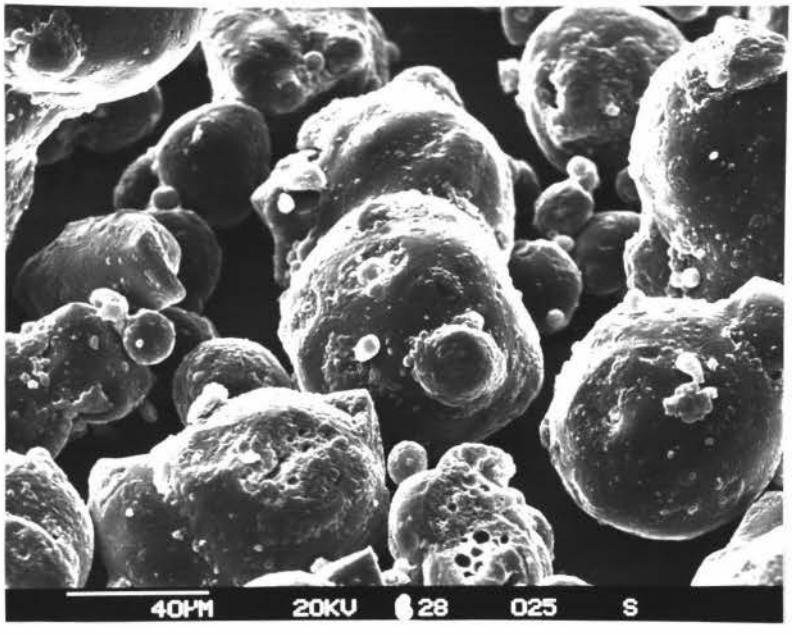

Plate 2 Scanning electron micrograph of high fat whey powder 500x

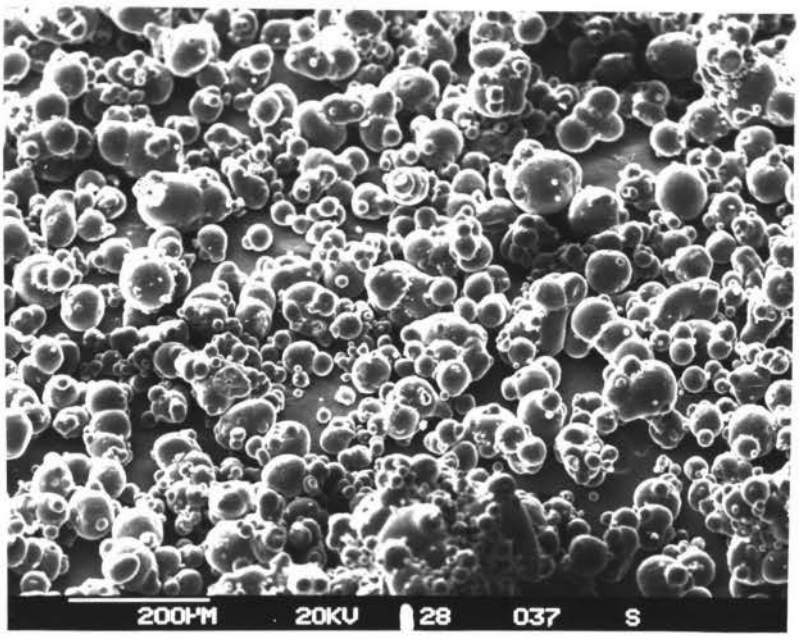

Plate 3 Scanning electron micrograph of whole milk powder 100x

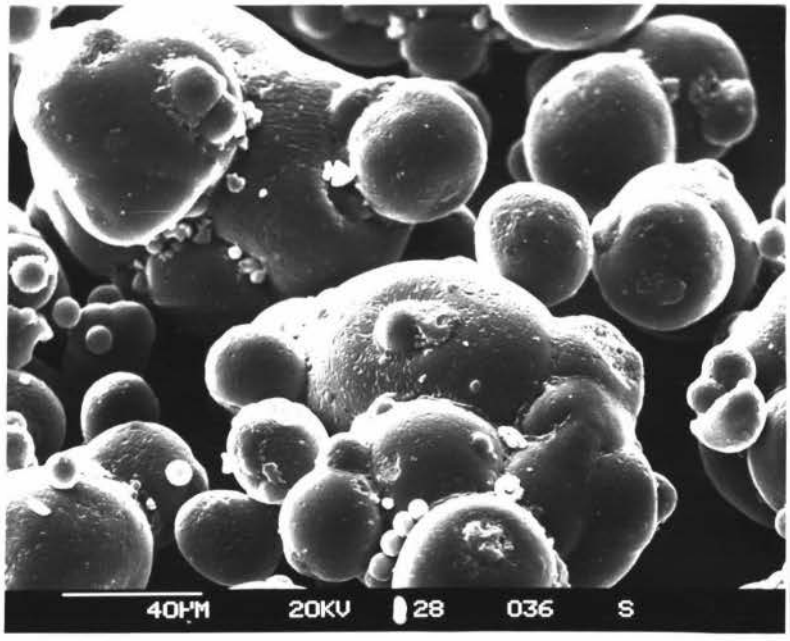

Plate 4 Scanning electron micrograph of whole milk powder 500x

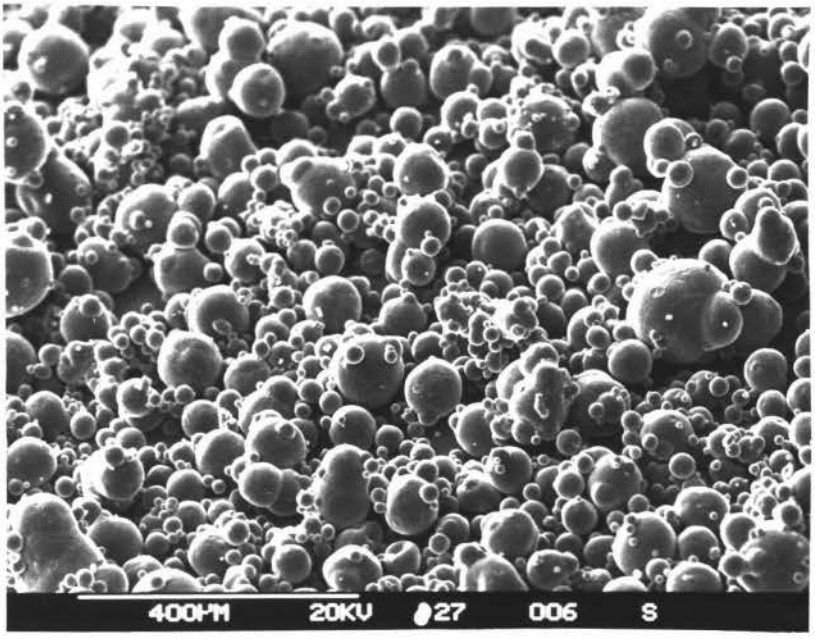

Plate 5 Scanning electron micrograph of base powder 100x

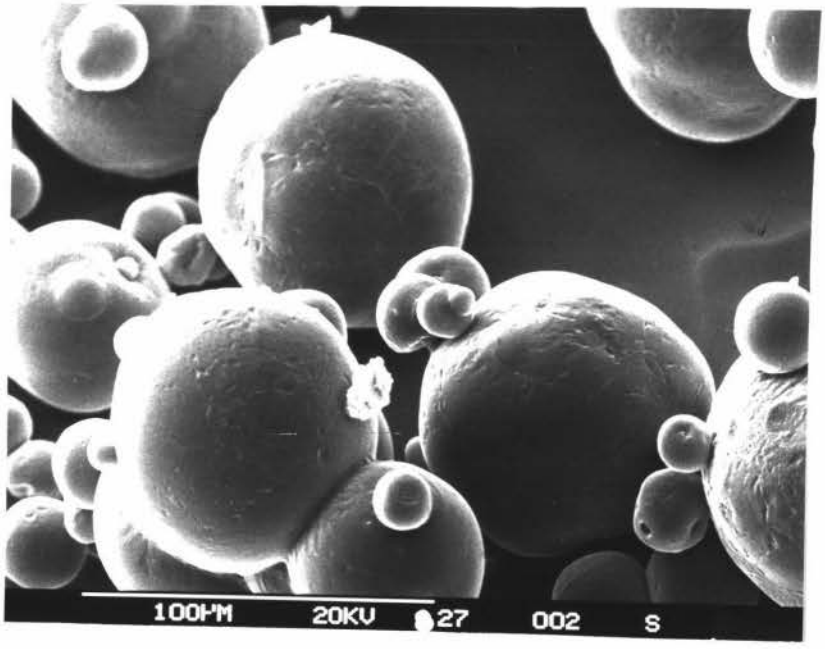

Plate 6 Scanning electron micrograph of base powder 500x

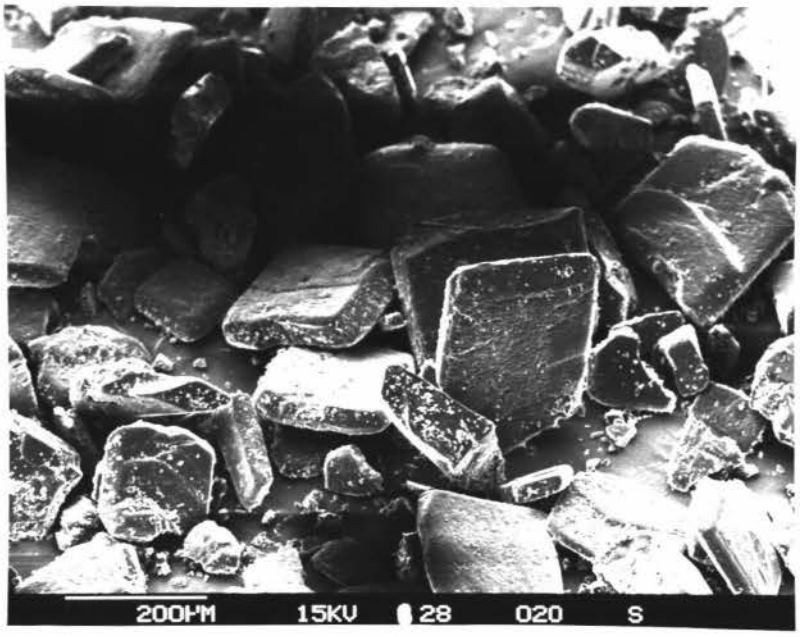

Plate 7 Scanning electron micrograph of ascorbic acid 100x

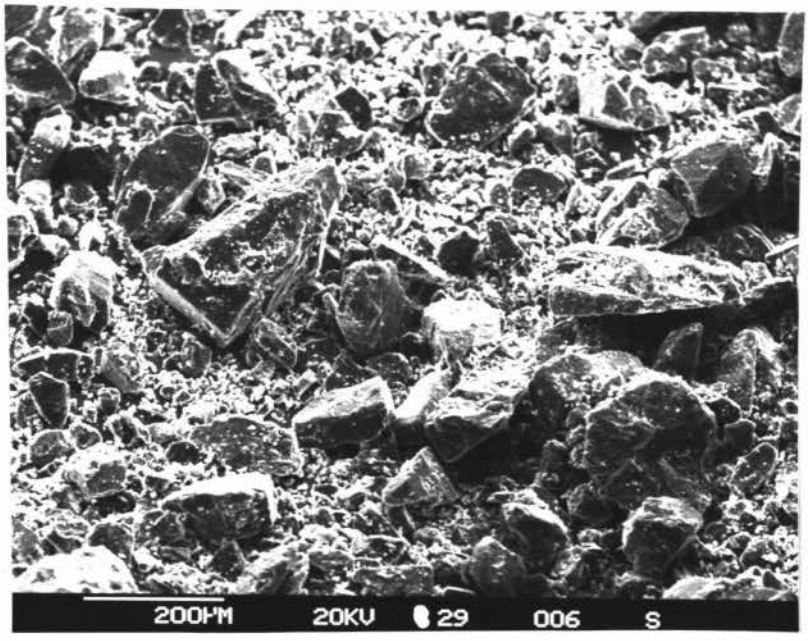

Plate 8 Scanning electron micrograph of lactose 100x

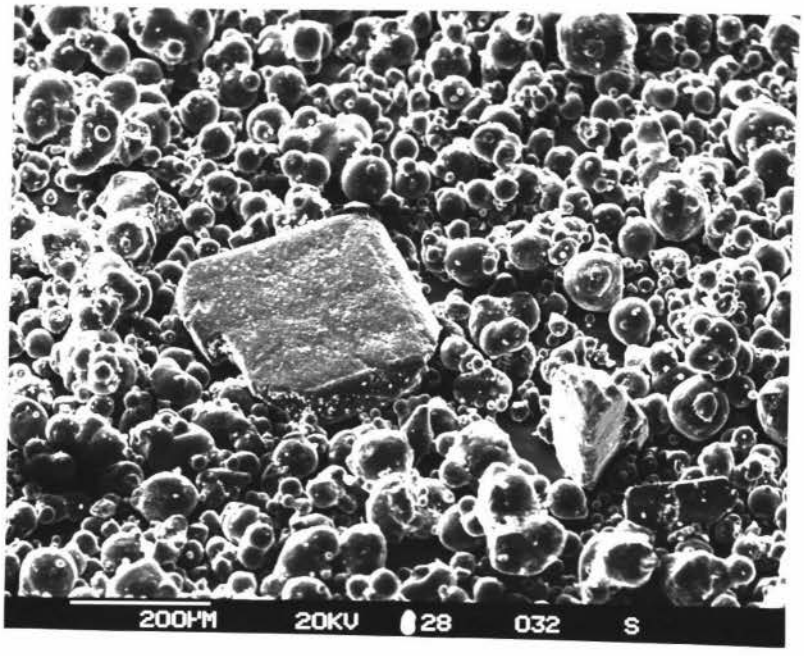

Plate 9 Scanning electron micrograph of the mixture of high fat whey powder, whole milk powder, lactose and ascorbic acid 100x

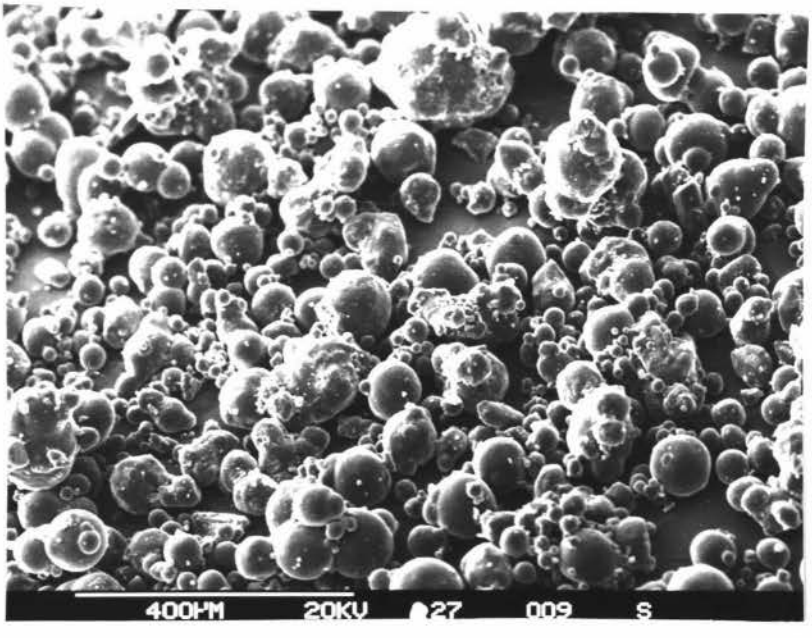

Plate 10 Scanning electron micrograph of the mixture of high fat whey powder, base powder, lactose and ascorbic acid 100x

4.4.3 Results and Discussion

4.4.3.1 High fat whey powder

The SEM picture in Plate 1 and Plate 2 reveal the irregular sphere shape of the whey powder particles which are caused by tomahawk-shaped crystals embedded in an amorphous matrix of the other components (Roetman, 1979) of the whey and milk fat. The particles are less clumped together but exist separately. More random voids are present in the mass.

4.4.3.2 Whole milk powder and base powder

The SEM pictures in Plate 3 and Plate 4 show the multiple-sphere shape of the whole milk powder particles. The smaller particles are riding on the surface of the larger particles. The relatively round shape is due to the amorphous state of lactose. Buma (1971) stated that there may be fat bridges between the particles. There are only fine cracks on the surfaces. Takada (1974) shows folds and vacuoles were found in SEM photos after fat extraction, which means the folds on the surfaces of the particles are fitted by the milk fat. More particles are found connected and less separated.

In Plate 5 and Plate 6, the particles of base powder are found well round and the surfaces are smoother than those of whole milk powder, The particles are agglomerated forming chain and pile structures. There are open structures in both whole milk and base powder.

4.4.3.3 Ascorbic acid and lactose

In Plate 7, the particles of ascorbic acid are found to be tetrahedral with rounded edges, In Plate 8, lactoses are found as irregular polygon. A few smaller particles adhere or are trapped on both surfaces.

4.4.3.4 The mixtures of high fat whey powder/whole milk powder and high fat whey powder/base powder

In Plate 9, the particles of high fat whey powder and whole milk powder are randomly mixed with each other. The agglomerates of whole milk powder are partly broken by whey powder. The same state exists in Plate 10, the micrograph of the mixture of whey powder and base powder. These three powders are not free-flowing powders but the positions of the particles in the mixtures are the results of motion sequence that is controlled by chance only. The fine particles mechanically attach with own species or others but can not form ordered structures.

4.5 General discussion

From Table 3.1, the moisture contents and the fat contents of high fat whey powder, whole milk powder and base powder are at a similar level. The free fat content of base powder is the lowest among the three powders. However, these three factors may not contribute to the difference of the cohesiveness between three powders. Buma (1971) stated that within the groups of powders with approximately the same particle size, the cohesiveness is independent of fat content in the range of 20 - 45% and there is no correlation between free fat content and cohesiveness of the powders. Buma (1971) further indicated that a small amount of surface fat is probably already sufficient to give a cohesive powder.

The major factor is the particle size and shape. High fat whey powder has irregular shaped spheres and larger particle size, whereas base powder has a relatively small particle size and more regular shaped spheres. The latter thus possesses higher surface energy.

The sucrose content is considered to be another factor contributing to the higher cohesiveness of base powder, which absorbs water at any a_w and the powder readily becomes soft and sticky.

When the lactose is crystallized before spray-drying, the usual tomahawk modification is formed. The crystals are often enclosed in the powder particles or partly covered with whey or milk solids. This will reduce the improved product properties like non-caking and free-flowing resulting from crystallization (Noyes, 1969). The compressibility of high fat whey powder is lower than whole milk powder and base powder due to its larger proportion of greater than 100 micron particle and irregular sphered shapes, but is slightly higher than that of lactose due to the coating of fat or proteins.

Staniforth (1981) stated that a binary mixture of particles having a diameter of 100 microns and above will be mainly random since for this particle size the gravitional force overcomes the particle electrostatic force. Mixtures of particles having 40 microns and less tend to be ordered mixtures because at this size range interparticle force becomes a decisive factor.

Table 4.4 Variations of particle sizes of high fat whey powder, whole milk powder, base powder, lactose and ascorbic acid the three powders (%)

	>100 µm	<40 µm
Whole milk powder	42.3	11.6
Base powder	22.6	17.6
High fat whey powder	41.9	14.1
Lactose	45.0	26.9
Ascorbic acid	82.4	5.8

The arithmetic mean diameters of these three powders are 96 - 76 microns which are between Staniforth's boundaries for order mixing and random mixing. However, there are relatively large variations in size, which are clear from both SEM graph and the testing results of Laser sizer. Table 4.4 shows the percentage of the particles under 40 microns and above 100 microns in the five powders. The particles above 100 microns tend to be randomized in mixing, whereas the particles under 40 microns tend to adhere on the surfaces of large particles. There is also a large proportion of particles with the size between 40 and 100 microns. The intermediate sized particles may show both tendencies. The cohesiveness due to the fat on the surface will provide resistance to segregation, which differ from a fully free-flowing system.

The mixtures of high fat whey powder with whole milk powder or with base powder can not be classified into any of the following types :random, partial random, ordered, partial ordered.

In practice, a fully randomized system will rarely be achieved with real powder components (Thiel, 1981). However, the degree of homogeneity conforming to a random mixture was frequently attained with cohesive, non-segregated powders (Egemann, 1980). In these two mixtures, the adhesions do not comply with the criteria for an ordered mix but rather featured the basic characteristic of random system. On the other hand, they do not show a fully disordered pattern according to random mixture. Therefore, this mixture system is termed a 'pseudorandom mixture' as suggested by Egemann (1981), which is mixed in a random fashion but is less prone to segregate.

Ascorbic acid and lactose are randomly distributed in the mass of

milk and whey powder and no evidence indicates the formation of ordered mixture. Therefore, the blended mixture is a combination of pseudorandom and random mixtures.

CHAPTER 5 CHARACTERIZATION OF EXPERIMENTAL BLENDER

5.1 Introduction

In blending infant formula, the operation should achieve two goals: it should homogeneously distribute the ingredients through the mass but should avoid adverse effects on nutrients by gentle operation for as short a time as possible.

