PHYSIO-CHEMICAL CHANGES IN INDIGENOUS WHOLE MILK POWDER BULK PACKED AND STORED UNDER COMMERCIAL CONDITIONS

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
(DEEMED UNIVERSITY)
IN PARTIAL FULFIMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTOR OF PHYLOSOPHY

IN

DAIRY CHEMISTRY

BY

RAVINDER KUMAR, M.Sc.

DIVISION OF DAIRY CHEMISTRY
SOUTHERN REGIONAL STATION
NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
BALGALORE – 560 030
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Dedicated to
My Beloved Parents

CERTIFICATE

This is certify that the thesis entitled "PHYSICO-CHEMICAL CHANGES IN INDIGENOUS WHOLE MILK POWDER BULK PACKED AND STORED UNDER COMMERCIAL CONDITIONS" submitted by MR.RAVINDRA KUMAR in partial fulfilment of the requirements for the Award of the DEGREE OF DOCTOR OF PHILOSOPHY IN DAIRY CHEMISTRY OF National Dairy Research Institute (Deemed University), Karnal (Haryana), India, is a bonafide research work carried out by him under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

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Ravindra Kumar

(RAVINDRA KUMAR)

PHYSICO-CHEMICAL CHANGES IN INDIGENOUS WHOLE MILK POWDER BULK PACKED AND STORED UNDER COMMERCIAL CONDITIONS

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ABSTRACT

Whole milk powders (WMP) of buffalo milk were packed in high density polyethylene (HDPE) bags encased in hessian laminated kraft paper bags and stored at 20°C as well as at ambient temperature and humidity for a period of 12 months. The ambient temperature ranged from 23°-38°C and relative humidity from 18 to 100%. The powders were examined initially and during storage for physico-chemical, bacteriological and organoleptic changes.

The WMPs had high initial organoleptic scores (8.5 to 8.8). The scores decreased with storage period. However, the powders stored at 20°C remained acceptable even at the end of 12 months of storage. The powders stored at ambient temperature developed or tended to develop off-flavour at the end of 9th month of storage. The moisture content of WMP increased at an average rate of 0.06% at 20°C and 0.09% at ambient temperature and it was not influenced by relative humidity. Development of off-flavour started when the moisture content of the powder exceeded about 4%. Based on initial moisture content, guidelines were suggested for predicting storage stability of WMP. The increase in titratable acidity was very small and caused no problem in stored WMP. The solubility index of WMP increased at a slow rate during storage. But it increased rapidly when the moisture in WMP exceeded 4%. Changes in solubility was due to browning reactions. The FFA content increased slowly and was influenced by the temperature of storage. Rapid increase occurred when the moisture content of WMP exceeded 4%. The lactic acid content did not increase as there was no bacterial activity in the powder during storage. The pH of WMP decreased gradually during storage. A sudden decrease in pH coincided with development of off-flavour in WMP. The heat stability decreased considerably at a steady rate which was influenced by the temperature of storage. Onset of off-flavour in WMP coincided with a sudden decrease in heat stability. The changes in viscosity was small as long as the powders remained organoleptically acceptable. A sudden change in viscosity occurred with the appearance of off-flavour. A large number of lactones present in the powder did not show any change during storage. Autoxidative changes occurred in WMP throughout the storage period as evidenced by changes in diene conjugation, peroxide value, TBA value, UV absorption at 280 nm and carbonyl profiles. Changes in diene conjugation was not helpful in predicting onset of oxidative spoilage in WMP. Peroxide values, determined by either iodometric or colorimetric method, were not consistent. A TBA value of above 0.05 could give detectable oxidised flavour in WMP. Determinations of total carbonyl value, monocarbonyl and steam volatile monocarbonyl contents were not helpful in knowing the oxidised status of fat of WMP. The levels of n-alkanals and unsaturated carbonyls help in detecting onset of oxidative spoilage in WMP. The OD at 280 nm showed high correlationship with organoleptic scores (r = - 0.96) and sudden increase in this value coincided with appearance of off-flavour. Free fat content increased markedly when the moisture content crossed 4%. The free fat was more prone to autoxidation than total fat. The peroxide value obtained on free fat was consistent. Browning changes occurred in WMP as revealed by increase in total hydroxymethylfurfural (HMF), free HMF, reducing capacity, colour index and decrease in SH- groups. Changes in total HMF and reducing capacity showed correlationship with the development of off-flavour. The number of bacteria decreased in the powder during storage.

The quality of WMP manufactured indigenously was of good quality and storage stability. Gas packing had no significant effect on shelf-life of WMP. The powder packed in HDPE bags remained as good as that packed in tins for nine months when stored at ambient temperature.

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INTRODUCTION

1.0 INTRODUCTION

Production of milk in our country is increasing considerably every year due to rapid development taking place in dairying. Production of milk being seasonal, manufacture of dried milk is the only practical means of conserving surplus milk. Hence, dry milk industry is developing in a big way in the country.

In order to meet the demands of market supply of milk uniformly throughout a year and in places of scarce milk production, it has become necessary to use milk powders for preparation of recombined milk or reconstituted milk to supplement the shortage of fresh milk. For this purpose at present mostly recombined milk is prepared from skim milk powder (SMP) and butter oil/ white butter. In the preparation of recombined milk use of a homogenizer is essential. Besides, for storing and transporting of butter oil or white butter, stringent cold storage conditions are necessary. Hence, its preparation in smaller dairies is not economical. However, the preparation of reconstituted milk from whole milk powder (WMP) is simple and economical. It does not need the use of a homogenizer and also it avoids problems associated with the storage and transport of butter oil or white butter needed in the case of recombined milk. Thus, there is a good scope for use of WMP in our country.

Storage stability of WMP is an important factor for its practical use in our commercial conditions. A stored powder should retain, as much as possible, its original flavour, taste, reconstitutable property and heat stability. The WMP, as compared to SMP, has a much lower shelf life mainly due to the susceptibility of milk fat to autoxidation during storage resulting in the development of off-flavours. There are a number of factors affecting the shelf-life of WMP such as temperature of storage, the degree of pre-heat treatment of milk, packaging, addition of antioxidants, the initial quality of milk, etc. In our country most of the WMP is made from buffalo milk and this may also show a significantly different shelf-life as compared to that of WMP of cow, as it is well established now that the keeping quality of cow milk fat is better than that of buffalo milk fat.

In packaging of WMP, the main consideration is to prevent absorption of moisture, oxygen and external odours by the powder from the atmosphere. Tin is the ideal packing for WMP as it fully meets all the above mentioned requirements and, in addition, in tins the powder can be gas packed. But tin packaging is very costly and uneconomical for bulk packing and transport of WMP. Alternatively, flexible packaging materials are much cheaper than tin. Recently PFA rules stipulated that WMP may be bulk packed in flexible bags encased in hessian laminated kraft paper bags, provided the packs are stored below 20°C. Flexible films may not provide complete barrier properties against absorption of moisture and gases by the powder under different conditions of relative humidity and temperature of storage. Also it is difficult to gas pack the powder in flexible bags. Therefore, the effect of these two limitations, in the case of flexible packaging, on the shelf-life of WMP has to be studied in relation to that of WMP stored in tins.

It is generally believed that gas packing of powder provides better oxidative stability to the powder. However, such differences in the oxidative stabilities between gas packed and nongas packed may become minimal if high preheating conditions are used before drying of milk into powder. Based on soluble whey protein nitrogen content (whey protein nitrogen index), which is inversely related to the intensity of preheat treatment, the powders are classified into low, medium or high heated type.

In order to introduce the use of WMP in our dairies for preparing reconstituted milk, the dairy industry in the country is keenly interested in knowing the suitability of using food grade polyethylene lined kraft paper bags for bulk packing, storing and transportation of indigenously produced WMP at ambient temperature as it is the most economical method of handling WMP. Also, in order to amend the present food laws (PFA 1955) to permit the use of food grade polyethylene lined bags for bulk packing and storage of WMP for use in reconstituted milk, data are required. Keeping in view of these above mentioned facts the present research work was undertaken.

2.0 REVIEW OF LITERATURE

The main objective of converting milk into dry powder is to enhance its keeping quality. The keeping quality of powder depends on the initial quality of raw milk used, processing variables, packaging and storage conditions. Considerable research work has been done on all these aspects which has been reviewed here giving critical comments wherever needed. The scope for further work on the subject is also indicated.

2.1 QUALITY OF RAW MILK AND SHELF LIFE OF DRIED MILK

Fresh raw milk with a low bacterial load is ideal for obtaining high quality dairy products including dried milk But in practice under commercial conditions, raw milk of varying initial quality would be available for manufacturing dried milk. The raw milk may have high developed acidity due to improper handling and lack of cold storage conditions. An initial high bacterial load in the raw milk results in the powder with high initial count (Findley *et al*. 1946).

Although initial lactate content in powder is of major concern, its development during storage is not significant. Apart from the presence of high amounts of lactate, the presence of several other substances in whole milk powder (WMP) may indicate the quality of raw milk used. Presence of high amount of ammonia (Helaine, 1977), histamine and tyramine (Askar, 1979) may serve as an additional quality index of raw milk used.

In properly cold stored raw milk, if the storage period is prolonged, although development of lactic acid is arrested other defects may arise due to growth of psychrotropic bacteria which are likely to produce heat stable proteolytic and lipolytic enzymes (Law, 1979). Sometimes raw milk having high developed acidity is neutralized before drying which may ultimately affect the shelf-life of dried powder.

The effect of above mentioned changes on the keeping quality of WMP during storage has not been fully understood. This aspect of study is particularly of interest in our country as our raw milk has not reached expected bacteriological standard (Reddy et al., 1989).

Jensen (1970) observed that storage of whole milk for 3 days at 5°C prior to spray drying promoted oxidation and lipolysis in WMP after 12 months of storage in tins at 30°C. Brush and Jaartsveed (1971) reported that powder made from high count milk developed tallowy flavour earlier than did the powder from low count milk. They attributed this to the possible presence of substance of bacterial origin.

Poulsen et al. (1971) compared the storage stability of WMP manufactured from fresh raw milk and milk kept at 5°C for 3 days. They found that after storage at 30°C for

12 months the powder manufactured from milk kept for 3 days had significantly lower score of flavour as compared to that of the powder made from fresh raw milk. Development of free fatty acids and compounds resulting from oxidation was also more pronounced in the powder made from the stored milk.

It appears from the limited data available that the quality of raw milk has an influence on the shelf-life of WMP, but further investigations are needed to establish definite correlation between these two aspects.

2.2 INFLUENCE OF PROCESSING VARIABLES ON THE INITIAL QUALITY OF WMP

The initial physico-chemical properties of WMP are considerably controlled by various processing steps involved in the conversion of milk into powder. Changes in these properties of powder during its storage may also be influenced by some of these processing variables.

2.2.1 PREHEAT TREATMENT

The raw milk is first subjected to preheat treatment with three objectives (1) to reduce bacterial load in the powder (2) to increase the heat stability of milk in subsequent concentration and drying steps and (3) to enhance autoxidative stability of WMP.

Various time-temperature combinations have been tried for preheating milk. Temperatures between 75 and 95°C with holding periods up to 30 min have normally been used

(Griffin et al., 1976). Higher preheating temperatures of 99 to 120°C with shorter holding periods of 30 to 120 s have also been applied commercially (Newstead et al., 1975).

De vilder and Rimbawan, (1978) observed that some of the initial physico-chemical properties of the powder such as free sulphydryls, reducing properties, HMF content and viscosity varied due to preheating conditions. They found that these parameters directly increased with the increase in preheating temperature from 70 to 110°C. They also found a marked increase in HMF value and viscosity with the increased heating of milk concentrate from 65 to 85°C.

The other chemical changes caused due to preheating of milk are denaturation and aggregation of the serum proteins (Davis, 1959), formation of colloidal calcium phosphate and consequent reduction in calcium ion concentration (Davis and White, 1959). The reduction of calcium ion concentration increases the heat stability of milk (Walstra and Jenness, 1984).

Preheating caused formation of disulphide bonds between β-lactoglobulin and k-casein (Lyser, 1972) which increased the heat stability of milk (Newstead et al., 1977). Preheating of milk also activate sulphur containing compounds especially SH-groups. Such groups have been intensively studied for their antioxidant effect (Mattick et al., 1945; Harland et al., 1952; Boon, 1976).

2.2.1.1 HEAT CLASSIFICATION

American Dry Milk Institute (ADMI, 1971) classified SMP on the basis of its content of denatured whey protein (WPNI, Whey Protein Nitrogen Index) which depends on the heat treatment given to milk:

Low heat : more than 6.0 mg N/g fat free dry matter
Medium heat: 1.51-5.99 mg N/g fat free dry matter
High heat : below 1.51 mg N/g fat free dry matter.

This classification was also applied to WMP by Baldwin *et al*. (1981) and on the basis of which the storage stability of WMP was predicted to be 4-6 months for low heat, 6-8 months for medium heat and more than 12 months for high heat powder.

2.2.1.2 PREHEAT TREATMENT AND STORAGE STABILITY

Poulsen *et al*. (1971) stated that a heat treatment of 95°C or above should be used for manufacture of WMP with a good storage stability. Jensen *et al*. (1973) preheated milk at 65° to 125°C for 200 to 20 s and the powder made was examined before and after 3 to 18 months of storage at 30°C. They observed that heating whole milk at 95°C or above was necessary to avoid oxidation and hydrolysis of fat during storage.

Boon (1976) found that a heat treatment of skim milk at 120°C/12 s combined with heating of cream at 93°C/10 s followed by 120°C/4 s resulted in a best stability of WMP.

Tuohy and Kelly, (1981) reported that a heat treatment of 120°C results in a better storage stability of WMP than one of 80 or 90°C.

Findley *et al*. (1946) observed that reduction of bacterial load in preheated milk was proportional to the temperature of preheating from 71-94°C for 20 s. The suitability of dried milk for reconstitution purposes depends upon viscosity and heat stability characteristics which are influenced by temperature of preheating (Mec Donala and Griffin, 1974).

2.2.2 CONCENTRATION

The preheated milk is concentrated to a total solids (TS) content ranging from 40 to 50 per cent before spray drying (Knipschiedt, 1986). This step of evaporating water in a vacuum pan or evaporator gives greater economy as compared with spray drying.

De Vilder *et al*. (1979) observed that heating the concentrate from 50 to 80°C lowered its viscosity which was substantial when the TS was high. The viscosity of the concentrate is of vital importance in determining various properties of WMP such as moisture content, solubility index, particle size distribution and free fat content (De Vilder *et al*., 1979; Baldwin *et al*., 1980; Knipschiedt, 1986; Ipsen, 1989).

Baldwin *et al*. (1980) noted that the level of concentration as well as holding time of concentrate before drying greatly influenced the viscosity of the concentrate. When the TS contents were 43.8 and 48.2 % the viscosities of the concentrates were 0.7 and 1.5 poise respectively and on holding this for 20 min the viscosities increased to 1.6 and 6.8 poise respectively.

2.2.2.1 INFLUENCE OF CONCENTRATION ON PROPERTIES OF WMP

Van Den Berg (1970) indicated that a higher viscosity of concentrate resulted in a powder of higher moisture content due to coarser atomization which caused difficulty in drying. De Vilder *et al*. (1979) observed that a change of viscosity from 0.2 to 6.4 poise of the concentrate increased the moisture content of dried milk from 3.4 to 3.8 %. Baldwin *et al*. (1980) observed that as the viscosity of the concentrate changed from 0.7 to 6.8 poise, there was only a small increase in the moisture from 2.1 to 2.6 %.

De Vilder *et al*. (1979) observed that as the viscosity of concentrate increased from 0.2 to 6.4 poise the solubility index increased from 0.05 to 0.60 ml. Baldwin *et al*. (1980) also observed that when the viscosity of the concentrate increased from 0.7 to 6.8 poise there was corresponding increase in solubility index from 0.4 to 2.6 ml. Salooja (1983) found an inverse relationship between viscosity of the concentrate and solubility index of the powder.

Strakhov and Favstova (1971) observed an increase in bulk density and mean particle diameter of the powder, as the viscosity of the concentrate increased. Jensen and Hansen (1974) observed that increase in viscosity of concentrate increased bulk density due of the powder to decrease in enclosed air and vacuole volumes. De Vilder *et al.* (1979) found that increase in viscosity of concentrate from 0.2 to 6.4 poise increased bulk density of WMP from 0.51 to 0.61 g/cm^3. Kerekrete (1980) found that concentrate with higher viscosity resulted powder with high bulk density. A slight increase in particle density of milk powder was observed when more viscous concentrate was employed for drying (Baldwin *et al.*, 1980; Snoren *et al.*, 1981).

De Vilder *et al.* (1979) reported that as the viscosity of the concentrate increased from 0.2 to 6.4 poise there was a decrease in the free fat content of WMP from 11.0 to 4.7 %.

2.2.3 HOMOGENIZATION

Homogenization of concentrate before spray drying is an optional step in manufacture of WMP. Homogenization mainly helps in reducing free fat content of the powder by reducing the size of the fat globules (Buma, 1971a). However, the homogenization effect in reducing the free fat is substantial, only when concentrates are having low TS content. At higher TS, its effect is reduced and it is not significant when the TS exceeded 50 to 52 % (De Vilder et al., 1974).

2.2.3.1 INFLUENCE OF HOMOGENIZATION ON PROPERTIES OF WMP

De Vilder et al. (1979) reported that homogenization caused only a small increase in viscosity when the TS in the concentrate was low (43.2 %). When the TS of the concentrate was higher (49.1 %), two stage homogenization caused a very little increase in the viscosity (0.8 to 1.0 poise) but one stage homogenization increased the viscosity drastically (0.8 to 8.3). However, when the TS content was very high (54.7 %) the change in the viscosity, either due to one stage or two stage homogenization, was very high (0.8 to 78 or 44 poise respectively). Hence homogenization cannot be adopted when the TS is very high.

Mol (1975) observed that homogenization of concentrate had an adverse effect on the solubility of WMP. This was attributed to the adsorption of casein micelles on the surface of newly formed minute fat globules derived from disruption of original fat globule, due to homogenization.

Hall and Hedrick (1975) reported that WMP made from homogenized concentrate had better keeping quality, prevented fat separation during reconstitution process and lower viscosity (of reconstituted milk) as compared to one made from unhomogenized concentrate. Zbikowski et al. (1978) determined certain chemical changes in the WMP made from homogenized concentrate milk (110-130 atm at 50-60°C) and control. There was decrease in free fat content from 17-37% to 5 to 6% of the powder, due to homogenization. After storing the powder for 6 months, the short chain fatty acid content was higher and the poly unsaturated fatty acid (PUFA) content was lower in WMP made from homogenized concentrate than in control.

Rotkiewicz (1979) that due to homogenization, the moisture content decreased from 3.99 to 3.54%, ferricyanide reducing value from 5.92 to 5.67 and free fat from 35.6 to 15.4 when compared to control. However, he observed that the WMP made from homogenized milk had a lower oxidative stability as compared to control. This is in contradiction to the observation made by Hall and Hedrick (1975). Mathur (1984) reported that homogenization should not cause undue increase in viscosity of the concentrate as it would cause problem during spray drying.

2.2.4 SPRAY DRYING

The conditions of spray drying such as inlet and outlet air temperatures, the type of atomizer, and the temperature to which the powder is cooled affect the physico-chemical properties of the dried milk. Recent innovations in the spray drying process such as two stage and three stage drying methods to achieve better thermal efficiency in drying process have also helped in obtaining powder with improved solubility and free fat characteristics (Ferrgusson, 1989).

2.2.4.1 EFFECT OF INLET AIR TEMPERATURE

The inlet air temperature determines the major energy input into the drier. Inlet air temperatures may range from 145° to 260°C. King and Sanderson (1970) indicated that a high inlet air temperature gave a powder with lower bulk density which they attributed to sudden evaporation of moisture.

Verney (1972) observed, during manufacture of SMP, that when the inlet air temperature was raised from 120 to 150°C, it increased the vacuole volume, moisture content and solubility index and decreased bulk density and mean particle density of the powder. It also decreased permeability of nitrogen into the powder. Bloore and Boag (1982) observed that higher inlet temperature (225°C) affected adversely the solubility index, bulk density, and particle density. They attributed this to greater heat damage to the powder at high inlet air temperature.

Knipschiedt (1986) observed that increasing inlet air temperature from 170 to 225°C and keeping the outlet temperature constant at 90°C caused an increase in moisture content (2.6 to 3.3%), solubility index (0.05 to

0.30 ml) and decrease in free fat content (6.6 to 4.1%) and bulk density (0.68 to 0.62 g/ml). The increase in solubility index and decrease in free fat content was attributed to the formation of hard particle surface of the powder due to higher inlet temperature. The decrease in bulk density with increasing inlet air temperature, inspite of increased moisture content, was believed to be due to increased entrapped air in the powder particles.

2.2.4.2 EFFECT OF OUTLET AIR TEMPERATURE

The outlet air temperatures of 72-100°C are employed in the manufacture of various dried milk products. Mol (1974) observed that an increase in moisture content and decrease in solubility index and bulk volume were due to lowering of outlet air temperature of drying.

De Vilder *et al.* (1976) noticed that increase in outlet air temperature from 70 to 105°C, while keeping the inlet air temperature at 195°C, decreased the moisture from 5.96 to 1.66%, bulk density from 0.66 to 0.54 g/cm^3, mean particle density from 1.14 to 0.98 and increased solubility index from 0.1 to 2.2, nitrogen penetration from 1.0 to 5.16 cm^3/ 100 g, vacuole volume from 8.25 to 24.43 cm^3/100 g and free fat from 7.42 to 29.44%.

Brummelhuis (1975) found that when the outlet air temperature was raised, there was an increase in solubility index and free fat content and decrease in moisture content and a particle density of the powder. At caused not only denaturation of whey proteins, which adversely affected the solubility index, but also caused expansion of the air vacuoles, which resulted in a lower bulk density and lower mean particle density. The high free fat content and high penetration of nitrogen into the powder was ascribed to vacuole expansion which caused formation of cracks on the surface of the particles of the powder.

In order to control the moisture content of the powder, a balance between inlet and outlet air temperatures has to be maintained. As a rule, the outlet air temperature must be increased by 1°C for each 5°C increase in inlet air temperature (Knipschiedt, 1986).

2.2.4.3 EFFECT OF ATOMIZATION

The atomization conditions are central to spray drying process, and they play an important role in defining such powder properties as particle size, particle density and bulk density through their influence on droplet size. These powder properties also depend on viscosity, temperature, concentration of the material being dried and inlet air temperature (Bloore and Boag, 1982).

The effects of atomizing pressure and nozzle orifice size on the physical characteristics of WMP were investigated by Tracy *et al.* (1951) using spraying system

SX type nozzles. They found that for a constant nozzle size, the bulk density increased with increasing atomizing pressure and decreased with increasing nozzle size at constant pressure. When the nozzle size was increased at constant feed rate and the pressure was allowed to fall, the bulk density increased.

De Vilder et al. (1976) obtained lower moisture content in the powder, without changing the inlet or outlet air temperature, but by increasing the degree of atomization of concentrated milk. This was attributed to larger surface area which caused a quicker evaporation of water. They also found that increasing the degree of atomization improved the solubility of the powder. However, free fat content and nitrogen penetration of the powder were unaffected.

2.2.4.4 EFFECT OF COOLING

Dry milks should be exposed to least amount of heat once the desired moisture content is reached. In WMP the cooling becomes indispensable as prolonged exposure to heat after manufacture induces discoloration and fast oxidative reaction during storage (Hunziker, 1949). Such exposure also significantly increased the free fat content of WMP (Hall and Hedrick, 1975).

Kunkel and Coulter (1947) showed that flavour of spray dried ice cream mix was adversely affected by holding the powder at 54°C for 36 hours or at 60°C for 24 *hours.* Bullock (1959) found that rapid cooling of the dried milk and subsequent conditioning treatment to melt the milk fat gave an improvement in the dispersability which lasted during 6 month storage at 6°C.

Kliman and Pallansch (1968) demonstrated that holding of SMP at elevated temperatures immediately after drying had no significant effect on its quality if the moisture content of the powder was less than 4%, provided holding temperatures was not more than 50°C and holding time not more than 24 hours.

2.3 CHARACTERISTICS OF WMP

The desirable characteristics of freshly made WMP may be described by a number of physico-chemical parameters. Mainly they are moisture content, pH, acidity, bulk density, particle density, wettability, dispersability, solubility, flowability, whey protein nitrogen index and free fat content. All these properties in the powder are controlled by adopting suitable processing variables (Woodhams and Murray, 1974; De Vilder et al., 1976).

2.3.1 CHEMICAL COMPOSITION

The quality of WMP is defined by certain specifications. These specifications are requirements in terms of maximum and minimum contents of some components, minimum level of desired characteristics and maximum levels of possible defects. In this regard different countries have evolved their own specifications either to meet the demands of international market or for utilization within the country to suit for intended final use.

Some of the national and international standards for spray dried whole milk powder are given below.

Table I: Indian Standards for spray dried WMP
(IS: 1165-1975; IS: 1165-1986)

Flavour and Odour	Clean	
Moisture per cent by mass,	max.	4.0
Total solids (milk solids and added salts) per cent by mass,	min.	96.0
Solubility index (ml)	max.	2.0
Total ash (on dry basis) per cent by mass,	max.	7.3
Fat, per cent by mass	min.	26.0
Titratable acid (lactic acid), percent by mass	max.	1.2
Bacterial count per gram	max.	50,000
Coliform count per gram	max.	90

Table II: ADMI Specifications for spray dried WMP
(ADMI,1971)

	Premium	Extra	Standard
Milk fat, %	26.00	26.00	26.00
Moisture, %	2.25	2.50	3.00
Titratable acidity, %	0.15	0.15	0.17
Bact. estimate per g.	30,000	50,000	100,000
Solubility Index, ml	0.50	0.50	1.00
Scorched Part, mg.	7.5	15.00	22.50
Copper, ppm	1.5	10.00	15.00
Iron, ppm	10.0	10.00	15.00
Oxygen, % *	2.0	3.00	-

* Requirements for gas packed products only.

2.3.2 MICROBIOLOGICAL QUALITY

The usual manufacturing procedure for WMP results in the destruction of most of the micro organisms present in the original milk. A greater destruction of micro organisms is brought about by the use of high preheat treatment of the milk or concentrate. Those micro organism which remain in the powder do not show any activity because of low moisture content of the powder (Findley et al., 1946).

The microflora of spray dried milk are made up of thermoduric micrococci, thermoduric streptococci and corynebacteria, aerobic spore formers and miscellaneous organisms. The Prevention of Food Adulteration Rules (1955) prescribe a maximum count of total bacteria of 50,000 and coliform of 90 per gram. Indian standards Institution (IS: 1165-1975) also specify similar microbiological standards for milk powder.

2.4 CHANGES IN PHYSICO-CHEMICAL CHARACTERISTICS OF DRIED MILKS DURING STORAGE

During storage the various characteristics or properties of WMP may change depending on the conditions of storage causing deterioration in the quality of the powder. The conditions of storage are temperature of storage, packaging and relative humidity. The changes in some of these parameters in dried milks are reviewed hereunder.

2.4.1 MOISTURE

Moisture appears to be the major factor which causes significant changes during storage. In general, for WMP to have good keeping quality the moisture content is kept below 4%. Packaging is the most important factor to prevent absorption of moisture from surroundings. The amorphous form of lactose in dried milk, makes the product hygroscopic (Hunziker, 1949).

Tarassuk and Jack (1948) found that moisture content of above 4% was not desirable as it caused accelerated oxidative and browning reactions in WMP, resulting in off-flavour development. Henry et al. (1948) stored SMP of high (7.3%), medium (4.9%) or low (2.9%) moisture contents at 20°, 28° and 30°C and concluded that moisture had the greatest effect on the storage life of the powder.

Absorption of moisture during storage has an adverse effect on the properties of the powder. Boon and Woodhams (1974) observed that absorption of high amounts of moisture (5.5 to 7.0%) during storage led to crystallization of lactose which in tern caused lumpiness and accelerated browning reactions.

Kopecky (1978) reported that absorption of moisture increased free fat, favoured browning reactions and also decreased solubility of WMP. Warburton and Pixton (1978) detected crystallization of lactose when the moisture content of the powder was between 7 and 9.5%.

Buma (1966) and Warburton and Pixton (1978) studied the physical structure of dried milk particles by Scanning Electron Microscope and found that microstructure of powder particle was considerably changed by absorption of moisture during handling and storage of the powder. Woodhams and Murray (1974) showed that large particles contained a high amounts of moisture. Thus, if WMP of large particles are produced during spray drying, either due to inadequate atomization or it is done deliberately, it contains high amounts of moisture.

Ardito et al. (1980) compared the relative rates of increase of moisture content in SMP packed in three different packaging materials namely nitrogen flushed tins, pigmented low density polyethylene film of 95 micron thick and paper LDPE-AL-LDPE laminates and stored at 23°C and 30°C with relative humidities 65 and 80% respectively. They observed, that after six months of storage, the moisture content did not increase in tins, but in other packagings it increased 2.8 to 5.3%.

In foods, water activity (a_w) rather than the total moisture content is more closely related to physico-chemical reactions and biological activity. The a_w of the food is defined as the ratio of vapour pressure of water in food to the vapour pressure of pure water at the same temperature (Labuza, 1980).

During storage of spray dried milk powder, lactose which is in amorphous state shifts towards a_w more crystalline thermodynamically stable state. As crystals bind less water than in the amorphous state, the vapour pressure of the powder shifts towards a higher value on crystallization, thereby increasing the water activity. Van Den Berg (1987) reported that a moisture content of 3.5% in milk powder corresponds to a_w of 0.1. Morr (1989) reported that dried milk products with a_w of about 0.1 were readily susceptible to lipid oxidation and associated off-flavour development.

2.4.2 TITRATABLE ACIDITY AND LACTIC ACID CONTENT

Determination of titratable acidity or lactic acid content is mainly concerned with the initial quality of dried milk, as bacterial activity and consequent production of lactic acid is not expected in dried milk during storage. The titratable acidity of WMP of good quality should not exceed 1.2% (IS: 1165-1975). Higher levels of titratable acidity indicate the presence of developed lactic acid in the raw milk used. However, titratable acidity is not dependable when neutralizers are used to reduce the acidity of raw milk. Therefore, determination of lactic acid content has the advantage that it is not affected by neutralization of raw milk (Lawrence, 1970). The EEC specification for SMP prescribed 1.50 mg lactic acid/100 g as maximum limit.

This corresponds to 1.1 mg lactic acid/100 g of WMP (Pisecky, 1987).

Henry et al. (1948) found that nitrogen packing and low storage temperature reduced the rate of change of pH during storage. However, there was a gradual increase in titratable acidity during storage. Coulter et al. (1951) attributed such increase in titratable acidity to the possible tying of amino groups of protein with lactose during storage.

Korhonen et al. (1976) found no change in lactic acid content during storage of dried milk packed in polyethylene for 3 months at 30°C and 43-84% RH. Pijanowski et al. (1978) found that the lactic acid contents of WMP manufactured in Poland ranged from 46 to 614 mg/100 g powder. Further they observed that the lactic acid content correlated directly with free fat content and inversely with solubility index.

Whole milk powder stored in polyethylene bags at room temperatures for 6 months showed practically no change in acidity (Vasic et al., 1971; Valent, 1977). Skim milk powder stored in 0.3 mm thick polyethylene films and in tins showed only a slight increase in acidity (Peschek, 1983). The WMP stored for 8 months at ambient temperature showed a very small increase (0.1%) in acidity. A gradual decrease in pH from 6.80 to 6.69 was noted in high heat SMP on storage for 12 months at 30°C (Kieseker and Clarke, 1984).

Mrithyunjaya and Bhanumurthi (1987) prepared five batches of WMP from buffalo milk and found that the titratable acidity ranged from 0.63 to 0.72%. Arora (1989) found that the titratable acidity widely ranged from 0.22 to 2.44% in different brands of spray dried SMP manufactured in India.

2.4.3 SOLUBILITY

Solubility is one of the most important properties of milk powder as it is directly related to the reconstitutability of the powder. Whole milk powder of very high solubility is now produced because of advances in spray drying process. Low solubility in powders is encountered only in the case of roller dried powders due to extensive denaturation of proteins (Woodhams and Murray, 1974; Baldwin *et al*., 1981; Ferrgusson, 1989).

Litman and Ashworth (1957) reported that solubility of WMP was unaffected when stored at low temperature but was decreased when stored at high temperature. The protein-carbohydrate interaction (Maillard browning) was reported to cause decrease in solubility during storage of dried milks (Henry *et al*., 1948; Litman *et al*., 1956; Cobos, 1979).

Mrithyunjaya (1982) observed an increase in solubility index from 1.07 to 1.80 ml in buffalo WMP on storage at 30°C for 6 months. An increase in solubility index from 0.09 to 0.77 ml was observed in low lactose infant formula on storage at 30°C for 12 months (Paul, 1984). Solubility index (expressed as Insolubility Index) in high heat SMP increased from 0.48 to 0.83 on storage at 30°C for 6 months (Muir *et al.*, 1986).

Kieseker and Clarke (1984) reported that initial solubility indices for low- and medium- heated SMP were less than 0.1 ml. This value did not change with low heat powder irrespective of storage temperature, while small increases were noted with medium- heat SMP held at higher storage temperatures. The high- heat SMP showed an initial solubility index of 0.2 ml which virtually remained unchanged after storage at 10° and 20°C for 12 months. The same powder held at 30°C showed slow increase in solubility index to 0.4 ml in 12 months, and powder held at 40°C reached 1.0 ml in 6-8 months.

2.4.4 FREE FAT

Usually free fat is defined as that fraction of the fat which can be extracted from WMP with organic solvent under standardized conditions. The free fat is also termed as unprotected fat or non globular fat (Buma, 1971a). Considerable importance has been given to the studies on initial free fat content and its development during storage in relation to the flavour deterioration in WMP (Buma, 1971d).