Three criteria are considered vital to the mixing operation: the properties of the materials, the profile of the blender and the operation parameters (Clump, 1967). The operation parameters include the rotating mode of the twin flights, the rotating speed, mixing time, and loading ratio.

Greathead (1957) reported that the homogeneity of a mixture of mineral and salt was improved with prolonged time in operating an industrial ribbon blender but the fluctuation in composition did not disappear. If the materials have different size and density segregation will probably occur which is more critical to free-flowing powders or less cohesive powders (Barbosa-Canovas et al,1985). Jiri Thyn and Karel Duffek (1976) reported that the loading ratio has significant effects on the mixing rate. With increasing load ratio, the mixing time necessary for obtaining the required value of homogeneity is also increasing. Load ratio is defined as the percentage of the blender diameter full of the powder. Most ribbon blenders are coaxial blenders which are mounted with opposite-rotating ribbons on the same shaft generating a counter flow of the material. A twin coaxial blender has been reported using co-rotation (Masiuk,

1987). However, the blender for this experiment can either co-rotate or counter-rotate. The effects of each parameter and their interactions on the homogeneity of the product must be taken into consideration together.

Response Surface Methodology (RSM) has been increasingly used in food science and technology. As the initial stage of RSM, factorial design is a powerful tool which is especially useful where interactions are commonplace. The results of the experiment which has been laid out as a factorial can be analysed to measure both main effects and interactions. The results can be further processed with the Path of Steepest Ascent (PSA) to optimise the dependent variables through systematic change of the independent variables. There are a number of papers to report the application of RSM in optimizing food products and food processes (Henselman, 1974; Agnilera et al, 1976).

The primary objectives of this work were to utilize factorial design and RSM to determine the relationship between variables and homogeneity of the product and to optimise the variables to generate an acceptable homogeneity.

5.2 Experimental design

Since rotating mode is a qualitative variable, the experiment was carried out with a two block 2^3 factorial design. One block was run co-rotating and the other counter-rotating. Three variables were mixing time, rotating speed and load ratio, each of which was set at two levels. In each block, one centre point was selected in replicate for identifying the error of the model. The matrices of the two blocks are shown in Table 5.1 and Table 5.2. The two blocks were compared to find the better rotating mode. The selected block was then further

processed with PSA.

5.3 Experimental

5.3.1 Blending

Whole milk powder and 25% fat whey powder were blended under the designed variables. Load ratio 0.4 corresponds to 1.25 kg high fat whey powder and 1.25 kg whole milk powder, 0.8 corresponds to 2.5 kg high fat whey powder and 2.5 kg whole milk powder. Ten approximately 10 gram samples were randomly taken when stopping the blender after mixing for the designed time. Plate 11 shows the experimental blender.

5.3.2 Testing of the homogeneity

The calcium content of the sample was tested with the method described in 3.3.2.1.

5.3.3 Data analysis

Since the ratio of powder was designed as 1:1, the designed ratio score, 50 was used as population mean. The mixing index was defined as the standard deviation of the designed ratio score divided by the acceptable standard deviation. The acceptable standard deviation was established according to 99.7% confidence interval and 15% tolerance of the designed level, (refer to Chapter 2.4). The equations are shown as follows:

$$\sigma = \sqrt{\sum (x_i - u)^2 / n}$$

$$\sigma_{acceptable} = 0.15u/3 = 0.025$$

$$MI = \sigma / \sigma_{acceptable}$$

Where: σ = standardard deviation
x = ratio of spot sample
u = designed ratio score or designed composition level
= 50 for 1:1 mixture
MI = mixing index, 1 or less is the acceptable mixing index.

1987). However, the blender for this experiment can either co-rotate or counter-rotate. The effects of each parameter and their interactions on the homogeneity of the product must be taken into consideration together.

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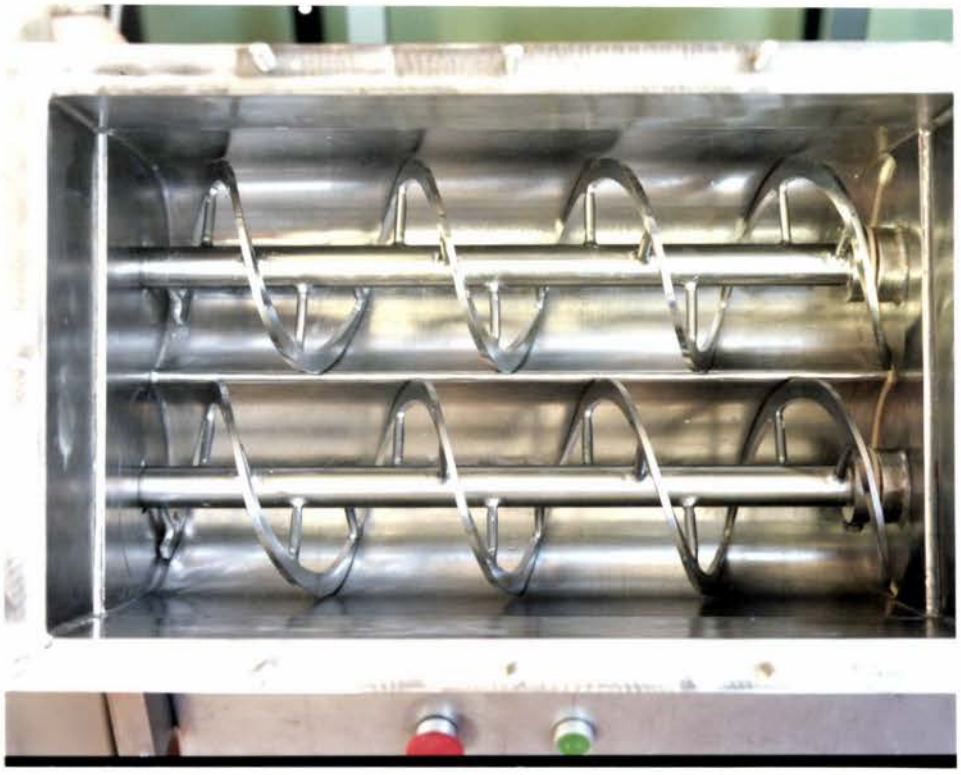

Plate 11 Photograph of experimental ribbon blender

processed with PSA.

5.3 Experimental

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Whole milk powder and 25% fat whey powder were blended under the designed variables. Load ratio 0.4 corresponds to 1.25 kg high fat whey powder and 1.25 kg whole milk powder, 0.8 corresponds to 2.5 kg high fat whey powder and 2.5 kg whole milk powder. Ten approximately 10 gram samples were randomly taken when stopping the blender after mixing for the designed time. Plate 11 shows the experimental blender.

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$$\sigma_{acceptable} = 0.15u/3 = 0.025$$

$$MI = \sigma / \sigma_{acceptable}$$

Where: σ = standardard deviation
x = ratio of spot sample
u = designed ratio score or designed composition level
= 50 for 1:1 mixture
MI = mixing index, 1 or less is the acceptable mixing index.

5.4 Results and discussion

The results of the trials are shown in Table 5.1 and Table 5.2.

Table 5.1 2^3 Factorial experimental design and results of counter-rotating block

	x_{1a}	x_{2b}	x_{3c}	MI
1	0.4	5	60	3.02
x_1	0.8	5	60	4.18
x_2	0.4	10	60	2.82
x_1x_2	0.8	10	60	7.44
x_3	0.4	5	80	2.78
x_1x_3	0.8	5	80	6.14
x_2x_3	0.4	10	80	2.82
$x_1x_2x_3$	0.8	10	80	4.14
Centre point	0.6	7.5	70	2.80
Centre point	0.6	7.5	70	2.62

a x_1 = Load ratio
b x_2 = Mixing time, minutes
c x_3 = Rotating speed, rpm

Table 5.2 2^3 Factorial experimental design and results of co-rotating block

	x_{1a}	x_{2b}	x_{3c}	MI
1	0.4	5	60	2.42
x_1	0.8	5	60	2.58
x_2	0.4	10	60	0.98
x_1x_2	0.8	10	60	1.24
x_3	0.4	5	80	2.02
x_1x_3	0.8	5	80	2.42
x_2x_3	0.4	10	80	0.92
$x_1x_2x_3$	0.8	10	80	1.64
Centre point	0.6	7.5	70	1.38
Centre point	0.6	7.5	70	1.30

a x_1 = Load ratio
b x_2 = Mixing time, minutes
c x_3 = Rotating speed, rpm

5.4.1 Selection of rotating mode

The results of two blocks were analysed with oneway ANOVA. There is a significant difference ($p < 0.001$) between two blocks. The statistical analysis is summarized in Appendix 4.

It can be concluded that counter-rotating exhibited inferior mixing to co-rotating. It was confirmed by the observation that the powder was mainly mixed at one end of each flight and less mixed from the other end to the front of the last part of the ribbon in which the powder was mainly under conveying. In co-rotating, the powder was not only mixed at the last ribbons but also transferred from the bottom to the top resulting in circulation, in which both diffusion and convection may be involved.

Therefore, the results of the co-rotating block was further analysed by regression and the co-rotating mode was selected for further experiments.

5.4.2 Analysis of co-rotating data

In RSM, the determination of optimum conditions was carried out by combining a special experimental design with modelling by first order and second order polynomial equations in a sequential testing procedure.

Initially in this study, a 2^3 factorial design with replicated centre points was performed with the assumption that there was a linear relationship between the variables and the response. The equation is expressed as follows:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3$$

To test the interactions between variables, the regression equation was first determined as follows:

$$Y = b_0 + bx_1 + bx_2 + bx_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3$$

The variables were coded as high level +1, low level -1, centre point, 0. It has been reported that the mixing of powders follows first order kinetics and many experimental results were in agreement with the observation. Assuming the same relationship is present in the results, the responses for fitting were expressed as the natural logarithm of the mixing index. Both were fed into Mutab computer program for regression analysis. The fitted equation for determining interactions is as follows:

$$\ln MI = 0.461 + 0.132x_1 - 0.352x_2 - 0.004x_3 + 0.071x_1x_2 + 0.057x_1x_3 + 0.058x_2x_3 + 0.028x_1x_2x_3 \quad (5.1)$$

Table 5.3 Coefficients and t values of the regression equation (5.1)

Column	Coeff	SD(Coeff)	t-value
constant	0.461	0.060	7.63
x_1	0.132	0.068	1.96
x_2	-0.352	0.068	-5.21
x_3	-0.004	0.068	-0.05
x_1x_2	0.071	0.068	1.05
x_1x_3	0.057	0.068	0.85
x_2x_3	0.058	0.068	0.85
$x_1x_2x_3$	0.028	0.068	0.42
R^2	94.40%		

x_1 = Load ratio
x_2 = Mixing time, in minutes
x_3 = Rotating speed, rpm

The first order model is dependent on the assumption that the effects of the variables are additive. In checking the interactions of the variables measured by the estimated coefficients, the first order terms, the effects of load ratio and time are larger than the interaction terms. Since $t_{1/2a\ 0.1} = 1.886$ for 2 degree freedom, the

coefficients of interaction terms and rotating speed are not significant at 80% confidence interval. The equation is thus approximated as follows:

$$\ln MI = 0.461 + 0.132x_1 - 0.352x_2 \qquad (5.2)$$

Table 5.4 Coefficients and t values of regression equation (5.2)

	Coeff	SD(Coeff)	t-value
constant	0.461	0.050	9.28
x_1	0.132	0.056	2.38
x_2	-0.352	0.056	-6.33
R^2	86.72%		

The R-squared = 86.72% indicates that this first order model is adequate to account for the variance of homogeneity for the experimental regions. The coefficients are significant at 95% confidence interval since $t_{1/2a\ 0.025} = 2.364$. There is no necessity for second order prediction. Continual RSM experimental work was in the direction of the predicted optimum homogeneity area.

The variables were coded as +1, -1 and 0 for the regression models. The decoded equation was thus calculated as the follows, the detailed decoding is shown in Appendix 5.

$$\ln MI = 1.121 + 0.66x_1 - 0.141x_2 \qquad (5.3)$$

The four variables were thus reduced to two variables, residence time and loading ratios. The equation shows that the standard deviation reduces with prolonged time and reduced loading ratio.

However, rotating speed is an important variable in operating a ribbon blender. Within the range of 60 - 80 rpm as used in this experiment, the effect of speed was found to be insignificant. It was eliminated due to the investigating range. However, at lower speed than 60 rpm, the powder moved with difficulty and some compaction occurred. The higher speed than 80 rpm may be too vigorous and may destabilize the fat and vitamin contents of the powders.

Equation (5.2) represents a simple plane to which the steepest ascent technique is used for optimisation. The detailed calculation of unit change is shown in Appendix 6. The unit changes of load coefficient and time and the estimated homogeneities are listed in Table 5.5. As a mixing index 1 is the acceptable homogeneity, the next experiment may be organized as a 2^2 factorial design with time variable (high level 12.5 minutes, low level 10 minutes, centre point 11.25 minutes) and load ratio (high level 0.52, low level 0.48, centre point 0.5).

Table 5.5 Predicted Mixing Index from PSA

Time (min)	Load ratio	Predicted ln MI	MI
8.75	0.575	0.269	1.308
10	0.550	0.076	1.079
11.25	0.500	-0.133	0.875
12.5	0.475	-0.326	0.722

5.5 Conclusion

With two block 2^3 plus centre point experimental design, the co-rotating operating mode was selected. Mixing time and load ratio have significant effects on the homogeneity. Rotating speed does not have a significant effect within the range chosen. The response of

homogeneity can be optimized through PSA by continuous inputting of unit changes of the independent variables until obtaining the acceptable homogeneity.

CHAPTER 6 STUDY ON MIXING RATE

6.1 Introduction

Whole milk powder, high fat whey powder and especially base powder are all cohesive powders. It has been reported that the more cohesive materials retards the mixing time to reach a certain degree of mixing, but segregation or demixing is less likely to occur (Barbosa-carovas, 1985).

The mixing rate has been described to follow first order kinetics. The rate constant is considered to be a function of the material variables, blender profile variables and operation variables.

In the experiments in RSM (refer to 5.4), the effects of operation variables on homogeneity has been revealed with whole milk powder mix. In this work on the mixing rate and homogeneity of minor components, the effects of load ratio on the mixing rate, the effects of time on homogeneity and the effects of cohesiveness of the powders on both the homogeneity and mixing rate were to be investigated.

6.2 Experimental Design

Whole milk powder and whey powder; Base powder and whey powder respectively were mixed with ascorbic acid at load ratio 0.4 and 0.8. The experiment equations were to be derived. The rate constants were compared to assess the effects of material variables and operation variables.

6.3 Experimental

6.3.1 Blending

The ratios of both whole milk and base powder to 25% fat whey powder were 50:50 (wt/wt).

Ascorbic acid was premixed with 100 mesh lactose in a bowl at the ratio of 1:3. The ascorbic acid content of the mixture was designed as 60 mg per 100g powder. The mixture of ascorbic acid and lactose was added into half of the powder through a 60 mesh sieve, then the rest of the powder was added and mixed at 60 rpm for 15 minutes.

The sample size was as the same as in 5.3.2. The samples were randomly withdrawn at 2.5 minute intervals in all the cases.

6.3.2 Testing

The ratios of milk powders to whey powder were tested using the method described in 3.2.1.

The ascorbic acid contents of both milk powder and base powder were tested using the method described in 3.2.3.

The ascorbic acid contents of the mixtures were tested using the method described 3.2.3.

6.4 Results and Discussion

The mixing indices of ascorbic acid of the mixtures were calculated using the equations in 5.3.3 where the designed level was 60 mg per 100 gram powder. The results are tabulated in Table 6.1 and Table 6.2 and showed in Fig. 6.1 - Fig. 6.4.

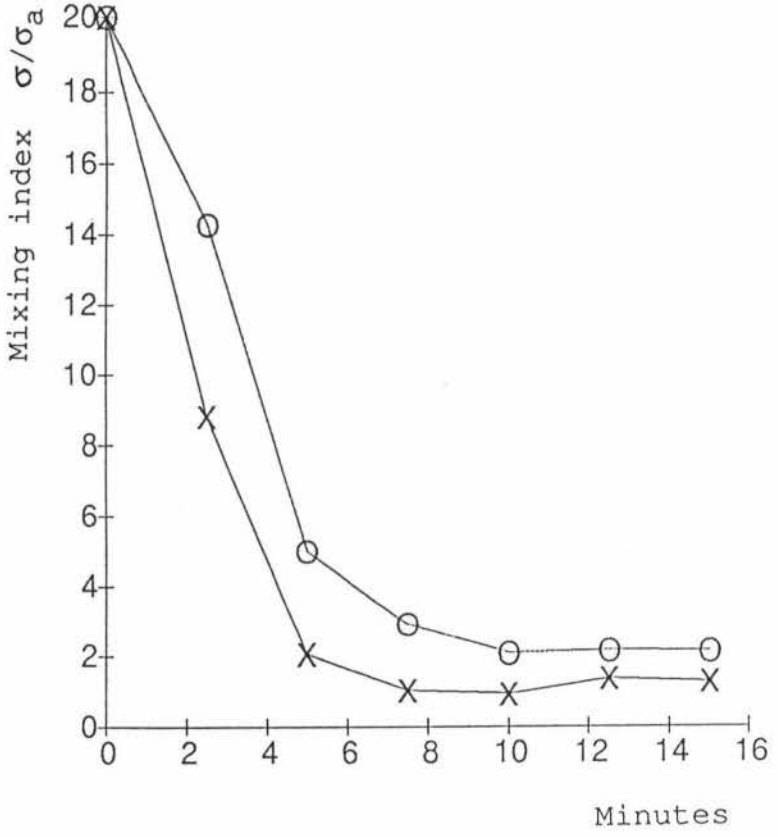

Fig. 6.1 Mixing indexes of ratio and ascorbic acid in whole milk powder mixture vs time at load ratio 0.4. Ratio (x), Ascorbic acid (o).

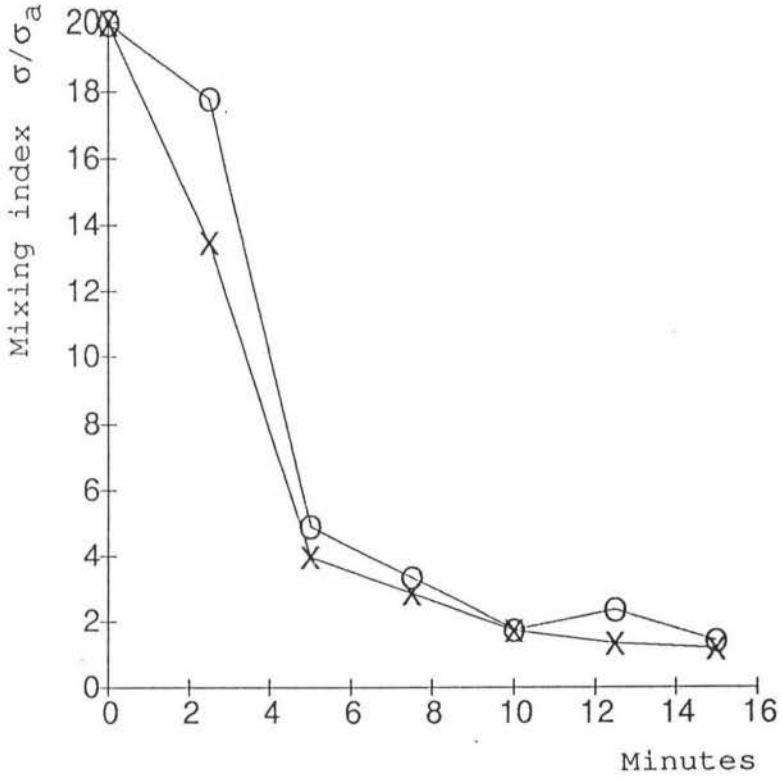

Fig. 6.2 Mixing indexes of ratio and ascorbic acid in whole milk powder mixture vs time at load ratio 0.8. Ratio (x), Ascorbic acid (o).

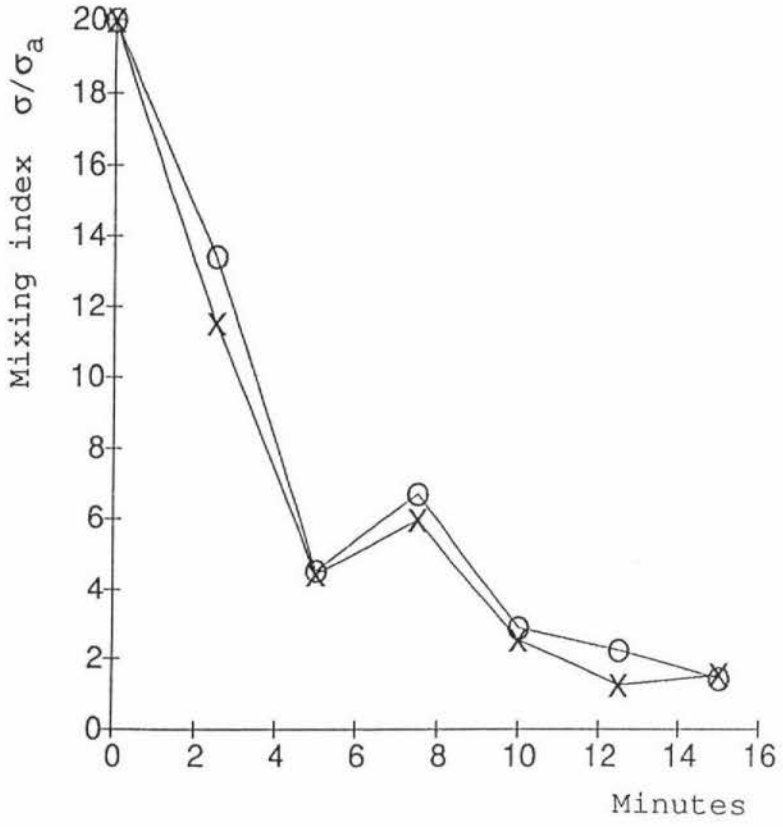

Fig. 6.3 Mixing indexes of ratio and ascorbic acid in base powder mixture vs time at load ratio 0.4. Ratio (x), Ascorbic acid (o).

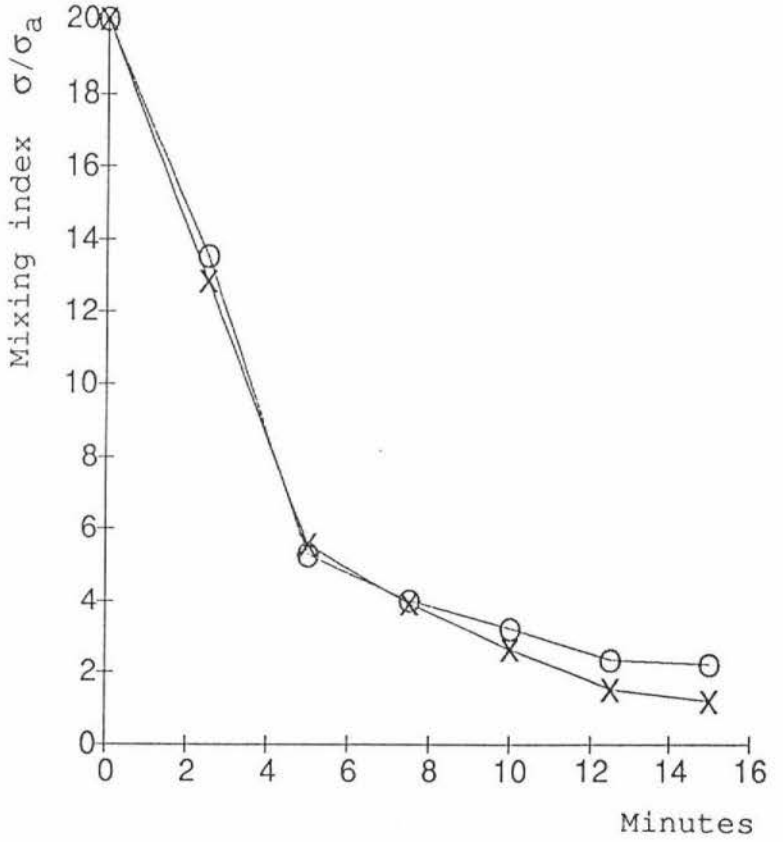

Fig. 6.4 Mixing indexes of ratio and ascorbic acid in base powder mixture vs time at load ratio 0.8. Ratio (x), Ascorbic acid (o).

Table 6.1 The ratio and ascorbic acid mixing indices of whole milk powder mix and high fat whey powder

Minutes	R-MI[a]	S^2[b]	A-MI[c]	S^2[d]
Loading ratio 0.4				
0	20.00		20.00	
2.5	8.82	418.25[e]	14.30	1467.44
5.0	2.06	13.59	5.02	225.32
7.5	1.04	2.83	2.92	76.68
10.0	0.94	5.39	2.08	33.10
12.5	1.38	11.65	2.16	41.06
15.0	1.28	8.25	2.14	33.51
Loading ratio 0.8				
0	20.00		20.00	
2.5	13.48	1118.41	17.80	1308.10
5.0	3.99	86.04	4.90	132.37
7.5	2.84	34.29	3.35	80.80
10.0	1.74	18.21	1.76	24.12
12.5	1.34	8.34	2.36	34.49
15.0	1.18	7.78	1.43	13.78

a Ratio mixing index
b Variance of ratio score
c Ascorbic acid mixing index
d Variance of ascorbic acid content
e Significantly different from that of the minimum MI point, (refer to 6.4.4)

Table 6.2 The ratio and ascorbic acid mixing indices of base powder mix and high fat whey powder

Minutes	R-MI[a]	S^2[b]	A-MI[c]	S^2[d]
Loading ratio 0.4				
0	20.00		20.00	
2.5	11.51	828.25[e]	13.44	786.54
5.0	4.46	109.71	4.56	126.12
7.5	5.98	210.53	6.72	212.34
10.0	2.56	40.81	2.90	55.15
12.5	1.28	10.28	2.26	39.48
15.0	1.56	14.98	1.46	19.13
Loading ratio 0.8				
0	20.00		20.00	
2.5	12.86	888.07	13.56	1323.30
5.0	5.58	192.41	5.32	148.78
7.5	3.96	67.52	4.0	105.31
10.0	2.66	29.98	3.24	69.64
12.5	1.54	12.54	2.36	38.83
15.0	1.22	8.88	2.24	29.55

a Ratio mixing index
b Variance of ratio score
c Ascorbic acid mixing index
d Variance of ascorbic acid content
e Refer to Table 6.1

6.4.1 Mixing rate equations

The empirical equations were derived from whole mixing time range from 0 to 15 minutes and reduced mixing time range from 0 to 10 minutes. The regression equations take the general form:

$$MI = MI_o \exp(-kt)$$

Where: MI = mixing index at time t
MI_o = initial mixing index
k = rate constant, 1/minute
t = mixing time, in minutes

The constants of the rate equations are shown in Table 6.3 and 6.4. The R^2s of the equations indicate that the models are adequate to account for the variance of the MI in all eight cases. The reduced range regression equations show a slight improvement of fit of the model, which would indicates the model may more applicable at the early stage of mixing.

Table 6.3 The coefficients of mixing rate equations (Whole time range)

Mixing Index	Load Ratio	ln MI_o	k	R^2 (%)
Ratio MW_a	0.4	2.285	0.182	68.3
Ratio MW	0.8	2.784	0.199	92.4
Ratio BW_b	0.4	2.830	0.180	91.4
Ratio BW	0.8	2.898	0.191	98.1
Ascorbic MW	0.4	2.733	0.162	84.7
Ascorbic MW	0.8	2.885	0.186	88.2
Ascorbic BW	0.4	2.883	0.170	92.5
Ascorbic BW	0.8	2.776	0.151	91.4

a MW = Whole milk powder and whey powder mixture.
b BW = Base powder and whey powder mixture.

Table 6.4 The coefficients of mixing rate equations (Reduced time range)

Mixing Index	Load Ratio	ln MI_o	k	R^2 (%)
Ratio MW_a	0.4	2.825	0.330	92.7
Ratio MW	0.8	3.003	0.258	95.6
Ratio BW_b	0.4	2.886	0.191	87.7
Ratio BW	0.8	2.967	0.209	97.6
Ascorbic MW	0.4	3.038	0.245	96.5
Ascorbic MW	0.8	3.154	0.261	94.7
Ascorbic BW	0.4	2.927	0.182	84.1
Ascorbic BW	0.8	2.939	0.194	93.8

a MW = Whole milk powder and whey powder mixture.
b BW = Base powder and whey powder mixture.

6.4.2 Effects of load ratio on homogeneity and mixing rate

The results of both whole milk powder mix and base powder mix (in Table 6.1 and Table 6.2) reveal that a reduced load ratio shortens the time to reach the minimum MI point, which is in agreement with the reports (Jini Thyn, 1976) and the findings in RSM experiment. Within the whole time range, the rate constants at lower ratio were gnerally smaller than those at higher load ratio. Within reduced time range, the rate constants at lower load ratio were larger than those at higher ratio in Whole milk powder mixing, but they were approximately the same in base powder mixing.

The differences between the ratio and ascorbic acid mixing indices of base powder mixes at two load ratios were not significant and neither was the difference between the ratio and ascorbic acid MI of whole milk powder mix at load ratio 0.8. There was a significant difference between the ratio and ascorbic acid mixing indices in whole milk powder mix at load ratio, 0.4 at early stages but both reached similar level at later stages. It indicates that ascorbic/lactose premix can be relatively evenly distributed through the bulk powder.

The results show that the minimum MI occurred at load ratio 0.4 and 10 minutes in whole milk powder mix, which was the only homogeneity that reached the acceptable MI 1. In base powder mix, the ratio homogeneity at ratio 0.4 was better than that at load ratio 0.8.

The homogeneities of ascorbic acid in all the cases were lower than those of ratio scores.

6.4.3 Effects of cohesiveness on homogeneity and mixing rate

The mixing rates of base powder mix at both load ratio 0.8 and 0.4 were lower than those of whole milk powder mix due to the higher cohesiveness. The homogeneity was also below those of whole milk powder mixes. The basic material properties of the mixing components which are cohesive would limit the degree of homogeneity that can be attained (Chowham, 1981). The moisture content of base powder is 3.82% which accounts for the highest cohesiveness in the three powders. The moisture content of sugar added milk powder is 2% in the milk product specification of the Ministry of Light Industry, China (MLI, 1982). A reduction of moisture content of the base powder will lead to the drop of cohesiveness which in turn allow the increase of mixing speed and improvement of the homogeneity. It should be mentioned that any factor related to cohesiveness may directly or indirectly affect the mixing rate and homogeneity of the mixture. The effect of temperature was not demonstrated in the experiment. However, in industrial production, the mechanical heat and the heat from environment to the powder will increase the cohesiveness which in turn slows down the mixing rate.

6.4.4 Fluctuations of homogeneity after the minimum MI points

There were some fluctuation after the minimum MI points in all the cases. As reported, there is less possibility of demixing cohesive

materials. The minimum MI point at the curve is considered as the reference standard for comparison. The comparison of the ratio of variance, S^2 of the fluctuation points with those of the minimum MI points can provide an indication for identifying whether the fluctuation is due to the change of mixing system or by chance (Clump, 1967). The sample testing results were found to have normal distributions by use of probability plotting (Croxton ,1960) with exceptions of the early stage of blending. The critical F value (9,9) at 99% level is 5.35. The significantly different ones are underlined in Table 6.1 and Table 6.2, all of which occurred before the minimum MI points. None of the S^2 ratio of the fluctuation points (after the minimum MI points) to the minimum MI point is larger than the critical F value. Therefore, the fluctuation is considered as random error by chance rather than a change in mixing. The fluctuation may be due to the dynamic nature of the mixture. The mixture is an agglomerate which is broken, formed broken and reformed under the mechanical operation. Around the minimum MI point, the breaking and reforming is thought to have reached an equilibrium, the homogeneity can be affected randomly by any factors such as temperature change or moisture absorption. However, the fluctuation magnitude is rather limited as showed by the results.

6.4.5 Effects of the profile of the blender

In one type of ribbon blender, the outer spiral blade moves the solids axially in one direction and the inner spiral moves the solids in the opposite direction. (Clump, 1967). In the blender used in the experiment, there is no inner spiral, the powder only can be moved at one direction, there is less axial mixing inside the powder. In

addition, axial mixing is less rapid than radial moving (Clump, 1967). This blender mixes the powders radially at the end blade and circulates them from the bottom to the top during which small scale mixing occurs at the boundaries between moving and stationary material. The equipment is thus not considered as an effective mixing machinery. Both inner spiral and radial elements will improve the efficiency to approach the acceptable homogeneity.

6.4.6 Sensory quality variance due to powder ratio

Reconstituted high fat whey powder was tasted sweeter and more watery than whole milk powder. The variance in powder ratio were assessed through the difference in sweetness and mouthfeel (Table 6.5). The results of twoway AVONA was shown in Appendix 7. At the 5% probability level, the least significant differences (LSD) were calculated. With the LSDs, the mixtures with powder ratios of 60:40 or 40:60 can be tested significantly different from 50:50 in terms of sweetness and mouthfeel. The powder with a ratio of 55:45 or 45:55 were not tested significantly different from 50:50 mixture. Appendix 8 lists the ratio scores of the mixtures of whole milk powder with high fat whey powder at load ratio 0.4 and 0.8. After 10 minutes mixing, most of the ratio scores were between 55:45 and 45:55. Therefore, such mixtures are not significaty different from the mixture with designed powder ratio.

Table 6.5 Variance of powder ratio and sensory scores

Powder ratio HFWP/WMP (%)	Mean Score Sweetness	Mouthfeeling
60:40	5.19	5.49
55:45	4.16	4.30
50:50	3.71	4.21
45:55	3.70	3.96
40:60	3.07	3.70
LSD	0.62	0.60

6.5 Conclusion

The reduced ratio shortened the time to reach an acceptable homogeneity and led to a better homogeneity in the mixing of macro components. The effects of reduced ratio wasaa not significant in the mixing of micro components. The mix of base powder results in a slower rate and inferior homogeneity due to higher cohesiveness. The results also indicated that the homogeneity will not be limitlessly improved with prolonged time. The fluctuation after the minimum MI point is not considered as demixing or segregation but error by chance. The profile of the blender needs to be altered for increasing mixing rate and approaching the acceptable homogeneity. The sensory quality of the mixture of whole milk powder and high fat whey powder did not show significant difference when the powder ratios were between 45:55 and 55:45 whose mixing indices were beyond the acceptable MI.

CHAPTER 7 STUDY ON PILOT BLENDER TRIAL

7.1 Introduction

The data from a number of experiments on a particular blender can not be used for another blender. The trials on an experimental blender are valuable for developing the methodology of blending and for understanding the relationship of the variables. However, the performance of a large scale blender needs to be reassessed. This work was aimed at finding the behaviour of base powder/whey powder mix and the premix of ascorbic and lactose in a pilot blender, and the effects of sampling from the inside and discharge of the blender.

7.2 Experimental

7.2.1 Description of the blender

The blender consists of two opposite-spiral ribbons on a shaft and three blades jointed vertically to the shaft between the spirals, which is shown in the plate 12.

7.2.2 Experimental procedure

Base powder 25 kg and high fat whey powder 25 kg correspond to a load ratio of 0.5. Ascorbic acid 30 g and lactose 90 g were premixed in a bowl. Base powder was first added in blender then the premix of ascorbic acid and lactose was added, followed by high fat whey powder. The material was mixed at 45 rpm. Ten samples were taken from both the discharge and the inside of the blender at designed time intervals.

7.3 Results and discussion

The results are tabulated in Table 7.1 and graphically expressed in Fig. 7.1- 7.3.

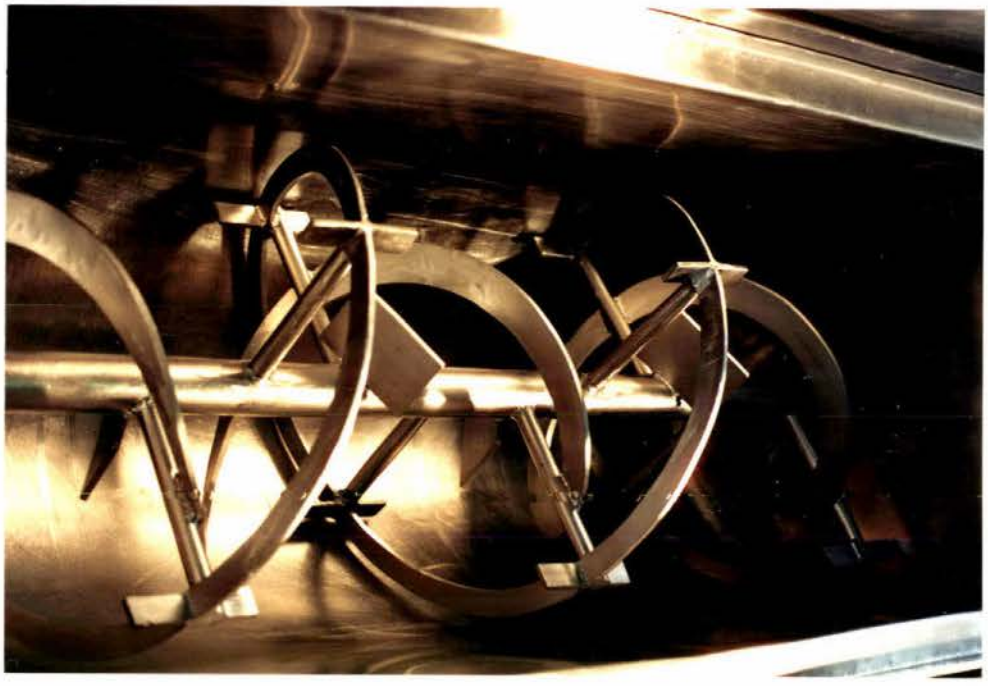

Plate 12 Photograph of pilot ribbon blender

Table 7.1 The ratio and ascorbic acid mixing indices in pilot blender trial

Minutes	R-MI[a]	S^2[b]	A-MI[c]	S^2[d]
Samples from discharge				
0	20.00		20.00	
5	7.50	350.05[e]	4.12	150.67
7.5	4.53	118.44	4.02	57.05
10	3.34	57.73	2.11	38.23
12.5	2.20	30.35	1.90	24.72
15	1.61	16.62	1.44	12.84
20	1.60	13.49	1.73	24.91
25	1.43	12.81	1.65	7.95
Samples from inside of blender				
0	20.00		20.00	
5	8.98	466.12	7.30	290.29
7.5	7.21	318.49	4.02	116.60
10	4.64	130.46	3.65	76.39
12.5	1.92	22.88	2.19	20.79
15	1.67	17.26	1.81	7.22
20	1.52	13.76	1.79	9.51
25	1.35	11.42	1.58	6.01
Samples pooled				
0	20.00		20.00	
5	8.28	414.46	7.62	428.29
7.5	6.02	218.53	3.36	92.42
10	4.05	94.60	2.98	64.00
12.5	2.07	26.65	2.05	23.73
15	1.64	16.84	1.58	12.41
20	1.56	13.77	1.76	25.72
25	1.39	12.13	1.61	6.98

a Ratio mixing index
b Variance of ratio score
c Ascorbic acid mixing index
d Variance of ascorbic acid content
e Refer to Table 6.1.

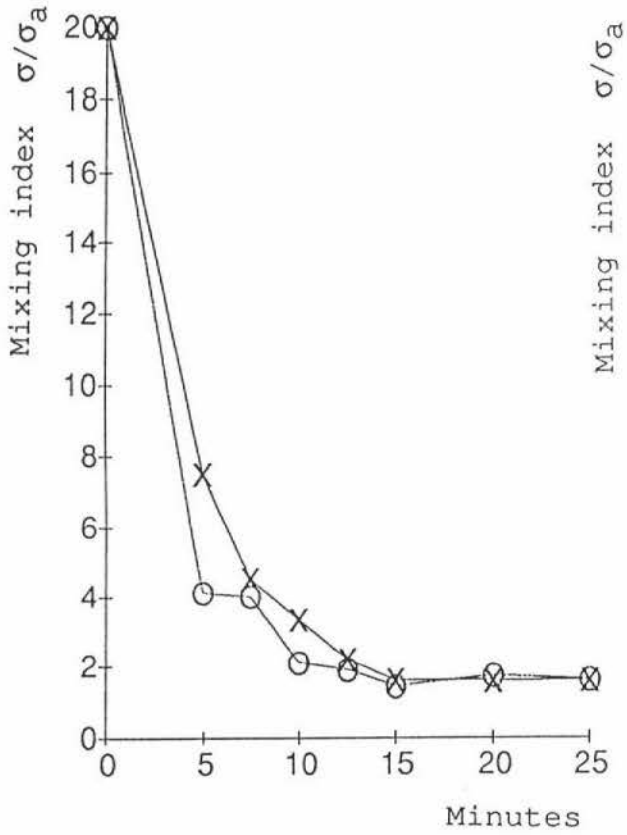

Fig. 7.1 Mixing indexes of ratio and ascorbic acid vs time in pilot trial, Sampled from discharge. Ratio (x), Ascorbic acid (o).

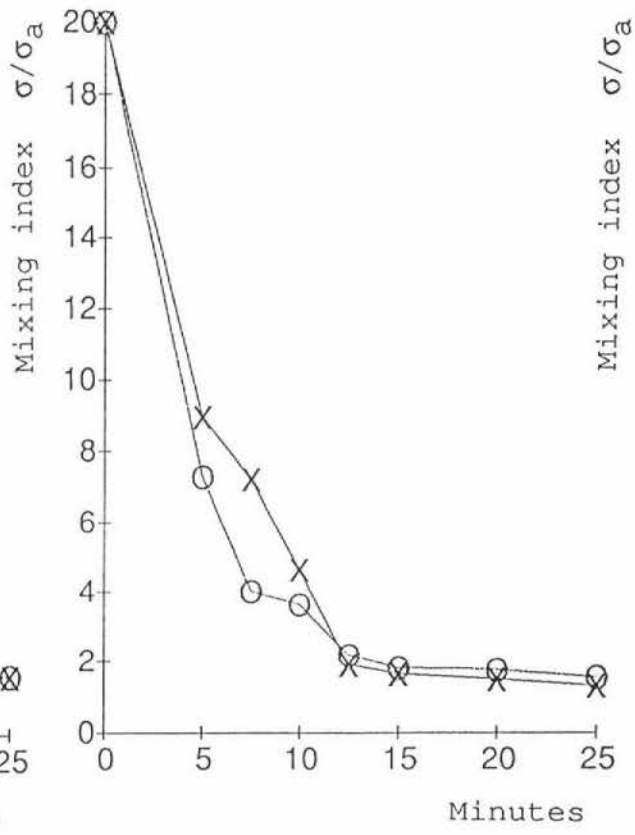

Fig. 7.2 Mixing indexes of ratio and ascorbic acid vs time in pilot trial, Sampled from discharge. Ratio (x), Ascorbic acid (o).