Litman and Ashworth (1957) reported that temperature of storage had a significant effect on the increase in the free fat content of WMP. Buma (1971c) observed that free fat content did not significantly influence the development of oxidation in WMP kept for 6 months in the presence of air at 30°C, but decreased solubility and wettability. Vasic et al. (1971) found no change in free fat content in WMP stored in polyethylene bags for 6 months at room temperature. Pijanowski et al. (1974) observed an inverse relationship between free fat content and wettability.

Puzikova and Efimova (1974) reported that the initial free fat content of WMP ranged from 0.6 to 22.7%. However, Miocinovic and Ostojic (1983) reported a much narrow range of 1.2 to 3.0%. Kopecky (1975) observed an increase in free fat content from 4 to 22% during storage with a considerable decrease in dispersability. Further, he found a higher rate of oxidation in free fat than in bound fat. However, increased free fat content does not have a definite effect on the quality and shelf-life of WMP stored under normal conditions.

Buma (1971b) reported that the free fat content was higher in roller dried than in spray dried milk powder. Truyen and Orsi (1977) observed that free fat contained more unsaturated fatty acids than did the bound fat. Tran The Truyen (1977) indicated that higher RH and higher temperature of storage significantly increased the free fat content of the WMP. Rotkiewicz et al. (1978) attributed this increase in free fat content to crystallization of lactose due to absorption of moisture by WMP from the surroundings. However, the moisture absorption during storage was directly related with temperature of storage, RH of the surroundings and packaging material. Further Rotkiewicz (1979) reported the absence of lactose crystals in fresh WMP. During storage of WMP in polyethylene bags at 20°C and RH of 88%, the moisture content increased from 3.80 to 5.0% and α-monohydrate crystal influenced not only the free fat content but also other physico-chemical properties of the powder.

Buma (1966) reported that particle size of milk powder strongly influenced the free fat content. Reinke et al. (1960) obtained WMP with low free fat level by adopting lower preheat treatment of milk, using whole milk instead of recombined milk, lower heat treatment of concentrated milk and using large nozzle orifice at low pressure during spraying.

2.4.5 HEAT STABILITY AND VISCOSITY

The stored powder when reconstituted should show not only acceptable organoleptical properties but also good heat stability and normal viscosity, as the reconstituted milk is expected to be used in manufacture of various products.

Heat stability of milk may be defined as the ability of milk proteins, particularly casein system, to remain in colloidal suspension when milk is subjected to heating.

It is determined as heat coagulation time (HCT) by heating the milk at a particular temperature (120°, 130° or 140°C), in temperature controlled oil bath till there is an appearance of first clot.

O' Sullivan (1971) indicated that an acceptable heat stability characteristics could be obtained by using high heat powders based on the classification established by the American Dry Milk Institute (1971). However, this method of selection was not reliable as the use of milk owders with a WPNI of less than 1.5 mg of undenatured whey protein nitrogen per gram of powder in many instances, resulted in evaporated milk with unacceptable viscosity or which coagulated during sterilization (Kieseker et al., 1984). Recently, Kieseker and Aitken (1988) developed an objective method for assessment of heat stability characteristic of both SMP and WMP. This method is based on determining the time required for 26% dispersion of WMP (8% fat and 18% SNF) to thicken or coagulate when held at 120°C. A minimum time of 21 min was found necessary for the manufacture of a product with acceptable viscosity and heat stability characteristics.

Kieseker and Clarke (1984) reported that powders which have been subjected to high temperature preheating during manufacture only have a satisfactory level of heat stability. Further, they reported a decrease in heat stability of high- heat SMP from 27 min to 9 min during maintained an acceptable standard of heat stability over the 12 months storage period.

Kieseker _et al_. (1984) observed that WMP made from unhomogenized milk had lower heat stability as compared to that made from homogenized milk. They concluded that while changes may occur in the heat stability characteristic of WMP, the powders can be stored satisfactorily at temperatures from 3 to 30°C over a 12 month period.

Sargent _et al_. (1959) studied the relation between the heat stability of reconstituted SMP and the degree of hardness of water used for reconstitution and found that the heat stability was adversely affected by high calcium and magnesium levels in water. Sweetsur (1976) examined the stability of instant SMP when added to hot coffee and showed that the formation of insoluble material was inversely related to the coagulation time of the reconstituted powder, measured at 140°C, and directly related to the casein number of milk protein.

The heat stability characteristics of milk powder are largely determined by the heat stability of original milk and the nature of processing treatment (Muir _et al_., 1978). Studies on variations in heat stability of milk and milk products have shown that there is a marked seasonal effect which limits the period during which heat stable SMP can be produced and this period may be further limited by changes in weather conditions, where periods of drought or prolonged wet weather may cause a decrease in heat stability (Kieseker and Pearce, 1978).

According to Muir *et al.* (1978) and Kelly (1981) the effect of pH on heat stability can be most marked, and small alterations in pH due to either seasonal variations or to heat treatment can be effective in changing the heat stability characteristics of milk. In fact the adjustment of pH either before or after preheating has been used with variable success to improve the heat stability of the powder. Other factors which may influence the heat stability of the powder, include adjustment of mineral content and composition (Newstead, 1977; Kieseker, 1982) addition of aldehydes and sugars (Holt *et al.*, 1978), addition of urea (Muir and Sweetsur, 1976; Muir *et al.*, 1978) and hydrolysing the lactose (Kelly, 1981).

The inherent viscosity of non fat milk powders, which is largely established during preheating stage in powder preparation, plays an essential role in the manufacture and subsequent viscosity pattern of reconstituted or recombined condensed milk. Unlike heat stability there exists no definite correlation between WPNI and viscosity. For this reason WPNI cannot be used as a reliable basis for selection of powder for manufacturing evaporated and condensed milk of desired viscosity (Kieseker, 1973).

Kieseker and Clarke (1984) showed that viscosity of SMP was affected during storage at different temperatures. The viscosity of low- heat powder, which was higher initially than medium- heat counter part, increased during storage at 40°C to such extent as to be unacceptable for further use within 4 months. The same powder held at 30°C reached the upper limits of the acceptable range of viscosity of 55-60 poise within 10 months. The medium-heat powder while affected to some extent by storage at 40°C remained practically unchanged when held at 20° and 30°C. Although the reason for change in viscosity during storage is not known, Newstead et al. (1978) attributed this to possible decrease in pH during reconstitution of the powder.

2.4.6 AUTOXIDATIVE CHANGES

Autoxidation of fat is reported to be the major cause of flavour deterioration in WMP during storage (Coulter et al., 1948; Radema, 1954; Boon, 1973; Baldwin and Humphries, 1976; Baldwin et al., 1981; Hall and Lingnert, 1984; Renner, 1988). The course of autoxidative changes of milk fat in WMP is expected to be same as the one that occurs in anhydrous milk fat (Coulter et al., 1951). But the factors affecting the rate of autoxidation could be different as the milk fat will be in intimate contact with other components of powder which may directly or indirectly interact with autoxidative reactions. For instance the solid-not-fat in the powder may compete with milk fat for oxygen absorption (Shipstead and Tarassuk, 1953). Thereby, showing greater absorption of oxygen in powder which develops oxidation slowly than by one which develops oxidation at a faster rate.

The autoxidative reactions in WMP are affected by a number of factors such as (i) quality of milk, (ii) preheat treatment given to the milk, (iii) addition of antioxidants, (iv) packaging and (v) temperature and RH during storage (Ipsen, 1989). The oxidative stability of WMP made from buffalo milk may be significantly different from that made from cow milk in view of the well established observation that cow milk fat is more resistant than buffalo milk fat against oxidation (Rama Murthy and Narayanan, 1974). This difference in oxidative stability has been attributed to the presence of higher amounts of tetraenes and pentaenes in buffalo than in cow milk fat and these fatty acids are highly susceptible to autoxidation (Rama Murthy and Narayanan, 1973).

The rate of autoxidation is also shown to be influenced by the degree and nature of unsaturation of fatty acid. For instance, the relative autoxidation rates quoted for methyl ester of oleic, linoleic and linolenic acid are 1:12:24 and 1:100:1000 for methyl esters of sterate, oleate and linoleate (Gunstone, 1958). Seawson and Stein (1970) found that the comparative autoxidation susceptibility of fatty acid esters with 0 to 6 nonconjugated double bonds was a linear function of the number of double bonds.

The typical pattern of behaviour of milk fat during storage is that it will show some inherent resistance against autoxidative changes during certain initial part of storage period. This part, during which there are no detectable autoxidative changes, is known as induction period. At the end of the induction period, autoxidation is initiated and thereafter the rate of autoxidation increases with increase in storage period (Badings, 1960; Frankel, 1979; Rossell, 1983).

2.4.6.1 MECHANISM OF AUTOXIDATION

The mechanism of autoxidation in milk fat is now well established. It is highly complex in nature which produces a large variety of primary and secondary oxidation products. The present concept of autoxidation is largely based on the hydroperoxide theory propounded by Farmer and Sutton (1943). According to this theory, oxidation of unsaturated fatty acids proceeds through a free- radical chain mechanism involving initiation, propagation, and termination steps.

The hydroperoxides are primary oxidation products. They do not possess any off-flavour. They are very unstable and readily undergo further reaction to give rise to mainly aldehydes belonging to the carbonyl classes of n-alkanals, alka-2-enals and alka-2,4-dienals. These carbonyls are mainly derived from PUFA during storage (Ellis *et al.*, 1961; El Negoumy *et al.*, 1961; Hammond and Hill, 1964; Raghuveer and Hammond, 1966; Kinsella, 1969).

Table III shows decomposition products of oleic, linoleic and linolenic acids. Hence, it is evident that different PUFA can produce different carbonyls (Badings, 1960). Among the carbonyls produced the unsaturated ones possess strong off-flavours (Patton *et al.*, 1959; Swoboda and Lea, 1965; Frankel, 1979).

Table III: Decomposition products of hydroperoxides of oleic, linoleic and linolenic acid

Fatty acid	Hydroperoxides	Secondary autoxidation products
Oleic acid	8-OOH	Decanal, 2-Undecenal
	9-OOH	Nonenal, 2-Decenal
	10-OOH	Octane, 1-Octanol, nonenal
	11-OOH	Heptane, 1-heptanol, octanal
Linoleic acid	9-OOH	3-Nonanal, 2,4-Decadienal
	13-OOH	Pentane, 1-pentanol, Hexanal
	10-OOH	2-Octene, 2,-Octen-1-ol, 3-nonenal
	12-OOH	Hexanal, 2-Heptenal
Linolenic acid	9-OOH	3,6-Nonadienal, 2,4,7 decatrienal
	12-OOH	3-Hexenal, 2,4-Heptadienal
	13-OOH	2-Pentene, 2-penten-1-ol, 3-hexenal
	16-OOH	Ethane, ethanol, propional
	10-OOH	2,5-Octadiene, 2,5-Octadien-1-ol, 3,6-nonadienal
	15-OOH	Propional, 2-Butenal

2.4.6.2 METHODS FOR DETERMINATION OF OXIDATIVE CHANGES

Measurement of autodixative changes in milk fat, in general, is carried out by either organoleptic examination which is subjective in nature or by chemical methods which give objective assessment of degree of oxidation. In the chemical methods either the primary oxidation products namely hydroperoxides or the secondary oxidation products mainly, the carbonyls are estimated. However, the detection of appearance of such products of oxidation, only indicate the end of induction period of milk fat and at that stage it would not be possible to prevent autoxidation which has already setin. Therefore, measurement of induction period by accelerated induction period tests is of more practical use than measuring the products of autoxidation (Frank et al., 1982).

2.4.6.2.1 INDUCTION PERIOD

Induction period in milk fat is measured by different accelerated tests such as, Schaal oven test, oxygen absorption method and active oxygen test (Williams, 1966; Rossell, 1983). The principle involved in these tests is the measurement of time required to start rapid absorption of oxygen when fat is exposed to oxygen at elevated temperatures and the extrapolation of the time observed to that at normal storage temperature of the product. These tests have been helpful in predicting shelf-life of stored milk fat (Hadorn and Zurcher, 1974; Frank et al., 1982). However, the application of these methods have not been successful in the case of WMP because of absorption of oxygen by SNF of the powder during the tests. The longer the induction period of the fat oxidation, more oxygen is relatively used up by the SNF fraction (Shipstead and Tarassuk, 1953).

2.4.6.2.2 DIENE CONJUGATION

Oxidation of PUFA is accompanied by conjugation of double bonds which can be measured by the increase in UV absorption at 233 nm due to diene conjugation. Farmer and Sutton (1943) indicated that diene conjugation increased proportionately to the uptake of oxygen and to the formation of peroxides in the early stages of oxidation. On the other hand, Privett and Blank (1962) indicated that the plateau of the UV absorption curve always occurred just prior to the end of the induction period. Thus, they concluded that diene conjugation preceded the actual formation of detectable amounts of hydroperoxides. Inspite of these interesting observations there has not been any method to measure oxidative changes based on changes in diene conjugation either applied to the milk fat or to the WMP.

Br"chkova (1976) determined the UV absorption of the fat extracted from dried milk products which were stored up to 30-60 days and observed a characteristic maximum at 277 nm for all the products. In the products containing milk fat, only the maximum tended to increase with the increase in duration of storage. But in the samples containing 15% vegetable fat, the maximum was lower in 60 days than in 30 day stored samples. No organoleptic changes were detected in the stored products.

Helal et al. (1976) observed an increase of diene conjugation in control as well as in samples containing Nordihydroguaiaretic acid (NDGA) plus citric acid and the Tween 60 during storage for 6 months. They found an increase in diene conjugation value to 1.81%, 1.29% and 1.45% respectively and concluded that addition of antioxidant or emulsifier to the milk powder retarded the formation of conjugated dienes. However, the emulsifier was less effective than antioxidant.

Radaeva et al. (1976) studied the changes in the ratio of saturated, unsaturated and polyunsaturated fatty acids in the fat of WMP during storage. They found a considerable decrease in the ratio of saturated and PUFA. El-Alamy et al. (1979) reported that buffalo WMP with increased content of PUFA showed a poor oxidative stability as measured by TBA value.

2.4.6.2.3 PEROXIDE VALUE

Peroxides in milk fat can be estimated either by the iodometric method of Swoboda and Lea (1958) or by the ferric thiocyanate method of Loftus Hills and Theil (1946). The iodometric method has been widely used in case of anhydrous milk fat or ghee because of good correlation between the developments of peroxides and off flavours

(Smith et al., 1958; Fioriti et al., 1974; Narasimha Murthy, 1983).

Coulter et al. (1951) doubted the suitability of applying iodometric method to WMP because of the problems encountered in the isolation of fat from the powder. The fat isolation may be incomplete or the peroxides may be destroyed during extraction.

Loftus Hills and Theil (1946) applied the ferric thiocyanate method of determination of peroxide value to WMP and found that the colorimetric method was suitable and also more sensitive than the iodometric method. Coulter et al. (1951) also recommended this method for applying to WMP.

Rotkiewicz (1979) reported an increase of peroxide value from 3.22 to 9.39 in WMP stored for 6 months at 20°C. However, Mrithyunjaya (1982) found no peroxide development during storage for 6 months at 37°C.

Kieseker et al. (1984) determined peroxide value by ferric thiocyanate method on fat isolated from low, medium and high- heat whole milk powders stored at 3°, 25° and 30°C for 12 months. Lowest values were associated with high heat powder. On the other hand low and medium- heat powders exhibited higher values which increased considerably as the storage period progressed. Within heat treatment, lowest peroxide values were associated with powder stored at 25°C with somewhat higher peroxide levels developing in the same powder stored at 3 and 30°C. Although they found a correlation between peroxide development and flavour score, the flavour deterioration was not specifically characterised as oxidized flavour.

During storage the flavour score decreased, but the high- heat powders were still regarded as acceptable even at the end of 12 month storage. The medium- heat powders became unacceptable after 8 months and low- heat powder after 4 months of storage. The flavour stability exhibited by high heat powder is presumably due to development of SH-groups during preheat treatment of the milk at high temperature.

Tuohy et al. (1981) studied the peroxide value of WMP at elevated temperature of 60°C. They reported a peroxide value of 31 after 8 months of storage. But they did not observe any relationship between peroxide value and development of oxidized flavour.

2.4.6.2.4 TBA VALUE

The thiobarbituric acid (TBA) value is another commonly recommended method for the measurement of lipid oxidation based on the estimation of malonaldehyde which is one of the secondary oxidation products (King, 1956; Gray, 1978; Ward, 1985; Pikul et al., 1989).

Hamn et al. (1968) found good correlation between TBA values and peroxide values during oxidation of stored milk fat. Gaba and Jain (1973) found higher initial TBA values in fresh buffalo than in cow milk fat.

Application of TBA value test to WMP has the advantage that it can be applied directly to the WMP without extraction of the fat, unlike in the case of peroxide estimation wherein fat has to be extracted. Mettler (1973) subjected WMP directly to steam distillation and applied TBA test to the distillate and suggested that the method was useful in following oxidative changes in WMP. However, he could not obtain a correlation between TBA value and oxidized flavour in the powder.

Abd El-Salam _et al_. (1973) found that TBA value of over 0.080 coincided with the first appearance of oxidative flavour in buffalo WMP and infant food and hence, suggested that a TBA value of over 0.080 could be taken as an index of oxidative rancidity in WMP.

Some authors (Hamn _et al_., 1968; Phole _et al_., 1964; Fioriti _et al_., 1974; Singh _et al_., 1979) found that the change in TBA value during storage of milk fat was quite erratic, inconsistent and showed no definite correlation with flavour and keeping quality of milk fat. In contrast, Dunkley and Jennings (1951), Gaba and Jain (1973) and Pokorny _et al_. (1985) reported that TBA value was satisfactory for evaluating autoxidation in milk fat during storage. Narasimha Murthy (1983) reported that the TBA value correlated well with the development of oxidative flavour in both cow and buffalo ghee during storage.

Dowrschak and Czuczy (1975) found a gradual increase in TBA value in baby food stored at room temperature for 2 years. However, they have not indicated any correlationship between TBA value and development of oxidized flavour.

Valent (1977) observed that the initial TBA value of WMP ranged from 6.8 to 7.7 and it increased to 17.4 - 27.7 during storage at 20°C for 7 months. In the powder stored at 37°C for the same period, the TBA value increased to 26.1-31.3. Rotkiewicz (1979) also observed changes in TBA value in WMP stored at 20°C for 6 months and the average increase in seven different samples was 0.068.

Tuohy _et al._ (1981) studied the oxidative changes in spray dried WMP packed under air in polyethylene bags and stored at 12°, 37° and 60°C. Very little change was observed in powder stored at 12°C even at the end of 12 months of storage. In the case of powder stored at 60°C, extensive oxidation was noted at the end of 8th week. The TBA value went up to 0.30 and peroxide value up to 31.0. In the powder stored at 37°C the oxidized flavour developed at the end of 9 months when the TBA value crossed 0.05.

Ipsen and Hansen (1988) observed increase of TBA value in WMP stored at 30° and 38°C. A significant interaction on the TBA value with raw milk quality, preheat treatment, storage temperature and storage time was recorded. They found that medium- heat powder can be stored for 70 weeks at 30°C without a significant deterioration in flavour quality.

2.4.6.2.5 CARBONYL CONTENT

Several carbonyls are the natural constituents of milk fat among which monocarbonyls comprising mostly methyl Ketones (80%), n-alkanals (12%), alk-2-enals (4%) and trace amount alka-2,4-dienal are the major classes of carbonyls (Bhat *et al.*, 1981). However, monocarbonyls are also produced during autoxida-tion of milk fat which are mainly made up of n-alkanals, alk-2-enals and alka-2,4-dienals (Lillard and Day, 1961; Lea and Swoboda, 1962; Boon *et al.*, 1976). Among the carbonyls produced during oxidation, the steam volatile n-alkanals and alka-2,4-dienals mainly contribute towards oxidized flavour. Therefore measurement of n-alkanals and alka-2,4-dienals may give a better index of oxidized flavour development in WMP (Mettler, 1973).

The investigations on carbonyls of WMP gained momentum and credibility after Schwartz *et al.* (1963) developed a method for direct isolation of carbonyls from milk fat. Rama Murthy and Jain (1973) modified Schwartz procedure for routine estimation of total carbonyls in milk fat. This modified method gives results which are reproducible to a higher degree than original Schwartz method.

Several workers have shown that carbonyl content increased during autoxidation of milk fat or ghee (Fross, 1964; Ahmed et al., 1969, Gaba and Jain, 1974; Bhat et al., 1981).

Keen et al. (1976) standardized a method for isolation, separation and quantitative estimation of classes of monocarbonyls from WMP during storage by adopting the method of Schwartz. They found that the concentration of monocarbonyls in three air packed WMP samples after storage for one hour, 16 months and 5 years were 7.1, 16.2, 48.8 uM/100 g respectively. However, they observed that saturated aldehydes constituted the major class of monocarbonyls isolated from each powder and it does not help in differentiating carbonyls of oxidized and unoxidized fat in powders.

Continuing this investigation, same group of workers (Boon et al., 1976) further separated the classes of carbonyls from WMP stored under nitrogen and air into individual components and identified them by thin layer chromatographic method. They identified a wide range of methyl ketones (C_3 to C_{15}) in powder stored in nitrogen or air for 16 months at 37°C. But the concentration of individual ketones in reconstituted milk from these powders were lower than their corresponding individual flavour threshold values. n-Alkanals (C_1 to C_{10}) comprised the major proportion of monocarbonyls in the oxidized powder but were in minor proportion in powder stored under nitrogen. The concentration of C_5 to C_7 n-alkanals in reconstituted milk from a 16 months old oxidized powder exceeded their individual flavour threshold values.

Unsaturated aldehydes were identified at low but organoleptically significant concentrations in oxidized powders. Undeca- 2,4-dienal was the most abundant unsaturated aldehyde identified, but other compounds considered to play a role in oxidized flavour included pent-2-enal, hept-2-enal, non-2-enal, hepta-2,4-dienal and nona-2,4-dienal. The authors, although, did not indicate the peroxide value, oxidized flavour score and levels of n- alkanals of WMPs at regular intervals during storage they drew a general conclusion that there existed a close relationship among the three parameter mentioned above and hence suggested that estimation of n-alkanals would provide a good basis for the objective assessment of flavour quality of WMP.

Temperature of storage was found to play a very important role in the production of carbonyl compounds during storage of WMP. Kopecky (1978) found that carbonyl content in WMP was more when stored at 30° to 60°C than in that stored at 20° to 23°C. This was obviously due to higher rate of oxidation at elevated temperatures.

Hall et al. (1985) studied the relationship between oxidized flavour and volatile compounds in stored WMP and found that the formation of n- hexanal correlated well with oxidized flavour. The relationship showed an initial linear phase which gradually changed to exponential phase after 30 weeks of storage at 25°C.

Tuohy *et al.* (1982) isolated and estimated total carbonyls of fat extracted from WMP and found it to range from about 1.0 mM/kg fat in fresh WMP up to 2.9 mM/kg fat in powder stored at 37°C for 44 weeks. The total carbonyls comprised mainly of saturated aldehydes. They also subjected milk powder to steam distillation and measured the UV absorption of distillate at 280 nm as a simple empherical measure of volatile carbonyl content. The UV absorption at 280 nm for fresh powder was about 0.02 absorbance unit and it increased up to 0.20 absorbance unit in powder stored at 37°C for 12 months. The volatile carbonyls also comprised mainly of saturated aldehydes.

Tuohy and Barrett (1982) estimated the individual volatile carbonyls in stored WMP using HPLC. Quantitative and qualitative changes in individual volatile compounds were observed in WMP stored at 37°C for 12 months. The following aldehydes were tentatively identified: acetaldehyde, propanal, butanal, isovaleraldehyde, pentanal, trans-2-hexanal, hexanal, octanal, nonanal, trans, trans-2,4-decadienal and decanal.

2.4.7 MEASURES TO ENHANCE OXIDATIVE STABILITY OF WMP

It was mentioned earlier (cf. 2.3.1.2) that the preheat treatment given to milk before drying is an important factor contributing to autoxidative stability of milk fat in WMP during storage. The other factors that may enhance the autoxidative stability of WMP during storage are addition of synthetic antioxidants and packing the WMP under inert atmosphere.

2.4.10.1 EFFECT OF PREHEATING

During preheat treatment of milk β-lactoglobulin and other whey proteins are denatured resulting in the activation of sulphur containing compounds, specially SH-groups, which act as antioxidants (Coulter *et al.*, 1951).

Tamsma *et al.* (1962) studied the changes in flavour of WMP prepared from preheated milk. They found that flavour stability of vacuum foam dried WMP packed in air at 27°C for 6 months can be improved by heating milk to 74°C for 30 min, 77°C for 6 min or 91° to 110°C for 15 s. Heating above these levels results in no further improvement of product since the high level of cooked flavour became objectionable.

Kisza and Rotkiewicz (1974) found inhibition of oxidation in WMP with a high content of free SH-groups but attributed the inhibition to the total reducing ability of the powder, rather than only to the presence of SH-groups. They also found a good correlation between the amount of SH-groups and denaturation of β-lactoglobulin. Maximum content of SH-groups was found at 90°C and holding time of 15 to 300 s. Kirch Mayer _et al_. (1984) also recommended a heat treatment of 90 to 95°C in order to maximize the content of free SH-groups in milk prior to drying.

Baldwin _et al_. (1981) proposed a method for predicting the autoxidative stability of WMP on the basis of WPNI of the powder; storage stability of 4 to 6 months for low-heat, 6 to 8 months for medium-heat and more than 12 months for high-heat powders. Kieseker _et al_. (1984) observed that the reconstituted milk prepared from high-heat WMP showed maximum flavour stability and it was independent of storage temperature. However, the flavour of reconstituted milk deteriorated as the storage period of the powder advanced, the powder was still regarded as acceptable at the end of 12 months trial.

Ipsen and Hansen (1988) reported that flavour of WMP depended significantly on the interaction between temperature of storage and heat treatment of milk. They indicated that the powder manufactured from milk given a severe heat treatment (WPNI 3.8 mg/g) had better storage stability interms of flavour than powder manufactured from milk given a less severe heat treatment (WPNI 5.9 mg/g).

This effect was most pronounced when the powder was stored at 30°C. At 20°C the heat treatment had little effect.

2.4.7.2 EFFECT OF GAS PACKING

To delay the onset of oxidised off-flavours, WMP for retail market has for many years been packed under inert gas to achieve low residual oxygen content of less than 2% in head-space which inhibited lipid oxidation (Coulter *et al.*, 1951, Tuohy, 1984).

Tamsma *et al.* (1961) reported that the shelf-life of WMP could be extended by reducing the oxygen levels in the packages below 0.5%. The WMP when packed in tins, which contained 0.1% oxygen, and stored for 6 months at 27°C was organoleptically superior to that packed in 1% oxygen or in air.

Boon (1973) used PVDC coated polyethylene bags for packaging WMP with nitrogen. The bags were stored at 30°C for 7 months. During this time the oxygen concentration in headspace increased from 2 to 8 %, and the samples were judged to be slightly better in flavour than control samples packed without nitrogen. Further storage (15 months at 30°C and 22 months at 22°C) only led to a slightly oxidized flavour.

Tuohy *et al.* (1981) mentioned a storage stability of 12 months at 12°C for WMP packed in tins with unchanged atmosphere. Samples packed in barrier film had a stability of about 38 weeks at 27°C. Instant WMP exhibited a stability of 18 to 22 weeks when packed in same films with unchanged atmosphere, a stability of 26 weeks when packed using vacuum, and a stability of more than 22 weeks when packed with nitrogen. It was concluded that WMP packed under nitrogen was protected better than that packed in the film package.

Driscoll _et al_. (1985) observed that gas packing became more important as the temperature of storage increased. Similarly, Hall and Anderson (1985) found significant changes in the odour and flavour profile of WMP stored at 25°C for 84 weeks. However, these changes were effectively retarded when the powder was stored under nitrogen.

2.4.7.3 EFFECT OF SYNTHETIC ANTIOXIDANTS

In India no antioxidant other than Butylated hydroxy anisole (BHA) is permitted in WMP under the provision of Prevention of Food Adulteration Rules (1955). Abbot and Waite (1962) found dodecyl gallate as a very effective antioxidant for WMP. Propyl gallate and NDGA also showed promising results. However, BHA was found to be ineffective towards improving the autoxidative stability.

Tamsma _et al_. (1963) studied the storage stability of WMP containing antioxidants. The relative effectiveness of antioxidants in reducing flavour deterioration in stored samples was found to decrease in the following order: lauryl gallate, propyl gallate, NDGA, ascorbyl palmitate, BHA, ascorbic acid, dihydroquercetin, sodium diethyldithio carbamate, thiodipropionic acid, quercetin, dilauryl thiodipropionate. Largest flavour stabilizing effect was observed in air packed samples stored at 27°C for six months. However, they concluded that none of the antioxidants tested allowed the production of powder with greater storage stability than those obtained by drying milk preheated at 74°C for 30 min.

Abbot (1971) found that a combination of citric acid and ascorbyl palmitate had a significant effect in improving the keeping quality of WMP stored at 37°C for 6 months. Dravid (1973) observed that addition of BHA less than 0.01 %, had no antioxygenic effect in WMP.

Radaeva et al. (1974) found that WMP containing added quercetin kept for 12 months at 20°C without development of any tallowy flavour. But the control powder developed the tallowy flavour after 8 months of storage. Helal et al. (1976) added NDGA (0.05%) plus citric acid and Tween (0.1%) separately to WMP and determined the changes in diene conjugation, TBA value and monocarbonyl content during storage. They concluded that addition of antioxidant enhanced the keeping quality whereas emulsifier adversely effected the storage stability of WMP.

Hall and Lingnert (1984) found that addition of Maillard Reaction Products (MRC), prepared from histidine and glucose, when added to WMP were as effective as BHA/BHT in retarding the development of off-flavours and off-odours. Mrithyunjaya and Bhanumurthi (1987) studied the autoxidative changes in spray dried buffalo WMP. They observed that TBA value increased from 0 to 0.05 in unprotected powder and 0 to 0.037 in powder containing BHA (0.01 %) on storage for 6 months.

2.4.8 HYDROLYTIC RANCIDITY

Formation of free fatty acids (FFA) due to hydrolysis of milk fat may result in the development of off-flavour which is termed as hydrolytic rancidity. Compared to oxidative rancidity, hydrolytic rancidity is not a serious problem in dairy products specially in WMP where the moisture content is very low. However, lipolytic enzymes can still be active in milk powders (Acker, 1962).

2.4.8.1 MEASUREMENT OF HYDROLYTIC RANCIDITY

Many methods have been suggested for estimation of hydrolytic rancidity in dairy products and all these methods are based on the solvent extraction of total lipids and subsequent titration of the extract against standard alkali (Thomas *et al.*, 1954; Frankel and Tarassuk, 1955; Deeth *et al.*, 1975; Salih *et al.*, 1977; Driessen *et al.*, 1977).

There are two main problems in these methods. One is the recovery of all the individual FFA quantitatively, specially the short chain fatty acids ($C_{4:0}$ and $C_{6:0}$) which are volatile and hydrophilic in nature. The second is the avoidance of lactic acid, citric acid and phospholipids coming into the extract. The solvent mixture of heptane/ isopropanol/ sulphuric acid (40:10:1) suggested by Dole and Menertz (1960) appears to over come these difficulties to a large extent. Deeth et al. (1975) modified the above method by suggesting a mixture of petroleum ether/isopropanol/4N sulphuric acids (40:10:1) to obtain better recovery of the fatty acid in the extract.

The spectroscopic methods suggested by several authors (Chakarbraty et al., 1969; Lowery and Tinsley, 1976; Koops and Klomp, 1977) based on reaction of copper soap of fatty acids, with dithio carbamate is of doubtful value for milk powder as these methods do not estimate short chain fatty acids which are directly responsible for rancid flavour and hence titration methods are more reliable indicators of hydrolytic rancidity in milk powders.

Although determination of individual free fatty acids by Gas Liquid Chromatographic method (Deeth et al., 1983; Needs et al., 1983; Spangelo et al. 1986) offers a quantitative determination of FFA yet the extraction step involved prior to GLC may give rise to inaccurate results.

2.4.8.2 CHANGES IN FFA

Nair (1930) found no lipase activity during storage of WMP made from milk preheated to 63° to 65°C for 30 min. Hetrick and Tracy (1948) found that milk powder made from milk without preheating showed residual lipase activity as indicated by high free fatty acid values which increased with storage time. Conversely, the powder made from milk preheated at 60°C for 30 min did not show any increase in lipase activity during storage.

Christensen et al. (1953) found that milk not preheated became rancid in vacuum pan and powder made from it was very rancid in taste. They further observed that heating milk to 65°C or higher for 20 min was sufficient to destroy lipase activity.

Rotkiewicz (1979) found an increase in FFA content from 0.97 to 1.74 during storage of WMP for 6 months at 20°C. The development of FFA in stored WMP was also observed by Steen (1978), De Vilder (1982), Tuohy et al. (1982), Allen (1983), Thompkinson (1984), and Ipsen and Hansen (1988).

Ipsen (1989) found that lipolysis in stored WMP was related to the lipolysis in the initial milk and to the total number of bacteria in the milk. This indicates that heat stable lipases of bacterial origin are responsible for the lipolysis in stored WMP. However, Rao and Murli (1989) observed a small increase FFA in egg powder during storage which was attributed to non enzymatic hydrolysis of lipids.

2.4.9 BROWNING CHANGES

Non-enzymatic browning changes occur in milk powders during storage because of their high concentration of lactose and proteins containing high levels of lysine. It is generally considered that next in importance to autoxidative changes are the browning changes, which are responsible for development of off-flavours in stored milk powder (Coulter et al., 1948; Labuza, 1972; Holsinger, 1973; De Vilder, 1982; Renner, 1988).

Nonenzymatic browning reactions in dairy products depending on the severity of heat treatment given could be classified as:

(a) Caramelization, that is, brown discolouration of sugar without the presence of amino component;

(b) Browning caused by Maillard reaction between lactose and amino component; and

(c) Oxidative browning (Patton, 1955).

Nonenzymatic browning via Maillard reaction was considered as an important mode of deterioration of powders during storage (O' Brien and Morrissey, 1989).