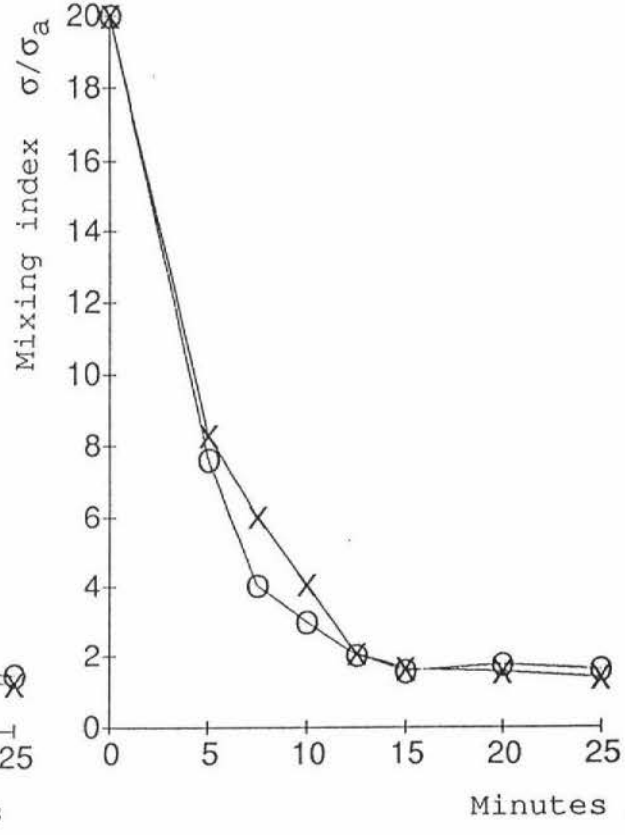

Fig. 7.3 Mixing indexes of ratio and ascorbic acid vs time in pilot trial, Pooled sample, Ratio (x), Ascorbic acid (o).

7.3.1 Sampling effects on homogeneity

The noticeable differences of mixing indices only occurred at early stages of mixing between the samples from the discharge and the inside of the blender. As a whole, the differences of ratio and ascorbic acid content between two groups are not significant at 5% probability level analysed by oneway ANOVA. It indicates that the homogeneity of the sample from the discharge and inside are in the same population. The two groups are pooled as 20 samples, by which the uncertainty of the estimated standard deviation to true standard deviation is reduced to 0.70 - 1.46 at 95% confidence interval or 0.65 - 1.78 at 99% confidence interval (Valentin, 1967). It indicates that in mixing of milk powder and whey powder, the sample can be withdrawn from the discharge. In many practical applications, it is preferred to sample the mixture during discharge from the mixer or from storage, because this gives more realistic assessment of the quality of the mixture (Schofield, 1981).

7.3.2 Validity of first order equation

From Table 7.2, 7.3, the R^2s of rate equations show that the mixing followed first order reaction at the reduced time range from 0 to 12.5 minutes, whereas, the R^2s of rate equations are lower in the whole time range. It shows that first order equation is less valid at the later stage of mixing of cohesive powders.

Table 7.2 Coefficients of mixing rate equations (whole time range)

Mixing Index	ln MI_o	k	R^2 (%)
Ratio (d)[a]	2.431	0.101	79.9
Ascorbic (d)	2.001	0.084	60.5
Ratio (i)[b]	2.691	0.115	86.9
Ascorbic (i)	2.415	0.097	81.5
Ratio (p)[c]	2.597	0.110	84.4
Ascorbic (p)	2.334	0.096	77.4

a Samples from the discharge of the blender.
b Samples from the inside of the blender.
c Pooled samples

Table 7.3 Coefficients of mixing rate equations (reduced time range)

Mixing Index	ln MI_o	k	R^2 (%)
Ratio (d)[a]	2.936	0.176	99.3
Ascorbic (d)	2.674	0.190	88.8
Ratio (i)[b]	3.095	0.175	95.0
Ascorbic (i)	2.907	0.174	97.5
Ratio (p)[c]	3.025	0.174	98.7
Ascorbic (p)	2.911	0.186	96.4

a Samples from the discharge of the blender.
b Samples from the inside of the blender.
c Pooled samples

7.3.3 Optimum zone of a mixing run

The minimum point of MI occurred at 15 minutes in the samples from discharge in ratio and ascorbic acid mixing, whereas, the deviations continued to reduce during the mixing in the samples from the inside of the blender. As the outcome of pooled results, the ratio mixing index was at a minimum at the end of mixing run but the ascorbic acid MI reached a minimum at 15 minutes, after which the fluctuations occurred. With S^2 test, the fluctuation points are not considered as demixing but some error by chance.

The standard deviation is the standard deviation due to mixing but

the measured value includes variations due to sampling and analysis. There is uncertainty as to whether the minimum point is the optimum point in the mixing. The minimum MI point is not necessarily superior to its neighbouring points. The difference is not significant at 5% probability level in the samples from 12.5 minutes to 25 minutes through testing with oneway ANOVA. Although the deviation of analysis can be established, the deviation of sampling has not been determined. In both experimental and pilot blender trials, the mixing rate reduced or the homogeneity fluctuated around the minimum points after a period of mixing. Therefore, it considered that there is an optimum zone rather than an optimum point. It is suggested that the optimum zone could be identified using the control chart methodology. It is constructed for ratio mixing as following:

1. The designed ratio score is selected as average level, u.
2. The standard deviation of the ratio score at minimum point serves as the standard deviation, σ for calculating of upper and lower limits.
3. The upper and lower limits for 99.7% confidence interval are calculated with the formula as:

   $u \pm 3\sigma/\sqrt{n}$ Where: n sample number
4. The ratio mean score is plotted at each mixing time.

The calculation is shown in Appendix 7. The optimum zone is shown in Fig 7.4. Since the optimum zone of ratio mixing started at 12.5 minutes, the mixing is thus not necessarily done for the time reaching the minimum MI but for the time within the zone.

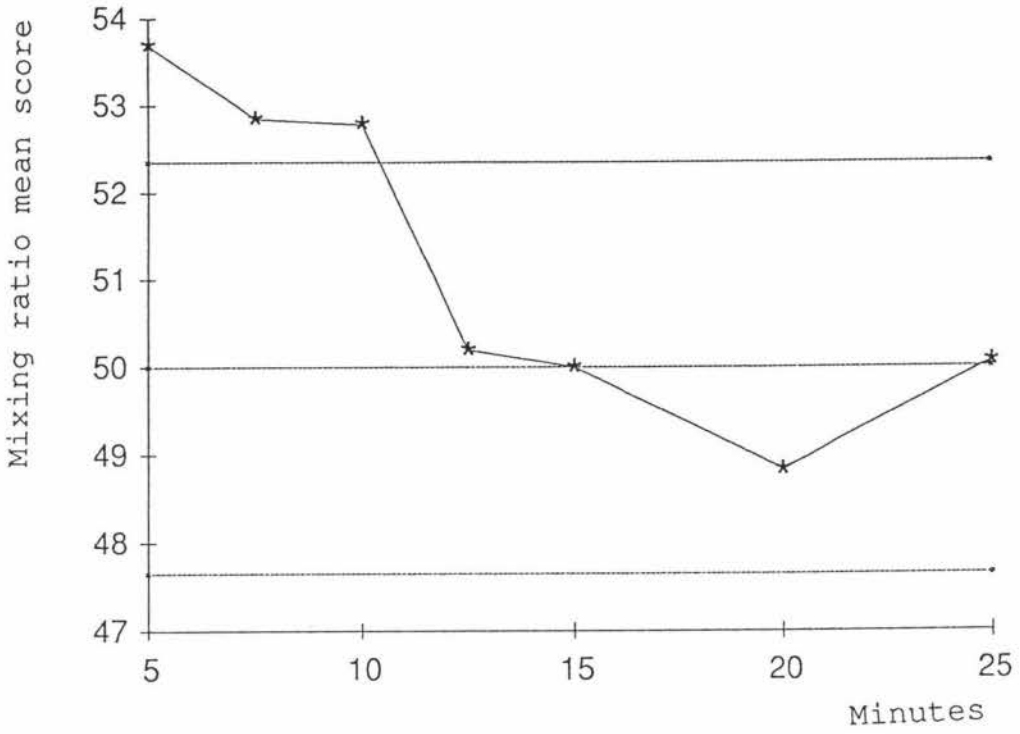

Fig. 7.4 Optimum zone of mixing high fat whey powder and base powder in pilot blender.

7.3.4 Mixing index and nutrient specification

The homogeneities of both ratio and ascorbic acid are still above the acceptable MI of 1. Doubtless, with acceptable MI, the nutrient quality can be ensured. However, the acceptability of infant formula is eventually evaluated with a nutrient specification not only mixing index. It should be mentioned that mixing of milk powder and whey powder differ from pharmaceutical application as milk powder and whey powder contain the same nutrients and some quantities are close in level, which is shown in Table 3.1. The homogeneities of the

composition elements are not equally affected by the variance of the powder ratio. The mixing indices of the composition of the pooled samples are calculated from the ratio score (Table 7.4). As an example, the calculation of protein mixing index at 5 minutes was given in Appendix 8. As to the macronutrient, protein, fat, carbohydrate, their mixing indices reached acceptable level 1 after 5 minute mixing due to the small composition difference between two powders. Although ash ratio of two powders is 1.87, the ash MI reaches the acceptable MI, 1. The MIs of casein/whey protein and saturated fatty acid/unsaturated fatty acid were above 1 due to the ratios of two powders are 12.12, 12.4 respectively. After mixing for 15 minutes, their MI reached below 2.

Table 7.4 Composition mixing indices of the pooled samples

Minutes	Protein	Fat	Carbohydrate	Ash	Casein/Whey protein	Saturated fat/ Unsaturated fat
5	0.98	0.84	0.44	2.58	11.24	9.66
7.5	0.50	0.64	0.32	1.88	6.62	9.14
10	0.34	0.20	0.20	1.26	5.62	3.92
12.5	0.18	0.10	0.12	0.62	2.48	2.32
15	0.14	0.08	0.08	0.48	1.82	1.90
20	0.12	0.08	0.08	0.48	1.66	1.76
25	0.12	0.06	0.08	0.42	1.66	1.60
Ratio$_a$	1.26	1.10	0.93	1.88	12.40	12.12

a The composition ratio of base powder to high fat whey powder.

7.3.5 Mixing micronutrients with bulk powders

From Table 7.2 and Table 7.3, it can be seen that the mixing rates of powder ratio and ascorbic acid are close. At later stages of the mixing, the homogeneities and mixing rates of ascorbic acid and ratio reached almost the same levels. Similar to pharmaceutical operation,

the milk powder and whey powder serves as an excipient for the vitamins and minerals as active agents. The preparation of premix and the addition mode, are vital to the mixing, which needs further study. There is no upper limit set up for vitamins in infant formula except vitamin A and vitamin D in the Codex standard (FAO Codex, 1984). In making infant formula by drying, vitamins are added in extra to cover the losses in processing and storage. Similarly, vitamins should be added in some flexibility for dry blended infant formula. The product can be evaluated at two stages. The MI is used firstly to assess the homogeneity for selected operation conditions. The specified contents of vitamins are used later to determine the acceptability of the product.

7.4 Conclusion

It appears that a first order relation is less significant or not significant at the later stage of mixing cohesive powders. In a mixing run, there is an optimum zone rather than an optimum point, which can be identified with control chart methodology. Evaluating with nutrient specification, the composition of the mixture can reach the acceptable MI at the mixing time in the optimum zone except for the ratio of casein to whey protein and the ratio of unsaturated fatty acid to saturated fatty acid. Ascorbic acid lactose premix was mixed with the bulk powder at nearly the same rate as ratio mixing.

CHAPTER 8 STUDY ON STORAGE TRIALS

8.1 Introduction

As well as mixture homogeneity, product stability is a major concern in assessing the feasibility of dry blending for infant formula manufacture. As mentioned, Krasheninin (1983) reported that destabilization of milk fat occurred under more severe conditions. The effects of gentle mixing on the fat stability of infant formula is unknown. Since the fat oxidation of milk powder is a relatively slow reaction (Touhy, 1981), it is desirable to carry out an accelerated shelf life test (ASLT) to compare the stability of the blended powders with the unblended. The basic assumption underlying ASLT is that the principles of chemical kinetics can be applied to quantify the effects which extrinsic factors such as temperature, humidity, gas atmosphere and light have on the rate of deteriorative reactions on foods. By subjecting the food to controlled environments in which one or more of the extrinsic factors is maintained at a higher than normal level, the rates of deterioration will be shorter than normal time for the food to become unacceptable and thus reach the end of its shelf life. Because the effects of extrinsic factors on deterioration can be quantified, the magnitude of the acceleration can be calculated and the 'true' shelf life of the product under normal conditions calculated.

The use of 2-thiobarbituric acid (TBA) for estimating the degree of lipid oxidation in milk products has been described by many workers (Dunkley and Jennings, 1951; Mitchell, 1955; King, 1962; Mettler,

1973). Mettler (1973) reported that reaction of steam distillates with TBA had been used as a routine procedure to assess deterioration of powdered milk products. When the level of oxidation is low, more sensitive peroxide methods may be employed (Hills and Thiel, 1946). Both TBA and Peroxide Value (PV) methods were used as chemical indexes to monitor autoxidation rates of milk fat (Hamm, 1968). Boon (1976) investigated the effects of preheating on the stability of whole milk powder with these two chemical indexes.

The rate of the Maillard reaction increases with the elevation of temperature. Blending is unlikely to bring about adverse effects on the stability with regard to the Maillard reaction but the reaction product is another source of off-flavour which can be detected by the taste panelists. Hydroxymethyl furfural is a product of the Maillard reaction at early stage. The compound formed on reaction between HMF and TBA showed an absorption maximum at 443 nm. In samples in which considerable Maillard reaction occurred, it was possible that the TBA_{530} value would be affected by reaction between HMF and TBA reagent (Mettler, 1973). Therefore, HMF was to be measured through the trials to identify the effects of the Maillard reaction on the stability of the samples.

The chemical tests (TBA, PV, HMF) can not substitute for sensory evaluation (Mettler, 1973; Downey, 1969). Quantitative Descriptive Analysis (QDA) is often used to quantitatively or qualitatively determine the characteristics of products. A comparison of profiles representing the same product before and after storage may be used to indicate changes in character and/or intensity over time (Dethmers, 1979). A linear graphic scale has been reported applied to various

food products (Stone, 1985). During the storage trial in this work, the QDA technique was used to determine the flavour changes of the samples.

A kinetic approach has been increasingly used in determining of the shelf life of food products. The fat oxidation of milk powder was reported to follow a zero order reaction at the initial stage, and then follow a first order reaction (Hall, 1984). The formation of HMF proceeds at a zero order reaction rate in most food products (Labuza, 1985). Through the use of kinetic models, the storage stability can be predicted.

The primary purpose of this trial was to distinguish the differences between blended powders and unblended powders as to fat stability, and establish the reaction kinetics of fat deterioration and Maillard browning of the experimental specimens.

8.2 Experimental Design

Assuming Q_{10} = 2 for assessing fat oxidation and a shelf life of 2 years (Labuza, 1985), high fat whey powder, whole milk powder and the blended samples were stored at 20°C, 30°C and 40°C for 180 days.

The samples were analyzed for TBA, PV, and HMF at monthly intervals.

High fat whey powder and whole milk powder were mixed in the ratio 1:1 as unblended samples immediately before sensory evaluation.

8.3 Experimental

8.3.1 Sample preparation

2.5 kg high fat whey powder and 2.5 kg whole milk powder were mixed at 60 rpm for 10 minutes in the experimental blender.

The blended mixture, high fat whey powder and whole milk powder

were packaged and heat sealed into paper/aluminum foil/polyethylene sachets. Each weighed 200 grams. The packs were stored at 20°C, 30°C and 40°C.

High fat whey powder and whole milk powder packaged as above were also stored at -20°C as reference samples.

8.3.2 Chemical testing

Blended samples, high fat whey powder and whole milk powder were tested for TBA values using the method described in 3.3.3.

The PV values of the three lots were determined using the methods described in 3.3.4.

The free HMF values of the three lots were determined using the methods described in 3.3.5.

The initial values were determined by duplicate measurements of three replicates and duplicate measurements of single samples at each time during storage.

8.3.3 Sensory evaluation

The methods of sensory evaluation are detailed in 3.3.7.

The sensory quality due to powder ratio variation was tested in addition to storage stability.

8.3.4 Data analysis

Twoway ANOVA was performed to determine if a significant difference existed between the blended and the unblended samples for the following:

1. Individual sensory scores;
2. Mean sensory scores;
3. Chemical testing results.

Regression analyses were performed for fitting zero and first order

kinetic reaction models to the data from chemical testing and sensory evaluation and consequently, for generating Arrhenius and linear equations to quantify the effect of temperature on the changes.

Twoway ANOVA and regression analysis were run using a Mutab computer program (Boag, 1988) on an IBM PC-AT computer (clone).

8.4 Results and Discussion

The results of chemical testing and sensory evaluation are summarised in Table 8.1 and 8.2. The initial values of the samples are presented in the tables. The results are also graphically presented in Fig. 8.1 - Fig. 8.13.

8.4.1 Stabilities of the powders assessed by chemical testing

8.4.1.1 TBA values

From Table 8.1, it can be seen that the TBA values increased with elevated temperature and the prolonged storage time at a constant temperature. After 180 days, TBA values of the whole milk powder at 20°C and 30°C increased 0.027 and 0.037 respectively. Touhy (1981) reported that the TBA value of whole milk powder reached above 0.05 after 20 - 24 week storage at 37°C and 0.29 after 10 week storage at 65°C. Only a slight decrease in the quality of whole milk powder kept at 12°C was found whereas TBA values greater than 0.05 after 24 week storage at 37°C were observed. The TBA of the sample stored at 40°C was 0.069 after 180 days. The results of this present study are in broad agreement with the results reported above.

The TBA values of high fat whey powder were, as would be expected, markedly higher than those of whole milk powder. After 180 days at 40°C, the TBA value reached 0.123. There are no reports in the literature on the fat stability of high fat whey powders.

Downey (1969) reported that the relationship between TBA (King method) and taste panel assessment of lipid oxidation in milk was: TBA 0.025, flavour quality acceptable, TBA 0.025-0.050 doubtful, TBA 0.050 unacceptable. These results may not be directly applicable to reconstituted whole milk and a whey powder mixture for two reasons: the flavour is different to that of fresh milk and the procedure used by King to measure TBA differs from the procedure followed in this work. However, taking a TBA of 0.050 as a rejection level, high fat whey powder at 40°C would be considered oxidised to an unacceptable degree after 90 days, and blended powder after 150 days at 30°C.

HMF is steam volatile freely soluble in water. The product formed on reaction between HMF and TBA showed an absorption maximum at 443 nm. In the samples in which considerable Maillard reaction had occurred, it was possible that TBA_{530} would be affected by the reaction between HMF and TBA reagent (Mettler, 1973). This may be one of the reasons leading to the higher readings in high fat whey powder.

The averaged chemical testing results of whole milk powder and high fat whey powder were termed 'unblended sample' and compared with the blended samples. Table 8.3 summarises the results of twoway ANOVA for the chemical testing of blended samples and unblended samples. There was no significant difference between the two sets of results at the 5% probability level.

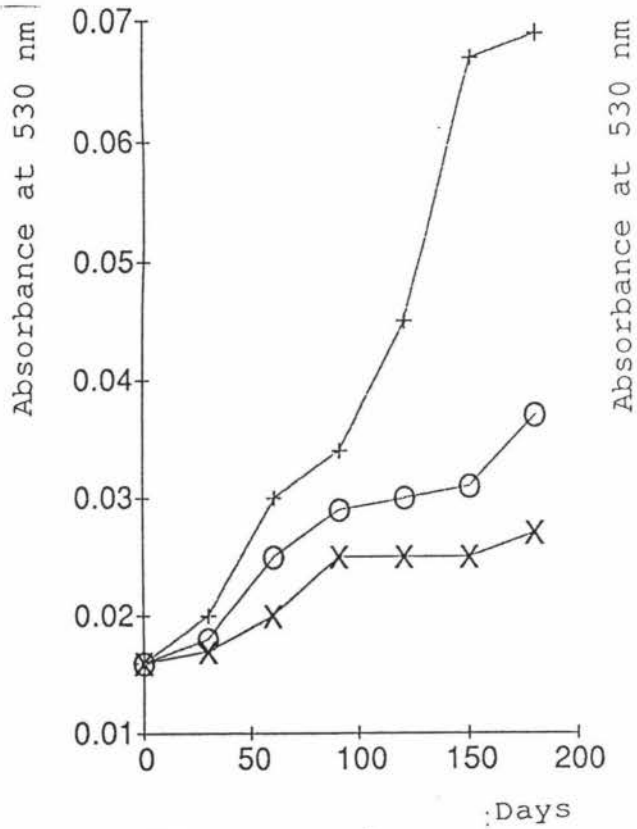

Fig. 8.1 Changes of TBA values in whole milk powder at 20°C (x), 30°C (o) and 40°C (+).