2.4.9.1 CHEMISTRY OF MAILLARD REACTION

Hodge (1953) integrated the available information into simplified scheme (Fig. I) which is still considered

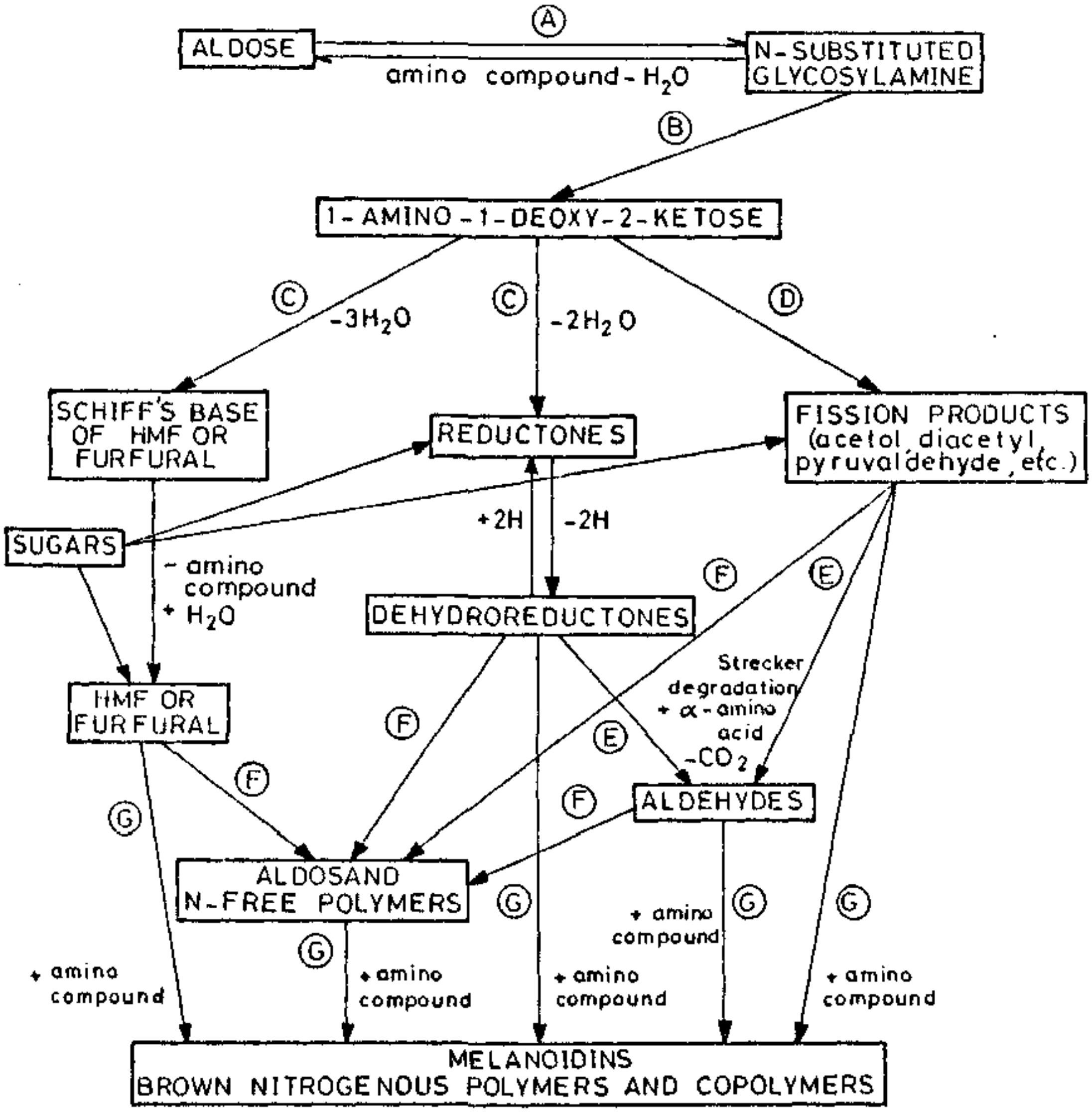

Fig. I. NON-ENZYMIC BROWNING. BASED ON HODGE.(1953)

to be the most appropriate description of Maillard reaction. The reaction has been shown to occur between aldo or keto sugar and amino group of an amino acid.

The Maillard reaction is conveniently divided into three stages. The initial reaction involves condensation of reducing sugar and amino compound to form Schiff base followed by the formation of an N-substituted glycosyl amine (A). This is followed by irreversible and spontaneous rearrangement of N- substituted glycosylamine via the Amadori or Heyns rearrangement (depending on whether the sugar is an aldose or ketose respectively) to form 1-amino-1-deoxy-2-ketose (B) or 2-amino-2-deoxyaldose respectively.

The intermediate stage comprises dehydration (C), either by loss of three molecules of water to furfurals or by loss of two molecules of water to reductones; fission (D), mainly by dealdolisation, and Strecker degradation (E), the interaction of amino acids and dicarbonyl compounds, which may be either dehydro reductones or dehydration or fission products.

The final stages of nonenzymatic browning involve the conversion of furfurals, fission products, reductones and Strecker aldehydes to high molecular weight melanoidins with further involvement of amines (F,G). Only limited information is available about the structure of the chromophores formed (O'Brien and Morrissey, 1988).

2.4.9.2 MEASUREMENT OF BROWNING

Tarassuk and Jack (1948) used arbitrary dry colour standard for estimating the extent of Maillard reaction in stored WMP. Henry et al. (1948) employed the Lovibond tintometer for measurement of colour in SMP. Keeney and Bassette (1959) suggested that estimation of HMF during storage of powder helped in detecting early symptoms of browning reactions before the actual formation of colour. They, further, gave a colorimetric method to determine either free HMF or potential HMF derived from browning intermediates. As this method was giving inconsistent results it was modified by Craig et al. (1961) and further by Della Monica et al. (1969). Pijanowski et al. (1970) and Valent (1975) also considered that determination of HMF as a most valuable objective test to determine the onset of browning changes in milk powder. Burton (1984) noted, that in the method of Keeney and Bassette the colour reaction was not specific to HMF as the colour reaction was shown by some of the intermediate compounds.

The HPLC method suggested by Bockel and Rehman (1987) has the advantage that it is possible to determine HMF specifically and the formation of coloured complex with TBA is not necessary because of strong UV absorption of HMF at 280 nm. O'Brien and Morrissey (1988) found that HPLC method for measuring HMF was more sensitive than the colorimetric method.

Palombo *et al.* (1984) directly measured the brown pigment produced due to browning in milk powders by a simplified enzymatic method. This involved liberation of brown pigment from protein by means of proteolytic enzyme pronase. After clarification, the browning index was determined spectrophotometrically. The repeatability of the method was claimed to be very high. Walstra and Jenness (1984) used reflectance meter and Christophe *et al.* (1988) used photoaccoustic spectroscopy for measurement of colour in dried milk products.

The manifestations of browning reactions are formation of HMF, increase in reducing capacity, colour change, production of off-flavours, production of water, lowering of pH, decrease in solubility, loss of vitamin C activity, production of fluorescence and loss of amino acids particularly lysine and sulphur containing amino acids, (Keeney and Bassette, 1959; Vasic *et al.*, 1971; Kisza and Boraski, 1983; Nurstein, 1986).

2.4.9.2.1 HMF CONTENT

Hydroxymethylfurfural is an intermediate in the Maillard reaction, being both formed and destroyed at the rates depending on the temperature (Burton, 1983). Generally it has been observed that HMF content gets accumulated in the dried milks during storage. Hence, levels of HMF in stored milk powder is used as an index of browning (Renolds, 1963; De Vilder, 1982; Golubovic *et al.*, 1983; Caric *et al.*, 1984).

Pijanowski et al. (1970) found an increase of 0.69 to 3.09 µM/l and 4.80 to 8.50 µM/l in free and potential HMF respectively in WMP during storage at 30°C. They recommended that determination of HMF in WMP can be used as a control of early deteriorative change in WMP. Rotkiewicz (1979) found a very small increase in potential HMF content from 7.50 to 8.44 µM/l 100g during storage of WMP for 6 months at 20°C.

De Vilder (1982) packed SMP in industrial milk powder bags and stored under different conditions of temperature and RH: i.e., at 20°C with 72% RH and at 30°C with 55 and 95% RH for a period of 12 months. In the powder stored at 95% RH, the HMF content increased rapidly at the end of 9 months storage when the level of moisture had reached 5%. In the other powders in which the levels of moisture was less than 5% even at the end of 12 months, no significant increase in HMF content was noticed.

Caric et al. (1984) observed increase of HMF content from 5.68 to 28.43 µM/100g in SMP stored for 8 months at room temperature. They also observed that addition of iron and Vitamin A to SMP significantly increased HMF values.

Sharma (1985) reported that HMF contents increased from 0.65 to 17.60 mM/l in roller dried SMP and from 0.54 to 5.9 mM/l in spray dried WMP on storage for 12 months at 37°C. Mrithyunjaya and Bhanumurthi (1987) observed an increase in HMF value from 2.47 to 7.64 mM/l in WMP stored at 37°C for 6 months. Ipsen and Hansen (1988) studied the influence of storage temperature on free HMF content and found that WMP when kept at 38°C for 70 weeks showed an increase from 3.7 to 7.6 µM/l, whereas in WMP stored at either 20°C or 30°C the increase in HMF content was insignificant.

2.4.9.2.2 REDUCING CAPACITY

The capacity of dried milks to reduce acid ferricyanide, increases on storage (Champman and Mc Farlane, 1945; Lea 1947; Robbertsen and Radema; 1975; Rotkiewicz, 1979; Renner, 1988). Lea (1947) and Crowe et al. (1948) have shown that this increase resulted from interaction of lactose with protein and probably also from decomposition of lactose catalyzed by the buffer salts of milk.

Coulter et al. (1948) found that moisture content of powder was the primary factor in influencing the production of acid ferricyanide reducing substances during storage. Higher moisture content in the powder (1.32 to 4.78%) was associated with greater reducing values. Decker et al. (1951) reported that acid ferricyanide reducing values were higher in the powder stored at 29°C than at 7°C, and higher in nitrogen than air packed at 29°C. The latter result was thought to be due to greater retention of ascorbic acid in the nitrogen packed samples.

Kopecky (1978) found a slow increase in ferricyanide reducing value in WMP samples with 2.03% moisture and stored at 20° to 23°C, but the increase was accelerated with increasing storage temperature and more so with increasing moisture content.

2.4.9.2.3 COLOUR

Change in colour during storage of dried milk is the most obvious observation and has often been used as the yardstick for measuring extent of browning. During storage, colour is not produced in the initial stage of Maillard reaction, only a little is produced in the intermediate stage and most of it results in the final stage (Feeney and Whitaker, 1982; Caric *et al.*, 1984; Nursten, 1986).

Henry *et al.* (1948) reported that dried milk with less than 5% moisture showed only very slight changes in colour even after two years of storage at 37°C. Labuza, *et al.* (1970) found that maximum development of brown colour occurred in dried food when the water activity ranged from 0.5 to 0.7. Caric *et al.* (1984) reported that browning reaction of Maillard type (colour development, off-flavour etc.) were intensified during storage of dried milk if the moisture content was 5% or higher. Kieseker and Clarke (1984) noted that powder with less than 4% moisture did not show any browning reaction even when stored at 40°C.

2.4.10 MICROBIAL QUALITY

Initial bacterial count of the milk powder depends on the bacterial load of raw milk used, conditions of preheat treatment and drying of milk. However, the bacterial activity is inhibited in dried milk powders because of its low moisture content (Findley *et al.*, 1946; Poulsen *et al.* 1971; Ipsen and Hansen, 1988).

Bacterial counts in milk powder tend to decrease considerably during storage. Reduction in bacterial number depends on condition of storage. Bacterial flora tend to die out more rapidly as the temperature of storage is raised. Total microbial counts in dried protein product decreased from 29,000 to 700 when stored at 8°C for 12 months and at 20°C for 9 months (Eremin, 1985). In spray dried SMP, standard plate count decreased from 1.2×10^{5} to 1.8×10^{4} after 6 months of storage at room temperature (Arun *et al.*, 1979).

A gradual decrease in *Micrococcus flavus* count from 7.5×10^{5} to 0.2×10^{5} was noted in spray dried skim milk on storage at 25°C for 36 weeks (Thompson *et al.*, 1978). *Streptococcus durans* tends to disappear fairly rapidly in dried milks, whilst *Streptococcus thermophilus* survived for longer periods. *Micrococcus* spp. which are more viable than the streptococci, constitute the bulk of the flora of many milk powder samples after 3 to 6 months of storage (Crossley and Johnson, 1942).

Muir *et al.* (1986) noted a considerable decrease in thermophilic count from 4.2×10^2 to 140 and thermoduric count from 4.4×10^3 to 1.7×10^3 in low heat SMP on storage at 30°C for 6 months. Rahman *et al.* (1988) analysed different samples of dried milk for total viable count, coliforms, staphylococci, yeast and molds and direct microscopic counts. They concluded that high direct microscopic count as well as total viable count in dry milk samples reflected poor sanitation.

Coliforms tend to die out rapidly on storage of milk powders. Viable coliform counts of 51 to 60/g decreased rapidly and diappeared after 6 months of storage of SMP (Arun *et al.*, 1980). Spores are relatively resistant during storage. Spore count in high heated SMP decreased from 4.5×10^2 to 4.0×10^2 on storage at 30°C for 6 months (Muir *et al.*, 1986).

Presence of pathogenic bacteria in dried milk has been reported from time to time (Schroeder, 1967; Marth, 1969; Craven, 1978; Sharp, 1986). The major cause of food poisoning due to contaminated milk powder was associated with *Salmonella* spp. (Mettler, 1989). However, Anderson (1955) and Schroeder (1967) reported two cases in which *Stapylococcus* spp. were associated with food poisoning due to dairy powders. Mettler (1989) in his recent review has emphasized the need for good manufacturing practices (GMP), equipment and their soundness, air quality, product and environment testing and critical control of all participatory systems in the manufacture of dried milk to ensure good bacteriological quality.

2.5 OFF-FLAVOURS IN WMP

The flavour of good quality WMP can be described as clean, rich, sweet and pleasant (Patel and Gupta, 1978). However, during storage, WMP may undergo various oxidative and non oxidative changes, which lead to development of off-flavours of various kind e.g., tallowy, metallic, stale, gluey, cardboardy, musty, green etc. (Bandings, 1980; Hall and Lingnert, 1984).

2.5.1 OFF-FLAVOUR DUE TO OXIDATIVE CHANGES

In WMP, because of its high fat content, autoxidation is the major cause of flavour deterioration during storage. It has been discussed in section 2.4.9.2.5 of this review that the carbonyls are the major end products of autoxidation of milk fat and they are directly responsible for several off-flavours observed in WMP.

Depending upon the concentration of carbonyl compounds, the off-flavours observed in milk fat are in the following order: metallic, fatty, fishy and tallowy (Badings, 1970). Both volatile and non-volatile carbonyl compounds have been shown to produce different off-flavour in WMP during storage (Coulter *et al.*, 1951; Keen *et al.*, 1976).

Boyd *et al*. (1965) reported that C_7 to C_{10} alkanals possessed an oily and tallowy odour, while C_7 to C_{11} alk-2-enals gave rise to painty odour. Fross *et al*. (1960a, 1960b) observed that in autoxidised milk fats, the presence of higher proportions of n-heptanal, n-octanal, n-nonanal, 2-heptanone, 2-heptanal and 2-nonanal imparted tallowy flavour to milk fat, while increase in the proportions of n-pentanal and C_5 to C_{10} alk-2-enals resulted in a painty odour. The total amount of carbonyl compounds was ten times greater in tallowy sample but 100 times greater in milk fat having painty odour, as compared to milk fat which exhibited a fishy odour.

Park and Patton (1961) found that badly deteriorated WMP contained a larger variety and higher concentrations of alkan-2-ones and n-alkanals than did the powder of good quality. They suggested that medium and high concentrations of ketones contribute to both stale and oxidized flavour respectively, whereas aldehydes cause these off-flavours at much lower concentrations. Unsaturated carbonyl compounds e.g., alk-2-enals and alka-2,4-dienals, although constitute a minor proportion (3.0 and 1.8 % respectively) of monocarbonyl classes of stored WMP, play a significant role towards the development of oxidized off-flavours (Boon *et al*., 1976). This was attributed to their low flavour threshold values (Day *et al*., 1963, Fross, 1979 .

Hall and Lingnert (1984) and Hall and Anderson (1985) studied the kinetics of the formation of volatile oxidation products of fat and showed the predictive relationship between flavour characteristics and volatile compounds. They concluded that the decreasing acceptance of WMP during storage was mainly due to autoxidation which produced long chain aldehydes, which imparted various types of off-flavours to WMP.

2.5.2 OFF FLAVOUR DUE TO LACTONES

Anhydrous milk fat and other dried products containing milk fat develop stale and coconut like off-flavours even when processed and stored under effective antioxygenic conditions. However, coconut flavour caused by lactones may develop during storage of WMP if the storage temperature and water content are high. It has been shown that the coconut like off-flavour is due to the formation of delta-deca and dodecalactones (Keeney and Patton, 1956; Patton, 1961; Tamsma *et al.*, 1975; Eriksen, 1976, Wadhwa and Jain, 1989).

Although pasteurization of milk produces lactones in barely detectable quantities, progressive development of lactones occur during storage of WMP. Patton (1962) reported the presence of C_{10} and C_{12} delta lactones in two samples of WMP packed in air and nitrogen. He found no difference in GLC patterns of volatiles from both samples confirming the formation of lactones due to non oxidative mechanism. However, Eriksen (1976) showed that the formation of lactone was of lipid origin due to oxidative changes.

Presence of both gamma- and delta- lactones have been reported in butter oil and ghee (Ellis, 1975; Wadhwa, *et al.*, 1979; Wadhwa and Jain, 1984). The buffalo milk fat contained higher proportions of C_{10} and C_{12} delta-lactones than cow milk fat (Wadhwa and Jain, 1980). They concluded that development of off-flavour in stored ghee could be possibly attributed to the increase in the levels of delta-lactone during storage.

2.5.3 OFF-FLAVOURS DUE TO NON-OXIDATIVE CHANGES

Although several steps such as high preheat treatment, addition of antioxidants and packing under inert atmosphere may be adopted to control off-flavours due to autoxidation, there may be development of other off-flavours arising due to non oxidative changes. The off-flavours originating from nonoxidative changes are primarily due to browning reactions, these have been termed as stale, gluey, musty etc. (Shipstead and Tarrasuk, 1953).

Ferretti and Flanagan (1972) found that combined effect of several compounds such as, 2-furaldehyde 2-furfuryl butyrate, methyl pyrazine, Methyl-2-formylpyrrole, O-cresol, benzaladehyde and salicyaldehyde produced during browning reactions contributed significantly to the stale flavour of dried milk during storage.

Park *et al.* (1964) isolated o-aminoacetophenone from dried milk which had developed a stale flavour and found that this compound possessed a definite stale flavour. Arnold *et al.* (1966) confirmed the above findings. However, on the basis of available evidence it seems reasonable to propose that stale flavour in dried milks is due to compounds arising from lipid deterioration as well as those resulting from the Maillard reaction (Henry *et al.*, 1948; Coulter *et al.*, 1951; Ramshaw and Dunstone, 1969a,b; Fross, 1979).

2.6 PACKAGING OF DRIED MILKS

The primary purpose of packaging of any food is to protect it from the external contamination and to contain it for bulk or retail distribution and sales. The economics of packaging material in relation to the shelf-life, expected of the product, is an important consideration. While selecting a suitable packaging material for WMP, the properties expected are that the packaging material should have good barrier properties against absorption of moisture, oxygen, odours and bacteria. It should also prevent exposure of powder to out side light (Cummins, 1976). Other factors in deciding the package include bulk density and cohesivity of milk powder (Dua, 1970; Lovell, 1972).

Crossley (1966) while discussing packaging materials for dried milks, concluded that no single packaging material can satisfy the full range of the above mentioned requirements. Further, he emphasized the need to study the physico-chemical properties of packaging materials in conjugation with various storage changes in dried milk.

The characteristics of a few packaging materials which are being used for dried milks are given below:

2.6.1 METAL CAN

Metal can is nearly perfect container for milk powder. Tin plate usually has a thickness varying from 0.15 to 0.5 mm and coated on both sides with thin protective film (0.4 to 2.5 mu) of commercially pure tin. Food grade lacquer (usually epoxy phenolic type coating) is applied on the food contact surfaces for preventing both rusting and interaction of tin with food.

The tin can was found to be the best for packaging of WMP (Kovalenko and Norkina, 1973; Tuohy, 1984) and for SMP (Ardito et al., 1980; Driscoll et al., 1985). Since tin is costlier than other packaging material, its use in food packaging increases the cost of the product considerably. (Malin, 1980).

2.6.2 FLEXIBLE PACKAGING MATERIAL

Flexible packaging materials are being increasingly used for bulk and retail packing of various dried milk storage is to be protected mainly against absorption of moisture, oxygen and exposure to light. These requirements can be achieved to a greater extent in a flexible packaging material through the process of laminating different materials, each one having one or more above mentioned properties in good measure. Coating of plastic material with metal is one example of this approach (Martin, 1986).

The flexible packaging materials which are being used for packaging of dried milk and their effects on the quality of dried milk during storage are discussed here.

2.6.2.1 METALLIZED POLYESTER

Metallized polyester film is made by coating polyester film with a metallic layer. Goddard (1980) surveyed potential and actual application of metallized polyester films for food, including dairy products and gave tabulated information for different metallized polyester films with respect to their barrier properties against moisture, oxygen and resistance to puncture. The metallized polyester films have been shown to possess excellent moisture and oxygen barrier properties.

Most package requirement need a thickness of 12 micron polyester (Dublin, 1978; Sahaar and Skodis, 1982). Mrithyunjaya (1982) reported that the cost of metallized polyester film was about 40% less than that of tin.

Promising results were obtained by Tuohy (1984) in packing WMP using polyethylene film laminates containing a metallized polyester film layer. Vacuum packaging of WMP in oxygen impermeable flexible metallized polyester film laminate offered about the same shelf-life as that obtained by canning under inert gas. Mrithyunjaya and Bhanumurthi (1987) packed WMP in metallized polyester bags and stored at 30°C for 6 months, found that WMP remained organoleptically acceptable even at the end of the storage period.

2.6.2.2 POLYETHYLENE

As the name implies polyethylene is an addition polymer of ethylene. The low density polyethylene (LDPE) is only structurally different from high density polyethylene (HDPE). The LDPE is tough, translucent, waxy solid with a density varying between 0.916 and 0.935 gm/ cm^2. It has high impact strength, good sealable property. It has moderate barrier property against moisture, but poor barrier property against gases (Bremer, 1987). The HDPE is tough, slightly translucent and less waxy than LDPE with a density usually greater than 0.941 gm/cm^2. The HDPE has good water barrier properties but only moderate barrier properties against oxygen. However, these two properties are better in HDPE than in LDPE (Agarwal, 1988).

Vasic et al. (1970) observed that moisture content in WMP packed in polyethylene bags and stored at room temperature for a period of 6 months increased from 3.1 to 5.3%. Vasic et al. (1971) in another study found no change in free fat, solubility, pH, reducing capacity, sulphydryl content and flavour in WMP packed in polyethylene bags and stored at room temperature and 60% RH for 6 months. Boon (1974) observed that the LDPE used for bulk packaging of milk powder did not offer much protection against external flavours.

Boon (1973) observed that WMP packed in vinylidene chloride coated polyethylene bags and stored at 30°C showed slight oxidation at the end of 7 months. Warburton and Pixton (1978) observed that dried milk stored in plastic lined paper sacks, which are tied at the neck absorbed very little moisture.

Rotkiewicz (1979) studied the changes in physico-chemical indices of WMP during storage when packed in tins and polyethylene bags. He found that when the powder was stored at 20°C and 75% RH, there was an increase in moisture from 3.80 to 4.64% in six months in the case of polyethylene packings and there was no change in moisture content in tin packings. The rate of increase of moisture was higher with higher RH. The increase in moisture content in the powder packed in polyethylene effected adversely the quality of powder by increasing ferricyanide reducing value, HMF content, free fat, FFA, TBA value and peroxide value. However the type of polyethylene used has not been indicated by the author.

Peschek (1983) found no significant change in acidity of dried skim milk stored at 20°C in 0.3 mm thick polyethylene film after a period of 38 months. Sanyal and Balachandran (1988) observed a very small change in organoleptic qualities of spray dried malted milk when packed in polyethylene pouches kept at 30°C for two months.

2.6.2.3 SARAN COATED FILM

Saran packaging films are made from copolymer that is predominantly vinylidene chloride (PVDC). Due to very high chloride content in polymer, unplasticized saran film is highly crystalline and offers extremely high barrier to passage of oxygen and other gases, water vapour, odours, aroma and flavour. It has high resistance to greases, oils, solvent and many chemicals which could a tack other packaging films (Ferguson, 1987). Saran films also possess high cling and gloss.

Boon (1973) observed that WMP when packed in saran coated bags and stored at 30°C showed only slight oxidation at the end of seven months. The same author in 1974 observed that when 50 g samples of dried milk were sealed in plastic bags and stored in tins with tallow, wool or apples for 4 weeks at 30°C, the LDPE did not protect the powder against absorbing any odour where nylon/polyethylene offered protection against fatty odours but not against apple odour in high humidity condition. The saran coated film was not permeable to oxygen or water soluble odours, but did not protect against fatty odour. He concluded that the ideal film for dried milk would be laminate of PVDC, polyethylene and nylon.

2.6.3 BULK PACKING OF DRIED MILKS

Bulk packing of dried milks is very important for economical handling and transportation in the industry. In India bulk packing of WMP is done in 10 kg tins under nitrogen. However SMP is bulk packed in LDPE bags encased in hessian laminated craft paper bags.

Bulk packing of WMP in flexible bags is permitted provided the powder bags are stored below 20°C (PFA, 1987). Although the above recommendations have been made for bulk packing of WMP in flexible packaging material, there are no data indicating the actual shelf-life of WMP in such packings when stored at different temperatures and relative humidities.

Sudhakar *et al.* (1987) suggested a bag of multilayer coextrution film of LD/HD/LD with 50 micron thickness for bulk packing SMP. The authors claimed that the powder packed in such bags, sealed and then put in primary bag gave total protection against moisture absorption and mechanical stress. They also stated that the bag worked out cheaper by about 15 to 20 % compared the bitumen laminated bag and weighed 300 g as compared to 700 g of laminated jute bag.

Foils with a non-continuous metal layer have been shown to protect the flavour of WMP packed in 25 kg bags for one year or more, when kept at 38°C and 60% RH. But these foils are unsuitable for packing of WMP when stored under high humid conditions (95% RH). Foils with continuous layer of metal are able to protect WMP when stored at 38°C and 60% RH and could be an alternative to tins (Ipsen and Hansen, 1988).

The above review of literature indicates that practically no data are available on the shelf-life of WMP produced indigenously. Research studies to obtain such data become necessary in view of the facts that WMP produced in the country is mostly from buffalo milk and the quality of raw milk used is only of average quality. The data will also help in formulating our food laws for packaging, storing and handling of indigenously produced WMP.

SCOPE AND PLAN OF WORK

3.0 SCOPE AND PLAN OF WORK

3.1 SCOPE OF STUDY

In the recent past large quantities of SMP and butteroil were received through international aid programmes. These materials are used for preparing recombined milk to supplement the market milk supply needs throughout the season in several parts of our country. The facilities for preparing recombined milk are available only in major dairies and has not been extended to smaller dairies because of consideration of large investment and economic viability. Alternatively, the preparation of reconstituted milk from WMP is a viable proposition to be adopted by smaller dairies to meet the demand for liquid milk. Hence, there is a growing new interest in our country to use WMP for the purpose of preparing reconstituted milk.

However, the process of preparing reconstituted milk from WMP requires bulk packaging and storing of WMP under commercial conditions for economical transportation and easy handling of the powder. Since the WMP is highly hygroscopic and prone to oxidative spoilage, a packaging material which can protect powder against absorption of moisture and oxygen is necessary so that WMP may remain organoleptically acceptable for a reasonable period of storage. Among the several flexible packaging materials available, HDPE appears to be suitable for bulk packaging of WMP because of its several advantages. The HDPE has excellent moisture barrier property. It has reasonably good oxygen barrier property, easily available in food grade, manufactured indigenously and also economical. The hessian laminated kraft paper bag when used as the outer bag gives adequate strength to package during handling and transportation. It also gives protection against light.

It is well known that the initial quality of milk and type of milk, either cow or buffalo can influence the shelf-life of WMP. From the literature reports given in the previous chapter, it is evident that there are no data available on the keeping quality of WMP commercially produced in our country when packed in different packaging materials and stored under variable atmospheric conditions. Hence, there was a need to conduct a systematic study for commercially produced WMP bulk packed in HDPE bags encased in hessian laminated kraft paper bags and stored under variable conditions.

The following objectives were set out for developing the plan of investigation:

1. To evaluate the feasibility of packing WMP in 25 kg bags and storing at controlled as well as ambient atmospheric conditions.
2. To study the various physico-chemical changes and their effects on organoleptic quality of WMP and its recons-tituted milk.
3. To develop a suitable chemical test for objective assessment of keeping quality of WMP during storage.

4. To evaluate the initial quality of WMP available in the market.
5. To recommend an optimum period under commercial conditions for storing WMP without spoilage, when bulk packed in HDPE bags.

3.2 PLAN OF WORK

To fulfill the above mentioned objectives, the following plan of work was adopted in this study.

3.2.1 PACKAGING OF WMP

The WMP commercially produced, at KMF product dairy Dharwar, was packed in 25 kg HDPE bags encased in hessian laminated kraft paper bags. The powder was also packed in 500 g tins. Three different trials were conducted.

3.2.2 STORAGE OF WMP

All the three batches of WMP were stored for 12 months; one set at ambient temperature at Dharwar and another set at 20± at NDRI, Bangalore.

3.2.3 EVALUATION OF QUALITY OF WMP AVAILABLE IN MARKET

Three different brands of WMP from market were collected for evaluation of quality of WMP.

3.4 EVALUATION OF SHELF-LIFE

The WMP was evaluated on the basis of different physico-chemical, microbiological and sensory properties, initially and during storage at monthly intervals.

3.2.4.1 PHYSICO-CHEMICAL PROPERTIES

The different physico-chemical tests performed to evaluate the quality of WMP initially and during storage were:

1. Moisture
2. Fat
3. Titratable acidity
4. pH
5. Solubility
6. Viscosity
7. Lactic acid
8. Free fatty acid content (FFA)
9. Whey protein nitrogen index
10. Heat stability
11. Lactones
12. Autoxidative changes

    a. Diene conjugation
    b. Peroxide value
    c. TBA value
    d. Carbonyls

        (i) Total carbonyl value

        (ii) Isolation and estimation of monocarbonyls by column chromatography.

        (iii) Steam volatile monocarbonyls and their class separation by TLC

    e. Development of a suitable chemical test for objective assessment of keeping quality of WMP,

    f. Rate of free fat liberation and the rate of autoxidation as compared to total fat.

    g. Fatty acid profile of free fat and total fat by GLC.

13. Browning changes

    a. Free and potential HMF
    b. Colour index
    c. Ferricyanide reducing value
    d. Free SH- groups

3.2.5 MICROBIOLOGICAL ANALYSIS

1. Total plate counts
2. Coliform counts

3.2.6 SENSORY EVALUATION

Reconstituted milk as well as milk powder were examined for the following sensory attributes on a nine point hedonic scale by a sensory panel.

1. Flavour
2. Body and texture
3. Appearance
4. Over all acceptability

MATERIALS AND METHODS

4.0 MATERIALS AND METHODS

4.1 SOURCE OF WHOLE MILK POWDER

The spray dried buffalo WMP produced at Karnataka Milk Federation (KMF) Product Dairy, Dharwar was used for the study. Different brands of WMP available in the market in 500 g tins were also collected.

4.2 PACKAGING MATERIAL

The WMP was bulk packed in 25 kg high density polyethylene (HDPE) bags encased in hessian laminated kraft paper bag and in 500 g tins, the details of which are given below:

4.2.1 KRAFT PAPER BAGS

1. Type: Multiwalled kraft paper bag lined with hessian cloth to pack 25 kg of milk powder.
2. Outer most ply is kraft paper of 80 GSM crepe paper lined with hessian cloth having mesh of 305/sq. meter.
3. Two inner plys are of plain sac kraft paper of 80 GSM of a reputed paper mill.

Dimensions

1. Outer most ply 56 x 96.6 cm.
2. Inner two plys 48 x 96.5 cm with quest 8 cm (4 cm on either side) the bottom of the bag is stitched by using closing thread of good quality.

4.3.2 HIGH DENSITY POLYETHYLENE BAGS

The HDPE bags were supplied by National Dairy Development Board (NDDB), Anand with following specifications:

1. Thickness - 80 micron.
2. WVTR (at 23°C/ 85% RH) - 8 g/m^2 . dia
3. Oxygen permeability (at 23°C) - 800 Cm^3/m^2 . dia. bar

4.3.3 TIN PLATE CANS

Five hundred gram capacity tin cans lacquered inside were used to pack WMP which served as control. The tins were supplied by M/s Fabricon Industries Pvt. Ltd., Bangalore with following specifications:

Thickness : 0.016 inch

Gas barrier properties : Very good

Grease resistance : Excellent

Size of the can : 10.5 cm dia x 13.5 cm height

4.3 STORAGE CONDITIONS:

Whole milk powder bulk packed in 25 kg HDPE bags encased in hessian laminated kraft paper bags was stored for not less than 12 months; one set at ambient temperature at Dharwar and another set at 20°C at NDRI, Bangalore. At Dharwar Dairy Plant the ambient temperature ranged from 22 to 38°C and relative humidity ranged from 18 to 100% in a year.

4.4 NUMBER OF TRIALS

Three trials of the study were carried out on batches of WMP produced on the following dates : 2.2.88, 2.3.88 and 2.4.88. Samples were drawn at monthly intervals from each batch and for each sample separate bag was used.

4.5 PRODUCTION OF SPRAY DRIED WMP

Standardized buffalo milk was preheated to 90 ± 2°C for 15 s in a tubular preheater. The preheated milk was concentrated to 42-45% total solids with the help of triplet effect evaporator. The concentrated milk was fed to spray dryer equipped with integrated fluidized bed dryer through a centrifugal spinning disc atomizer. The inlet air temperature was maintained at 180 ± 2°C and outlet air temperature at 75 ± 2°C. The powder was cooled to 30°C and packed in 25 kg bags.

4.6 PURIFICATION OF SOLVENTS

4.6.1 PREPARATION OF CARBONYL FREE HEXANE

Contaminant carbonyl compounds in hexane were removed using the method of Schwartz and Parks (1961). One gram of 2,4-dinitrophenyl hydrazine (DNPH) was dissolved in 12 ml of 85% o-phosphoric acid by grinding in a 6" mortar. About 8 ml of water were added to the clear yellow solution and the precipitate formed was dissolved by further grinding. Then 20 g of celite-545 were added and ground until a homogenous damp preparation was obtained.

The bright yellow impregnated celite was poured into a chromatographic column (2.0 x 25 cm) containing a plug of glasswool at the constricted portion of the tube and filled with hexane. Five milliliters of hexane were allowed to pass through the column by opening the stopcock. The slurry was packed by gentle pressure using a flattened glass rod. The hexane in the column was drained and 100 ml of benzene were flushed through the column material using gentle pressure in order to remove the colour impurities which behaved like aliphatic monocarbonyl DNP hydrazones. Hexane was stirred with excess DNPH reagent for 15 min and an aliquot was removed to serve as blank. The treated hexane was passed through the coloumn at the flow rate of 4 ml per min. The first 50 ml of the effluent were discarded and the remaining portion of the effluent was collected and distilled to get carbonyl free hexane.

4.6.2 PETROLEUM ETHER (40-60°)

The main impurity in petroleum ether is of unsaturated aromatic hydrocarbons. This was removed by shaking the petroleum ether with 10% of the volume of concentrated sulphuric acid. Vigorous shaking was then continued with successive portions of a concentrated solution of potassium permanganate in 10% sulphuric acid until the colour of the permanganate remains unchanged. The solvent was then thoroughly washed with water, dried over anhydrous calcium chloride and distilled.

4.6.3 ETHYL ACETATE

The ethyl acetate 95-98% grade usually contains some water, ethanol and acetic acid and may be purified in the following manner: To one litre of the solvent 100 ml of acetic anhydride and 10 drops of concentrated sulphuric acid were added. The mixture was heated under reflux for four hours and then fractionated. The distillate was shaken with 20-30 g of anhydrous potassium carbonate, filtered and redistilled.

4.6.4 ACETONITRILE

Acetonitrile LR was kept over phosphorus pentaoxide overnight and distilled at 82°C.

4.7 ANALYTICAL METHODS

4.7.1 MOISTURE/TOTAL SOLIDS

Moisture/total solids content in dried whole milk was determined by the method as detailed in IS : 11623-1986.

4.7.2 FAT

Fat in the powder was determined as detailed in IS: 11786 -1986. For routine tests the Gerber method was used (IS: 1224 - 1977).

4.7.3 MILK SOLIDS-NOT-FAT (SNF)

The SNF content of reconstituted milk (RCM) was estimated according to method outlined in IS : 10083-1982.

4.7.4 TITRATABLE ACIDITY

The titratable acidity of the whole milk powder was determined as detailed in IS : 1165-1986 (Appendix A).

4.7.5 SOLUBILITY INDEX

Solubility index of powder samples was estimated as per ISI method given in SP : 18 : Part-XI (1981).

4.7.6 TOTAL ASH

Total ash of WMP was determined as detailed in IS : 1547-1985 (Appendix C).

4.7.7 LACTIC ACID

A modified Ling method (1951) based on a procedure developed by American Dry Milk Institute (Velasco & Moats, 1959) for determination lactic acid content was used.

In this procedure,5.38 g of dry milk were reconstituted in 50 ml of water and a 25 ml of aliquot from this was taken. This was deproteinized by the successive addition of 10 ml of barium chloride solution (98.8 g/litre), 5 ml of zinc sulphate solution (225 g/litre) and 10 ml of 0.66 N sodium hydroxide and filtered. One millilitre of 1 % ferric chloride in 0.025 N hydrochloric acid was added to 10 ml of the above filtrate. The colour was read after 30 min at 425 and 650 nm. The reading at 650 nm was to correct for turbidity which frequently developed in the final solution. The difference in the optical density at these wavelengths was used to determine the lactic acid present.

The calibration curve was prepared by adding known amounts of calcium lactate to milk and measuring the increase in optical density.

4.7.8 FREE FATTY ACIDS (FFA)

The FFA content was determined by the method of Thomas *et al.* (1954) with slight modifications. Two g of WMP were ground in a mortar with the help of hot distilled water and transferred to Mojonnier fat extraction tube. To this 10 ml of ethyl alcohol (95%) were added. The contents of the flask were shaken vigorously for two minutes. Twenty ml of ethyl ether and 30 ml of petroleum ether (40-60°C) were added and the contents of the flask were vigorously shaken and kept aside for half an hour for complete separation of two layers. The ethereal layer was transferred from Mojonnier flask to 250 ml conical flask. The extraction of fatty acids was repeated with half the quantities of petroleum ether and diethyl ether used in first extraction. Both the extracts were added and to this 1-2 g of sodium sulphate was added. The extract was decanted into another 250 ml conical flask and titrated against 0.01 N alcoholic sodium hydroxide solution using 1 ml of phenolphthalein (0.5% in alcohol) solution as indicator.

The results were expressed in uM per gram fat.

$$\text{FFA (uM/g fat)} = \frac{10 \times \text{Volume of 0.01N NaOH used}}{\text{Weight of the fat in powder}}$$

4.7.9 pH

Thirteen grams of WMP was reconstituted with 100 ml distilled water. The pH of the reconstituted milk was determined with the help of Elico pH meter (Model LI-120) at room temperature.

4.7.10 HEAT STABILITY

Heat stability of reconstituted milk (12.5 % TS) was determined according to the method developed by Davies and White (1966) and as modified by Jairam *et al*. (1976). One ml of milk was pipetted into a pyrex tube of 10 cm length and internal diameter of 0.8 cm of which one end was already closed with synthetic rubber cork. The other end of the tube was then closed with a similar cork. After shaking the tube gently, the tubes were clamped crosswise on an aluminium carriage of 10 tube capacity. The carriage was tilted so that the sample solution could wet all the inner surface of the tube. The carriage was placed in a liquid paraffin bath maintained at 130°C. Simultaneously a stop watch and the rock mechanism (8 cycle/min) were started. The tubes were illuminated from above by a table lamp. As soon as moving particles or clots were seen throughout the milk the timer was stopped and the exact time was noted. The time in minutes was taken as heat coagulation time (HCT) or heat stability.

4.7.11 WHEY PROTEIN NITROGEN INDEX (WPNI)

Whey protein nitrogen index was done by the method prescribed in Niro Atomizer (1978) which is based on the original method of Harland-Ashworth, reported by Kuramoto *et al.* (1959). The brief description of the method is given below.

In a test tube (8 x 1"), 2.70 g of WMP were reconstituted with 20 ml of distilled water. To this 8 g of sodium chloride were added and the contents were incubated for 30 min in a water bath maintained at 37°C. The contents of the tube were shaken for 8-10 times during the first 15 min of incubation period, to ensure complete saturation of the sample with sodium chloride. The test tube was taken out and the contents were filtered through a Whatmann 42 filter paper. Five ml of the filtrate were collected. One ml aliquot of the filtrate was taken into a cuvette. To this 10 ml of saturated sodium chloride solution was added. The cuvette was stoppered with a rubber stopper and inverted to facilitate mixing. Two drops (delivered from a 5 ml volumetric pipette) of the HCl solution (10 g/100 ml) were added and turbidity was developed. Again the contents of cuvette were mixed with inversion. While inverting care was taken to avoid the formation of foam. After 5-10 min, the cuvette contents were inverted again for mixing and turbidity was measured at 420 nm in a Bausch & Lomb spectrophotometer. Diluted casein free filtrate made by diluting 1 ml of original filtrate with 10 ml of saturated sodium chloride solution was used as blank.

Results were calculated from standard curve and expressed as mg undenatured whey protein nitrogen/g powder.

4.7.12 VISCOSITY

The viscosity of reconstituted milk (12.5% TS) was determined with Hoppler viscometer (type BH, manufactured by Vebprufgerate Werk Medingen, Dresden, Germany). The temperature of sample was kept constant at 30°C with the help of Haake Ultrathermostate (Germany). After leveling the viscometer, the measuring tube was filled with the sample, the ball No. 3 was inserted through the open end of the tube and it was closed with a screw cap. Time required for the ball to move 100 mm distance was recorded for three such events and average value was considered for the final calculation of viscosity. The specific gravity of the sample was also determined using a specific gravity bottle at 30°C. The dynamic viscosity of the sample was calculated from the formula based on Stoke's law.

$$n = F(Sk - Sf) K$$

Where, n = Dynamic Viscosity in centipoise.

F = Fall time of the ball in seconds

Sk = Specific gravity of the ball

Sf = Specific gravity of the sample

K = Ball constant.

4.7.13 FREE FAT

Free fat from the WMP was extracted by using the method of Tamsma *et al.* (1959) modified by Niro Atomizer (1978). At room temperature, 50 ml of CCl_4 was added to 10 g of WMP in a 125 ml glass stoppered Erlenmeyer flask. The mixture was then shaken for 15 min on a shaking device (Griffin flask shaker, U.K). The degree of shaking was regulated so that the splashing can be avoided. Immediately it was filtered through Whatmann No.42 filter paper. A 25 ml aliquot of the filtrate was transferred to a preweighed aluminium evaporating dish and the solvent was removed by keeping the dishes on a hot plate at 135°C, then in a hot air oven maintained at 105°C for about one hour. The dishes were cooled in a desiccator and the fat residue was weighed and the weight was multiplied by two. The free fat was expressed as percentage of total fat in the dry milk.

4.7.14 FATTY ACID PROFILE

The fatty acid profile of the total fat (cf 4.7.16.1) and free fat (cf 4.7.16.8) were determined by gas liquid chromatographic technique after converting the samples in to their methyl esters by the method of De Mann (1964) as follows:

4.7.14.1 PREPARATION OF METHYL ESTERS

Two hundred mg of dry milk fat were transferred to a freeze dry tube and 0.4 ml of 0.025 N sodium methoxide solution in absolute methanol was added. The tube was sealed and placed in a water bath at 60°C for 30 min to 1 hour. Complete esterification was indicated by the disappearance of the two layers.

4.7.14.2 ELUTION OF FATTY ACID IN GLC

The methyl esters obtained from above were injected (0.2-0.3 µl) with the help of a Hamilton microsyringe into the injection port of the Pye-Unicam series 304 dual column gas chromatograh equipped with flame ionisation detector, recorder, and automatic temperature programmer. The 5 ft x 1/4 inch column was packed with 20 % diethylene glycolsuccinate (Lac 728, F and M corporation) on diatoport P (F and M corporation). Three µl of the methyl ester mixture were injected at a column temperature of 80°C. After a 3 minutes run, the column temperature was raised at the rate of 18°C/min to 195°C. The separation of butyrate peak from solvent peak was facilitated by maintaining the column temperature at 80°C in the initial stages. The temperatures of both injection port and detector were 250°C. The rate of flow of the carrier gas namely, nitrogen was 70 ml/min.

The percentage proportions of fatty acids were calculated from the areas of the peaks obtained for various fatty acids. The area of the peaks were measured by triangulation. It was assumed that the area under each peak was proportional to the weight of the corresponding fatty acid methyl ester. The percentage of fatty acid methyl ester, thus obtained converted to per cent fatty acids and values for only the major fatty acids have been reported.

4.7.14.3 IDENTIFICATION OF FATTY ACIDS

The fatty acid peaks were identified by comparing the retention times of the peaks of the fatty acid methyl esters with those obtained for the standard fatty acid esters separated under the identical conditions. Methyl esters of fatty acids 4:0 and 6:0 were prepared by refluxing them with methanol containing 5% concentrated sulphuric acid. Mixture of fatty acid methyl esters 8:0 to 18:0, 18:1, 18:2 and 18:3 were procured from Sigma Co., U.S.A.

4.7.15 ISOLATION AND ESTIMATION OF LACTONES

Method of Wadhwa and Jain (1984) was used for the analysis of lactones from milk powder which is as follows:

4.7.15.1 COLUMN CHROMATOGRAPHY

The glass column 2.0 x 55 cm was packed in the sequence of glass wool, treated acid alumina (6 g), anhydrous sodium sulphate (35 g) and mixture of celite-545 (35 g), digitonin 240 mg dissolved in 1 ml hot water and fat (10 g). The packed column was eluted with acetonitrile at the rate of 0.80 ml/min until 200 ml effluent were collected. The solvent was evaporated with a rotary flash evaporator to about 10 ml. From this the fat layer was separated and the supernatant effluent was again concentrated under a fine stream of nitrogen to get crude lactonic isolate of fat.

4.7.15.2 PURIFICATION OVER SILICA PLATES

The concentrated effluent (crude lactonic fraction) from the column was spotted on 0.25 mm silica gel G plates (silica gel G:water: 2:1), activated at 110°C for 1 hr. The plates were developed at 25°C in the solvent system benzene:ethyl acetate (9:1) saturated with water acidified with one drop of HCl. After development, the plates were placed in iodine chamber and the lactonic region was scrapped. The scrapped material was eluted with 25, 15 and 10 ml of acetonitrile, filtered through Whatmann filter No. 40 to remove silica gel particles followed by evaporation under rotary flash evaporator to get the lactonic isolate of milk fat extracted from WMP.

4.7.15.3 FRACTIONATION OF LACTONES BY GLC

Pyne Unicam series 304 flame ionization gas chromatograph dual column was used. Model mixture of (C_8 - C_{16}, C_{18} and gamma - C_6, C_{12}, C_{15}, C_{16}, C_{18} lactones (each about 1%) in acetonitrile solvent was prepared. Both delta- and gamma- lactones up to C_{12} were fractionated at 160°C and those above C_{12} were fractionated at 195°C . Model mixture of lactones and lactonic isolates of ghee were fractionated under the conditions as follows:

Column stationary phase : 10 % DEGS coated on 100 - 120 mesh Diatomite CCAW

Column dimensions : 1 M x 4 mm

Column temperature : Initial temperature 160°C / 40 min.

Rate 15°C / 1 min

Upper temperature 195°C / 60 min.

Injector temperature : 210°C

Detector temperature : 225°C

Range : 100

Attenuation : 16

Flow rate of gases : Carrier gas N_2 = 30 ml/min

Hydrogen = 33 ml /min

Air = 330 ml / min.

4.7.15.4 CHARACTERIZATION AND ESTIMATION OF LACTONES

Characterization was done through retention time. Gamma C_7 lactone was chosen as the internal standard (IS) to be added to the lactonic isolate of different samples, 25 ug of this internal standard was added to lactonic isolate from 10 g fat. The whole material was dissolved in 0.05 ml (50 ul) of acetonitrile, 4 ul of this solution was injected into the column under the standardised conditions of fractionation of lactones described earlier. Individual lactones of fat were quantified according to the equation.

$$\text{ppm of lactones} = \frac{2 \times \text{peak area of lactone} \times 50}{\text{peak area of internal standard} \times 4 \times 10}$$

$$= 2.5 \times \frac{\text{peak area of lactone}}{\text{peak area of internal standard}}$$

Peak areas were calculated by the triangulation method.

4.7.16 MEASUREMENT OF AUTOXIDATION

4.7.16.1 EXTRACTION OF FAT FROM WHOLE MILK POWDER

A method described by Kontson *et al.* (1973) was adopted with some modifications. Dry milk (50 g) and powdered ice (5 g) were mixed vigorously with a spoon for 10 to 15 min. The homogeneous mixture was then transferred to the column of an extractor. The extraction tube was attached to a 500 ml round bottom flask and 200 ml of methylene chloride were added to the extractor. The flask was heated on a water bath maintained at 55-60°C. The solvent was refluxed at this temperature for a period of 4-5 hours . The filtration of the solvent from the extractor to the flask was regulated at a rate of 6 ml per min with the help of the teflon plug stop cock, so as to maintain the solvent above the level of powder in the extraction tube. On completion of extraction, all the solvents in the tube was drained into the flask. The solvent was then evaporated under vacuum and finally in the presence of nitrogen at 40-45°C.

4.7.16.2 PEROXIDE VALUE BY IODOMETRIC METHOD

Peroxide value was determined by adopting the iodometric method of Lea (1931). One g of fat sample was accurately weighed into a 100 ml conical flask and dissolved in 20 ml solvent mixture (two volumes of glacial acetic acid and one volume of chloroform). One g of potassium iodide was added to the mixture and the flask was closed with a standard glass joint with a opening. The contents were immediately heated on a boiling water bath in such a way that it starts boiling within 30 s and kept in boiling condition for 30 s. The contents were then cooled under tap water closing the opening of the standard glass joint. Approximately 30 ml of distilled water and 1 ml of freshly made starch indicator (1% solution) were added to the flask. The contents were immediately titrated against 0.002N sodium thiosulphate solution. Similarly a blank titration was also carried out. The peroxide value was calculated and expressed as mM of oxygen/kg of fat.

4.7.16.2.1 PEROXIDE VALUE BY IODOMETRIC METHOD AFTER PASSING THROUGH COLUMN OF SILICA GEL

To remove the substances in the fat which interfere in the peroxide value determination by iodometric method, the fat was treated as follows:

About 1.5 g of fat dissolved in 10 ml methylene chloride was passed through a column of silica gel (60-120 mesh, activated at 110°C for overnight and desiccated). Two hundred ml of methylene chloride, required for total recovery of fat, were used for elution. The solvent was then evaporated under vacuum and finally in the presence of nitrogen at 40-45°C. The fat thus obtained was used for the determination of peroxide value as described in section 4.7.16.2.

4.7.16.3 PEROXIDE VALUE BY COLORIMETRIC METHOD

The method of Newstead and Headifen (1981) as modified by Rogers and Kieseker (1986) was used for estimating peroxides developed in milk powder during storage. The standard method used to determine the peroxide content of milk fat which has been adopted by the International Dairy Federation (1974) is based on the method of Loftus Hills and Thiel (1946). This basic method was adopted for use with milk powders following the development of suitable fat extraction procedure by Pont (1955). The method used is as follows:

Ten g of WMP were mixed with 20 ml water at 50°C in a 50 ml beaker using a glass rod. The reconstituted milk was transferred to a 150 x 25 mm diameter glass stoppered test tube and 0.5 g of sodium borate was added and tubes were kept in a water bath at 50°C for 15 min. To this 15 ml of deemulsifying reagent (50 g sodium salicylate and 50 g sodium tricitrate were dissolved in distilled water, mixed with 86 ml of n-Butanol and the volume was made up to 450 ml) were added and incubated at 70°C in a water bath for 3 min. The contents were then centrifuged at 1000 rpm for 3 min. To the tubes sufficient distilled water at 60°C was added and the fat level was raised to neck. The tubes were further centrifuged for 2 min and left standing in a water bath at 50°C untill the fat layers were separated clearly.

The fat samples equivalent to 0.2 ml fat were weighed in a 150 x 18 mm diameter glass stoppered test tubes. To this 9.7 ml of solvent mixture (70 : 30 : Chloroform : Methanol) were added and mixed thoroughly. The colour was developed by adding 0.05 ml of ammonium thiocyanate (3 g in 10 ml) and 0.05 ml of ferrous chloride (freshly prepared) followed by shaking and allowing to stand for 5-10 min. A reagent blank with no fat was also prepared. The developed colour was measured against blank at 500 nm in a Busch and Lomb spectrophotometer. The results were expressed in m. eq. of oxygen/kg fat by using the standard curve and the following formula:

$$\text{Peroxide value} = \frac{\text{Net } \mu g \text{ iron/10 ml test solution}}{\text{Weight of the fat} \times 55.84}$$

4.7.16.4 DIENE CONJUGATION

Conjugation of non-conjugated polyunsaturated fatty acids of milk fat taking place due to autoxidation during storage was measured by the spectrophotometric method of AOCS (1971).

4.7.16.5 TOTAL CARBONYL VALUE

The method of Rama Murthy and Jain (1973) was used for determination of total carbonyl value of free fat extracted from the powder.

About 0.1 g of accurately weighed fat sample was dissolved in 0.5 ml carbonyl free hexane in a 25 ml glass stoppered flask and 0.5 g of celite-DNPH phosphoric acid mixture was added. The flask was stoppered and shaken well, allowed to stand at room temperature for 24 hr and 9.5 ml of carbonyl-free hexane were added to make up the total volume to 10 ml. The concentration of DNP hydrazones in the solution was estimated by measuring the optical density of the solution at 345 nm and converting the reading to μM, using E = 22,500. A blank experiment was carried out simultaneously.

4.7.16.6 THIOBARBITURIC ACID (TBA) VALUE

The method of Mettler (1973) was adopted for the estimation. Milk powder (15 g) was dispersed by swirling with 75 ml cold distilled water in a clean 500 ml round bottom flask. To this 7.0 ml of 3 N HCl were added and the contents were mixed. The distillation apparatus was assembled and a stop watch was started when steam first came into contact with the acidified milk. The distillation was continued at a controlled rate to collect 50 ml of distillate in 10-15 min.

The distillate was then mixed thoroughly. Twenty ml of the distillate was pipetted into a 50 ml boiling tube and 20 ml of TBA reagent (0.67% in glacial acetic acid) were added. The contents were mixed and heated in a boiling water bath for 35 min. During boiling the open end of the test tube was covered with an inverted 2.0 ml beaker. A reagent blank was prepared in the same manner with 20 ml of distilled water replacing the steam distillate. The contents were cooled and absorbance was read at 530 nm. The TBA value was reported as absorbance at 530 nm.

4.7.16.7 UV ABSORPTION OF STEAM DISTILLATE AT 280 nm

A simple method for determination of oxidative changes occurring in milk powder during storage was developed and was adopted in the study. Whole milk powder (15 g) was dispersed by swirling with 75 ml distilled water in a clean 500 ml round bottom flask and 4-5 drops of silicone oil were added to prevent the formation of foam during distillation. To this 7 ml of 3 N HCl were added and the contents were mixed. The round bottom flask was fixed to vacuum distillation assembly and the distillation was started. Simultaneously a stop watch was also started when the steam first came into contact with the milk powder slurry. The distillation was continued at a control rate to collect 50 ml of distillate in 8-10 min.

The steam distillate was read in a spectrophotometer at 280 nm against distilled water blank and the results were expressed directly.

4.7.16.8 EXTRACTION OF FREE FAT FOR OXIDATIVE CHANGES

Free fat for the determination oxidative changes was extracted by the procedure described by Wewala & Baldwin (1982). To 20 g of sample in 250 ml conical flask 100 ml of petroleum ether (40-60 boiling range) were added. The contents were shaken on a mechanical shaker for 5 min. The solvent layer was then filtered through Whatmann No.42 filter paper and collected in a round bottom flask. Extraction procedure was repeated once more. The collected solvent containing the fat, was evaporated in a vacuum rotary evaporator at 45°C . The final traces of solvent were evaporated under a fine jet of nitrogen. The fat so obtained was used for the estimation of various oxidative changes occurring during storage.

4.7.16.9 ANALYSIS OF CARBONYLS

Carbonyls compounds in the fat extracted from the powder were converted into their 2,4-DNP hydrazones and were analysed adopting the method described by Schwartz *et al*. (1963) as mentioned below:

4.7.16.9.1 Isolation and estimation of total carbonyls as their 2,4-DNP hydrazones (Step 1)

A column of celite- 545 impregnated with 2,4-DNPH, phospnoric acid and water was prepared as described below:

One g of 2,4-DNPH was dissolved in 12 ml of 85% O-phosphoric acid by grinding in a 6" mortar, 8 ml of distilled water were added to the clear yellow solution and the precipitate formed was redisolved by further grinding. Twenty grams of celite-545 were then ground into the solution until a homogeneous damp powder was obtained. The bright yellow impregnated celite was then packed into a chromatographic column (20 x 2.5 cm) containing hexane and a plug of glass wool at the constricted portion of the tube. The column was flushed with 50 ml of benzene followed by carbonyl free hexane until a colour less effluent was obtained. A 20% milk fat solution was prepared in carbonyl free hexane and was passed through the column. The flow rate of the effluent was maintained at 2 ml/min. When the last portion of the solution had just entered the column the sides of the tube were washed with 50 ml of carbonyl free hexane, which drained into the column by gravity. The column was then flushed with carbonyl free hexane until the effluent emerged colourless. The concentration of total carbonyls as their 2,4-DNP hydrazones in the effluent was estimated by measuring the absorbance of the solution at 340 nm and converting the reading to u mole using E = 22,500. A blank experiment was performed simultaneously.

4.7.16.9.2 Adsorption of 2,4-DNP hydrazines on magnesia celite column (Step 2)

A mixture of magnesium oxide activated by the addition of 6% moisture and celite 545 (1:1) was slurred in a Erlenmayer flask with hexane. The slurry was then poured through a funnel into a chromatographic column (1.5 x20 cm) containing a plug of glass wool at the constricted portion of the tube. The slurry was packed immediately under moderate air pressure leaving about 1 cm of hexane above the surface of the bed. The sides of the tube were washed down with hexane, free of solid material. A plug of glass wool was placed on the surface of the adsorbent to prevent disturbance of the adsorbent while pouring the sample solution. The effluent from step 1 was carefully passed through the column. A gentle air pressure was applied to the top of the column to ensure an unbroken stream of the effluent issuing out of the column. The adsorbed 2,4-DNP hydrazones appeared as a deep purple zone on the top of the column. The fat solution was drained and the sides of the tube were carefully washed down with hexane. Washing with hexane was continued until the effluent was free from fat, which was confirmed by drying a drop of the effluent on a filter paper. The adsorbed 2,4-DNP hydrazones were then eluted with a mixture of nitromethane and chloroform (1:3). Elution was continued till a colourless effluent emerged from the column. The effluent containing 2,4-DNP hydrazones of monocarbonyls and ketoglycerides was evaporated on a steam bath under reduced pressure. The residue was extracted with a little amount of hexane.

4.7.16.9.3 Separation of 2,4-DNP hydrazones of monocarbonyls and ketoglycerides on alumina column

Neutral aluminium oxide was deactivated by the addition of 6% moisture 20 hours before use. A column (1.5 x 10 cm) was packed using a slurry of deactivated alumina (5 g) in hexane. A plug of glass wool was placed on the surface of the adsorbent in the column. The hexane solution of the 2,4-DNP hydrazones from step 2 was passed through the column. When the last portion of hexane solution had passed into the column, the sides of the tube were washed down with hexane and the monocarbonyl fraction was eluted with a mixture of benzene-hexane (1:1). The slow moving Ketoglyceride band was completely eluted with benzene. The effluents were evaporated over steam bath and the residues were extracted with analytical grade chloroform. The concentration of monocarbonyls and keto glycerides were estimated by measuring the absorbance of the solution at 360 nm and converting the reading to micromoles by using E = 22,500.

4.7.16.9.4 TLC separation of 2,4-DNP hydrazones of monocarbonyls into different classes and their estimation

The 2,4-DNP hydrazones of monocarbonyls were separated into four classes namely methylketones, alkanals, alk-2-enals and alka-2,4-dienals, using the TLC method of Schwartz et al. (1968) which is described below:

Three grams of MgO light (EM) and 7 g celite-545 were vigorously shaken with 50 ml distill water in a stoppered 200 ml Erlenmayer flask until a lump free suspension was obtained. The slurry obtained was coated on a glass plate (20 x 20 cm) with the help of an applicator (Desaga) to obtain a coating with a thickness of 0.5 mm. The plates were held at room temperature open to the air for 48 hours The hydrazones were spotted closely from the benzene solution with the help of micropipette at about 1 cm from the base of the plate. The plate was developed for about 90 min with a mixture of hexane and chloroform (95:25). The hydrazone derivatives were separated into four bands with distinct colours, light gray , brown, light pink and dark pink and these were identified as methyl ketones, n-alkanals, alk-2-enals and alka-2,4-dienals classes respectively. The plate was removed from the TLC chamber and each class was scraped with a razor blade. The hydrazone derivatives of each class were then extracted with minimum quantities of analytical grade chloroform and filtered through Whatmann No.1 filter paper. The purity of the derivatives of each class was determined by measuring the absorption maxima of the chloroform solution. The absorption maxima for derivatives of authentic classes being 363, 355, 370 and 390 nm for methyl ketones, n-alkanals, alk-2-enals, and alka-2,4-dienals respectively. If the derivatives separated were impure they were once again subjected to TLC as described above. The percentage of each class of monocarbonyls fractions was obtained by measuring the absorbance of the derivatives at respective maxima and converting it to micromoles using E = 22,500, 22,200, 27,000 and 37,000 respectively for methyl ketones, n-alkanals, alk-2-enals and alka-2,4-dienals.

4.7.16.9.5 Isolation and estimation of steam volatile monocarbonyls

Isolation and estimation of steam volatile monocarbonyls (SVMC) from WMP was done by the method of Vijayender Rao (1981).

4.7.16.9.5.1 Preparation of 2,4-DNPH solutions

A saturated solution of 2,4-DNPH was prepared by dissolving 1.5 g of DNPH in 200 ml of 2N HCl. The clear solution was washed with 3 x 25 ml of carbonyl free hexane in order to remove the impurities which behave like DNP - hydrazones of aliphatic carbonyls.

4.7.16.9.5.2 Conversion of steam volatile carbonyl compounds in milk into their 2,4-DNP hydrazones

Hundred ml of reconstituted milk were taken in a 500 ml round bottom distillation flask and 4-5 drops of silicone oil were added to prevent the formation of foam during distillation. The flask was connected to a glass distillation unit, the receiving end of which was kept in a container with 50 ml of saturated DNPH solution. About 100 ml of the distillate were collected in order to get the complete recovery of carbonyl compounds. Foaming was reduced by slow distillation and cooling the flask with wet cloth whenever excess foam formation was noticed. The distillate was kept over night and the monocarbonyl 2,4-DNP hydrazones were extracted with 4 x 50 ml carbonyl free hexane.

The optical density of the resulting yellow solution was measured at 340 nm and the concentration of the solution with respect to DNP hydrazones (in uM) was calculated, using $E = 22{,}500$. A blank experiment was run simultaneously. The SVMC hydrazones obtained were further separated into classes and by the TLC method as described in the text (cf 4.7.16.9.4).

4.7.17 MEASUREMENT OF BROWNING

4.7.17.1 HYDROXYMETHYL FURFURAL (HMF)

The method of Keeney and Bassette (1959) as modified by Craig *et al.* (1961) and Della Monica *et al.* modified was used. Using this method total as well as free HMF can be determined.

4.7.17.1.1 Total HMF

In this method WMP was reconstituted to a level of 12% TS at 40-45°C. Ten ml aliquot of the reconstituted milk were pipetted into a 50 ml test tube. To this 5 ml of 0.3 N oxalic acid were added and the contents were mixed. The tube was covered with an inverted 20 ml beaker and the contents were digested by placing the tube in a boiling water both for 1 hour. Then it was removed from the water bath and cooled in an ice water bath to room temperature. Five ml of 40 % trichloroacetic acid (w/v) from a burette were added and the contents were mixed and filtered through Whatmann NO.42 filter paper. Four ml of this filtrate were pipetted into a test tube. To this 1.0 ml of 0.05 M TBA (0.72 g of thiobarbituric acid in 100 ml of water by warming slightly and cooling to 25°C before use) was added. The tube was placed in a water bath at 40°C for 40 min and then cooled to room temperature. The absorbance of the solution was measured at 443 nm against a blank (prepared in the same way as the sample but subst-ituting water for milk) using Gilford spectrophotometer.

Calculations

$$\text{Total HMF } (\mu\text{M/litre}) = (\text{Absorbance} - 0.055) \times 87.5$$

4.7.17.1.2 Free HMF

Free HMF from the sample was estimated by adopting the above procedure with the omission of the digestion step. Free HMF was then calculated by the following manner.

Free HMF (μM/litre) = (Absorbance - 0.015) x 81

4.7.17.2 COLOUR INDEX

The method of Palombo *et al.* (1984) was used with slight modifications. In this method 1 g of WMP was dispersed and mixed thoroughly in 5 ml deionized distilled water at 45°C . Three ml of this were transferred to a test tube which contained 0.8 ml of proteolytic enzyme pronase (SIGMA P-6911, 5-8 units/mg solid) solution (3.75 mg enzyme/ml in tris - HCl buffer pH 7.2). The test tubes were incubated for 180 min at 45°C in a water bath. The tubes were then cooled in an ice water bath and 0.3 ml of 100% trichloroacetic acid were added to each tube. Later the tubes were centrifuged at 7000 rpm for 20 min and the solutions were filtered through Whatmann No.1 filter papers. Optical density of the filtrate was measured in a Gilford 250 spectrophotometer at 420 nm and 550 nm using distilled water as a blank. The reading at 550 nm was used for correction due to turbidity. The colour index, OD calculated as

$$OD = A^{420} - A^{550}$$

The colour index was expressed as OD/g dry solid.

4.7.17.3 FERRICYANIDE REDUCING VALUE

The method of Crowe et al. (1948) as improved by Robbertsen and Radema (1975) was used for determination of ferricyanide reducing value. One hundred mg sample of milk powder were weighed and transferred into a test tube fitted with a ground glass stopper. To this 5 ml of water at 50°C were added and mixed thoroughly. Then 5 ml of 0.02M phosphate buffer with a pH of 6.6 and 5 ml of 1% potassium ferricyanide solutions were added. The contents were heated for exactly 20 min in a continuously agitated water bath maintained at 50°C. The test tubes were cooled immediately to 25°C or lower in an ice water bath. Five ml of 10% trichloroacetic acid solution were added and filtered through Whatmann No.40 filter paper. Five ml of the filtrate were mixed with 5 ml of 1% solution of sodium carboxymethyl cellulose (low viscosity: 80% substitution level) and 1 ml of 0.1% freshly prepared ferric chloride solution. The optical density was read in a Gilford 250 spectrophotometer at 660 nm against a reagent blank exactly after 10 min. The preparation of reagent blank was similar to that of sample, with the exception that the addition of milk powder was omitted. Cysteine hydrochloride was used as a reference standard for calibration curve. The values of the reducing substances were expressed in terms of mg cysteine hydrochloride per gram of the sample.