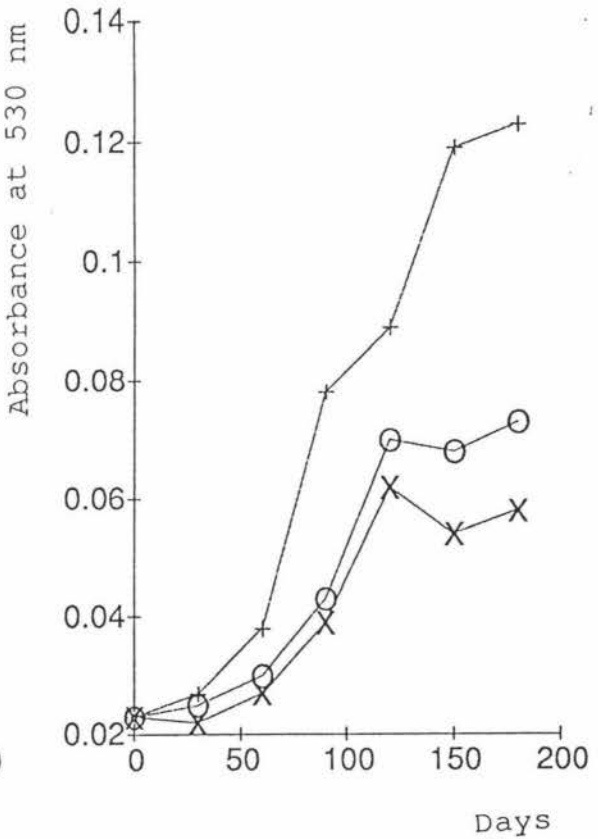

Fig. 8.2 Changes of TBA values in high fat whey powder at 20°C (x), 30°C (o) and 40°C (+).

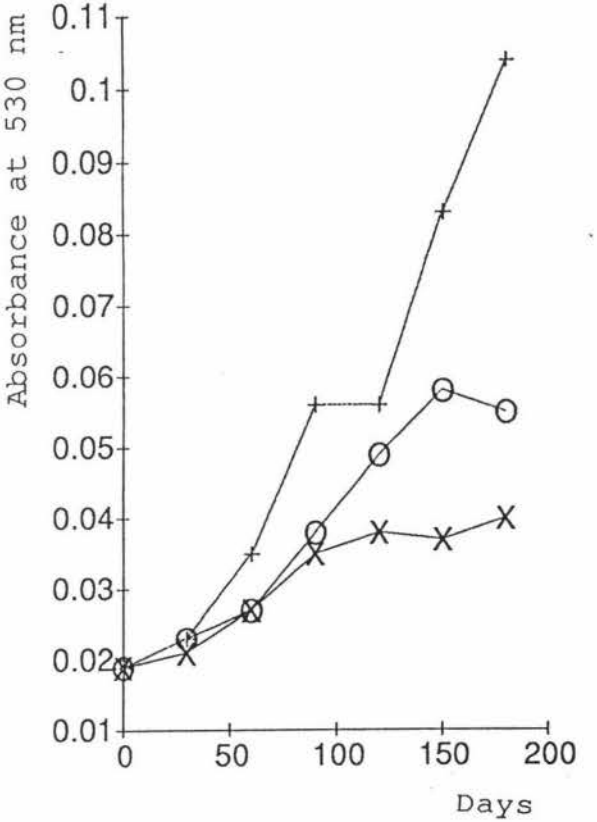

Fig. 8.3 Changes of TBA values in the blended samples at 20°C (x), 30°C (o) and 40°C (+).

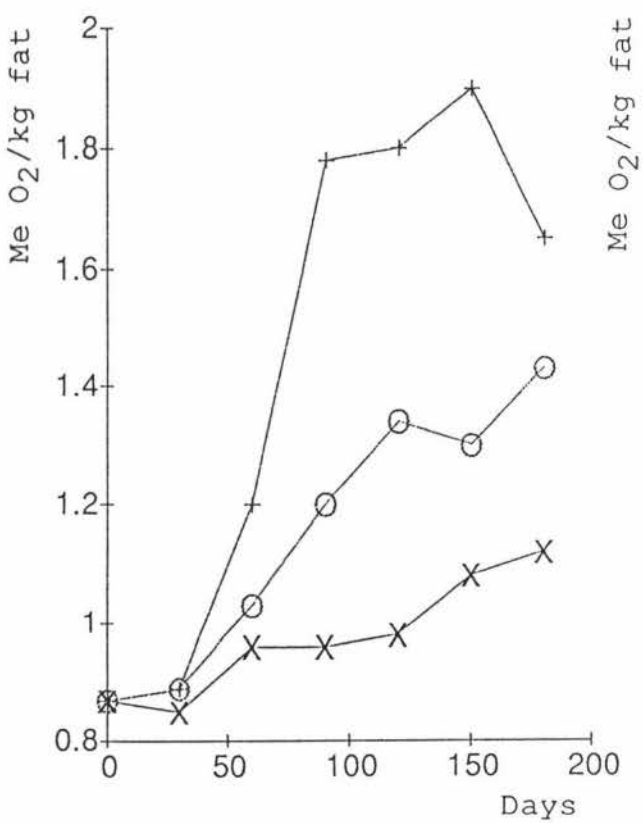

Fig. 8.4 Changes in PV of whole milk powder at 20°C (x), 30°C (o) and 40°C (+).

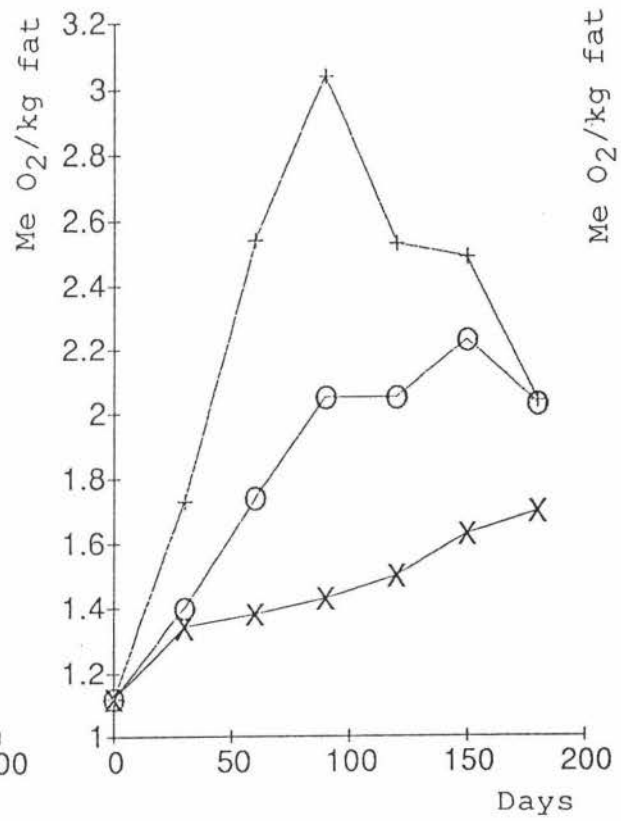

Fig. 8.5 Changes in PV of high fat whey powder at 20°C (x), 30°C (o) and 40°C (+).

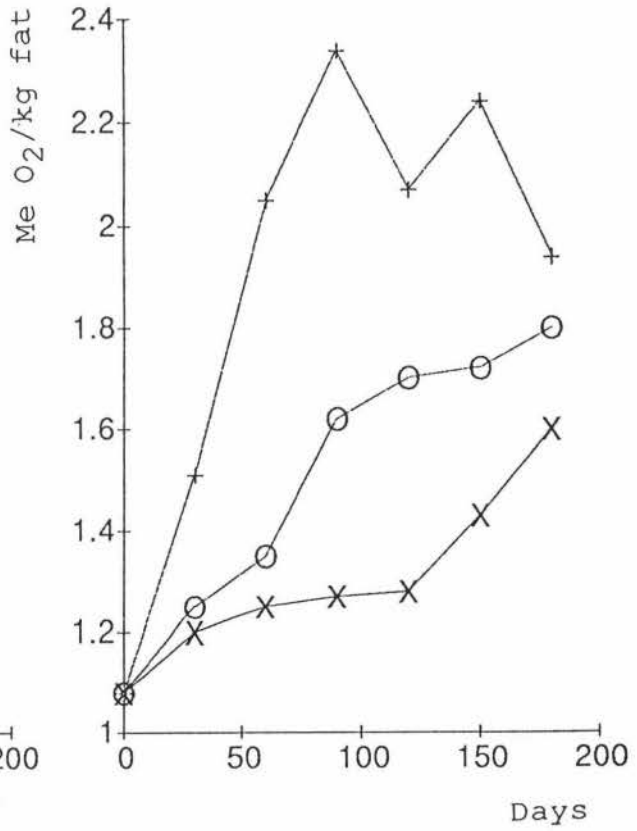

Fig. 8.6 Changes in PV of the blended sample at 20°C (x), 30°C (o) and 40°C (+).

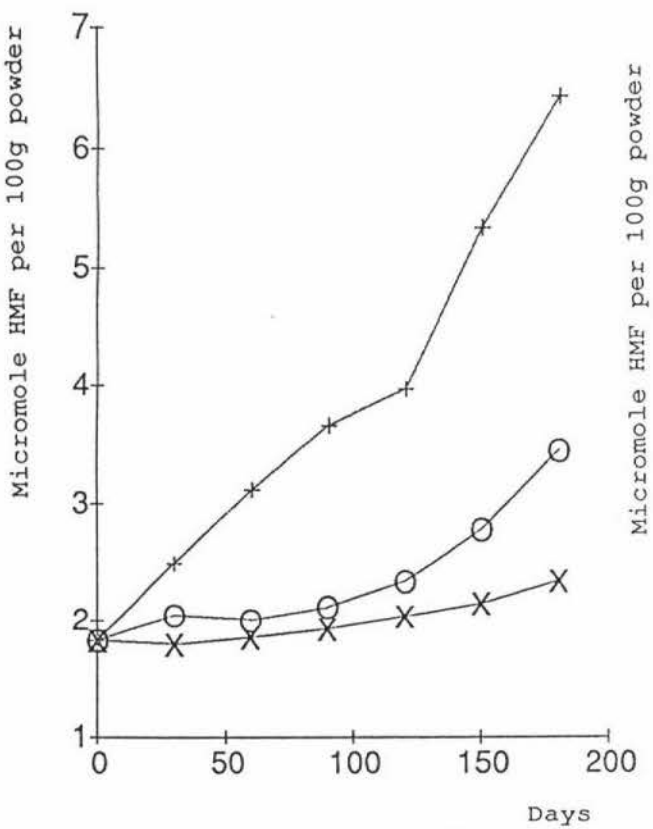

Fig. 8.7 Changes of HMF in whole milk powder at 20°c (x), 30°c (o) and 40°c (+).

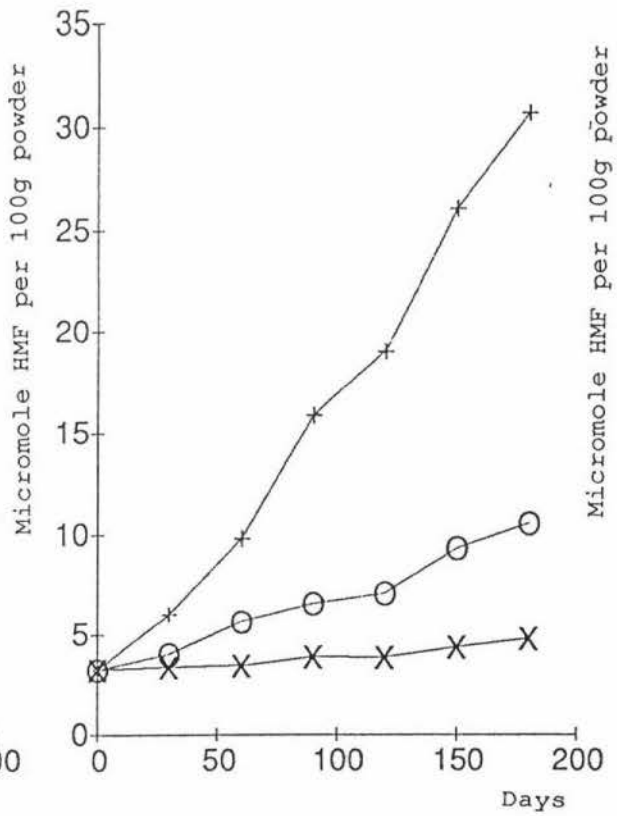

Fig. 8.8 Changes of HMF in high fat whey powder at 20°c (x), 30°c (o) and 40°c (+).

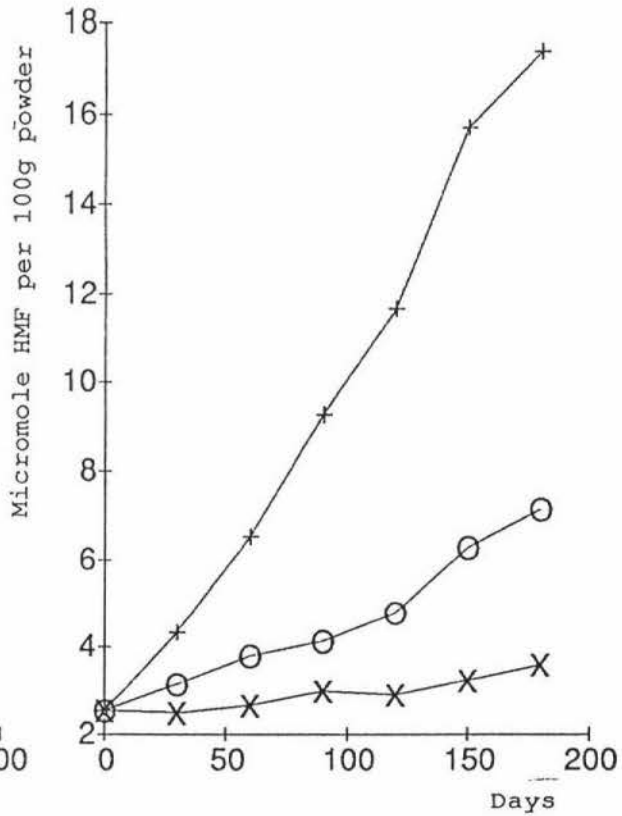

Fig. 8.9 Changes of HMF in the blended sample at 20°c (x), 30°c (o) and 40°c (+).

Table 8.1 Chemical testing results

	Temperature °C	Storage time Days	TBA_a	PV_b	HMF_c
Whole milk powder		0	0.016	0.87	1.83
Whey powder		0	0.023	1.12	3.28
Blended mixture		0	0.019	1.08	2.53
Whole milk powder	20	30	0.017	0.85	1.80
Whey powder	20	30	0.022	1.34	3.39
Blended mixture	20	30	0.021	1.20	2.48
Whole milk powder	30	30	0.018	0.89	2.05
Whey powder	30	30	0.025	1.40	4.04
Blended mixture	30	30	0.023	1.25	3.15
Whole milk powder	40	30	0.020	0.96	2.50
Whey powder	40	30	0.027	1.73	6.03
Blended mixture	40	30	0.023	1.51	4.34
Whole milk powder	20	60	0.020	0.96	1.86
Whey powder	20	60	0.027	1.38	3.47
Blended mixture	20	60	0.025	1.25	2.65
Whole milk powder	30	60	0.025	1.03	2.01
Whey powder	30	60	0.030	1.74	5.65
Blended mixture	30	60	0.027	1.35	3.80
Whole milk powder	40	60	0.030	1.50	3.12
Whey powder	40	60	0.038	2.54	9.83
Blended mixture	40	60	0.035	2.05	6.53
Whole milk powder	20	90	0.025	0.96	1.93
Whey powder	20	90	0.039	1.43	3.92
Blended mixture	20	90	0.035	1.27	2.98
Whole milk powder	30	90	0.029	1.20	2.12
Whey powder	30	90	0.043	2.05	6.58
Blended mixture	30	90	0.038	1.62	4.13
Whole milk powder	40	90	0.034	1.93	3.67
Whey powder	40	90	0.078	3.04	15.90
Blended mixture	40	90	0.056	2.34	9.29

Table 8.1 continued.

	Temperature °C	Storage time Days	TBA_a	PV_b	HMF_c
Whole milk powder	20	120	0.025	0.98	2.04
Whey powder	20	120	0.062	1.50	3.87
Blended mixture	20	120	0.037	1.28	2.90
Whole milk powder	30	120	0.030	1.34	2.34
Whey powder	30	120	0.070	2.05	7.09
Blended mixture	30	120	0.049	1.70	4.79
Whole milk powder	40	120	0.045	1.95	3.97
Whey powder	40	120	0.089	2.53	19.04
Blended mixture	40	120	0.056	2.07	11.67
Whole milk powder	20	150	0.025	1.08	2.15
Whey powder	20	150	0.054	1.63	4.38
Blended mixture	20	150	0.037	1.43	3.24
Whole milk powder	30	150	0.031	1.30	2.78
Whey powder	30	150	0.068	2.23	9.34
Blended mixture	30	150	0.058	1.72	6.29
Whole milk powder	40	150	0.067	1.85	5.34
Whey powder	40	150	0.119	2.49	26.07
Blended mixture	40	150	0.083	2.24	15.70
Whole milk powder	20	180	0.027	1.12	2.34
Whey powder	20	180	0.058	1.70	4.79
Blended mixture	20	180	0.040	1.60	3.60
Whole milk powder	30	180	0.037	1.43	3.45
Whey powder	30	180	0.073	2.03	10.56
Blended mixture	30	180	0.055	1.80	7.15
Whole milk powder	40	180	0.069	1.93	6.43
Whey powder	40	180	0.123	2.04	30.70
Blended mixture	40	180	0.104	1.94	17.39

a Absorbance at 530 nm
b Milliequivalent of O_2 per kg fat
c Micromole HMF per 100 g powder

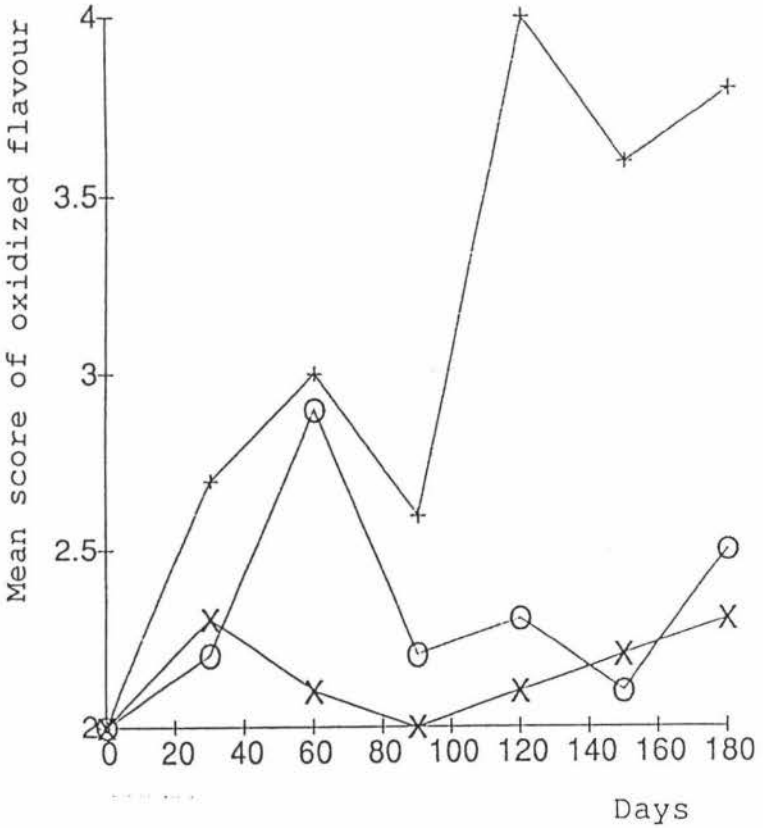

Fig. 8.10 Changes of sensory mean score of oxidised flavour in the blended samples at 20°C (x), 30°C (o) and 40°C (+).