4.7.17.4 FREE SULPHYDRYLS

The method of Narang *et al*. (1967) was used with a slight modification to improve sensitivity and applicability to dry milk. The procedure adopted was as follows:

Four sets of reaction mixture were prepared. The first set contained 5 g of WMP, 10 ml of distilled water and 10 ml of 0.5 molar phosphate buffer. The reaction mixture was shaken well and set aside for 2 hours. To this 5 ml of 50% trichloroaceticacid (w/v) were added and the contents were filtered through a Whatmann No.42 filter paper. The second set contained 10 ml of distilled water and 10 ml of phosphate buffer. To this 5 ml of 50%, TCA were added after 2 hours. The third set contained 5 g of milk powder, 10 ml of distilled water, 7 ml of phosphate buffer and 3 ml of N-ethyl maleimide (NEM). This was subjected to the same treatment as in set one. Similarly the fourth set contained 10 ml of distilled water, 7 ml of phosphate buffer and 3 ml of NEM. This was subjected to same treatment given to the set two. The absorbance of the filtrate from set one was measured against that from set two and that of set three against set four at 310 nm. The difference in two absorbance was proportional to the concentration of free-SH. Narang *et el*. (1967) measured absorbance at 300 nm. However, the use of 310 was preferred where the absorbance is only 5% less but there is little interference from other substances that absorb at 300 nm (Habeeb, 1972).

Since the molar absorptivity of NEM is 620 the micro moles of sulphydryls per ml of the reaction mixture were calculated by the following formula.

$$\text{Free -SH } \mu\text{M/ ml of reaction mixture} = \frac{\text{OD}}{620{,}000}$$

For calculating sulphydryl concentration in the original sample, the dilution factor was duly taken into consideration. In this case the total reaction volume was 25 ml and weight of the powder was 5 g, therefore

$$\text{Free -SH } \mu\text{M/ g powder} = \frac{\text{OD}}{620{,}000} \times \frac{25}{5}$$

4.8 MICROBIOLOGICAL ANALYSIS

Whole milk powder samples were examined for standard plate count and coliform count according to ISI methods as mentioned in SP : 18 : Part. XI (1981).

4.9 ORGANOLEPTIC EVALUATION

Thirteen grams of WMP were was reconstituted with 100 ml of potable water at room temperature and served to expert panel of judges to evaluate for its appearance, flavour, body texture and overall acceptably on nine point Hedonic Scale, with a score of nine representing "like extremely" and that of one as "dislike extremely". A score of six and above wad for the same parameters simultaneously.

4.10 STATISTICAL ANALYSIS

The data were statistically analysed using the STATGRAF utility in a PC/XT Computer.

RESULTS AND DISCUSSION

5.0 RESULTS AND DISCUSSION

The initial physico-chemical, bacteriological and organoleptic qualities of the three batches of freshly made WMP as required under BIS specification are shown in Table 1. The quality of all the samples conformed to Indian Standards (IS: 1165-1986) in all respect. In addition to these, other initial properties of the WMP samples are given in Table 2. The changes occurring in each of these properties during storage are presented and discussed below.

5.1 ORGANOLEPTIC QUALITY

The three batches of WMP bulk packed in HDPE bags encased in hessian laminated craft paper bags stored at 20°C and ambient temperature were organoleptically evaluated initially and at the end of every month of storage. The averages of the scores given by different judges of the panel for each of the sample are given in Fig. 1. The statistical analysis of the data is given in Table 3.

The initial scores of freshly made powder ranged from 8.5 to 8.8 which indicated that the freshly made WMPs were of good quality and highly acceptable. During storage the organoleptic score decreased gradually in all the samples, the rate of decrease being significantly higher ($P < 0.01$) at ambient temperature than at 20°C. This indicated that

Table 1: Initial quality of WMP as per BIS Specification.

Sl. No.	Property	Batch Number 1	2	3
1.	Moisture, % by mass	3.73	2.62	3.33
2.	Total solids, % by mass	96.27	97.38	96.67
3.	Solubility index, in ml	0.10	0.10	0.30
4.	Total ash, % by mass	5.62	5.41	5.69
5.	Fat, % by mass	26.0	26.1	26.2
6.	Titratable acidity (lactic acid), % by mass	0.98	0.94	0.97
7.	Standard plate count/ g	10,500	10,700	4,500
8.	Coliform count/ g	NIL	NIL	NIL
9.	Organoleptic scores *	8.7	8.8	8.5

* Analysed on a nine point Hedonic scale (Appendix - I).

Table 2: Physico-chemical properties which are not included in BIS Specification of fresh WMP at zero days of storage.

1. by iodometric method; 2. by colorimetric method

Sl. No.	Property	Batch Number		
		1	2	3
1.	Free fatty acids (mM/100 g fat)	0.937	0.926	0.531
2.	Lactic acid (mg/g)	1.92	1.54	2.88
3.	Heat stability (min, at 130°C)	31	31	30
4.	pH	6.70	6.71	6.70
5.	Viscosity (centipoise)	1.36	1.36	1.34
6.	Total HMF (µM/l RCM)	3.46	3.15	3.15
7.	Free HMF (µM/l RCM)	0.446	0.405	0.447
8.	Colour index (OD/g dry solid)	0.141	0.147	0.134
9.	Total reducing capacity (Cyst. HCl/g)	1.52	1.60	1.42
10.	Free SH-group (µM/g)	3.16	3.16	3.16
11.	Peroxide value[1] (mM O_2/kg total fat)	NIL	NIL	NIL
12.	Peroxide value[2] (m. eq. of O_2/kg total fat)	0.228	0.236	0.170
13.	TBA value (A_{530})	0.002	0.008	0.004
14.	Conjugated dienes (% of total fat)	0.756	0.894	0.705
15.	Total carbonyl content (µM/g total fat)	6.660	8.010	6.780
16.	SVMC (µM/100 g RCM)	1.380	1.490	1.210
17.	OD at 280 nm	0.067	0.070	0.064
18.	Free fat (% total fat)	3.480	2.820	5.610
19.	Peroxide value[2] (m.eq. of O_2/kg free fat)	0.346	0.344	0.312
20.	Conjugated dienes (% of free fat)	0.755	0.895	0.702
21.	Total carbonyl content (µM/g free fat)	6.830	8.240	7.020

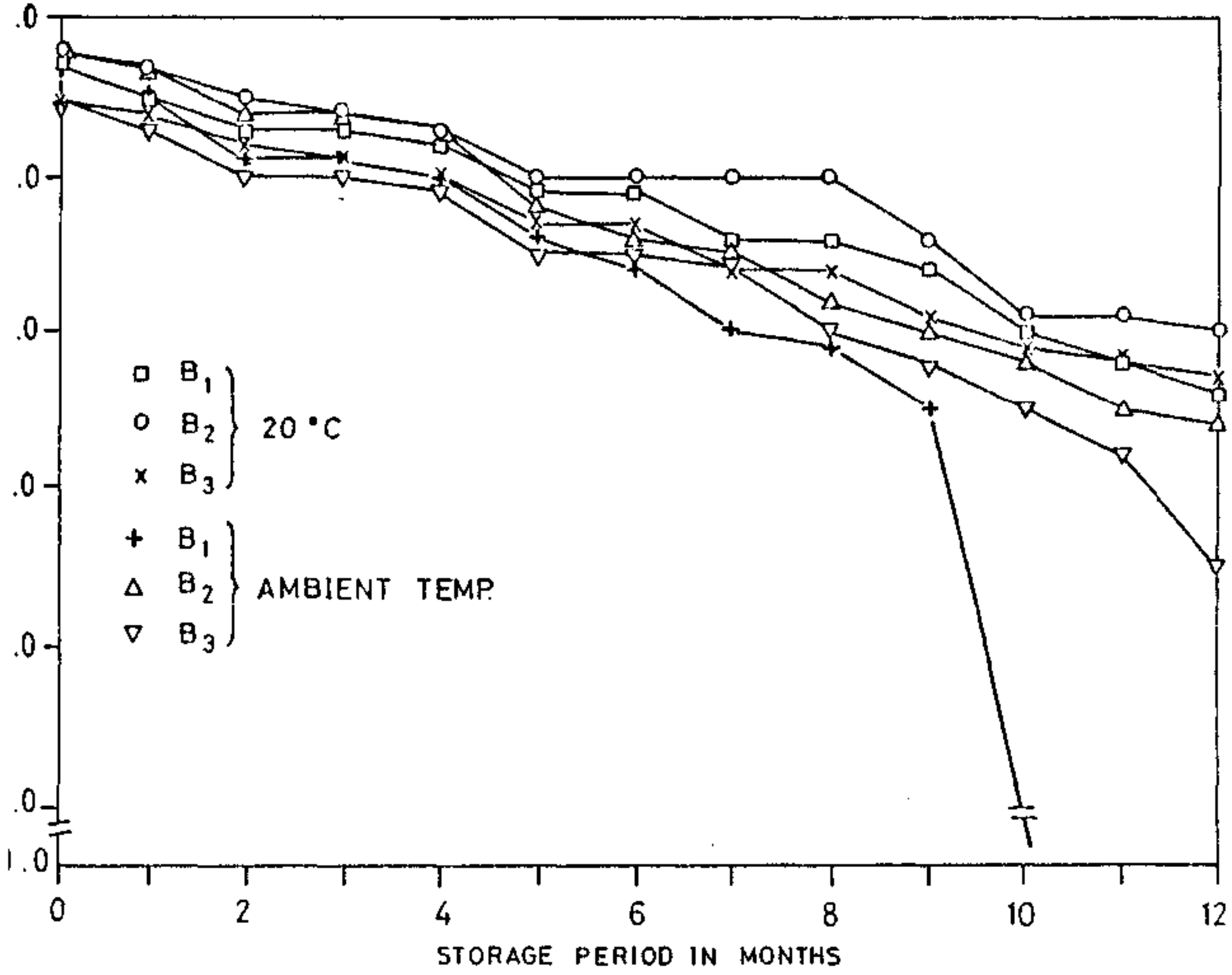

FIG. 1. CHANGES IN MEAN ORGANOLEPTIC SCORE OF WMP DURING STORAGE.

Table 3: ANOVA for the effect of temperature and period storage on the organoleptic characteristics of WMP.

Source of Variation	d.f.	m.s.s	F Ratio
Between Trials	2	2.53	6.61**
Between Temperature (A)	1	6.66	17.39**
Between Storage period (B)	12	5.31	13.87**
Interaction (A X B)	12	$6.72x10^{-1}$	1.753
Error	50	$3.83x10^{-1}$	

** Significant at 1.0 per cent level.

the flavour deterioration occurred continuously during storage and the rate of deterioration increased with increasing temperature. The rate of change of flavour score significantly differed as the storage period progressed and also it was significant among replicates (Table 3).

All the samples stored at 20°C showed scores between 6.6 to 6.7 even at the end of 12 months of storage indicating that they all remained clean and acceptable. In the samples stored at ambient temperature the rate of decrease of score was gradual up to the end of 9th month of storage and thereafter the rate of change varied widely among the sample. Thus the powder of batch No. 2 showed a score of 6.4 at the end of 12th month of storage indicating that it was clean and acceptable. The powder of batch No. 3 showed a score of 5.5 at the end of 12 months of storage indicating that the powder was on the verge of developing off-flavour and becoming unacceptable. The powder of batch No.1 showed a marked decrease in score after the 9th month of storage and reached a score of 3.9 when the powder was examined at the end of 10th month. In general, the WMP samples stored at ambient temperature and 20°C remained acceptable for a period of 9 months and 12 months respectively. Taking into consideration the variation in scores among the individual judges it was necessary to reduce the above mentioned periods by two months so that it becomes safer to predict the period for which the powder could be stored without spoilage.

For organoleptic evaluation, the WMP as well as the reconstituted milk (RCM) were taken for examination. The scores obtained for dry powders were in general higher than that obtained for RCM. This indicated that judging of RCM was more sensitive than the dry powder for organoleptic evaluation. Hence, in the study examination of RCM was preferred over that of dry powder for organoleptic evaluation.

5.2 CHANGES IN PHYSICO-CHEMICAL PROPERTIES

5.2.1 MOISTURE

The moisture content of the three batches of WMP samples stored at 20°C and ambient temperature, obtained at the end of every month of storage up to a period of 12 months is shown in Fig. 2. The increase in moisture during first 2 to 6 months of storage was not significant (the mean differences were less than the critical difference of storage periods). During subsequent period of storage the rate of increase was significant and rapid (Table 4). The initial period during which the powder showed resistance to absorption of moisture depended on the initial moisture content of the powder. Thus the powder of batch No.2 which had the lowest moisture content of 2.62% showed a shortest period of two months during which the moisture absorption was not significant.

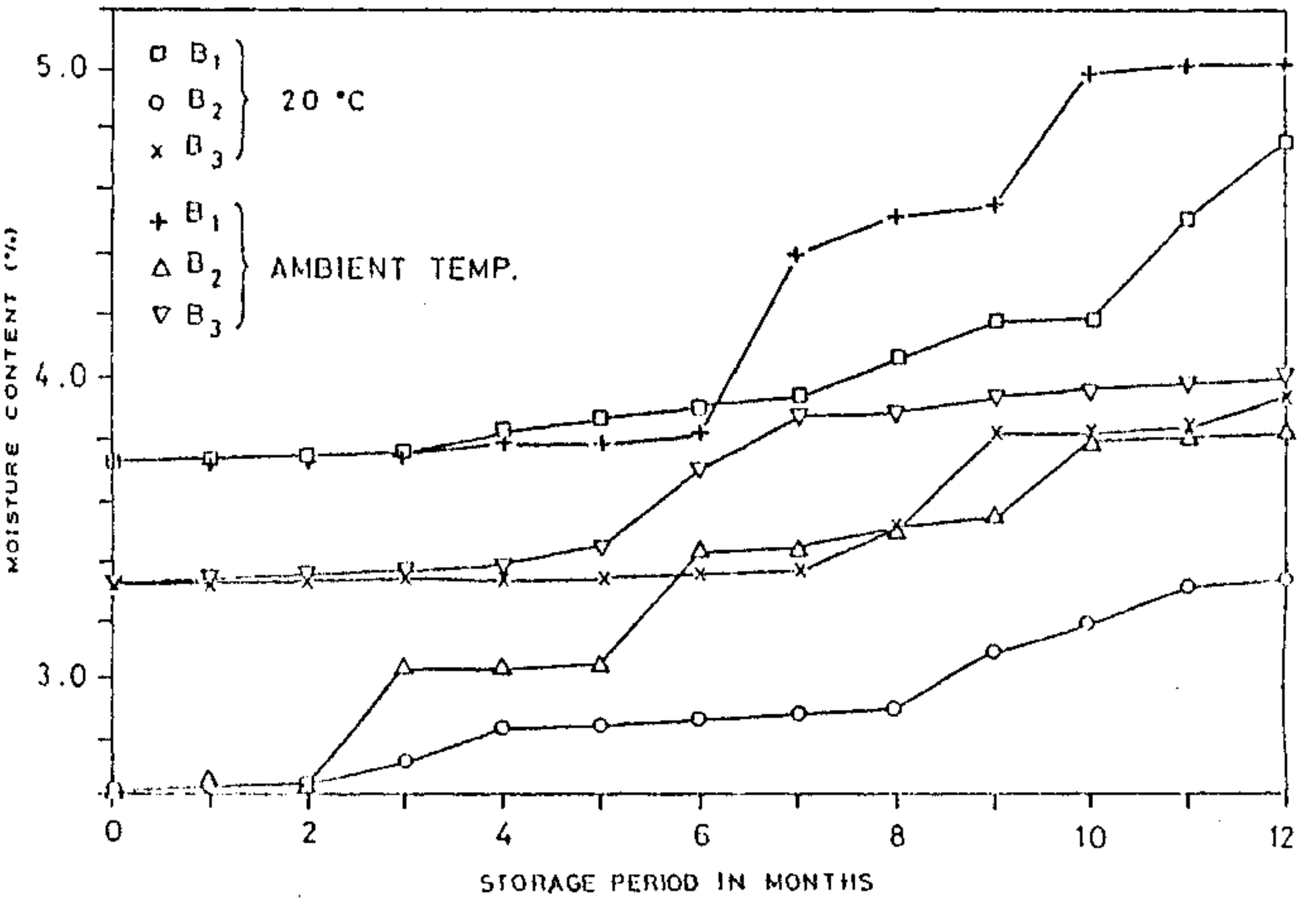

FIG. 2. CHANGES IN MOISTURE CONTENT OF WMP DURING STORAGE.

Table 4: ANOVA for the effect of temperature and period of storage on moisture, titratable acidity (TA), solubility index (SI) and free fatty acids (FFA) of WMP.

Source of Variation	d.f.	Mean sum of squares			
		Moisture	TA	SI	FFA
Between Replicate	2	6.68**	3.48×10^{-2} **	4.14×10^{-1} **	1.20×10^{-1} **
Between Temperature (A)	1	1.15**	3.66×10^{-2} **	7.52×10^{-1} **	6.74×10^{-1} **
Between Storage period (B)	12	6.69×10^{-1} **	8.03×10^{-3} **	7.39×10^{-2} **	1.59 **
Interaction (A X B)	12	6.58×10^{-2} *	7.17×10^{-4}	5.44×10^{-2} **	1.82×10^{-2}
Error	50	2.60×10^{-2}	9.47×10^{-4}	1.25×10^{-2}	2.2×10^{-2}

* Significant at 5.0 per cent level
** Significant at 1.0 per cent level

The powder of batch No.3 which had the highest initial moisture content of 3.33% showed a period of 4 months whereas powder of batch No.1 showed a period of 5 months. After this initial period the level of moisture in powder increased significantly ($P < 0.01\%$) with the storage period. The rate of change of moisture was significantly higher in samples stored at ambient temperature than in those stored at 20°C (Table 4). The average rate of increase of moisture in all the samples was 0.06 per month at 20°C and 0.09 at ambient temperature.

A comparison of monthly average increase of moisture with corresponding RH at Dharwar is given in Fig. 3. There was no definite trend between the rates of moisture absorption and RH of place which even touched 100% during certain months. This showed that the rate of absorption of moisture under the packaging conditions of the study was influenced by ambient temperature and very little by RH. Agarwal (1988) reported that the Water Vapour Transmission Rate (WVTR) of HDPE to be 0.3 g/m^2 at 23°C/85% RH. It can be inferred that HDPE possessed sufficiently good moisture barrier properties even under high humid conditions. Belcher (1983) also observed that HDPE had good moisture barrier property as compare to LDPE and LLDPE.

In WMP of batch No. 1 stored at ambient temperature the sudden development of off-flavour after 9th month of storage coincided with the level of moisture becoming more

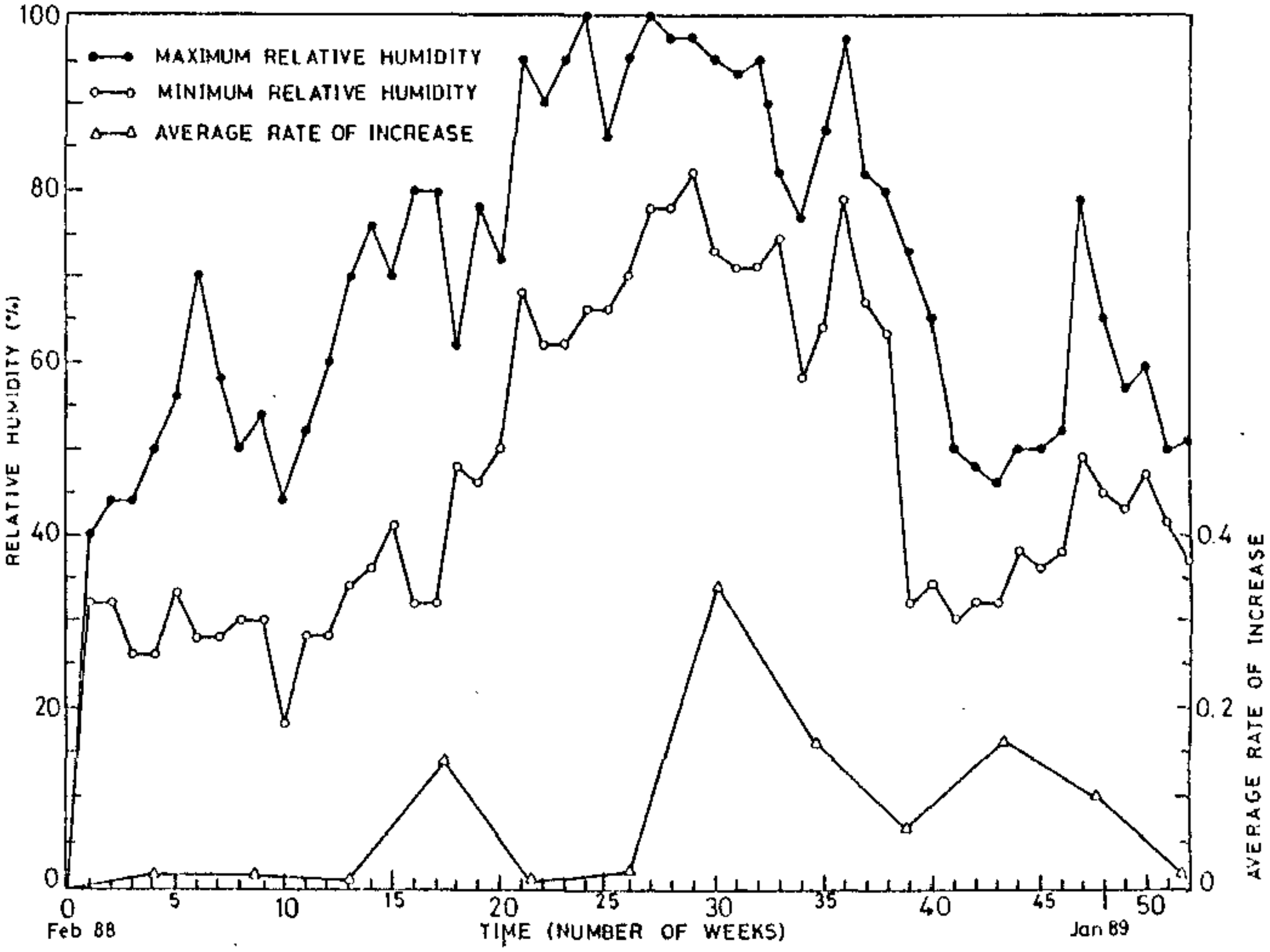

Fig. 3. WEEKLY MAXIMUM & MINIMUM RELATIVE HUMIDITY IN DHARWAD DURING A YEAR AND AVERAGE RATE OF INCREASE OF MOISTURE IN WMP.

than 4%. Thus a moisture level of around 4% appears to be very critical in WMP in accelerating the development of off-flavour. Tarassuk and Jack (1948) also reported that a moisture content of 4% was not acceptable in WMP as the moisture of above this level had been found to accelerate the development of off-flavour.

The following guidelines can be given to predict the storage period in respect of moisture content of WMP based on its initial moisture.

Initial Moisture	Period of Storage	
	Ambient	20°C
4.0	Cannot be Stored	Cannot be stored
3.5	Upto 5 months	Upto 12 months
3.0	Upto 10 months	More than 10 months

5.2.2 TITRATABLE ACIDITY

Initial titratable acidity (TA) of all the samples of WMP ranged between the narrow limits of 0.94 to 0.98. The TA showed a small increase which was gradual during storage (Fig. 4). The rate of increase was slightly higher at ambient temperature than at 20°C, but the difference was significant (Table 4). The average rate of increase in TA of the samples stored at ambient temperature was 0.035% and at 20°C it was 0.019%.

Vasic *et al.* (1971) and Valent (1977) observed practically no change in TA in WMP stored in polyethylene

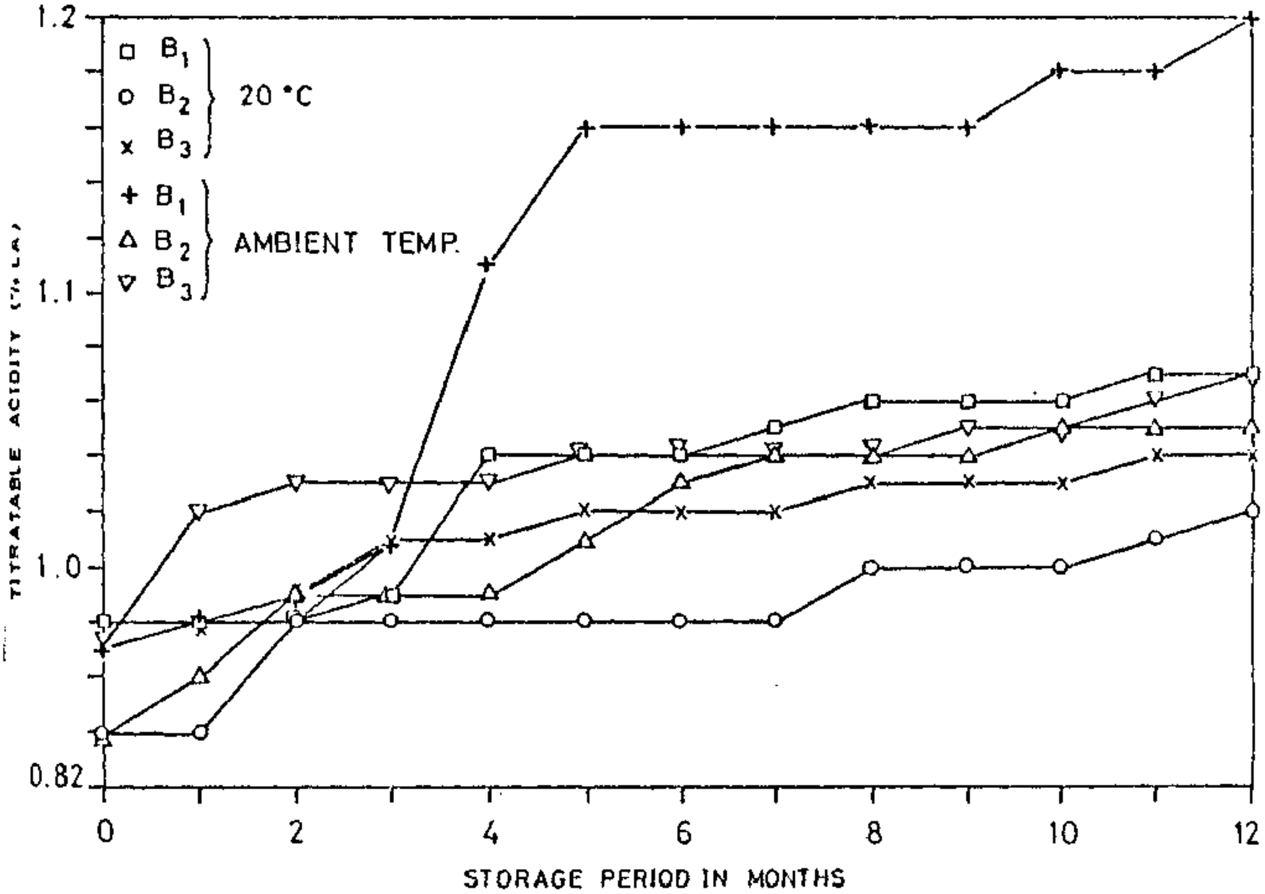

Fig. 4. CHANGES IN TITRATABLE ACIDITY OF WMP DURING STORAGE.

bags at ambient temperature for 6 months. Kieseker et al. (1984) also observed a very small increase in TA in WMP stored for 8 months at ambient temperatures.

As there was no bacterial activity in WMP during storage, production of lactic acid is not expected and hence the increase in TA could not be attributed to the production of lactic acid. Coulter et al. (1951) attributed such increase in TA to the possible tying of amino groups of protein with aldehyde group of lactose thereby resulting in an increase in carboxyl group for titration.

A knowledge of initial TA of the powder is very important as it indicates the bacteriological quality of raw milk used for manufacture of WMP, unless alkali is used for neutralizing the developed acidity of milk. Such addition of neutralizers are not permitted by PFA rules (PFA, 1987). Since the rate of increase of TA in WMP during storage is very small even at ambient temperature, the possibility of TA exceeding the maximum permitted limit of 1.2% (IS: 1165-1986) is very less. In fact in none of the samples the TA exceeded this limit even after storing the powder for 12 months. Hence, increase in TA of WMP during storage is not of much importance.

5.2.3 SOLUBILITY INDEX

The initial solubility index (SI) of all the three batches of WMP ranged from 0.10 to 0.30 ml which indicated that powder possessed very high solubility (99.9 - 99.7%

solubility). The most important condition needed to obtain milk powders of high solubility (low SI) is that the colloidal state of the casein micelles should not be destabilized during drying process. This condition is almost achieved due to high advances made in spray drying process (Fergusson, 1989).

The rate of increase of SI of WMP during storage is given in Fig. 5. In general SI increased gradually throughout the storage period. The rate of increase of SI of the powder was significantly higher in samples stored at ambient temperature than in those stored at 20°C (Table 4). The increase in SI can be attributed to gradual browning reactions that might be taking place during storage. Thus the conditions such as increase in moisture content and storage at high temperature, which favour browning reactions, influence the change in solubility. Litman and Ashworth (1957) reported that the rate of change in solubility of WMP greatly affected by the temperature of storage. Mrithyunjaya (1982) observed an increase in SI from 1.07 to 1.80 ml in buffalo WMP on storage at 30°C for 6 months. Kieseker and Clarke (1984) reported that initial SI for low- and medium- heat SMP were less than 0.1 ml. This value did not change with low heat powder irrespective of temperature of storage, while small increases were noted with medium- heat SMP held at higher temperatures of storage. The high- heat SMP showed an initial SI of 0.2 ml which was virtually unchanged

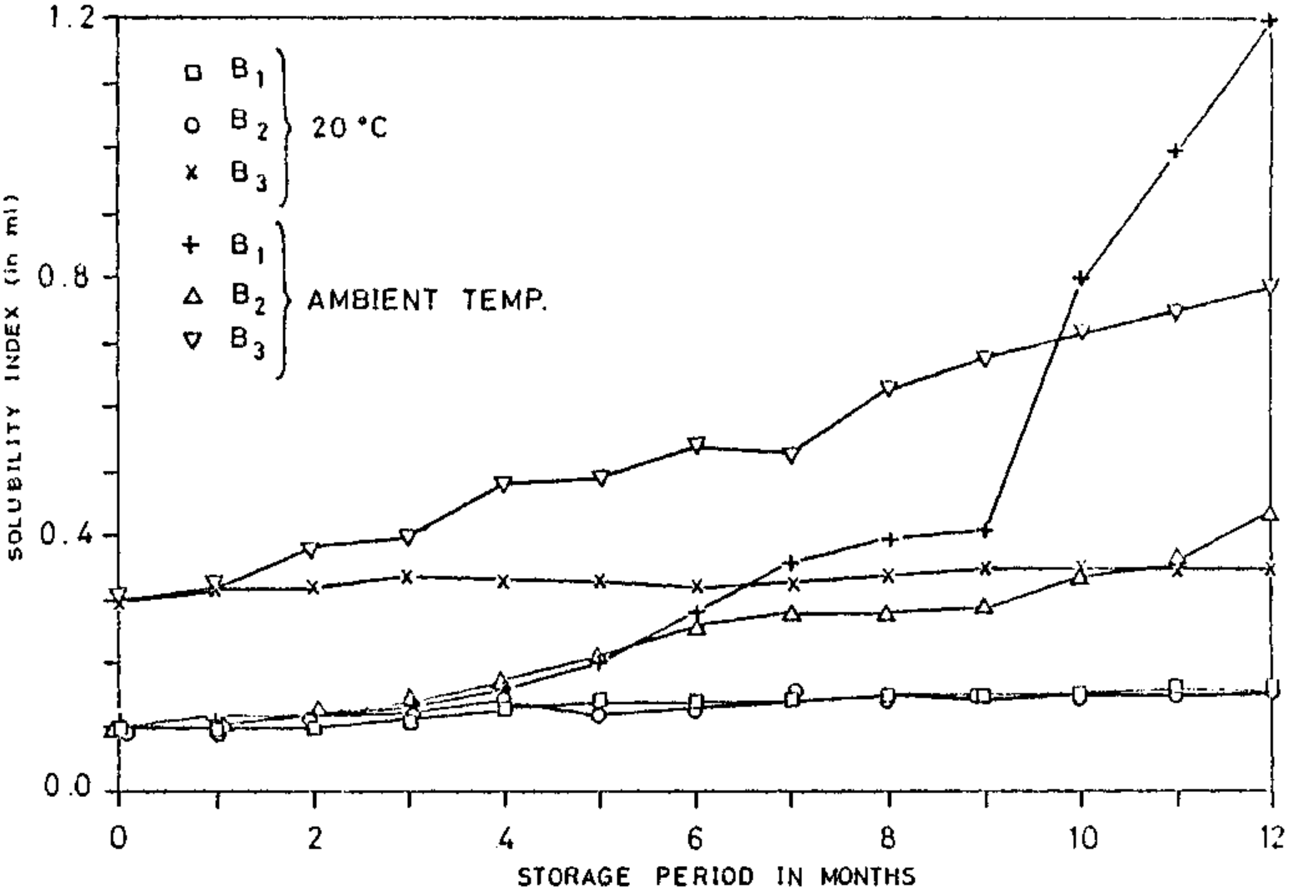

Fig. 5. CHANGES IN SOLUBILITY INDEX OF WMP DURING STORAGE

after storage at 10° and 20°C for 12 months. The same powder held at 30°C increased slowly in SI to 0.4 ml over the same time, and powder held at 40°C reached a value of 1.0 ml in 6-8 months. This showed that conditions used for preheating milk also significantly affected the change in solubility of powder during storage. The small changes in SI observed in the present study during storage are in accordance with the above mentioned observations as the powders were of medium heated type (Table 2).

In all the samples, except in the sample of batch No.1, stored at ambient temperature which became organoleptically unacceptable after 9th month of storage, the net increase in SI even at the end of 12 months of storage was only 0.42 at ambient temperature and 0.05 at 20°C. This showed that the rate of browning reactions in stored WMP are greatly controlled either because of low moisture or because of low temperature of storage. In the sample of batch No.1 stored at ambient temperature the increase in SI was gradual up to the end of 9th month of storage, but thereafter the increase was sudden and rapid. The reason for such rapid increase could be attributed to increased level of moisture and higher temperature of storage, resulting in accelerated browning reaction. Since, the standard prescribed for SI as per Indian Standards is 1.5 ml (IS: 1165-1986). The maximum value of SI observed among the stored sample in the present study was only 1.2 ml. Thus the chances of SI exceeding the prescribed limits of 1.5 ml is very remote. Therefore, changes in SI in WMP during storage are not of major concern.

5.2.4 FREE FATTY ACIDS

The initial free fatty acids (FFA) content varied widely from 0.53 to 0.94 mM/100 g fat among the samples, indicating the presence of varying amount of FFA in raw milk used for the manufacture of the powders. However, these levels of FFA did not adversely affect the flavour of the powders, as all the freshly made powders showed very high organoleptic scores which ranged from 8.5 to 8.8. Presence of high levels of FFA in freshly made powders can be related to the production of heat stable lipases in raw milk, cold stored for long periods (Ipsen and Hansen, 1988).

The changes in FFA content of WMP during storage are given in Fig.6. The FFA content increased gradually, the rate of increase being higher in samples stored at ambient than in those stored at 20°C. However, in the sample of batch No.1 stored at ambient temperature, the increase in FFA was gradual up to 9th month of storage, thereafter it was sudden and rapid reaching a value of 3.15 mM/100 g fat at the end of 10th month of storage. This rapid increase in FFA coincided with high increase in the level of moisture as well as development of off-flavour in this particular sample. However, the off-flavour developed

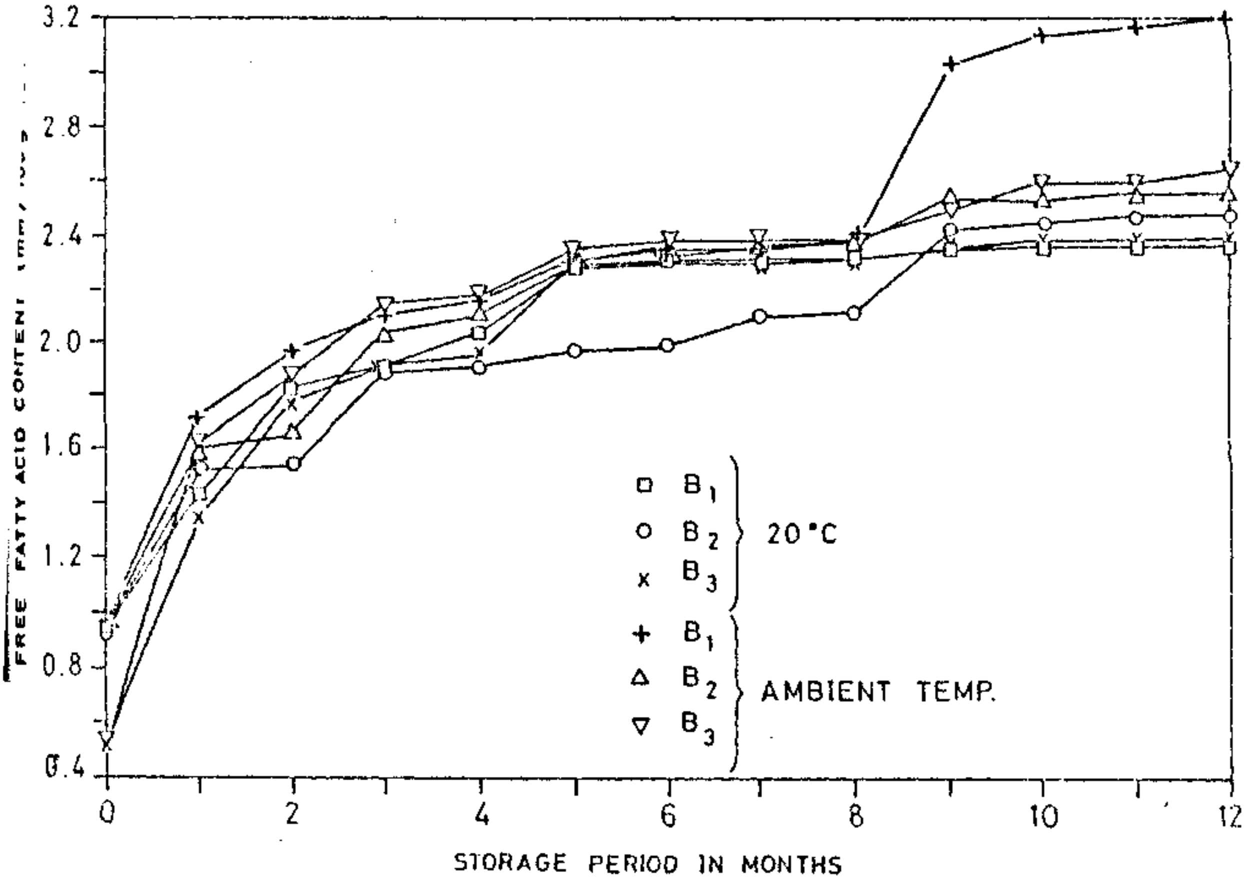

Fig. 6. CHANGES IN FREE FATTY ACID CONTENT OF WMP DURING STORAGE.

could not be characterised as rancid flavour. This showed that high levels of moisture in the powder favoured rapid hydrolysis of fat. The hydrolysis of fat in WMP can be brought about by both enzymatic and nonenzymatic changes (Ipsen, 1989; Rao and Murli, 1989).

In the other samples of WMP, which remained organoleptically acceptable till the end of the storage study of 12 months, the maximum increase in FFA was 1.78 mM/100 g fat, showing that even this increase in FFA did not contribute to detectable off-flavour. Among the total FFA the lower chain fatty acids only contribute to off-flavour. Since the threshold values of these fatty acids are high (Robards et al., 1988), considerable amounts of these fatty acids are to be produced before they contribute to rancid flavour.

5.2.5 LACTIC ACID

The initial Lactic Acid (LA) content of the three different batches of WMP ranged from 1.54 to 2.88 (Table 2). This level of LA did not affect significantly the flavour quality of the powder, as all the three batches of freshly made powders were organoleptically of very high quality (Table 1). The initial LA content of the powder depends on the levels of lactic acid in the raw milk used for the manufacture of powder. The initial LA content of the powder observed here falls within the range of LA content of 0.064 to 6.14 mg/g found in WMP manufactured in Poland (Pijanowski et al., 1978). Lawrence (1970) found 0.5 to 1.0 mg/g LA in freshly made SMP which were very low compared to values found in this study.

The LA contents of WMP during storage is shown in Fig.7. The LA content of the WMP samples showed practically no increase during storage at 20°C and ambient temperature. This is obvious as there was little bacterial activity in the WMP samples during storage. Earlier works also did not show any development of lactic acid in milk powder during storage (Lawrence, 1970; Korhonen et al., 1976).

Determination of LA content in powder helps in knowing the presence of neutralizers and the hygienic quality of raw milk used for the manufacture of dry milk. Although the LA content may not have any affect on the off-flavour of the powder, yet it may influence the solubility and free fat content of the powder (Pijanowski et al., 1978).

In this study LA content was estimated by the modified method of Ling (1951) based on a procedure developed by ADMI (Velasco and Moats, 1959) as this method gave more reproducible results as compared to the method of Davidson (1949). This may be due to interference of copper sulphate which is used for precipitating proteins of milk in the latter method.

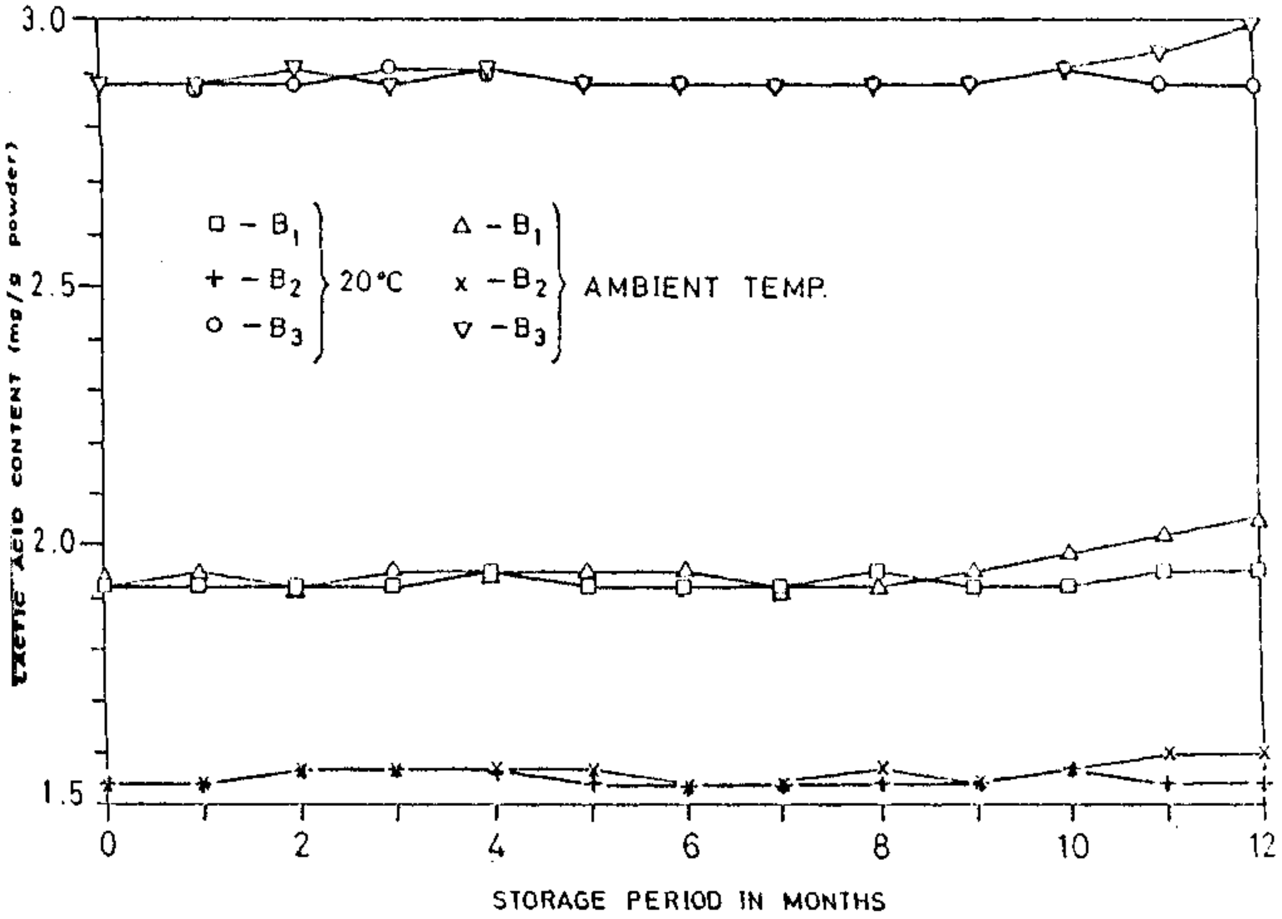

Fig. 7. CHANGES IN LACTIC ACID CONTENT OF WMP DURING STORAGE.

5.2.6 pH

The initial pH and change in pH values of all the three batches of powder during storage are shown in Fig.8. The initial pH values were same in all the WMP samples (6.7). The value was similar to the pH shown by normal cow or buffalo milk. Kieseker and Clarke (1984) reported that the initial pH value of low- medium- and high- heat powders were 6.75, 6.74 and 6.78 respectively.

The pH decreased gradually in all the samples which remained organoleptically acceptable even after 12 months of storage and the net change ranged from 0.08 to 0.12 units. The temperature of storage had practically no effect on the rate of change of pH although it was statistically significant (Table 5).

In the case of sample of batch No.1 stored at ambient temperature the pH changed gradually, as observed in other samples, up to the end of 9th month of storage and thereafter the rate of change was sudden and it reached a vale of 6.50 at the end of 10th month of storage. Thus the net decrease in pH at the end of 10th month was 0.2 units and the net change at the end of 12th month was 0.24 units. The results here demonstrate that sudden change in pH of WMP during storage indicates the onset of spoilage in the powder or development of off-flavour.

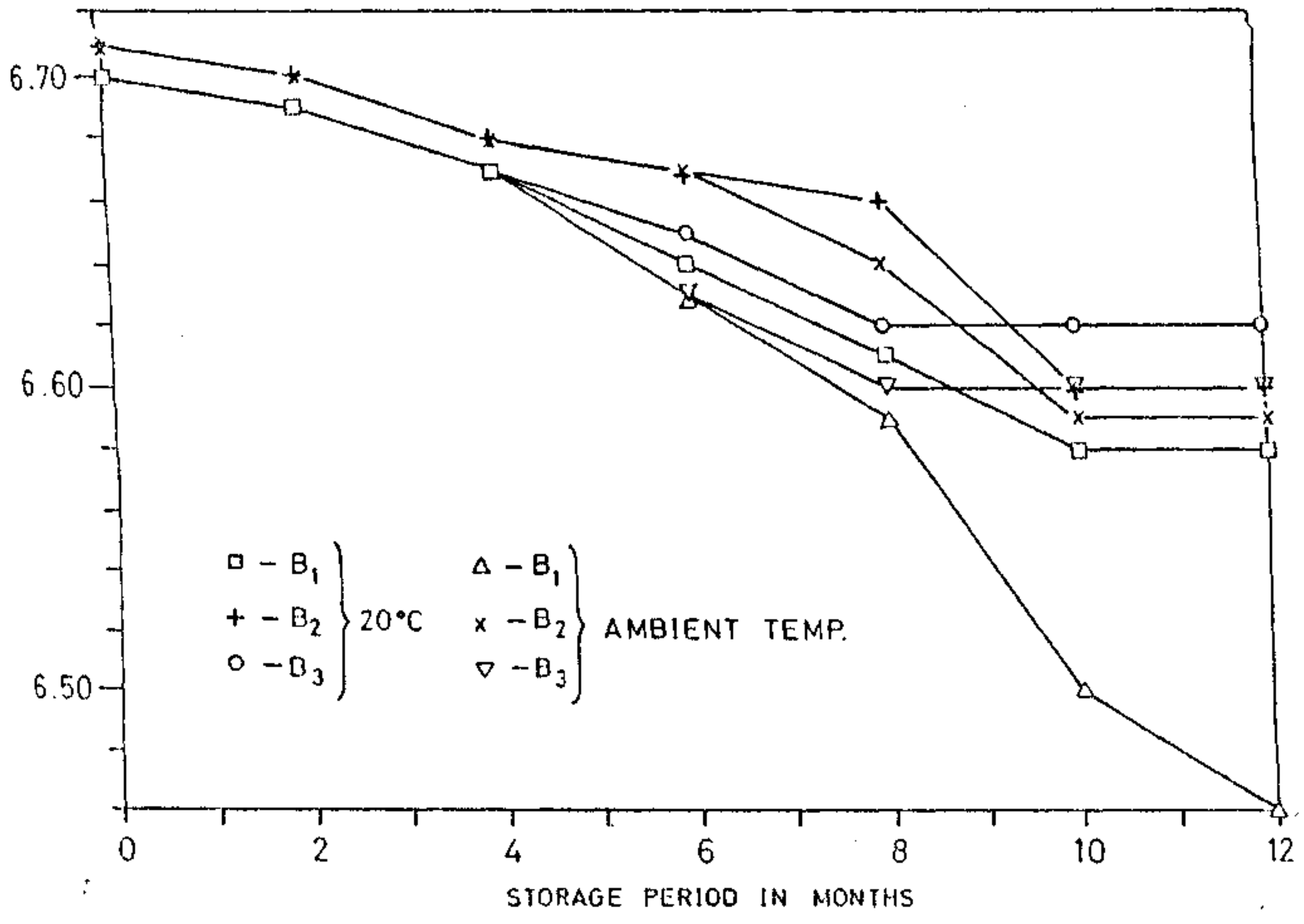

Fig. 8. CHANGES IN pH OF WMP DURING STORAGE.

Table 5: ANOVA for effect of temperature and period of storage on heat stability, pH and viscosity of WMP.

Source of Variation	d.f.	Mean Sum of Squares		
		Heat stability	pH	Viscosity
Between Replicate	2	52.09 **	4.55×10^{-3} **	1.61×10^{-3}
Between Temperature (A)	1	38.09 *	2.92×10^{-3} *	2.62×10^{-3}
Between Storage period (B)	6	305.19 **	1.60×10^{-2} **	5.36×10^{-2} **
Interaction (A X B)	6	7.54	6×10^{-2}	9.89×10^{-4}
Error	26	7.30	5.96×10^{-4}	1.07×10^{-3}

* Significant at 5.0 per cent level
** Significant at 1.0 per cent level

5.2.7 HEAT STABILITY

The initial heat stability of the powders were almost same and it was 30 to 31 min at 130°C. This heat stability is comparable to the heat stability shown by normal milk of either cow or buffalo (Satheesh and Unnikrishan, 1985). The changes in heat stability of WMP during storage are shown in Fig.9. The heat stability decreased considerably at a steady rate in all the samples, which remained organoleptically acceptable even at the end of storage study of 12 months. The rate of decrease in heat stability was significantly higher in sample stored at ambient temperature than in those stored at 20°C (Table 5).

A highly significant correlation (r = 0.97) between changes in pH and heat stability of the powder was found. Thus decrease in heat stability can mainly be attributed to the change in pH (Fig.8). The net change in heat stability at the end of 12th month of storage in all the above mentioned samples, ranged from 14 to 17 min which is very substantial. Thus it is very interesting to note that although there was no development of off-flavour in the samples yet there was considerable decrease in their heat stability. Hence, the heat stability of reconstituted milk is greatly influenced by the age of the WMP.

The sample of batch No.1 stored at ambient temperature behaved differently. The sample showed, a

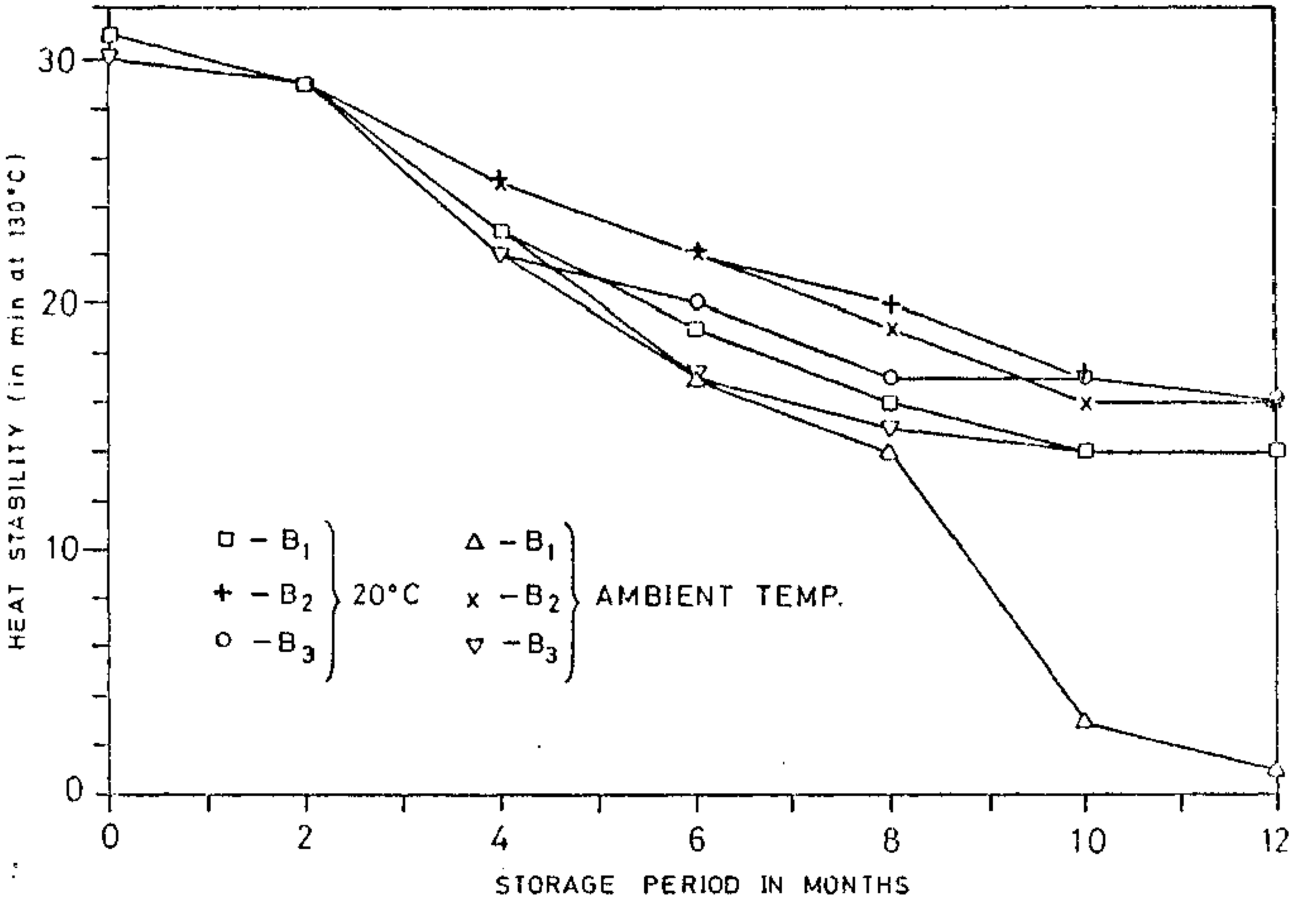

Fig. 9. CHANGES IN HEAT STABILITY OF WMP DURING STORAGE.

gradual decrease in pH till the end of 9th month of storage, like in other samples, but thereafter it showed a sudden decrease in heat stability coinciding with the sudden drop in pH as well as development of off-flavour. Therefore, the results show that the sudden decrease in heat stability is indicative of onset of spoilage in WMP during storage.

5.2.8 VISCOSITY

The initial viscosity of reconstituted milk made from the three batches of freshly manufactured WMP ranged from 1.344 to 1.356 centipoise. The changes in viscosity values due to storage of the powders are shown in Fig. 10. The viscosity of normal milk has been reported to range from 1.640 to 1.857 centipoise at 30°C (Jenness and Patton, 1959). During storage of WMP, the viscosity values increased gradually throughout the storage period in all the samples which remained organoleptically acceptable even at the end of 12 months of storage. The temperature of storage had no significant effect on the rate of increase of viscosity (Table 5). The net average change of viscosity over a period of 12 months was only 0.24 centipoise. Kieseker and Clarke (1984) reported that at higher storage temperatures (30° - 40°C) there was an increase in inherent viscosity of reconstituted powders, which was accompanied by change in solubility indices and pH values. The reason for increase in viscosity is not known. It has been demonstrated that small changes in pH

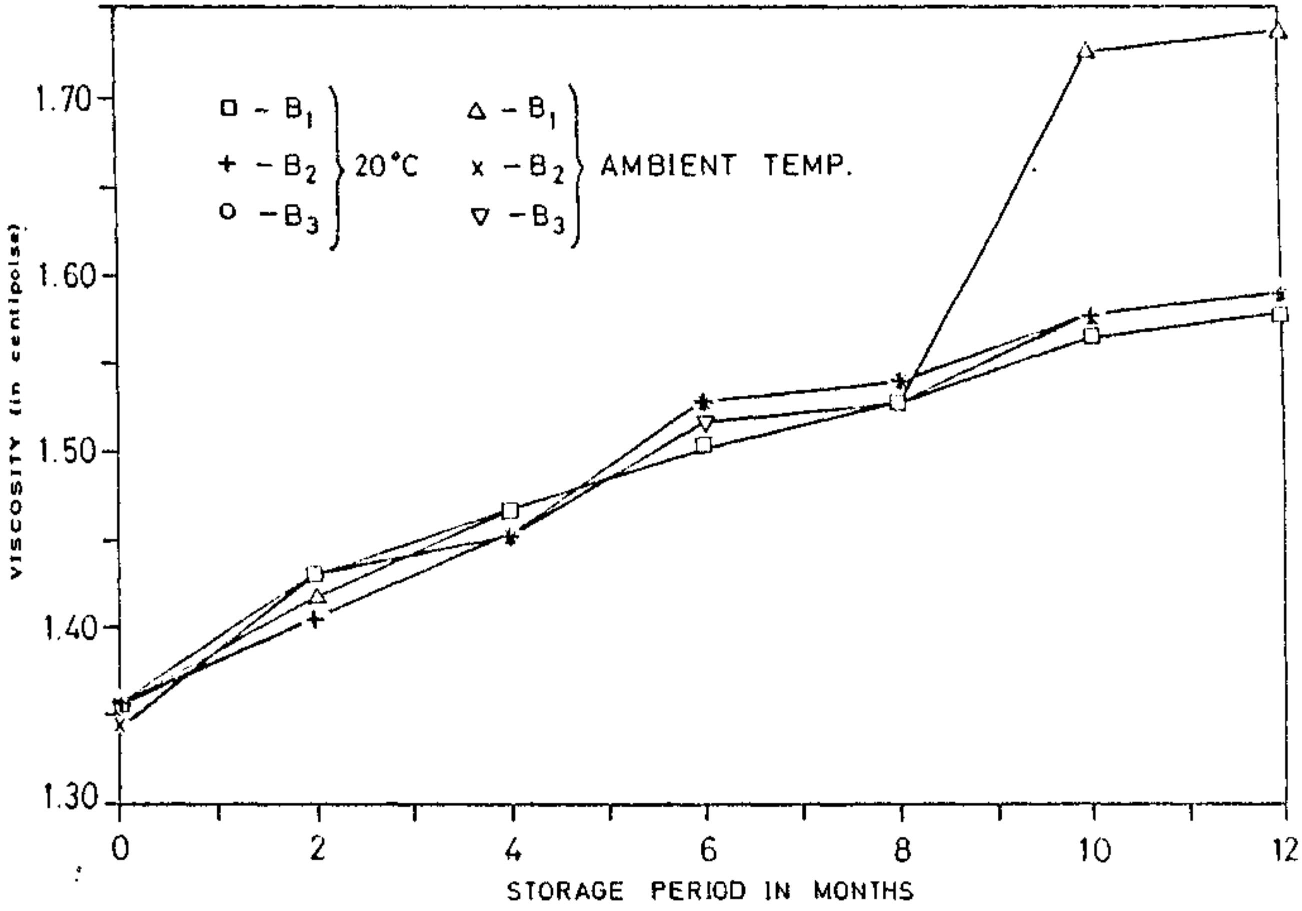

Fig. 10. CHANGES IN VISCOSITY OF WMP DURING STORAGE.

(0.05 - 0.10) during preparation of reconstituted milk can influence the inherent viscosity of powder (Newstead et al., 1978).

In the case of sample of batch No.1 stored at ambient temperature, which developed off-flavour after 9th month of storage, the viscosity suddenly changed coinciding with the development of off-flavour. The viscosity reached a value as high as 1.702 in the case of the spoiled sample. Thus the results of the study demonstrated that when the samples remained good during storage the viscosity of the reconstituted milk showed values which were comparable to those of normal cow or buffalo milk. In the case of spoiled sample the viscosity tended to increase abnormally.

5.2.9 LACTONES

The chromatogram obtained for the GLC separation of the lactone extract isolated from the fat of fresh WMP showed 44 peaks. The lactone pattern obtained from the WMP at the end of storage period also showed 44 peaks. Of these, 20 peaks were identified as delta- C_8 - C_{16}, C_{18} and gamma- C_6 - C_{12}, C_{15}, C_{16}, C_{18} lactones on the basis of their retention times with those of the standard lactones. Similarly four peaks were tentatively identified as delta- C_6 delta- C_7 and gamma- C_{13} and C_{14} lactones. The average concentrations of these identified individual lactones (24 peaks) and the total unidentified lactones

(20 peaks) of three batches of WMP are given in Table 6. The average concentration of delta- , gamma- and unidentified lactones were found to be 11.54, 1.29 and 3.34 ppm respectively. The major lactones in WMP were found to be delta- C_{10} (1.10 ppm) delta- C_{12} (3.07 ppm) and delta- C_{14} (4.86 ppm). Patton (1961) also observed that delta- C_{10} and C_{12} lactones were prominent flavour components in dried whole milk. The average total lactone concentration observed in WMP (16.16 ppm), in this study, was lower than that observed in ghee (30.26 ppm) as reported by Wadhwa and Jain (1984). This difference in the concentrations may be attributed to the differences in the heat treatments to which the products are subjected during their manufacture. Basic studies on the formation of lactones in milk products have shown that lactones are produced by heat in the presence of water from their precursor hydroxy fatty acids and the concentration of lactones increases with the increase in the heat treatment (Boldingh and Taylor, 1962; Eriksen, 1976; Wadhwa <u>et al</u>., 1979; Walstra and Jenness, 1984). During storage there was no significant change in the composition of lactones either qualitatively or quantitatively.

5.2.10 AUTOXIDATIVE CHANGES

Now it is well established that milk fat undergoes a typical pattern of autoxidative changes. The first stage is the period during which milk fat shows resistance towards autoxidation. After this stage the peroxides,

Table 6: Saturated n-aliphatic lactones (ppm) in fresh WMP samples*

Carbon No.	Delta-lactones	Gamma-lactones
6	0.43	t
7	t	-
8	0.84	t
9	t	t
10	1.10	0.030
11	0.13	t
12	3.07	0.089
13	0.16	0.074
14	4.86	0.027
15	0.06	0.104
16	0.74	0.148
18	0.15	0.150
Total	11.54	1.288

Total unidentified lactones (20 peaks)	3.34
Total delta- and gamma- lactones (24 peaks)	12.83
Gross (44 peaks)	16.17

* = Average of three batches
t = traces (< 0.01 ppm).

which are primary products of oxidation, are formed. Finally the peroxides are broken down to give rise to various secondary autoxidation products. In order to study the possible oxidative changes occurring in the fat of WMP in respect of each of the stages the following parameters were determined during storage of WMP: (i) Diene Conjugation, (ii) Peroxide value (iii) TBA value (iv) Total carbonyl value (v) UV absorption at 280 nm.

5.2.10.1 DIENE CONJUGATION

The initial diene conjugation values of milk fat extracted from WMP ranged from 0.705 to 0.894%. The diene conjugation values for cow and buffalo milk fats have been reported to be 0.821 and 0.725% respectively (Rama Murthy and Narayanan, 1972). The changes in diene conjugation in WMP during storage are shown in Fig.11. The changes in diene conjugation showed a typical pattern. It decreased during the first month of storage and then gradually increased, reaching a maximum at the end of about 7 months of storage. Thereafter, the diene conjugation decreased gradually. This trend was seen in all the individual samples. The temperature of storage, although statistically significant, had very slight effect on the rate of change of diene conjugation (Table 7). There was no significant difference in the rates of change of diene conjugation between samples which remained organoleptically acceptable till the end of 12 months of storage and that which was spoiled during storage.

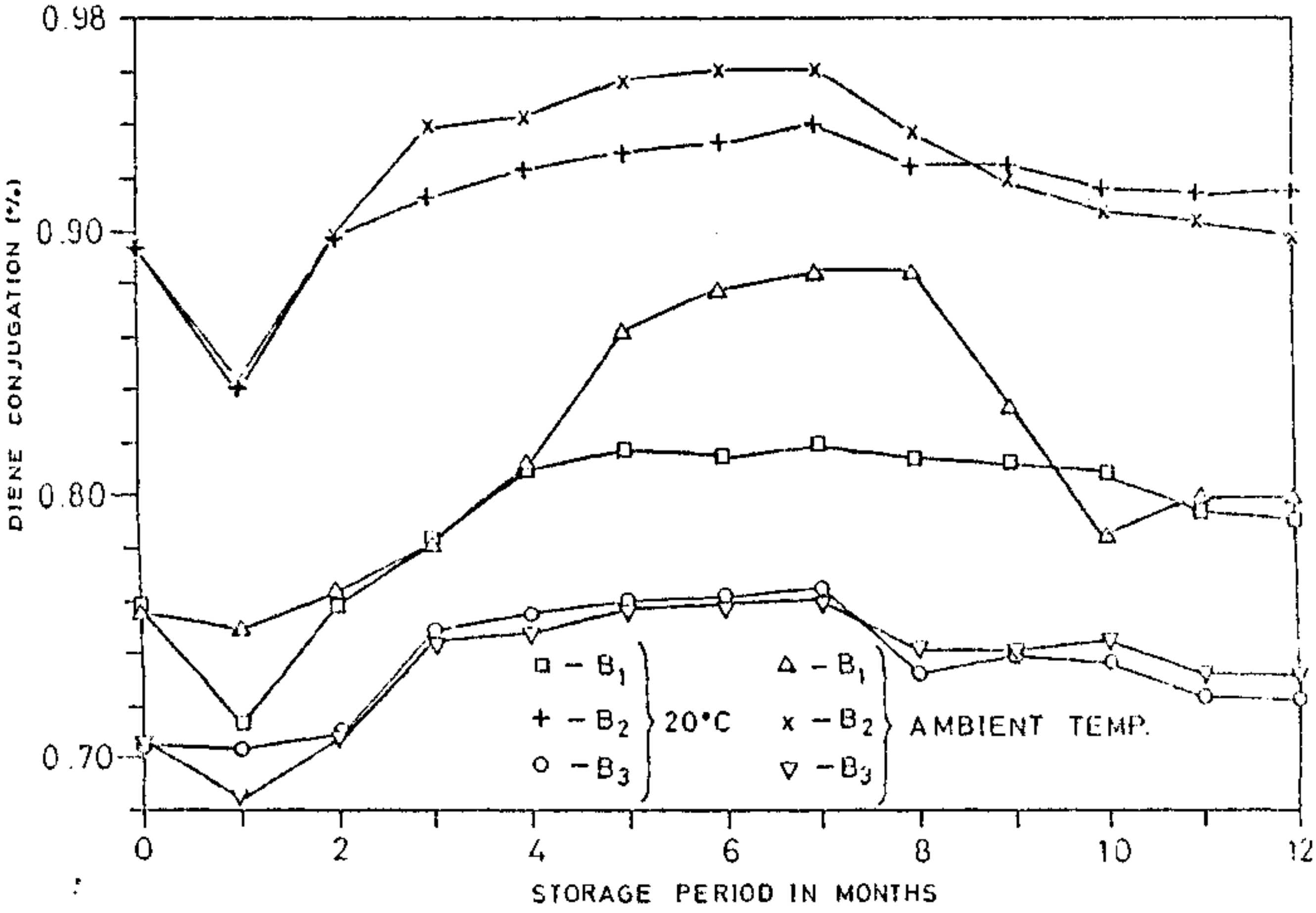

Fig. 11. CHANGES IN DIENE CONJUGATION OF WMP DURING STORAGE.