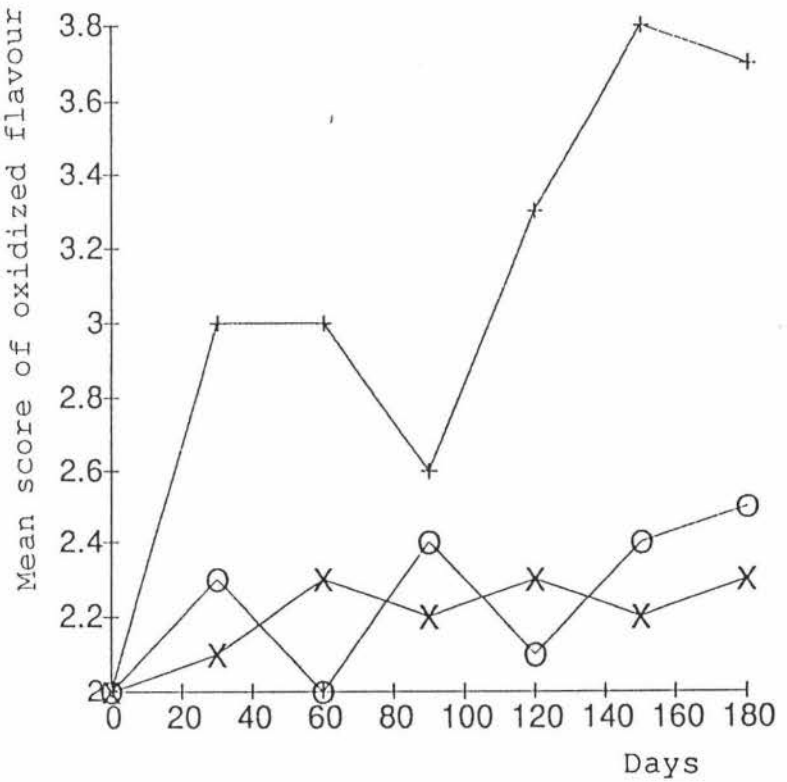

Fig. 8.11 Changes of sensory mean score of oxidised flavour in the unblended samples at 20°C (x), 30°C (o) and 40°C (+).

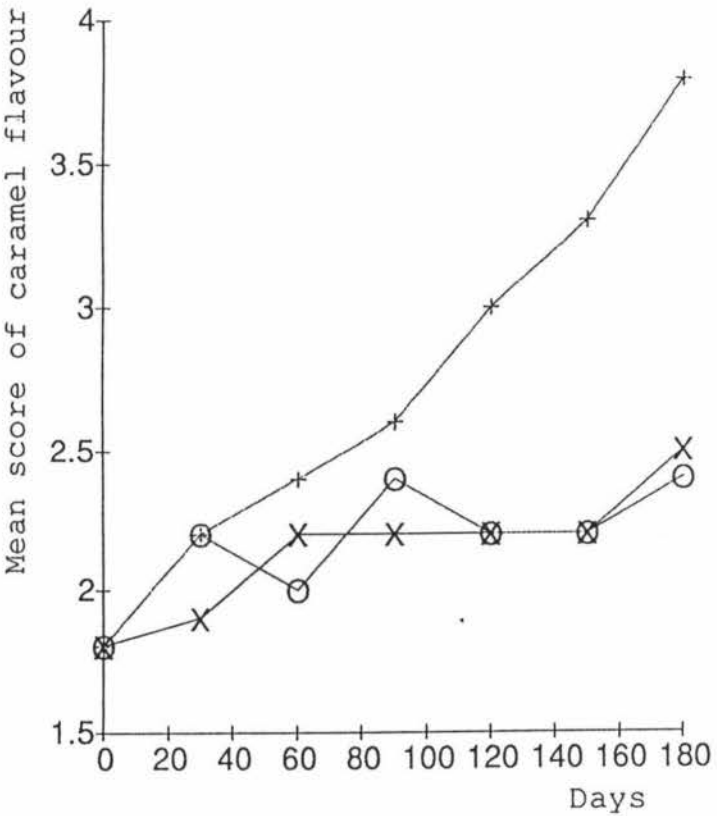

Fig. 8.12 Changes of sensory mean score of caramel flavour in the blended samples at 20°c (x), 30°c (o) and 40°c (+).

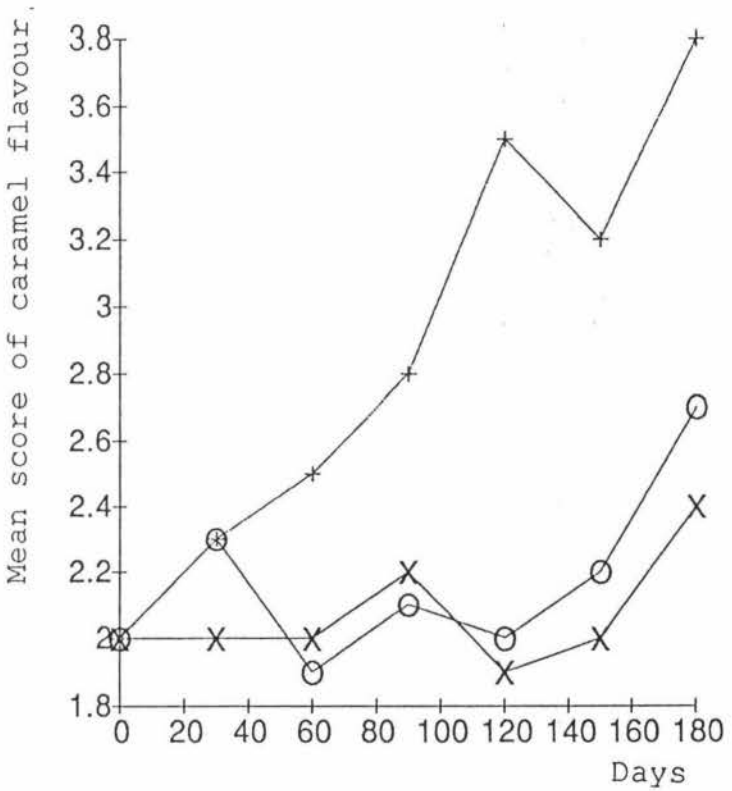

Fig. 8.13 Changes of sensory mean score of caramel flavour in the unblended samples at 20°c (x), 30°c (o) and 40°c (+).

Table 8.2 Mean scores of sensory evaluation

	0 days		30 Days		60 Days		90 Days		120 Days		150 Days		180 Days	
	B_a	Unb_b	B	Unb	B	Unb	B	Unb	B	Unb	B	Unb	B	Unb
20°C														
Caramel flavour	2.0	2.0	2.3	2.1	2.0	2.3	2.1	2.2	2.2	2.3	2.2	2.2	2.3	2.3
Oxidised flavour	1.8	2.0	1.9	2.3	2.2	2.0	2.2	2.2	2.2	1.9	2.2	2.0	2.5	2.4
30°C														
Caramel flavour			2.2	2.3	2.9	2.0	2.2	2.4	2.3	2.1	2.1	2.4	2.5	3.0
Oxidised flavour			2.2	2.3	2.0	1.9	2.4	2.1	2.2	2.0	2.2	2.2	2.4	2.7
40°C														
Caramel flavour			2.7	3.0	3.0	3.0	2.6	2.6	4.0	3.3	3.6	3.8	3.8	3.7
Oxidised flavour			2.2	2.3	2.4	2.5	2.6	2.8	3.0	3.5	3.2	3.2	3.8	3.7

a Blended samples
b Unblended samples

Table 8.3 F Ratios of chemical testing results by twoway ANOVA

	Treatments	Months
20°C		
TBA	1.030	32.479 ***
PV	27.522 ***	43.119 ***
HMF	1.165	65.270 ***
30°C		
TBA	1.057	70.374 ***
PV	1.269	92.307 ***
HMF	3.588	145.729 ***
40°C		
TBA	0.127	70.545 ***
PV	0.000	63.329 ***
HMF	4.217	420.712 ***

*** Significant at p = 0.001 level
The others are not significant at p = 0.05 level.

8.4.1.2 Peroxide values (PVs)

The PVs of the three lots showed faster increases at 30°C than those at 20°C. The development of PV did not parallel the changes of TBA. The PV of whey powder at 40°C reached a peak value of 3.04 after 90 days followed by a fall off, which is typical of peroxide decomposition in the course of fat oxidation (labuza, 1971). The PV of whey powder at 30°C showed a slight drop after 180 days. The PVs of the other samples showed consistent increases during storage. Touhy (1981) reported that a maximum PV of 4.6 was reached at 65°C after 12 weeks and dropped on further storage. Boon (1976) reported that the PV of whole milk powder was 2.32 after one year's storage at 30°C.

A peroxide value of 2 has been suggested as the rejection level for butter (Downey, 1969) but none has been suggested for milk powder. The maximum PV of the blended sample was reached after 60 days at 40 °C, declining over the next 120 days.

The PVs of blended and unblended samples at 20°C were below 2 after 180 days, although there was a significant difference ($p = 0.001$) between two lots. The differences in PV are not significant at the 5% probability level in the lots stored at 30°C and 40°C.

Both TBA values and PVs of whey powder are higher than those of whole milk powder. One possible reason is that whole milk powder received a more severe heat treatment than high fat whey powder during the manufacture. Heat treatment of milk causes the denature of β-lactoglobulin and other whey proteins, resulting in activation of sulphur-containing compounds, especially SH-groups. Such groups have anti-oxidant effects and have been intensively studied. Touhy (1981)

reported that a heat treatment of 120°C resulting in a better storage stability than one of 80 or 90 °C. Ipsen (1988) reported that the development of TBA in whole milk powder stored at 30°C was dependent on the heat treatment. In the manufacturing of the whole milk powder and high fat whey powder used in the storage trial, the milk was preheated at 115°c for 45 seconds whereas the whey was preheated at 80-85°c for 30 seconds (Scott, 1989; Cook, 1989). The lower heat treatment would likely be one of the causes of the reduced stability of high fat whey powder.

8.4.1.3 HMF

The results show that the concentration of HMF increases with temperature and storage time. The concentration of free HMF was higher in the powder kept at 38°C than at 20°C (Ipsen, 1988). No visible discoloration developed in the three lots at the three storage temperatures. The whole milk powder exhibited higher stability in that the increment of HMF at 40°C was 4.60 micromole/100 g powder, compared with the high fat whey powder increment of 27.42 micromole/100 g powder. As a possible explanation of the difference between whey powder and whole milk powder, the heat treatment exerts an influence on the content of HMF in powder. Ipsen (1988) reported that powder which received increasing heat treatment contained less free HMF after storage of 27 weeks. Labuza (1981) stated that whey powder contained a relatively large percentage of lactose and lysine so that the Maillard reaction readily occurred.

Water activity (a_w) is another important factor influencing the rate of the Maillard reaction. Loncin (1968) reported that dried milk powder stored at 40°C lost lysine and formed brown pigment at a_w 0.6 -

0.7 range. Labuza (1981) reported that the maximum rates of browning and protein quality loss during the storage of whey powder occurred at a_w 0.44 at both constant temperature (25, 35, or 40°C) and fluctuating temperatures (25-45°C). The effect of water activity was minor in that the a_w of high fat whey powder was 0.28 and whole milk powder 0.25 at 20°C calculated using the BET equation in 4.3.3. The reason why the extent of the Maillard reaction was so great in whey powder requires further investigation.

The effect of the Maillard reaction on fat oxidation has been mentioned (Renner, 1988, Labuza, 1981). The product of the Maillard reaction added to a milk mix before drying, exhibited antioxidant effects (Ipsen, 1988). The Maillard reaction progresses more rapidly at higher temperatures and would thus be expected to provide greater protection to the powder stored at higher temperatures. The results of this experiment did not provide evidence for this expectation.

The data in Table 8.3 indicate that there was no significant difference between the blended and unblended samples at the 5% probability level.

8.4.2 The storage stabilities of the powders assessed by sensory evaluation

The F ratios of twoway ANOVA for the individual scores (two factors were treatments and panelists) are presented in Table 8.4.

Table 8.4 F ratios of sensory evaluation results

	30 days		60 days		90 days		120 days		150 days		180 days	
	Treat.	Panels	Treat.	Panels	Treat.	Panels	Treat.	Panels	Treat.	Panels	Treat.	Panels
20°C												
Caramel flavour	0.537	0.996	1.462	1.760	0.383	10.713**	0.044	1.443	0.043	0.783	0.639	4.220*
Oxidised flavour	5.990*	4.800*	0.430	1.091	7.417*	1.482	2.645	2.281	1.427	0.660	0.028	1.502
30°C												
Caramel flavour	0.231	4.692*	9.426*	1.554	0.570	1.668	1.131	0.494	0.144	1.109	0.002	1.348
Oxidised flavour	0.134	3.275	0.082	1.216	0.079	0.718	8.260	0.796	0.006	1.610	0.188	0.838
40°C												
Caramel flavour	0.423	0.616	0.002	1.698	0.003	0.806	0.038	3.846	4.732	0.953	0.042	2.636
Oxidised flavour	0.082	1.216	0.066	0.862	0.563	1.306	1.896	0.411	0.053	1.618	0.081	3.063

* Significant at p = 0.05 level

** Significant at p = 0.01 level

The others are not significant at p = 0.05 level

In the majority of the cases, there were no significant differences among the panelists at the 5% probability level. However, the cases where there was a significant difference indicate the deviations of the panelists from the sensitivities and the understanding of the descriptors and scales. In most cases, there was no significant difference between the blended and unblended samples at the 5% probability level. Neither the blended nor the unblended samples were judged as being superior.

The F ratios of twoway ANOVA for the mean scores (two factors are treatments and times) are presented in Table 8.5.

Table 8.5 F ratios of the mean scores of sensory evaluation

	Treatments	Months
20°C		
Caramel flavour	0.577	0.526
Oxidised flavour	0.234	1.094
30°C		
Caramel flavour	0.221	6.316
Oxidised flavour	0.145	3.435
40°C		
Caramel flavour	0.015	8.112*
Oxidised flavour	2.520	32.476***

* Significant at p = 0.05 level
*** Significant at p = 0.001 level
The others are not significant at p = 0.05 level.

There was no significant difference between the blended and unblended samples on the descriptors. The difference among months are significant at the 5% or less probability for the descriptors, oxidised flavour and caramel flavour for the samples held at 40°C.

Table 8.6 indicates that the oxidised flavour and caramel flavour score correlate well with TBA and HMF measurements for both the blended and unblended samples held at 40°C. The only other high correlation coefficient was with TBA of the blended sample held at 20°C. The results imply that the flavour changes at 30°C and 20°C were below the taste panelists' thresholds of detection.

Table 8.6 Correlation coefficients between sensory evaluation scores and chemical testing results

	20°C	30°C	40°C
Oxidised flavour - TBA			
Blended mixture	0.8667	0.6516	0.9747
Unblended mixture	0.0300	0.4679	0.9233
Caramel flavour - HMF			
Blended mixture	0.4761	0.1688	0.8464
Unblended mixture	0.5728	0.7039	0.8388

8.4.3 Kinetics of TBA, PV, HMF and sensory scores

The kinetic approach is based on the rate of deteriorative reactions which are correlated with environmental and compositional factors (Saguy, 1980). In this study, the effects of temperature and time were investigated. Through the use of kinetic models, the deterioration in food quality can be calculated and predicted. The schemma of prediction of shelf lives through kinetic approach is shown in Fig. 8.14.

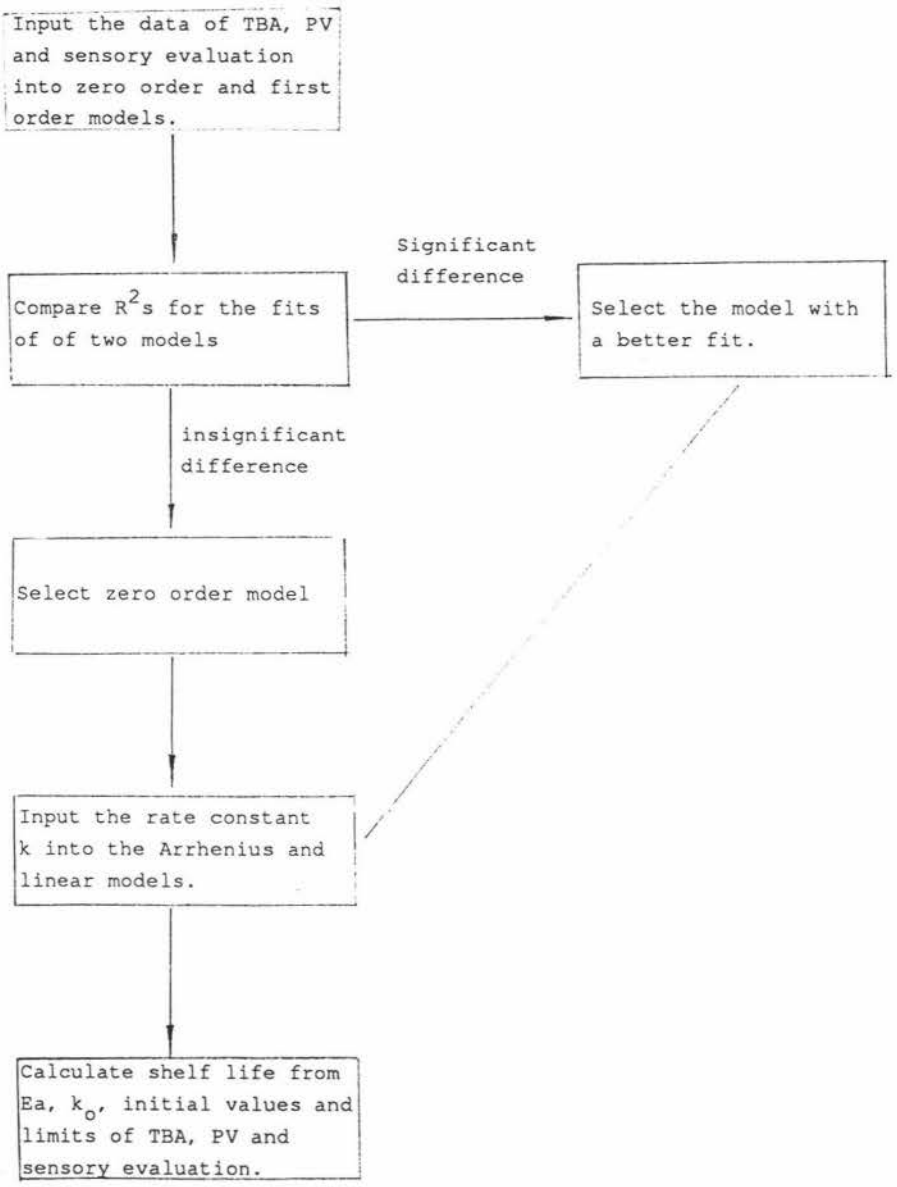

Fig. 8.14 Schema of the prediction of shelf life through kinetic approach

8.4.3.1 TBA and PV

TBA and PV are two chemical indexes for monitoring fat oxidation. Hamm (1968) reported that the logarithms of the indexes have a linear relationship with time, i.e. that they follow first order kinetics. Labuza (1979) stated that noninhibited fat oxidation was a first order reaction, and inhibited fat oxidation a zero order reaction. Hall (1985) reported that fat oxidation in whole milk powder in darkness at ±25°C has an initial linear phase which gradually changed to an exponential phase. The oxidation change from the first phase to the second phase was found to be 37 weeks. As both phases may be involved in this experiment, both zero and first order reaction models were are fitted to the experimental of TBA and PV data. The zero order relationship is defined as follows:

$$B = B_o + k_z t$$

Where: B = TBA or PV concentration at time t
B_o = TBA or PV concentration at $t = 0$
k_z = zero order rate constant OD day^{-1}
t = time in days

The first order relationship is defined as following:

$$A = A_o \exp(-k_f t)$$

Where: A = TBA or PV concentration at time t
A_o = TBA or PV concentration at $t = 0$
k_f = first order rate constant day^{-1}
t = time in days

The coefficients of zero and first order equations are presented in Tables 8.7 and 8.8. Since PVs of high fat whey powder and the blended sample declined at 30°C and 40°C, the models only fit well for all the PVs at 20°C. Therefore, the PV data from the start to the peak values were fed into the models for high fat whey powder and blended samples.

Table 8.7 TBA reaction rate constants based on regression

	°C	Zero Order B_o x10^{-4}	k_z (OD Day^{-1})	R^2 (%)	First Order $\ln A_o$	k_f x10^{-3} (Day^{-1})	R^2 (%)
Whole milk powder	20	0.016	0.64	89.1	-4.103	3.05	88.0
	30	0.016	1.12	94.6	-4.071	4.50	91.6
	40	0.011	3.19	94.8	-4.117	8.58	97.9
High fat whey powder	20	0.018	2.23	95.5	-3.863	6.05	94.7
	30	0.018	3.29	90.4	-3.831	7.51	92.3
	40	0.014	6.37	95.5	-3.783	11.00	94.1
Blended mixture	20	0.017	1.43	96.4	-3.928	4.94	95.1
	30	0.017	2.20	95.0	-3.947	6.40	94.9
	40	0.013	4.78	96.6	-3.933	9.78	96.7
Unblended mixture[a]	20	0.016	1.81	90.0	-3.958	5.61	94.1
	30	0.017	2.38	94.3	-3.944	6.71	94.9
	40	0.011	4.71	95.0	-3.958	9.69	97.4

a The rate constants are calculated with the averages of the results of whole milk powder and high fat whey powder.