Table 7: ANOVA for effect of temperature and period of storage on diene conjugation (D.C), peroxide value by iodometric method (P.V1), peroxide value by colorimetric method (P.V2) and TBA value of WMP.

Source of Variation	d.f.	Mean Sum of Squares			
		D.C	P.V1	P.V2	TBA
Between Replicate	2	2.17×10^{-1} **	6.17×10^{-2} *	1.66×10^{-2}	6.17×10^{-2} *
Between Temperature (A)	1	1.99×10^{-3} **	1.85×10^{-1} **	1.08×10^{-2}	1.85×10^{-1} **
Between Storage period (B)	12	5.04×10^{-3} **	9.01×10^{-1} **	2.85×10^{-1} **	9.01×10^{-1} **
Interaction (A X B)	12	2.54×10^{-4}	6.51×10^{-3}	1.05×10^{-2}	6.51×10^{-3}
Error	50	2.75×10^{-4}	1.53×10^{-2}	3.90×10^{-2}	1.53×10^{-2}

* Significant at 5.0 per cent level
** Significant at 1.0 per cent level

Initial diene conjugation observed in milk fat is due to the presence of conjugated dienoic acid to the extent of 0.704 to 1.10% in cow milk fat and 0.575 to 0.861% in buffalo milk fat. Decrease in diene conjugation during first month of storage could not be explained. The experiments of Privett and Blank (1962) showed that changes in diene conjugation could be due to both oxidative and non oxidative changes. They studied the rate of change in diene conjugation versus oxygen absorption in methyl esters of PUFA kept at 40°C. During the initial period where there was no detectable or measurable uptake of oxygen, the diene conjugation increased gradually. At the end of induction period the changes in diene conjugation showed a plateau and subsequently showed a sharp increase in UV absorption. This was attributed to the formation of stable hydroperoxides (conjugated acid). Addition of antioxidant had no effect on the rate of change of diene conjugation during induction period, but it had the effect of reducing the rate of diene conjugation during the subsequent period. Thus, they attributed UV absorption during induction period to both oxidative and non oxidative changes.

The decrease in diene conjugation observed at the end of 7th month of storage in all the samples could be attributed to the breakdown of conjugated hydroperoxides at a rate faster than that of its formation. Since, the change in diene conjugation was not significant in the initial period of storage, its determination did not serve to predict the onset of oxidative changes as indicated by peroxide value, TBA value, Total carbonyl value and UV absorption at 280 nm. The changes in diene conjugation is an evidence of autoxidative changes taking place in WMP during storage. But these oxidative changes appear to be insufficient to give detectable oxidative flavour to the powders.

5.2.10.2 PEROXIDE VALUE BY IODOMETRIC METHOD

The peroxide values of fat isolated from WMP during storage, as determined by iodometric method, are given in Fig.12. The initial peroxide values of all the fresh WMP samples were zero. Peroxide development was detected at the end of 2 to 3 months of storage. However, the rate of development of peroxides was very slow, even after 12 months of storage it reached a value of 0.9 to 1.3 in samples stored at 20°C and 1.2 to 1.5 in those stored at ambient temperature. Although the rate of development of peroxide was significantly higher at ambient temperature than at 20°C, the difference was very small (Table 7). Increase in peroxide value even to a level of 1.0 to 1.5 mM O_2/kg fat did not show any oxidized off-flavour either in WMP or in the reconstituted milk made from the same powder. The sample of batch No.1 stored at ambient temperature, although developed off-flavour at the end of 10th month of storage, showed only a peroxide value Of 1.2

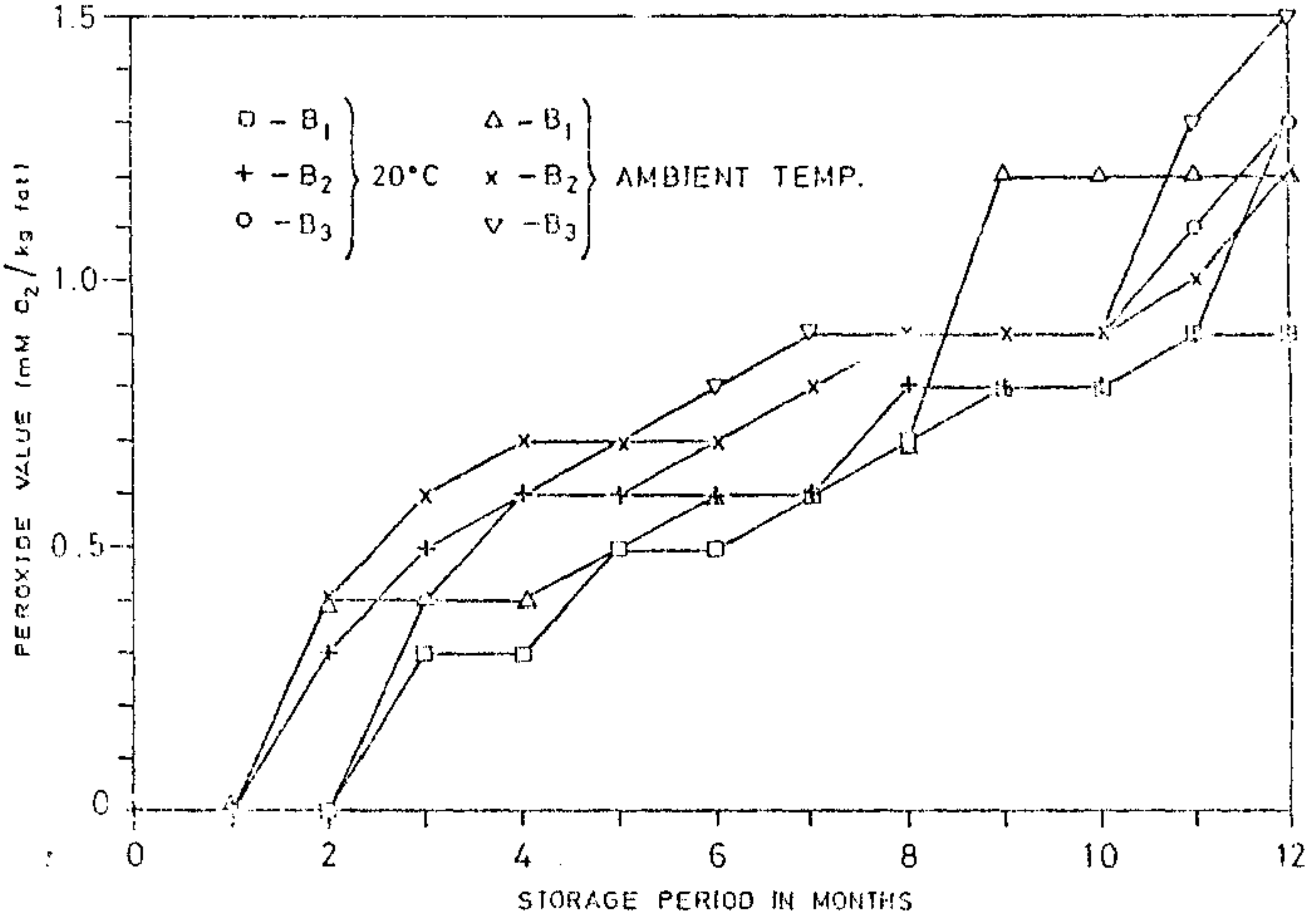

Fig. 12. CHANGES IN PEROXIDE VALUE (Iodometric method) OF WMP DURING STORAGE.

mM O_2/kg fat. Thus, measurement of peroxide value was not a reliable index of development of oxidized flavour in WMP.

On the other hand relationship between peroxide value and oxidized flavour development is very sensitive in the case of pure milk fat or ghee. Even development of peroxide value of 0.2 (mM O_2/kg fat) gives a detectable oxidized flavour (Rama murthy et al. 1969). However, such relationship between peroxide value and oxidised flavour was not found in the case of WMP. This difference between pure milk fat and fat extracted from WMP could be attributed possibly to some interfering substances extracted from non-fat portion of milk powder alongwith the fat, and these substances may be behaving like peroxides in reducing potassium iodide to Iodine in the method. This was demonstrated by a simple experiment performed in the study. When the fat isolated from WMP was passed through a column of silica gel it showed a substantial reduction in peroxide value (Table 8). An oxidized pure milk fat, with known peroxide value, when passed through the column under identical conditions, did not show any reduction in peroxide value, indicating that the column treatment of the fat did not remove any peroxide but only the interfering substances. This explains as to why peroxide method was applicable to milk fat but not to WMP. It appears that interfering substances are not present during the first two to three months of storage of the powder as the peroxide value was

Table 8: Effect of passing milk fat through a column of silica gel on peroxide value.

Samples analysed	Peroxide value before passing (mM O_2/kg fat)	Peroxide value after passing (mM O_2/kg fat)	% Decrease in peroxide value after passing
	<u>Fat extracted from WMP</u>		
1.	1.10	0.20	81.82
2.	0.90	0.10	88.89
3.	1.3	0.20	84.62
4.	1.3	0.20	84.62
5.	1.5	0.30	80.00
6.	1.2	0.20	83.33
7.	1.4	0.20	85.71
8.	1.0	0.10	90.00
9.	1.0	0.10	90.00
10.	1.4	0.30	78.57
	<u>Oxidized milk fat sample</u>		
1.	1.4	1.4	Nil
2.	3.2	3.2	Nil
3.	1.9	1.9	Nil

zero during this period and probably these interfering substances are produced later during storage due to browning reactions.

5.2.10.3 PEROXIDE VALUE BY COLORIMETRIC METHOD

The peroxide value of the fat isolated from WMP, as determined by colorimetric method, is shown in Fig.13. The initial peroxide value of the WMP samples ranged from 0.170 to 0.228 m.eq. of O_2 / kg fat. The values obtained for the WMP, as may be seen from the Fig.13, were very erratic and also no difference in the rates of oxidation due to temperature of storage was observed (Table 7). This observation is contrary to the oxidative changes expected in a milk fat during its storage. The inconsistent values obtained could be attributed to some unknown reducing substances coming into fat during extraction from WMP and these substances may be behaving like peroxides in oxidizing the Fe^{+2} to Fe^{+3} during analysis. Therefore, estimation of peroxide value by colorimetric method is not dependable in knowing the oxidized status of fat extracted from WMP and requires modification to remove these unknown reducing substantaces from the extracted fat before applying the method.

Kieseker *et al*. (1984), using the colorimetric method for estimating peroxide value in WMP, reported increase in peroxide value during storage which were more consistent than the values observed in this study.

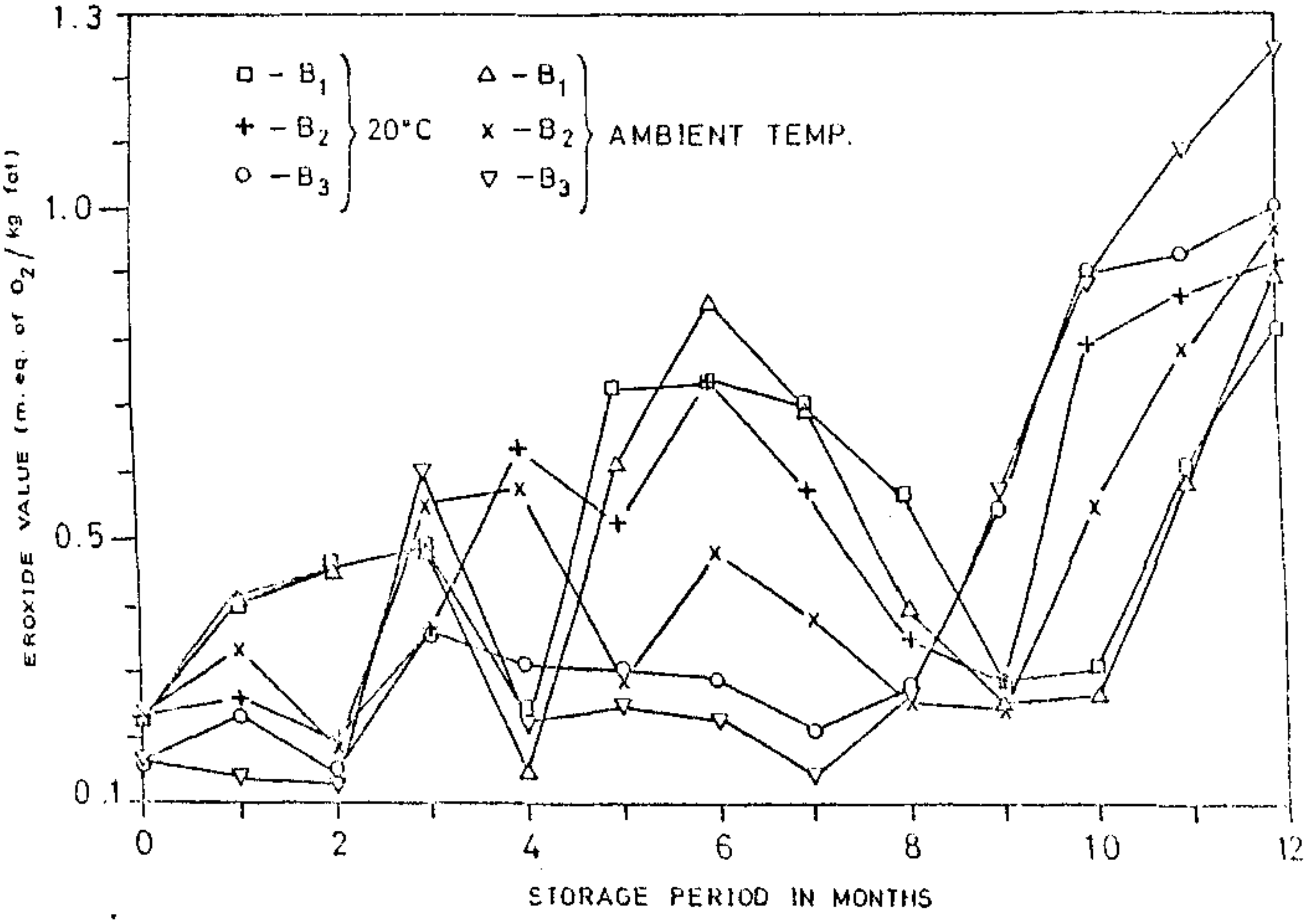

Fig. 13. CHANGES IN PEROXIDE VALUE (Colorimetric method) OF WMP DURING STORAGE.

5.2.10.4 TBA VALUE

The changes in TBA value of the WMP during storage are shown in Fig.14. The TBA value increased gradually throughout the storage period. The rate of increase was significantly higher in samples stored at ambient temperature than in those stored at 20°C (Table 7). However, in the case of sample of batch No.1 stored at ambient temperature the TBA value increased gradually till the end of 9th month of storage and thereafter the rate of increase in TBA value was sudden and rapid which coincided with the development of distinct off-flavour in the sample at the end of 10th month of storage. The corresponding TBA value was 0.068.

The sudden increase in TBA value is indicative of rapid oxidative changes occurring as a result of formation of high amount of free fat due to increase in moisture in the power and such free fat is known to be highly susceptible to autoxidation (Kopecky, 1975; Truyen and Orsi, 1977; Rotkiewicz, 1979). However, the sample, when organoleptically examined at this stage, did not show any definite oxidized flavour. This can be explained to the fact that at this stage browning changes are also rapid because of sudden increase in moisture in the powder. The off-flavour caused by browning reactions which is characterized as "gluey flavour" may be masking the oxidized flavour of the powder. An experiment was conducted to verify this contention. The fat extracted

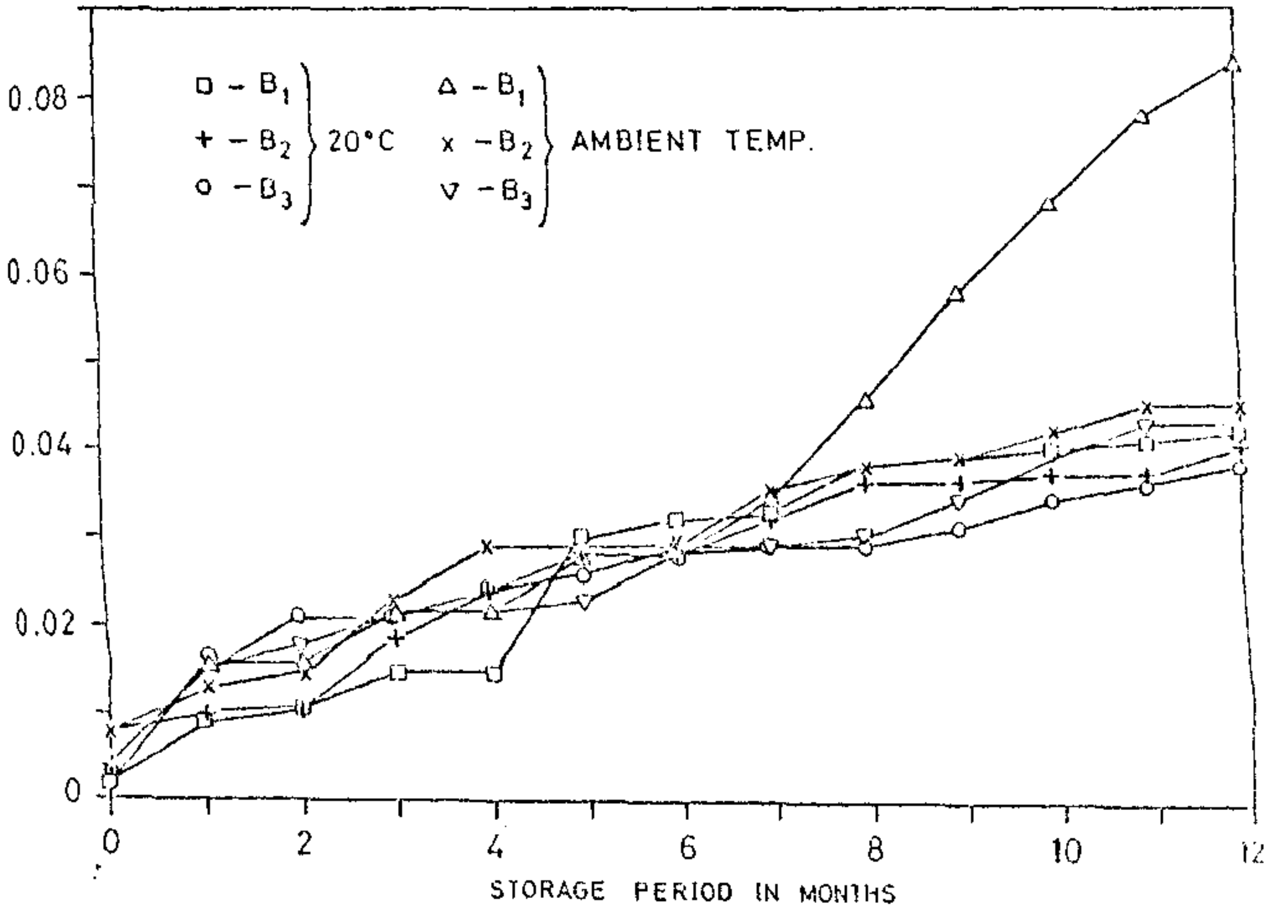

Fig. 14. CHANGES IN TBA VALUE OF WMP DURING STORAGE.

from the WMP, which had developed off-flavour (like gluey), was passed through a column of silica gel and eluted with non polar solvent like dichloromethane, the substances responsible for gluey flavour, which are mainly polar in nature (Park *et al.*, 1964; Arnold *et al.*, 1966; Ramshaw and Dunstone, 1969a; Ferretti and Flanagan, 1972) were retained on the column and the fat eluted out was free from such substances. The eluted fat, after removal of the solvent, showed a slight oxidized flavour, confirming that oxidized flavour was masked by gluey flavour in such samples.

In all the samples, which remained organoleptically acceptable, the TBA values were less than 0.045 even at the end of 12 months of storage. In the case of spoiled sample the TBA value was 0.068 at the end of 10th month when the sample had just started showing off-flavour. Therefore, TBA value of 0.05 in WMP can be taken as the limit to which powder can be stored without the development of detectable oxidized flavour. In another study conducted in this laboratory on the storage of WMP, it was observed that development of off-flavour in WMP samples coincided with higher TBA values ranging from 0.064 to 0.070. Therefore, it can be concluded that the TBA value is more reliable than peroxide value determined either by colorimetric or iodometric method in estimating oxidative changes in WMP.

5.2.9.5 TOTAL CARBONYLS

The total carbonyl values of fat isolated from WMP during storage are shown in Fig.15. The initial total carbonyl (TC) value ranged from 6.66 to 8.01 μM/g fat and this represents the carbonyls which are naturally present in milk fat. These carbonyls are made up of ketoglycerides and monocarbonyls. Again the monocarbonyls are mostly made up of methyl Ketones (Bhat et al., 1981). However, some carbonyls are produced as end products of oxidative deterioration of milk fat. Therefore, increase in TC value was related the extent of oxidative changes in the powder.

During storage, the TC value increased gradually in all the samples. The rate of increase was markedly higher in samples stored at ambient temperature than in those stored at 20°C (Table 9) indicating that the rate of oxidation was distinctly affected by the storage temperature. In the case of sample of batch No.1 stored at ambient temperature which developed off-flavour after 9th month of storage and which was shown to be partly due to oxidative changes (cf. 5.2.9.2) had TC value similar to those which did not show any off-flavour. This could be attributed to wide variation in the initial total carbonyl values. Hence, determination of TC value is not helpful in knowing the degree of autoxidation in WMP specially when the initial value is not known.

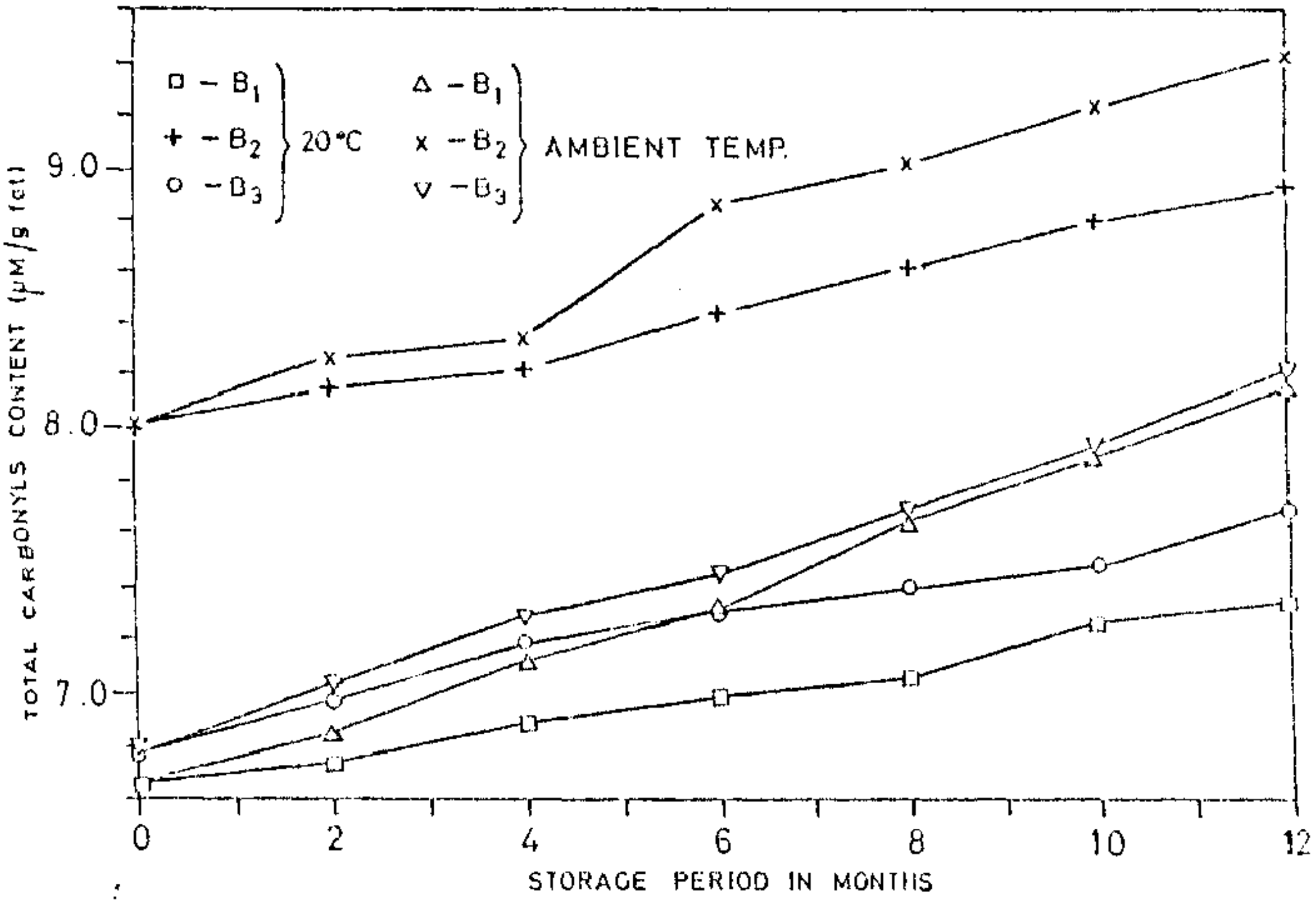

Fig. 15 CHANGES IN TOTAL CARBONYLS CONTENT OF WMP DURING STORAGE.

Table 9: ANOVA for the effect of temperature and period of storage on total carbonyls (T.C), monocarbonyls (M.C), SVMC and OD at 280 nm of WMP.

Source of variation	d.f	Mean sum of squares			
		T.C	M.C	SVMC	OD 280
Between replicate	2	8.17 **	7.77×10^{-2} **	2.59×10^{-2} **	2.64×10^{-4}
Between temperature (A)	1	9.45×10^{-1} **	1.96×10^{-2} **	1.68×10^{-1} **	1.34×10^{-3} **
Between storage period (B)	6	1.01 **	2.64×10^{-2} **	2.83×10^{-1} **	1.25×10^{-3} **
Interaction (A X B)	6	7.69×10^{-2} **	2.53×10^{-3}	2.45×10^{-2} **	2.84×10^{-4}
Error	26	5.62×10^{-3}	8.65×10^{-4}	5.47×10^{-4}	1.66×10^{-4}

** Significant at 1.0 per cent level

5.2.10.5.1 Monocarbonyls and Ketoglycerides

The total carbonyls isolated from fat extracted from WMP, at different intervals of storage period, was separated into monocarbonyls and ketoglycerides and then estimated. The quantities of these two fractions are given in Table 10. The initial monocarbonyl contents varied widely from 0.450 to 0.614. The monocarbonyl contents increased gradually during storage, the increase being significantly higher at ambient temperature than at 20°C (Table 9). The Ketoglyceride content did not show any significant change during storage. Therefore, the increase in TC value during storage was mainly due to increase in monocarbonyls.

In the case of sample of batch No.1 which had developed a slight oxidized flavour after the 9th month of storage showed a monocarbonyl content of 0.71. This value is even less than the value shown by some of the samples which had no developed off-flavour. Hence, it was difficult to judge the extent of autoxidation in WMP on the basis of monocarbonyl content.

5.2.10.5.2 Steam volatile monocarbonyls

The steam volatile monocarbonyls carbonyls (SVMC) estimated during storage of WMP are shown in Fig.16. The initial SVMC content ranged from 1.21 to 1.49 µM/100 g of recon-stituted milk (12% TS). The SVMC content increased gradually throughout the storage period in all

Table 10: Monocarbonyls and ketoglycerides from WMP

Period of storage (in months)		Batch No.1		Batch No.2		Batch No.3	
		Temperature of storage					
		20°C	Ambient	20°C	Ambient	20°C	Ambient
Initial	1*	0.45	0.45	0.61	0.61	0.49	0.49
	2**	2.01	2.01	2.35	2.35	2.19	2.19
Two	1	0.47	0.48	0.63	0.64	0.52	0.53
	2	2.11	2.14	2.36	2.37	2.19	2.20
Four	1	0.50	0.51	0.64	0.68	0.54	0.56
	2	2.11	2.14	2.36	2.37	2.20	2.21
Six	1	0.52	0.53	0.66	0.70	0.56	0.59
	2	2.12	2.11	2.37	2.38	2.21	2.21
Eight	1	0.53	0.55	0.68	0.73	0.58	0.63
	2	2.11	2.11	2.37	2.38	2.22	2.23
Ten	1	0.57	0.67	0.70	0.77	0.59	0.66
	2	2.10	2.10	2.38	2.38	2.23	2.23
Twelve	1	0.59	0.80	0.74	0.81	0.62	0.67
	2	2.12	2.12	2.40	2.40	2.24	2.25

* Monocarbonyl contents µM/g fat

** Ketoglyceride contents µM/g fat.

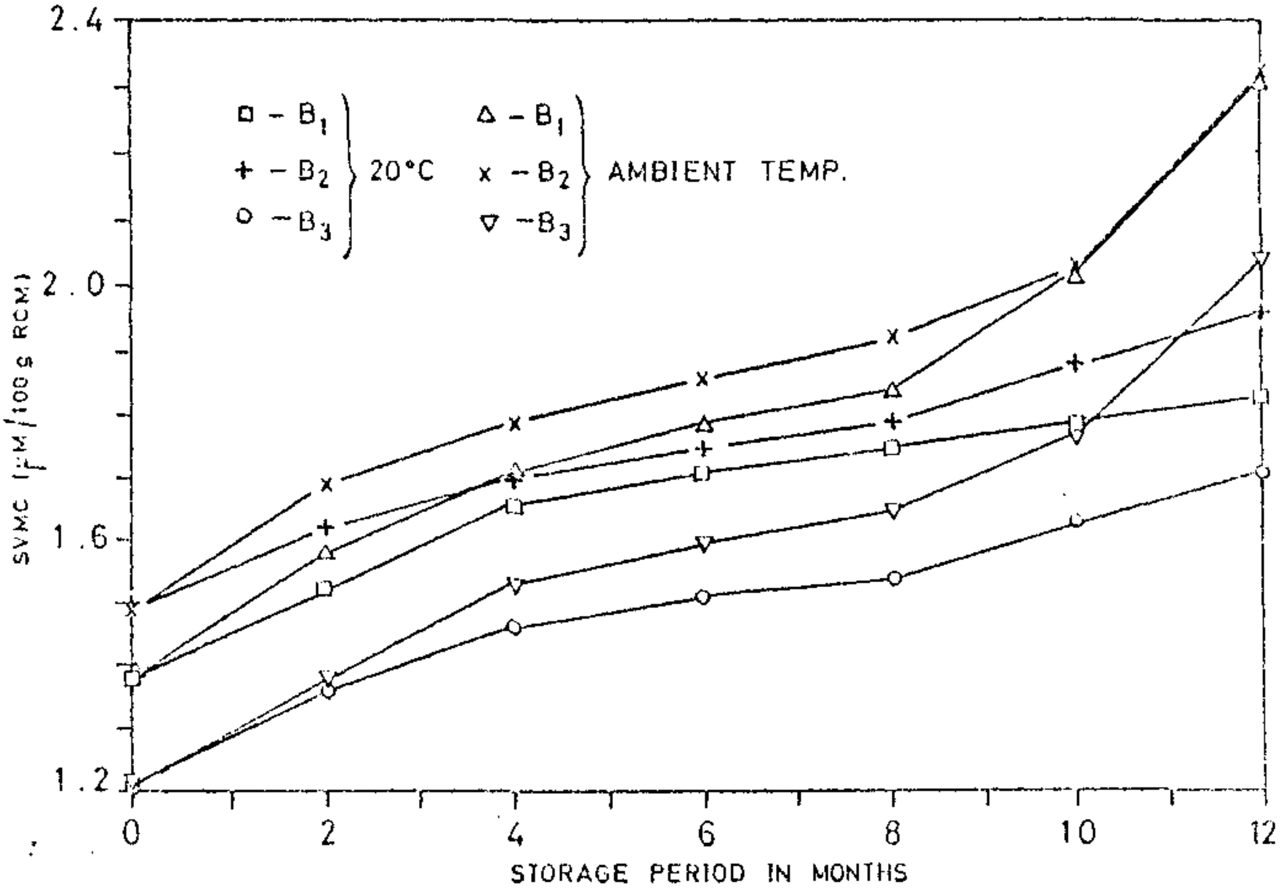

Fig. 16. CHANGES IN SVMC OF WMP DURING STORAGE.

the samples, the rate of increase being significantly higher at ambient temperature than at 20°C (Table 9). The net increase in the SVMC content was 0.64 which was slightly higher than the net increase shown by the samples which were found organoleptically acceptable even at the end of 12 months of storage period. Since the difference in the SVMC content between organoleptically acceptable and spoiled one was very small, the determination of total SVMC did not help in identifying the powders which had started developing off-flavours.

The total SVMC was fractionated into methyl ketones, n-alkanals and alk-2-enals and their quantities were estimated. The percentage proportions of these classes of SVMC from WMP samples which remained organoleptically acceptable the sample which showed off-flavour are given in Table 11.