Table 8.8 PV reaction rate constants based on regression

	°C	Zero Order B_o x10^{-3}	k_z (Me O_2/kg Day^{-1})	R^2 (%)	First Order $\ln A_o$	k_f x10^{-3} (Day^{-1})	R^2 (%)
Whole milk powder	20	0.84	1.46	91.3	-0.17	1.50	91.3
	30	0.85	3.35	94.0	-0.15	2.99	93.2
	40	0.99	6.44	77.4	-0.02	4.72	76.3
High fat whey powder	20	1.18	2.91	95.2	0.17	2.06	93.0
	30	1.21	7.43	93.8	0.20	4.53	90.7
	40	1.12	22.00	99.3	0.17	11.00	97.0
Blended mixture	20	1.06	2.04	88.8	0.02	1.82	90.7
	30	1.33	4.11	93.6	0.14	2.86	91.7
	40	1.10	14.00	98.8	0.12	8.75	96.8
Unblended mixturea	20	1.02	2.18	97.6	0.02	1.82	97.3
	30	1.08	4.47	88.7	0.08	3.25	86.6
	40	0.94	14.50	98.7	0.003	11.00	98.6

a The rate constants are calculated with the averages of the results of whole milk powder and high fat whey powder.

Table 8.9 HMF reaction rate constants based on regression

	°C	Zero Order B_o x10^{-5}	k_z (mole/100g Day^{-1})	R^2 (%)	First Order lnA_o	k_f x10^{-3} (Day^{-1})	R^2 (%)
Whole milk powder	20	1.74	2 87	89.1	0.56	1.41	90.6
	30	1.66	7.92	82.2	0.56	3.17	87.2
	40	1.66	24.00	96.1	0.68	6.58	98.1
High fat whey powder	20	3.13	8.23	92.3	1.16	2.09	94.1
	30	3.02	40.00	97.8	1.24	6.44	97.9
	40	1.74	157.00	98.8	1.42	12.00	95.6
Blended mixture	20	2.43	5.55	94.8	0.90	2.00	94.2
	30	2.33	24.00	97.0	0.96	5.49	98.9
	40	1.70	90.00	98.7	1.13	11.00	96.8
Unblended mixture[a]	20	2.38	5.93	89.8	0.88	2.00	91.2
	30	2.30	25.00	95.9	0.95	5.62	98.5
	40	1.87	86.00	99.0	1.12	11.00	96.2

a The rate constants are calculated with the averages of the results of whole milk powder and high fat whey powder.

Little difference in the R^2 values between the two models was observed. Labuza (1983) stated that the extent of a reaction must be 50% or more to be able to distinguish any difference between zero and first order models. The observations of this experiment indicate that at 20°C, the oxidation of high fat whey powder, whole milk powder and the blended mixture were still at the linear stage or the induction stage; at 30°C, the oxidation of whey powder was at the transition stage from zero order to first order reaction; at 40°C, the oxidation of whey powder possibly entered the exponential phase. The elevated temperature reduces the length of the linear phase. However, the short storage time did not allow the development of the exponential phase or the first order reaction in the stored samples, with exception of the whey powder at 40°C. The conversion rates of fat oxidation in milk

powder and high fat whey powder are not able to estimated, but the minor difference between the fits of the zero order and first order models indicats that the extent of the conversion of fat oxidation may be below 50%.

The temperature dependence of the rates of chemical reactions can be expressed by the Arrhenius model which has been applied to chemical reactions in foods (Labuza, 1980). Within a narrow temperature range, a linear approximation also can be applied, which was claimed to have the advantage of simplity in industrial cases (Olley and Ratkowsky, 1973). However, Saguy and Karel (1980) suggested that the Arrhenius equation gave the best correlation and was applicable over the widest temperature range. In this study, the reaction rate constants k were fitted to both the Arrhenius and the linear models.

The Arrhenius model is expressed as follows:

$$k = k_o \exp(-Ea/RT)$$

Where: k = rate constant of TBA, PV concentration change, day^{-1}
k_o = pre-exponential or frequency factor, day^{-1}
Ea = activation energy, $kJ\ mol^{-1}$
R = gas constant, $kJ\ mol^{-1}\ K^{-1}$
T = absolute temperature, K

The Linear model is expressed as follows:

$$k = k_o \exp(-bt)$$

Where: k = rate constant of TBA, PV concentration change, day^{-1}
k_o = pre-exponential or frequency factor day^{-1}
b = constant $day^{-1}\ K^{-1}$
t = absolute temperature K

The activation energy (Ea) and frequency factor (k_o) were determined by linear regression for each set of data and are presented in Tables 8.10 and 8.11. Higher R^2 values were obtained for

Arrhenius and linear models for all TBA values indicating a good fit for all quality parameters over 20°C - 40°C. However, the two models fit all the PV data of whole milk powder and the data of high fat whey powder before the decline in values, (refer to Fig. 8.5). Hamm (1968) reported that the Ea of the peroxide reaction was 43.05 kJ/mol and for the TBA reaction was 58.10 kJ/mol in milk fat oxidation. In the present study, the Ea of TBA reaction was 41.0 kJ/mol and 63.3 kJ/mol for high fat whey powder and whole milk powder, respectively. While the Ea of peroxide reaction was 77.0 kJ/mol and 56.9 kJ/mol for high fat whey powder and whole milk powder respectively. The higher Ea that means more energy is needed for the reaction to proceed in the sample, which may be more stable than the one with a lower Ea. However, the shelf life of a product is determined not only by Ea but also by frequency factor, k_o and the initial concentration of the criterion. Table 8.12 shows the effects of analytical precision of the method on the estimation of the rate constant for chemical reaction, which indicates that for a minor change in species monitored, analytical precision significantly influences the error of the chemical reaction estimation. Since the precision of TBA determination is ±7.3% and that of PV determination is ±5.3%, and conversion rates are assumed of between 20% and 50%, the errors would be between ±15% and ±50%. The storage experiment results are thus in agreement with the values reported by Hamm (1968).

Table 8.10 Constants of Arrhenius equations for TBA, PV and HFM

	$\ln k_o$	Ea kJ mol^{-1}	R^2 (%)	Q_{10} 20-30°C	Q_{10} 30-40°C
TBA					
Whole milk powder	25.43	63.3	96.4	1.7	2.9
High fat whey powder	17.57	41.0	97.2	1.2	1.9
Blended mixture	19.83	47.6	96.7	1.9	2.0
Unblended mixture	16.44	38.9	93.3	1.4	2.2
PV					
Whole milk powder	23.76	56.9	99.8	2.3	1.9
High fat whey powder	32.59	77.0	99.6	2.6	3.0
Blended mixture	33.78	80.7	96.4	2.0	3.4
Unblended mixture	31.64	75.6	96.9	2.1	3.2
HMF					
Whole milk powder	34.29	81.0	99.8	2.8	3.0
High fat whey powder	48.31	112.5	99.9	4.6	3.9
Blended mixture	43.67	106.2	99.9	4.2	3.4
Unblended mixture	45.31	102.0	99.9	4.3	3.8

Table 8.11 Constants of linear equations for TBA, HFM and PV

	$\ln k_o$	k	R^2 (%)
TBA			
Whole milk powder	-24.06	0.080	97.0
High fat whey powder	-14.62	0.052	97.8
Blended mixture	-17.38	0.060	97.3
Unblended mixture	-13.49	0.048	94.2
PV			
Whole milk powder	-21.33	0.074	99.5
High fat whey powder	-28.59	0.101	99.8
Blended mixture	-27.59	0.096	97.6
Unblended mixture	-29.41	0.103	97.1
HMF			
Whole milk powder	-30.08	0.106	99.9
High fat whey powder	-41.05	0.147	99.8
Blended mixture	-39.08	0.139	99.9
Unblended mixture	-37.37	0.134	99.8

Table 8.12 Effects of analytical precision of the method on the estimation of the rate constant for chemical reations

Analytical precision (%)	% Error in reaction rate constant k at the following % change in reactant species monitored						
	1%	5%	10%	20%	30%	40%	50%
± 0.1	14	2.8	1.4	0.7	0.5	0.4	0.3
± 0.5	70	14	7	3.5	2.5	2	1.5
± 1.0	>100	28	14	7	5	4	3
± 2.0	>100	56	28	14	10	8	6
± 5.0	>10	>100	70	35	25	20	15
±10.0	>100	>100	>100	70	50	40	30

Source: From Benson (1960)

The temperature quotient or Q_{10} was computed, this describes the sensitivity of the reaction rate to a 10°C change in temperature, defined as :

$$Q_{10} = k \text{ at}(T + 10^{o}C)/k \text{ at } (T^{o}C)$$

The Q_{10} values were computed using the above equation for the temperature range of 20 - 40°C. The Q_{10}s of TBA, PV and HMF are presented in Table 8.10. The Q_{10}s of PV reactions at 20 - 40°C are above the assumed Q_{10} of 2. The higher Q_{10}, the more sensitive the reaction is to changes in temperature. The magnitude sequence of Q_{10} over the range of 20 - 30$_{o}$C is as: HMF > PV > TBA. The similar trend exits over the range of 30 - 40°C. As mentioned in 8.4.1.1, the product of HMF with TBA may interfere with the TBA reading for fat oxidation and consequently, the Q_{10} of TBA value should have been greater than that of PV. However, this is not evident from the experimental results which requires further investigation.

8.4.3.2 HMF

The zero and the first order models were fitted to the HMF data

(Table 8.9). Regression analysis shows that a good fit for the HMF data was obtained for both zero and first order models. Little difference in R^2 was observed which means that the reaction conversion may be below 50%. The Arrhenius and linear models were fitted to the rate data the same as above and the Ea and Q_{10} were calculated accordingly (Table 8.11). Labuza (1981) reported that Ea of whey powder browning was in the range of 102.8 - 136.3 kJ/mol at a_w 0.33 - 0.65. The Ea of whole milk powder and high fat whey powder were found to be 83.6 - 114.1 kJ/mol, respectively. The difference between these results and those of Labuza may be due to due to the different specimen tested and different testing methods. Labuza (1981) evaluated brown pigment formation in whey powder by a three enzyme modification (Choi et al, 1949). Free HMF was determined in this experiment through trichloro acetic acid extraction and reaction with TBA reagent (Keeney, 1959).

8.4.3.3 Sensory scores

A zero and a first order model were fitted to the oxidised flavour and caramel flavour data (Table 8.13). Regression analysis showed a good fit for the data at 40°C. The R^2 indicates that zero order and first order models did not fit well to the data of both oxidised and caramel flavour at 20°C and 30°C. However, the Arrhenius and linear equations were fitted to the k values derived from the zero order reaction rate data to determine approximately the temperature dependence and shelf lives (Table 8.14 and Table 8.15). Although the Ea of caramel flavour of the unblended sample was quite different from that of blended sample, it is not meaningful since blending would not bring either positive or negative effects on the stabilities of the

powders against the Maillard reaction. The difference is largely due to the poor fits of zero order and first order models to the caramel flavour data at 20°C and 30°C.

Table 8.13 Sensory Score Change Rate Constants

	°C	B_z	Zero Order k x10^{-3} (unit Day^{-1})	Zero Order R^2 (%)	lnA_o	First Order k_f x10^{-3} (Day^{-1})	First Order R^2 (%)
Caramel flavour							
Blended mixture	20	2.06	1.07	29.8	0.72	0.51	30.6
	30	2.24	0.83	3.2	0.80	0.41	4.6
	40	2.22	9.76	75.5	0.81	3.32	75.6
Unblended mixture	20	2.08	1.31	54.0	0.73	0.61	54.6
	30	2.05	2.14	45.0	0.72	0.96	44.3
	40	1.85	3.21	82.1	0.84	2.87	70.8
Oxidised flavour							
Blended mixture	20	1.85	3.21	82.1	0.62	1.52	81.0
	30	1.96	2.38	52.1	0.67	1.14	52.6
	40	1.79	10.00	98.3	0.63	3.90	98.8
Unblended mixture	20	2.20	0.95	0.7	0.78	0.26	0.8
	30	1.96	2.38	32.9	0.67	1.03	32.0
	40	1.99	10.00	92.6	0.72	3.48	93.7

Table 8.14 Constants of Arrhenius eqauations of sensory scores

	lnk_o	Ea KJ $mole^{-1}$	R^2 (%)
Caramel flavour			
Blended mixture	51.78	128.50	64.7
Unblended mixture	14.32	34.21	99.9
Oxidised flavour			
Blended mixture	32.64	78.43	64.4
Unblended mixture	37.36	91.36	97.9

Table 8.15 Constants of linear equations of sensory scores

	$\ln k_o$	k	R^2 (%)
Caramel flavour			
Blended mixture	-32.77	0.111	66.5
Unblended mixture	-12.85	0.045	99.7
Oxidised flavour			
Blended mixture	-15.77	0.057	56.3
Unblended mixture	-34.62	0.118	98.4

8.4.3.5 Predication of shelf life

The shelf life was defined as the period between manufacture and retail purchase of a food product during which the product is of satisfactory quality (IFT, 1974). In this work, the shelf life is expressed as the time for reaching certain values of chemical and sensory criteria. The shelf lives of stored samples were determined using the following equations: (Labuza, 1983)

$$k = C/\Theta_s$$

Where: C = amount lost at time t_s
$= C_o - C_s$ for zero order
$= \ln(C_o/C_s)$ for first order

In case of TBA and PV, and oxidized flavour,
$C = C_s - C_o$ for zero order
$C = \ln(C_s/C_o)$ for first order

Since
$$\ln k = \ln C - \ln\Theta_s = \ln k_o - Ea/RT$$
$$\ln k = \ln C + \ln(1/\Theta_s)$$
Thus
$$-\ln\Theta_s = -\ln C + \ln k_o - Ea/RT$$

Where: Θ_s = shelf life for a certain quality change.

There is no authorized objective criteria of TBA, PV, HMF and sensory scores for whole milk powder and high fat whey powder. A TBA value of 0.05 is considered unacceptable for milk (Downey, 1969) and a

PV of 2 milliequivalents O_2 per kg fat for butter (Downey, 1969), which were taken as objective reference criteria. An oxidised flavour score of 3.5 was set for the objective reference score. These three values were used as C_s in the above equations. HMF was not taken as a criterion for determining the shelf life since this storage trial was designed mainly for assessing the fat stability. The calculated results are shown in Table 8.16. The linear relationships between the reciprocals of the temperatures and the shelf lives are presented in Fig. 8.15 - Fig. 8.19.

Table 8.16 Shelf lives estimated with criteria of TBA, PV and oxidized flavour scores (days)

	TBA	PV	Oxidized flavour
Whole milk powder			
20°C	592	762	/a
30°C	251	352	/
40°C	112	171	/
High fat whey powder			
20°C	128	329	/
30°C	73	116	/
40°C	44	43	/
Blended mixture			
20°C	231	466	1090
30°C	121	156	376
40°C	66	55	139
Unblended mixture			
20°C	190	480	1628
30°C	112	160	534
40°C	68	57	158

a Oxidised flavour scores of whole milk powder and high fat whey powder were not determined.

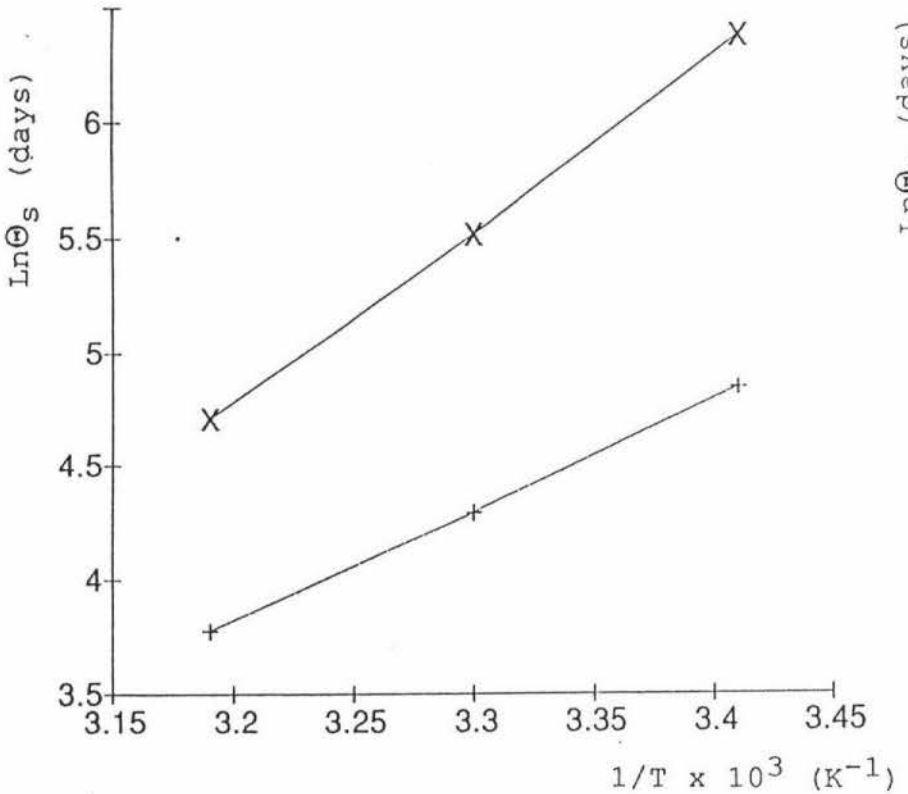

Fig. 8.15 Shelf lives as a function of temperature of whole milk powder (x) and high fat whey powder (+) estimated by TBA.

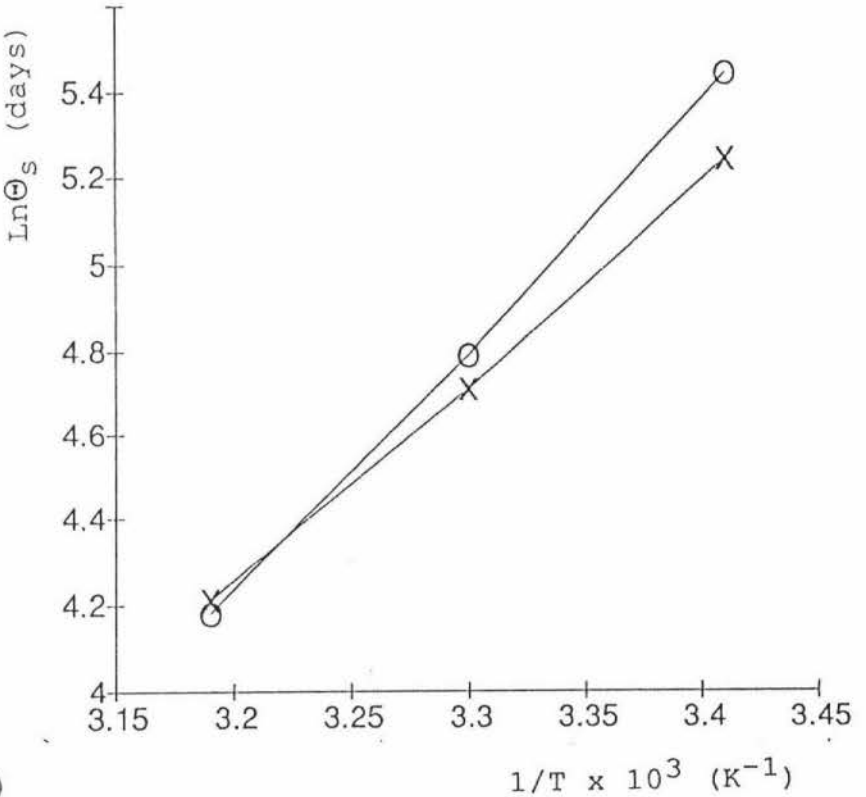

Fig. 8.16 Shelf lives as a function of temperature of the blended samples (o) and unblended samples (x) estimated by TBA.

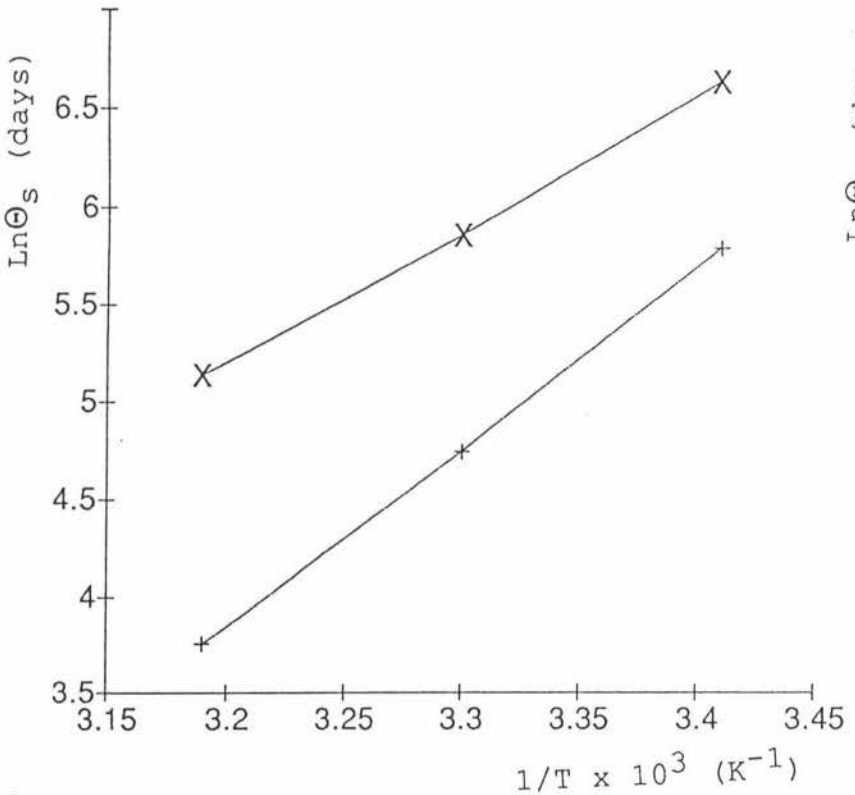

Fig. 8.17 Shelf lives as a function of temperature of whole milk powder (x) and high fat whey powder (+) estimated by PV.

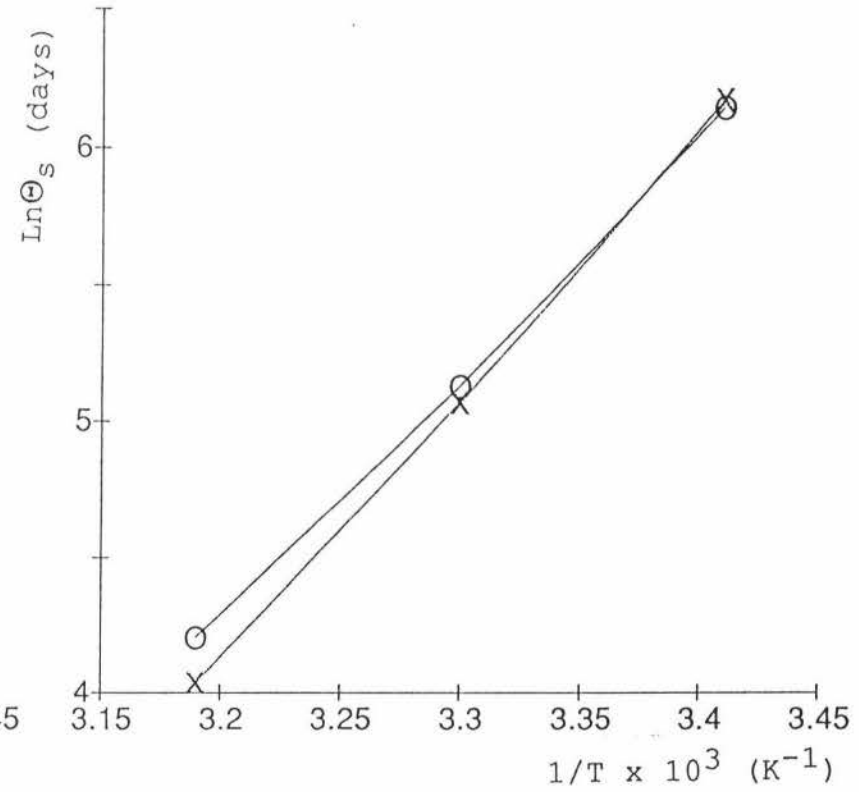

Fig. 8.18 Shelf lives as a function of temperature of the blended samples (o) and the unblended samples (x) estimated by PV.

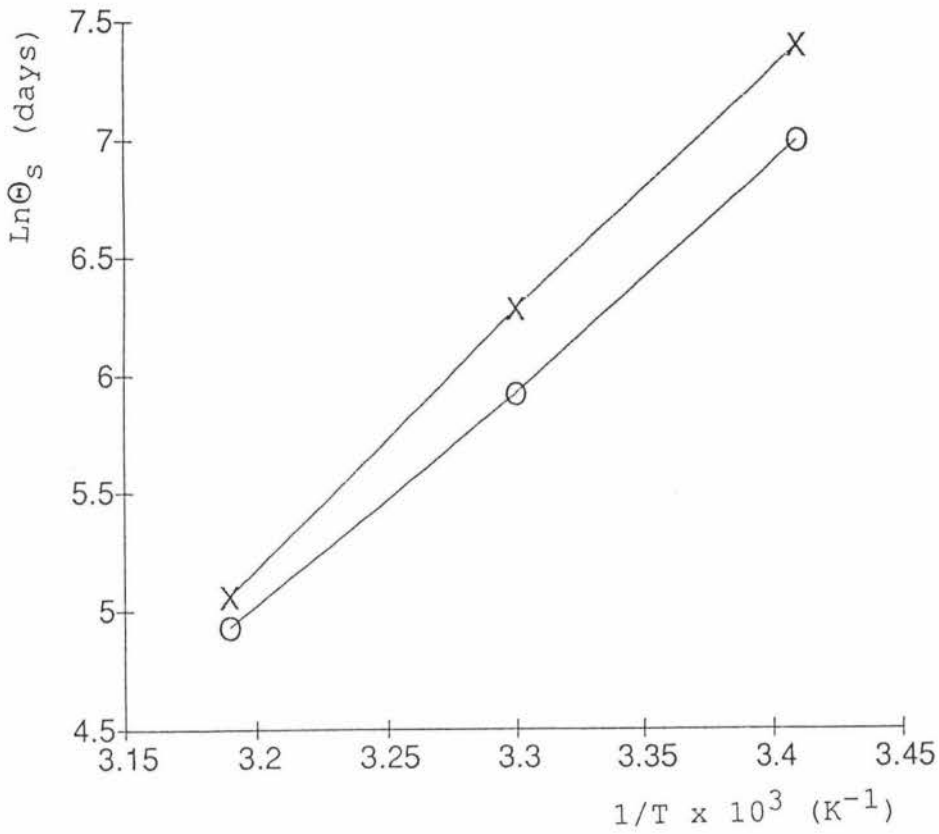

Fig. 8.19 Shelf lives as a function of temperature of the blended samples (o) and the unblended samples (x) estimated by oxidised flavour score.

The shelf lives predicted using TBA and PV criteria do not agree with those based on sensory scores. The longer shelf lives predicted using the sensory scores indicates that the panelists were not sensitive enough to detect the minor change during storage. It also implies that a TBA 0.05 and a PV of 2 milliequivalents O_2 per kg fat may be too low a level to be used as limiting criteria for the acceptability of milk powder and high fat whey powder. It is suggested that if the powder mixture is sold directly as infant formula for

immediate consumption, the sensory scores are important and should be the criteria used; if the mixture is to used as ingredient in formulated products, the sensory scores are relatively unimportant and chemical criteria should be used.

The shelf lives of blended and unblended mixtures are quite similar to each other, predicted using TBA and PV. The shelf life of the blended mixture, estimated with sensory scores, is shorter than that of the unblended mixture. The influence of error in the estimation of reaction rate must be taken into consideration. The error in sensory evaluation on the reaction rates could not be assessed as those of chemical reactions. The fit of the kinetic models is not as good as those of TBA and PV. However, the Arrhenius approach provides an approximate indication of shelf life (Labuza, 1983). The prediction of shelf life using sensory evaluation provides the shelf life range of the sample.

The HMF data indicates that considerabel Maillard reaction occurred during storage. HMF interferes with the TBA reagent (for fat oxidation), which leads to a shorter shelf life predicted by TBA. However, the shelf lives of the blended and unblended samples predicted by TBA are within the same range.

8.5 Conclusion

During 180 days of accelerated storage, significant deterioration occurred in the samples stored at 40°C as assessed by TBA, PV, HMF testing and sensory evaluation. The changes in the samples at 20°C and 30°C were not able to be distinguished by the sensory panels at the 5% probability level during the storage trial.

The blended samples did not significantly differ from unblended

samples at the 5% probability level in terms of TBA, PV, HMF and sensory descriptors.

Kinetic models were developed for TBA, PV, HMF and sensory scores data. Both the zero and the first order reactions fitted to the TBA and HMF data well. The zero and the first order reactions were fitted to the data of PV before it decreased. The two temperature models only fitted to the sensory data well at 40°C. Their temperature dependencies were expressed by the Arrhenius and linear models.

The shelf lives of blended and unblended samples predicted using PV were over 450 days at 20°C, and those predicted by sensory evaluation were in the range of 1100 - 1600 days.

Product stability depends on multiple factors. The quality of raw milk, the processing procedure, and the packaging and storage conditions may well be significant than the effects of blending. To successfully make blended infant formula, all these factors are required to be considered together in addition to the blending operation.

CHAPTER 9 SUMMARY AND CONCLUSION

The compressibility of food powder is an indication of cohesiveness. The compressibilitites of experimental specimens were determined using an Instron testing machine to give the following decreasing order: base powder, whole milk powder, high fat whey powder, lactose and ascorbic acid, i.e., base powder has the largest compressibility.

The particle sizes of the powders were determined using a laser particle sizer. The sequence of arithmetic diameters of the powders is the reverse of the above, i.e., base powder has the smallest particle size.

The scanning electron microscopy revealed the sizes, shapes of the particles and the microstructure of the mixture of high fat whey powder/whole milk powder, high fat whey powder/base powder, in which lactose and ascorbic acid were mixed. No evidence was shown to suggest that either the mixture of whey powder with milk powder or the mixture of the powders with ascorbic acid is an ordered mixture. The mixtures did not exhibit segregation or demixing. They are described as 'pseudorandom mixtures'.

The blending was carried out in an experimental ribbon blender and a pilot ribbon blender. Mixtures were designed with a ratio of 50:50 for either high fat whey powder to base powder or high fat whey powder to whole milk powder. Response Surface Methodology was used for characterizing the experimental blender. Load ratio and mixing time were found to have significant effects on the homogeneity. Rotation

speed had no influence in the experimental region chosen. At load ratio 0.4, the time for reaching a certain homogeneity was shorter than that at load ratio 0.8 in mixing high fat whey powder with whole milk powder or base powder. This effect is not apparent in mixing ascorbic acid with the powders. Due to higher cohesiveness, the mixing of base powder with high fat whey powder showed a slower rate and poorer homogeneity than that of whole milk powder.

The homogeneity cannot be improved limitlessly with prolonged time. Limited fluctuations occurred after reaching minimum mixing index (MI) points. The variance ratios of the fluctuation point to the minimum point were less than the critical F value, which indicate that there were no significant changes in the mixing system.

The mixing rate followed a first order reaction but the fit of the model declined when the time was prolonged due to fluctuation. In the mixing operation, it is considered that there is an optimum zone rather than an optimum point. The optimum zone is established using control chart methodology.

As to protein, fat and carbohydrate, the mixtures reached the acceptable MI. The secondary requirements such as the ratio of whey protein to casein and the ratio of saturated fatty acid to unsaturated fatty acid were affected by the variance of the powder ratio.

In terms of sweetness and mouthfeel, the mixture of a powder ratio 40:60 or 60:40 was assessed as significantly different from that of 50:50 at the 5% probability level. After mixing for 10 minutes, the powder ratios were between 40:60 and 60:40 and the differences could not be detected by sensory evaluation.

The blended samples and unblended samples of whole milk powder and high fat whey powder were tested through a 180 day storage trial. The

sensory quality changes were not significant at 20°C and 30°C, but were at 40°C. There was no significant difference between unblended and blended samples on the criteria of TBA, PV, HMF and mean sensory scores of oxidized flavour and caramel flavour at the 5% probability level.

Both zero order and first order reactions were fitted to the data of TBA, PV and HMF at three temperatures. Using the Arrhenius approach, the influence of temperature was assessed by deriving the Ea of TBA, PV and HMF. From this, the shelf lives of the samples were estimated.

At 20°C, the shelf lives of unblended and blended samples are estimated as 1628 days and 1090 days respectively, based on an oxidised flavour limit of 3.5 out of 7 points and 480 days and 466 days respectively, based on a PV limit of 2 milliequivalents O_2 per kg fat. Because HMF may interfere with the TBA reagent (for fat oxidation), the shelf lives of unblended and blended samples estimated by TBA were shorter than those estimated by PV and oxidized flavour scores.

In conclusion, dry blending of a 50:50 mixture of high fat whey powder and milk powder can produce satisfactory mixing of the major components. The mixing rate of ascorbic acid with the powders was closed to that of powder mixing. The effects of blending on fat stability is insignificant under the experimental conditions and the shelf life of blended samples can be expected over 1 year at 20°C. Therefore, the technique of manufacturing infant formula through dry blending is well worth investigation in commercial blenders.

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Appendix 1

Questionnaire of sensory evaluation for storage stability

Name _____ Date _____

Thank you for coming to this taste panel. You will be tasting a blend of reconstituted whey/milk powder. Please evaluate the sample for particular characteristic listed. Place a mark (x) at the point on the line which best describes the sample. Identify the mark (x) by writing its code above it.

Sample code _____ _____ _____ _____

Caramel Flavour

How strong is the caramel flavour?

1	2	3	4	5	6	7
No caramel flavour						Extremely caramel flavour

Oxidized Flavour

How strong is the oxidized flavour?

1	2	3	4	5	6	7
No oxidized flavour						Extremely oxidized flavour

Comments:

Questionnaire of sensory evaluation for powder ratio variance

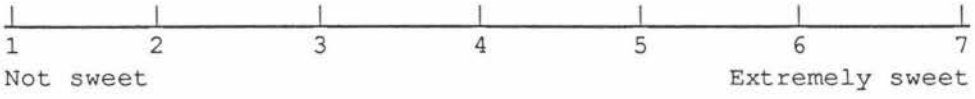
Name _____ Date _____

Thank you for coming to this taste panel. You will be tasting a blend of reconstituted whey/milk powder. Please evaluate the sample for particular characteristic listed. Place a mark (x) at the point on the line which best describes the sample. Identify the mark (x) by writing its code above it.

Sample code _____ _____ _____ _____

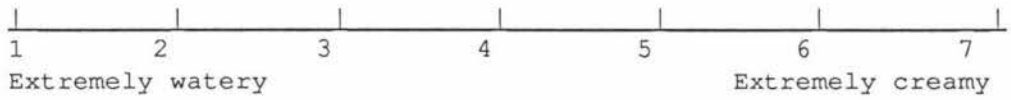
Sweetness

How sweet is the sample?



Mouthfeel

How does the sample feel in your mouth?



Comments:

Appendix 2

Sample preparation for sensory evaluation

1. Samples for estimation of powder ratio variance

Whole milk powder and high fat whey powder were mixed as the following ratio:

Whole milk powder	20	22.5	25	27.5	30	gram
High fat whey powder	30	27.5	25	22.5	20	gram

50 gram mixture was reconstituted with 350 ml 50°c distilled water.

2. Samples for evaluation of storage stability

The unblended sample was made from 25 gram stored whole milk powder and 25 gram stored high fat whey powder.

50 gram blended or unblended sample was reconstituted with 350 ml 50°c distilled water.

3. Samples for panelist training

Oxidized whole milk powder was obtained from NZDRI sensory evaluation section.

Caramelized sample was made by heating whole milk powder in oven at 70°c for 72 hours.

10 gram oxidized or caramelized sample was mixed with 20 gram high fat whey powder and 20 gram whole milk powder. This mixture was reconstituted with 350 ml 50°c distilled water.

4. Reference samples

Twenty five gram high fat whey powder and whole milk powder, both of which were stored at -20°c, were reconstituted with 350 ml 50 °c distilled water.

Two hours were allowed for hydration for all four samples.

Appendix 3

An example of calculation of root mean square

Take whole milk powder as example to show the calculation of root mean square with the following equation.

$$\% \text{ RMS} = [(Wi - Wi^*)/Wi]^2/n \times 100$$

Where: Wi = experimental water content
Wi^* = calculated water content.
n = number of experimental points

Calculation:

Wi	Wi^*	$[(Wi-Wi^*)/Wi]^2$
2.947	3.143	0.004
3.540	3.710	0.002
4.416	4.231	0.002
5.337	4.831	0.009
5.550	5.684	0.001
5.936	5.895	0.000
7.129	8.621	0.044
13.368	11.184	0.027
	Sum	0.089

$$\text{RMS} = (0.089/8)^{0.5} \times 100 = 10.54\%$$

Appendix 4

Analysis of Variance between Two Blocks

Due to	DF	SS	MS	Ft_a	Fc_b
Factor	1	23.893	23.893	4.41	15.14
Error	18	28.406	1.578		
Total	19	52.299			

a. Ft, F value is from 95th and 99th Percentile values of the F distribution (George, 1967).
b. Fc, F value is calculated from ANOVA results.

Appendix 5

Decoding of regression equation (5.2)

The coded variable is replaced by the following:

$$\text{Coded variable} = \frac{\text{Actual} - (\text{Hi level} + \text{Lo level})/2}{(\text{Hi level} - \text{Lo level})/2}$$

Therefore equation (5.2) would be rewritten as:

$$\ln MI = 0.461 + 0.132\left[\frac{x_1-(0.8+0.4)/2}{(0.8-0.4)/2}\right] - 0.352\left[\frac{x_2-(10+5)/2}{(10-5)/2}\right]$$

The decoded equation :

$$\ln MI = 1.121 + 0.66x_1 - 0.141x_2$$

Appendix 6

PSA for predicting the mixing index

The model is given in Appendix 4.

The steepest ascent vector is calculated as:

	x_1	x_2
a. Base level	0.6	7.5
b. Unit change (Hi level - Lo level)/2	0.2	3.75
c. Estimated slope	0.132	-0.352
d. Calcn. of steepest ascent (c/0.352)	0.375	-1
e. Steepest ascent changes (d x b/3)	0.025	1.25
f. Actual descent to minimum Y	-0.025	1.25

Therefore points on the steepest 'descent' vector are:

	x_1	x_2
(1)	0.575	8.75
(2)	0.550	10.00
(3)	0.500	11.25
(4)	0.475	12.50

Appendix 7

ANOVA results and calculation for LSD for sensory quality

variance due to powder ratio

Analysis of variance for sweetness

Due to	DF	SS	MS	Ft_a	Fc_b
C2	4	13.280	3.320	2.78	10.47
C3	6	3.983	0.664	2.51	2.09
Error	24	7.600	0.317		
Total	34	24.863			

a Ft, F value is from 95th and 99th Percentile values of the F distribution (George, 1967).
b Fc, F value is calculated from ANOVA results.

$LDS = t\sqrt{(2MS_E/n)} = 2.064\sqrt{(2x0.317/7)} = 0.62$

Analysis of variance for mouthfeel

Due to	DF	SS	MS	Ft_a	Fc_b
C2	4	13.201	3.300	2.78	11.70
C3	6	3.667	0.611	2.51	2.17
Error	24	6.767	0.282		
Total	34	23.635			

a Ft, F value is from 95th and 99th Percentile values of the F distribution (George, 1967).
b Fc, F value is calculated from ANOVA results.

$LDS = t\sqrt{(2MS_E/n)} = 2.064\sqrt{(2x0.282/7)} = 0.60$

Appendix 8

Ratio scores of the mixture of whole milk podwer and high fat whey powder after mixing for 10 minutes

Load ratio	10 minutes	12.5 minutes	15 minutes
0.4			
	49.0	51.5	50.3
	47.1	50.9	49.6
	48.4	45.9	50.9
	51.5	54.6	55.2
	51.5	50.3	48.4
	49.0	50.3	45.9
	54.0	51.5	50.3
	49.6	52.7	55.8
	49.0	54.0	50.2
	54.6	43.0	54.6
0.8			
	55.8	55.8	52.1
	50.9	46.5	48.4
	45.3	50.9	53.4
	50.9	49.0	50.3
	42.2	49.0	47.8
	55.8	53.4	51.5
	55.8	50.8	57.7
	50.3	55.2	50.3
	51.5	54.6	50.3
	50.3	51.5	48.4

Appendix 9

An example of calculation of the upper and lower limits of optimum zone

$u \pm 3\sigma/\sqrt{n}$

u, the averge level is determined as the designed ratio score 50.

The upper and lower limits are calculated as:

σ, the standard deviation of the minimum MI point is 3.5.

n, the sample number is 20

The upper and lower limits are calculated as:

$3x3.5/\sqrt{20} = 2.35$

50 ± 2.35

Appendix 10

An example of calculation of composition mixing indexes

The designed level of protein content of 50:50 mixture is calculated as follows:

$$P_{m\,d}\ \% = (P_{bp} + P_{hfwp})/2$$

$$= (16.30 + 20.69)/2 = 18.49$$

The protein content in the blended mixture is calculated as follows:

$$P_{m\,i}\ \% = [P_{bp} x s_i + P_{hfwp} x (100 - s_i)]/100$$

$$MI = [(P_{mi} - P_{md})^2/n]/P_{md}$$

Where: s_i = mixing ratio score of n_i spot sample
P_{mi} = Protein content in the n_i sample of the mixture
P_{md} = designed protein content of the mixture
P_{bp} = Protein content in base powder
P_{hfwp} = Protein content in high fat whey powder

The ratio scores of 20 samples mixed for 5 minutes are as follows:

76.42 58.28 51.81 73.83 20.72 72.53 24.61 49.22 37.56 46.63 55.69

81.60 47.92 59.58 75.12 60.87 22.02 23.32 90.67 45.34

The MI is calculated as 0.049