The initial SVMC content represents the carbonyls naturally present in WMP which are mostly made up of methyl ketones. The methyl ketones do not change significantly during storage as they are not produced during oxidative changes. On the other hand the proportion of n-alkanals as well as that of alk-2-enals increased gradually in WMP samples during storage. Their production during storage is attributed to the oxidative changes. It was noticed that the rate of increase of n-alkanals was distinctly higher in the case of samples which developed off-flavour during storage as compared to

Table 11: Percentage proportion of different classes of steam volatile monocarbonyls (SVMC)* from WMP during storage.

Monocarbonyl classes	Initial	4	8	12
	Organoleptically acceptable sample			
Methyl Ketones	76.8	75.2	74.3	73.6
n-Alkanals	15.7	17.0	17.7	18.4
Alk-2-enals	7.5	7.7	7.8	8.0
	Spoiled sample			
Methyl Ketones	75.1	73.5	72.7	61.3
n-Alkanals	17.4	19.0	19.5	29.5
Alk-2-enals	7.4	7.6	7.7	9.4

Period of storage (in months)

* DNP - hydrazones of SVMC obtained from 3 WMP samples pooled together.

the other samples which remained organoleptically acceptable even at the end of the study period. The rate of development of n-alkanals, in these two types of samples have been separately shown in Fig.17. Therefore, it appears that determination of steam volatile n-alkanals and unsaturated carbonyls help in identifying the onset of spoilage in WMP during storage.

5.2.10.6 UV ABSORPTION OF STEAM DISTILLATE AT 280 nm

It is known that certain volatile compounds show absorption maxima in UV light at 280 nm wavelength. Since it has been shown in the previous chapter that significant amounts of SVMC are produced in WMP during storage, an attempt was made to relate OD at 280 nm of steam distillate of powder to off-flavour development.

The UV absorption at 280 nm of steam distillate of WMP samples at different periods of storage are given in Fig. 18. The initial UV absorption values ranged from 0.064 to 0.070 and these values increased gradually, the rate of change being significantly higher at ambient temperature than at 20°C (Table 9). The rate of increase was very small in all the samples which remained organoleptically acceptable. However, the rate of increase of this value was very rapid once the powder started developing off-flavour. Thus, in all the samples which remained organoleptically acceptable the increase was only 0.03 at the end of 12 months of storage whereas the powder

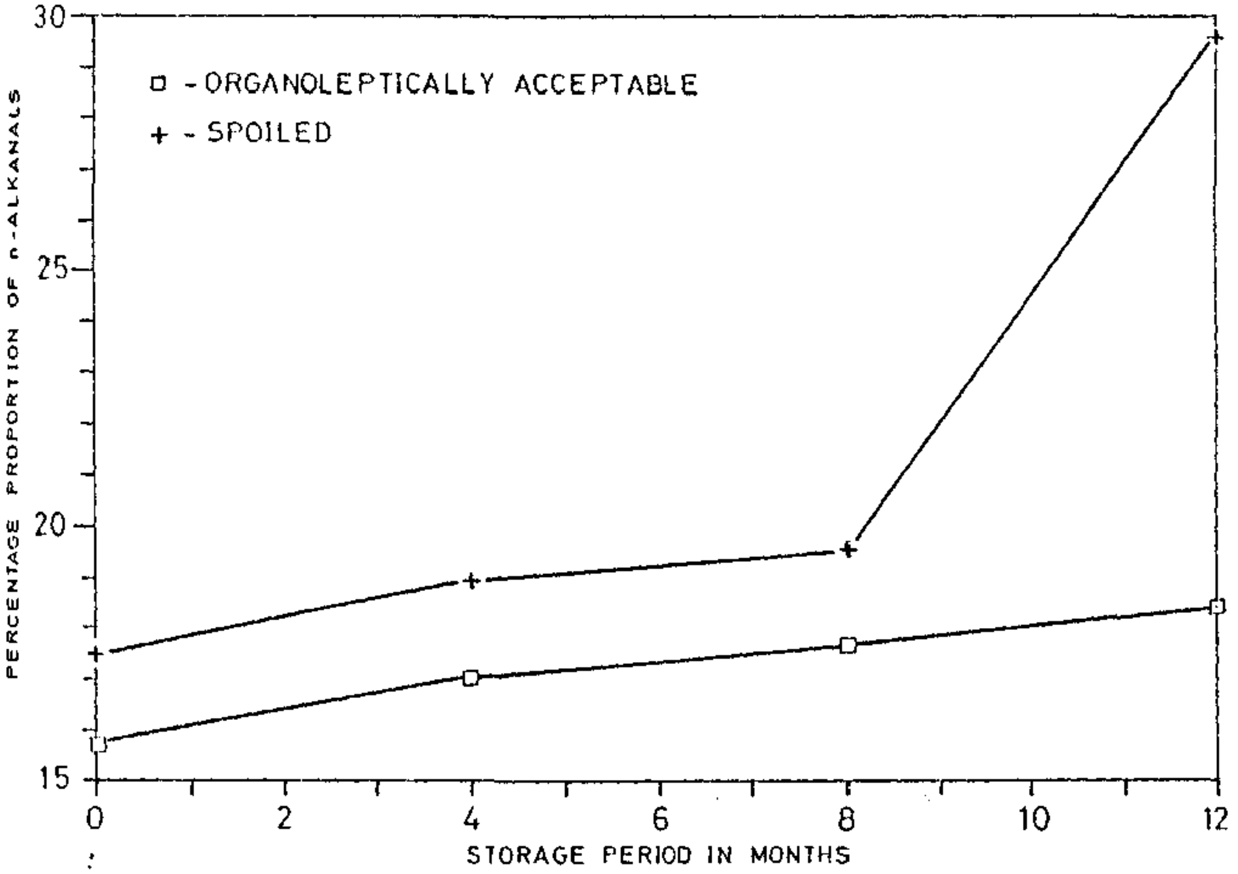

Fig. 17. PERCENTAGE PROPORTION OF n-ALKANALS FROM ACCEPTABLE AND SPOILED SAMPLES OF WMP.

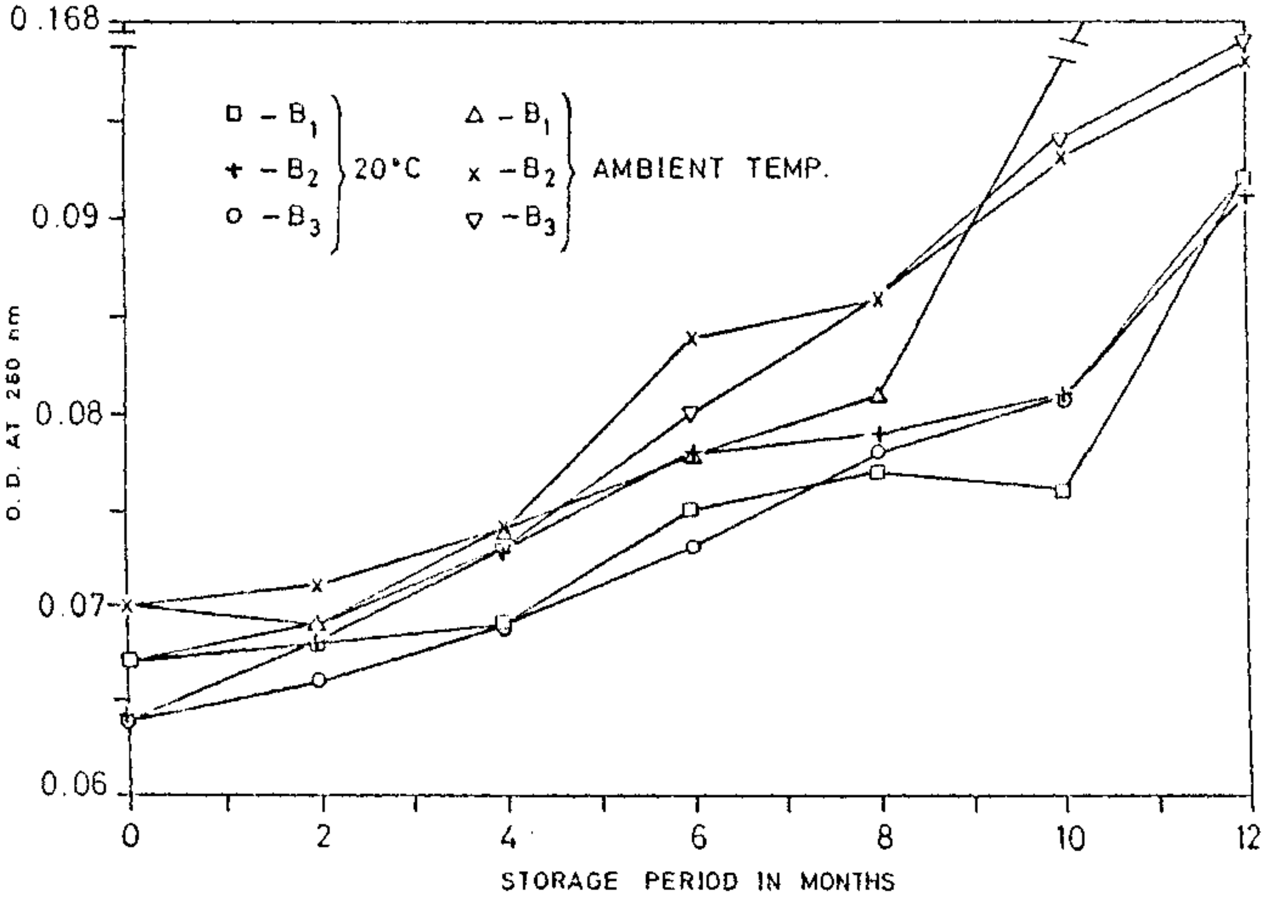

Fig. 18. CHANGES IN O. D. AT 280 nm OF WMP DURING STORAGE.

which developed off-flavour (batch No.1 stored at ambient temperature) after the 9th month of storage showed an increase of 0.074 at the end of 10 months and 0.110 at the end of 12 months. A highly significant correlationship was found between OD 280 nm and organoleptic scores (r= -0.96). On the basis of this observation it can be suggested that WMP which shows more than 0.050 increase in this value or reach a value of 0.150 may not be suitable for further storage. The method was applied on 10 samples of WMP of different ages, the relationship between OD 280 nm and flavour score is shown in Table 12. It is clear from the data that OD 280 nm correlated well with organoleptic scores (r= -0.98) confirming the applicability of method in evaluating the storage stability of WMP.

The UV absorption at 280 nm can be attributed to production of n-alkanals and alka-2,4-dienals (Mettler, 1973) which are products of autoxidation of milk fat (Vijayendra Rao and Rama Murthy, 1982). Keen *et al.* (1976) identified organoleptically significant amount of undeca-2,4-dienals in oxidized WMP. The other compounds which were considered to play an important role in oxidized flavour included pent-2-enal, hept-2-enal, non-2-enal, hepta-2,4-dienal and nona-2,4-dienal. Hall *et al.* (1985) found that the formation of n-hexanal correlated well with the oxidized flavour. Therefore, the measurement of OD at 280 nm of steam distillate of powder

Table 12: Average organoleptic scores and OD at 280 nm of steam distillate from different samples of WMP.

Sample No.	Average organoleptic scores	OD at 280 nm of steam distillate
1.	8.7	0.067
2.	7.4	0.084
3.	8.1	0.074
4.	6.6	0.092
5.	8.3	0.068
6.	3.6	0.145
7.	2.1	0.176
8.	1.3	0.194
9.	3.2	0.153
10.	2.3	0.169

Correlation coefficient (r) = - 0.98

is a quantita-tive estimate of such compounds in stored WMP samples.

5.2.11 FREE FAT

In dried whole milk the milk fat is expected to be present in its original globular form as found in raw milk. However, due to some mechanical stress on the globules, some of them may get ruptured during the manufacturing process. The fat coming out of the ruptured globule is considered as free fat and this fat is easily extractable by organic solvents. During storage also some more globule may get ruptured resulting in further increase in free fat. The free fat may have a significant influence on the development of off-flavour in stored WMP (Kopecky, 1975).

The free fat content of WMP samples, estimated under standard conditions, initially and during storage are shown in Fig.19. The initial free fat content of WMP samples ranged from 2.82 to 5.61% of total fat. The free fat content increased gradually and the rate of increase was significantly higher at ambient temperature than at 20°C (Table 13). However, in the case of sample of batch No.1 stored at ambient temperature, after 9 months of storage the free fat content increased suddenly and reached a value of 81.54% when analysed at the end of 10 months of storage. This change coincided with the sudden development of off-flavour at this stage. In all other

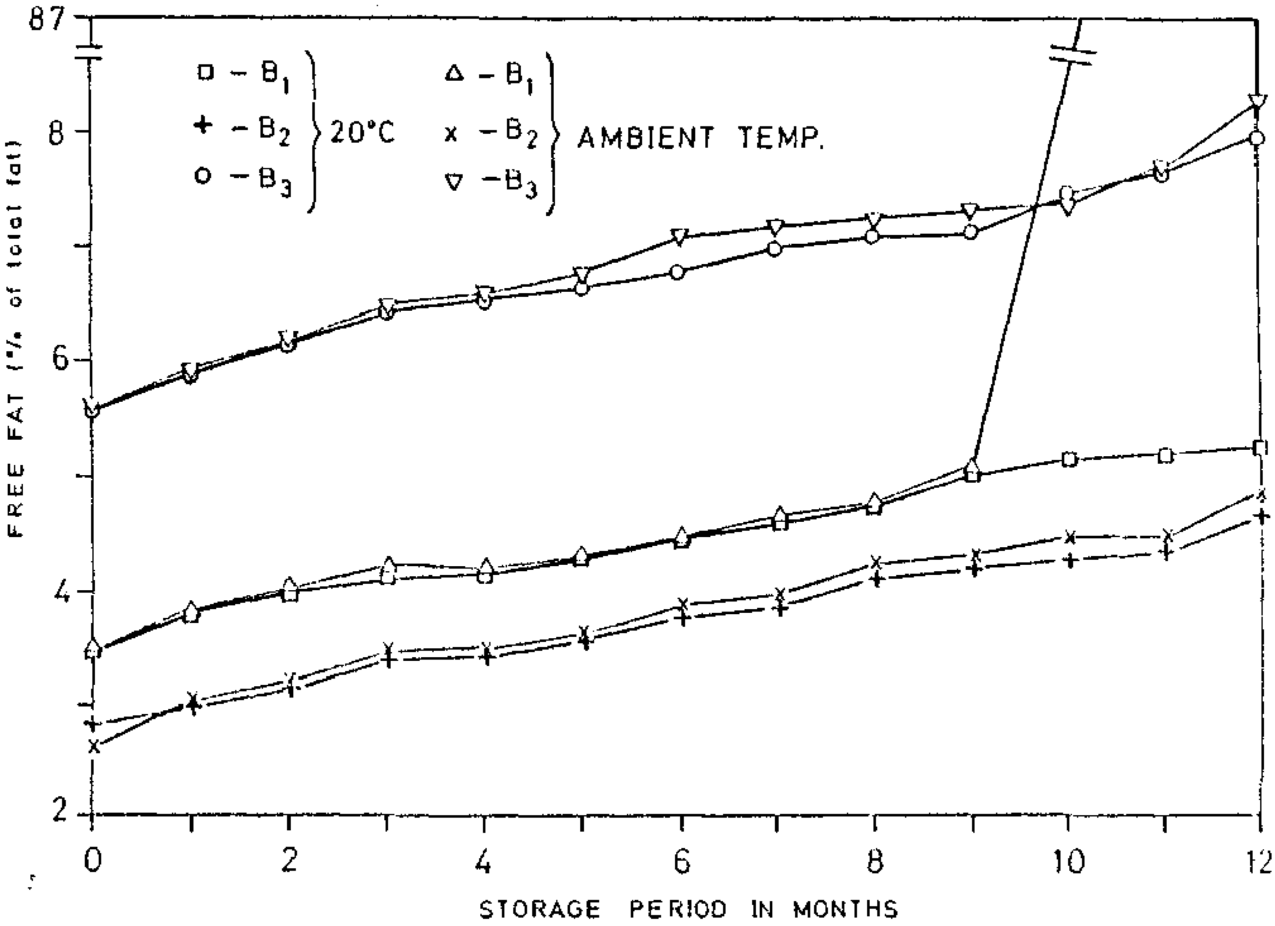

Fig. 19. CHANGES IN FREE FAT CONTENT OF WMP DURING STORAGE.

Table 13: ANOVA for the effect of storage temperature and storage period on the free fat development in WMP.

Source of variation	d.f.	m.s.s	F Ratio
Between trials	2	643.96	3.22*
Between temperature (A)	1	821.72	4.10*
Between storage period (B)	12	397.21	1.98*
Interaction (A X B)	12	195.87	0.98
Error	50	200.24	-

Significant at 1.0 per cent level

samples, which remained organoleptically acceptable even at the end of 12 months of storage, there was no such sudden change in free fat content during storage. The average net increase at the end of 12 months in these samples was only 2.14%. Therefore, increase in free fat content is a good index to judge the spoilage of powder.

The free fat content in WMP, reported by other workers ranged from 0.6 to 22.7% (Puzikova and Efimova, 1974). Therefore, free fat content found in the present study is comparatively less. Kopecky (1975) observed an increase in free fat content which ranged from 4 to 22 %. However, he has not indicated any relationship between increase in free fat content and development of off-flavour during storage. Increase in free fat during storage was attributed to the change in the physical state of lactose i.e., from amorphous to crystalline form as a result of absorption of moisture from the surroundings and rupturing of fat globules by crystals of lactose (Rotkiewicz _et al._, 1978). This observation was confirmed in this study by experiments in which known quantities of moisture were added to WMP and kept for different periods to observe for both sudden increase in free fat and formation of lactose crystals. The different levels of moisture added and the period after which the sudden increase in free fat content and formation of lactose crystals were observed are given in Table 14. It may be seen from the table that increase in free fat content or

Table 14: Effect of added moisture on the free fat development lactose crystallization and organoleptic scores during storage of WMP

Moisture %	Period of storage (in days)	Free fat (% total fat)	Appearance of lactose crystals	Organoleptic scores
3.70 (initial)	15	4.41	-	8.30
	30	4.52	-	8.20
4.5	15	4.62	-	7.96
	30	4.74	-	7.74
4.99	10	4.58	-	7.89
	20	82.14	+	3.24
5.50	5	4.63	-	7.90
	9	80.11	+	3.14
5.98	2	4.66	-	7.87
	4	80.26	+	3.20
6.60	1	4.57	-	7.81
	2	83.94	+	7.81

+ Indicate lactose crystals in powder.

appearance of lactose crystals was dependent on a combination of time of storage and level of moisture. Thus, as the moisture in the powder increased the time required to observe sudden increase in free fat content or appearance of lactose crystals decreased significantly. The appearance of lactose crystals coincided with the sudden increase in free fat content. Interestingly in all the cases, the formation of crystals of lactose was accompanied by increase in free fat content, development of off-flavour and brown colour. The development off-flavour as well as that of colour was attributed to browning reactions which are accelerated by the presence of high amounts of moisture and temperature of storage (30°C).

5.2.11.1 COMPARISON OF OXIDATIVE CHANGES IN FREE FAT WITH TOTAL FAT

It was of interest to establish the difference between free fat and total fat towards autoxidation during storage of WMP. The average changes in peroxide value, total carbonyl content and diene conjugation of free fat and total fat within each type of storage conditions are presented in Tables 15, 16 and 17. The TBA estimation of free fat has not been carried out, since the method was directly done on powder without extracting the fat.

The peroxide value of free fat of WMP measured by colorimetric method increased gradually during storage, the rate of increase being higher at ambient temperature

Table 15: Changes in peroxide value (by colorimetric method) of total fat and free fat extracted from WMP during storage.

Period of storage (In months)	Peroxide Value m.eq O_2/kg total fat			Peroxide Value m.eq O_2/kg free fat		
	20°C[1]	Ambient[2]	Ambient[3]	20°C[1]	Ambient[2]	Ambient[3]
Initial	0.21	0.20	0.23	0.33	0.33	0.35
1	0.30	0.24	0.42	0.46	0.47	0.45
2	0.27	0.16	0.50	0.68	0.71	0.74
3	0.41	0.56	0.49	0.86	0.91	0.95
4	0.40	0.40	0.15	1.00	1.11	1.13
5	0.52	0.27	0.61	1.21	1.37	1.35
6	0.59	0.36	0.85	1.33	1.53	1.60
7	0.49	0.27	0.70	1.53	1.76	1.79
8	0.40	0.26	0.40	1.74	1.91	1.99
9	0.38	0.41	0.26	1.85	2.10	2.17
10	0.67	0.71	0.27	1.86	2.22	2.77
11	0.80	0.93	0.58	1.91	2.42	3.00
12	0.91	1.10	0.90	1.94	2.48	3.36

1. Average of peroxide value obtained for three batch of WMP stored at 20°C
2. Average of peroxide value obtained for two batch of WMP stored at ambient temperature
3. Peroxide value of sample which developed off-flavour during storage.

Table 16: Changes in total carbonyl value of total fat and free fat extracted from WMP during storage

Period of storage (In months)	Total carbonyl value mM/g fat			Total carbonyl value mM/g fat		
	TOTAL FAT			FREE FAT		
	1 20°C	2 Ambient	3 Ambient	1 20°C	2 Ambient	3 Ambient
Initial	7.15	7.44	6.66	7.36	7.36	6.83
2	7.29	7.65	6.86	7.55	7.92	7.08
4	7.42	7.82	7.12	7.65	8.02	7.16
6	7.58	8.16	7.32	7.80	8.24	7.48
8	7.69	8.36	7.64	7.98	8.44	7.89
10	7.84	8.58	7.89	8.19	8.85	8.29
12	7.98	8.82	8.14	8.34	9.06	8.41
Net increase	0.83	1.42	1.48	1.02	1.43	1.58

1. Average of total carbonyl value obtained for three batches of WMP stored at 20°C.

2. Average of total carbonyl value obtained for two batches of WMP stored at ambient temperature.

3. Total carbonyl value of sample which developed off-flavour during storage.

Table 17: Changes in diene conjugation of total fat and free fat during storage.

Storage Period	Diene conjugation (%) (Total fat)		Diene conjugation (%) (Free fat)	
	Storage Temperature		Storage Temperature	
	20°C	Ambient	20°C	Ambient
0	0.785	0.785	0.784	0.784
1	0.752	0.759	0.783	0.769
2	0.787	0.789	0.800	0.798
3	0.815	0.822	0.808	0.810
4	0.829	0.834	0.826	0.827
5	0.835	0.859	0.839	0.843
6	0.836	0.866	0.846	0.865
7	0.841	0.869	0.849	0.867
8	0.825	0.855	0.841	0.851
9	0.826	0.832	0.830	0.834
10	0.820	0.812	0.831	0.826
11	0.810	0.812	0.828	0.828
12	0.809	0.809	0.828	0.822

than at 20°C. The values obtained were consistent unlike the observation made in the case of total fat extracted from WMP (Fig.13). This difference could be attributed to the fact that the free fat is rapidly extracted by using a highly non polar solvent which may avoid the extraction of interfering reducing substances present in the powder. On the other hand total fat is extracted by a somewhat polar solvent system, which may extract interfering reducing substances alongwith the fat from the powder.

It can be concluded that to know the degree of oxidation in WMP by estimation of peroxides, either by colorimetric or iodometric method, the analysis should be performed on the free fat and not on the total fat isolated from the powder.

The pattern and the rate of change in diene conjugation was similar to that in total fat. Therefore, determination of diene conjugation on free fat had no advantage over that determined on total fat. It has already been shown (cf. 5.2.9.1) that determination of diene conjugation on total fat was not helpful in knowing the onset of oxidative changes in WMP during storage.

Total carbonyls increased at a faster rate in free fat than in total fat at both the conditions of storage. However, the differences were very small. In addition, the free fat formed only 2.82 to 5.32% of total fat in the powder studied here. Hence, the slightly higher rate of oxidation in free fat as compared to that of total fat may not have significant impact on the total oxidative changes. In the case of samples with high free fat content, this fact should be taken into account in predicting the storage stability of WMP during storage.

10.2 FATTY ACID COMPOSITION OF FREE FAT AND TOTAL FAT

Since the free fat behaved differently with regard to autoxidative stability when compared to total fat of WMP during storage, it was of interest to characterize the differences in the fatty acid composition of these two types of fat. The average fatty acid compositions of total fat and free fat are given in Table 18. In general, it was observed that the free fat contained significantly lower amounts of saturated fatty acids 14:0, 16:0 and 18:0 and higher amount of unsaturated fatty acids viz., 18:1 and 18:2 as compared to total fat. Thus the total amount of unsaturated fatty acids in free fat was 35.5% whereas it was 28.9% in total fat. This is one of the reasons for the free fat to be more prone to oxidative changes. Truyen and Orsi (1977) reported a much higher difference in the total unsaturated fatty acid contents between free fat and total fat.

5.2.12 BROWNING CHANGES

Dried milks are succeptable to browning changes due to reaction between lactose and protein during storage. These changes are mainly influenced by moisture content

Table 18: Average fatty acid composition (% W) of total fat and free fat

Fatty Acids	Total Fat	Free Fat
4:0	4.29	4.16
6:0	1.44	1.83
8:0	0.85	0.84
10:0	1.41	1.91
10:1	0.07	0.07
12:0	2.51	1.97
14:0 Br	0.25	0.17
14:0	12.30	10.37
14:1	1.40	1.78
15:0	0.89	0.98
16:0 Br	0.33	0.42
16:0	35.10	32.22
16:1	1.72	1.79
17:0	0.39	0.42
18:0 Br	0.24	0.24
18:0	11.18	9.05
18:1	23.81	29.14
18:2	1.07	1.95
18:3	0.79	0.79
Total unsaturated fatty acid	28.86	35.52

and temperature of storage. Some of the manifestation of browning changes are production of HMF, increase in reducing capacity, reduction of sulphydryl groups and development of colour.

5.2.12.1 TOTAL HMF

The changes in total HMF content in WMP during storage are shown in Fig.20. The total HMF increased considerably in all the samples stored at ambient temperature as well as at 20°C during storage. The rate of increase was gradual and significantly higher at ambient temperature than at 20°C (Table 19). In sample of batch No.1 stored at ambient temperature the rate of increase in total HMF was gradual up to the end of 9th month of storage but subsequently the rate of increase was rapid. The HMF value, which was 21.26 at the end of 9th month became 29.10 µM/ 100 g powder at the end of 10th month (net increase = 7.84) and then reached a value as high as 62.30 at the end of 12 months. This rapid increase in total HMF after the 9th month of storage coincided with the rapid increase in moisture and development of off-flavour, rendering the sample organoleptically unacceptable. In other samples stored at ambient temperature, which remained organoleptically acceptable till the end of the storage study, the net average change of total HMF between 9th and 10th months of storage was only 0.36 and the average total HMF content in these samples at the end of 12 months of storage was 20.74.

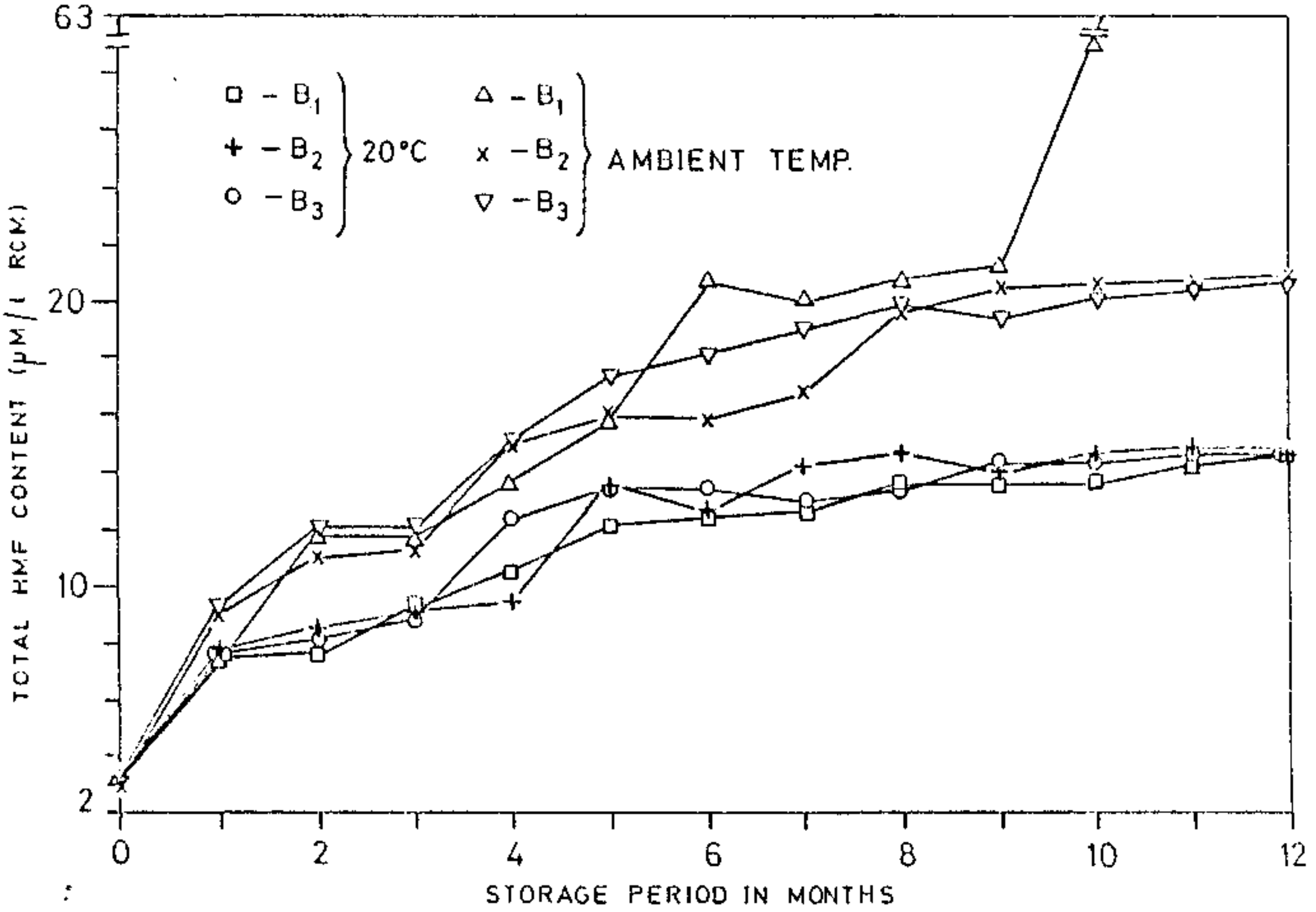

Fig. 20. CHANGES IN TOTAL HMF CONTENT OF WMP DURING STORAGE.

Table 19: ANOVA for the effect of temperature and period of storage on total HMF, free HMF, total reducing capacity (TRC), free SH- groups and colour.

Source of variation	d.f.	Mean Sum of Squares				
		HMF(T)	HMF(F)	TRC	Free SH	Colour
Between replicate	2	54.78	4.86×10^{-1}	3.11×10^{-1}	2.09×10^{-2} **	1.30×10^{-4} *
Between temperature (A)	1	639.21**	27.59**	3.27**	1.78×10^{-3}	1.35×10^{-3} **
Between storage period (B)	12	189.39**	5.44**	4.51×10^{-1} **	1.86**	2.65×10^{-3} **
Interaction (A X B)	12	40.51	4.90×10^{-1}	2.58×10^{-1}	9.18×10^{-3}	4.10×10^{-5}
Error	50	26.11	5.54×10^{-1}	1.19×10^{-1}	4.11×10^{-3}	3.33×10^{-5}

* Significant at 5.0 per cent level
** Significant at 1.0 per cent level

The rate of increase of HMF was greatly influenced by the combined effect of moisture, when it was more than 4%, and temperature of storage. In the sample of batch No. 1 stored at 20°C, although the moisture level exceeded 4% yet the total HMF content was only 14.61 at the end of 12 months of storage showing that storage at 20°C was low enough to prevent rapid browning reactions. The changes in HMF showed a highly significant inverse relationship with organoleptic scores during storage of WMP (r= -0.91). Hence, increase in HMF content can be taken as a measure of browning changes occurring in WMP. Therefore, high levels of HMF in milk powder is not desirable. On the basis of these results it may be suggested that a powder having HMF content of above 20 µM/100 g can be considered as prone to rapid browning and development of off-flavour on further storage.

Pijanowski et al. (1970) observed only small changes (from 4.8 to 8.5) in HMF during storage of WMP at 30°C for 6 months. However, they also recommended that determination of HMF in WMP is useful in detecting early deteriorative changes in WMP. Caric et al. (1984) observed substantial increase in HMF content from 5.68 to 28.43 is SMP stored for 8 months at room temperature. But they did not correlate the increase in HMF content with development of off-flavour in the powder. De Vilder (1982) observed that relative humidity had a significant influence in increasing total HMF content of SMP packed in flexible bags and stored at 30°C. But when the temperature of storage was 20°C, no significant increase in HMF content was noticed.

5.2.12.2 FREE HMF

The changes in free HMF content of WMP during storage are shown in Fig.21. The initial free HMF ranged from 0.405 to 0.497 μM/100 g powder. The rate of change of free HMF was significantly higher in samples stored at ambient temperature than in those stored at 20°C (Table 19). Ipsen and Hansen (1988) studied the influence of storage temperature on free HMF content and found that in WMP stored at 38°C for 70 weeks the free HMF content increased from 3.7 to 7.6 μM/100 g powder, whereas in WMP stored at 20° or 30°C the increase was insignificant.

Although the free HMF content fluctuated during storage yet the increase was significant ($P < 0.01$). Hence, determination of free HMF did not give much information on the extent of browning in the WMP.

5.2.12.3 TOTAL REDUCING CAPACITY

The changes in total reducing capacity of the powders during storage are shown in Fig.22. The initial reducing capacity of the three batches of WMP ranged from 1.42 to 1.60 mg cysteine HCl/g powder. It may be seen from the figure that in samples stored at 20°C the changes in total

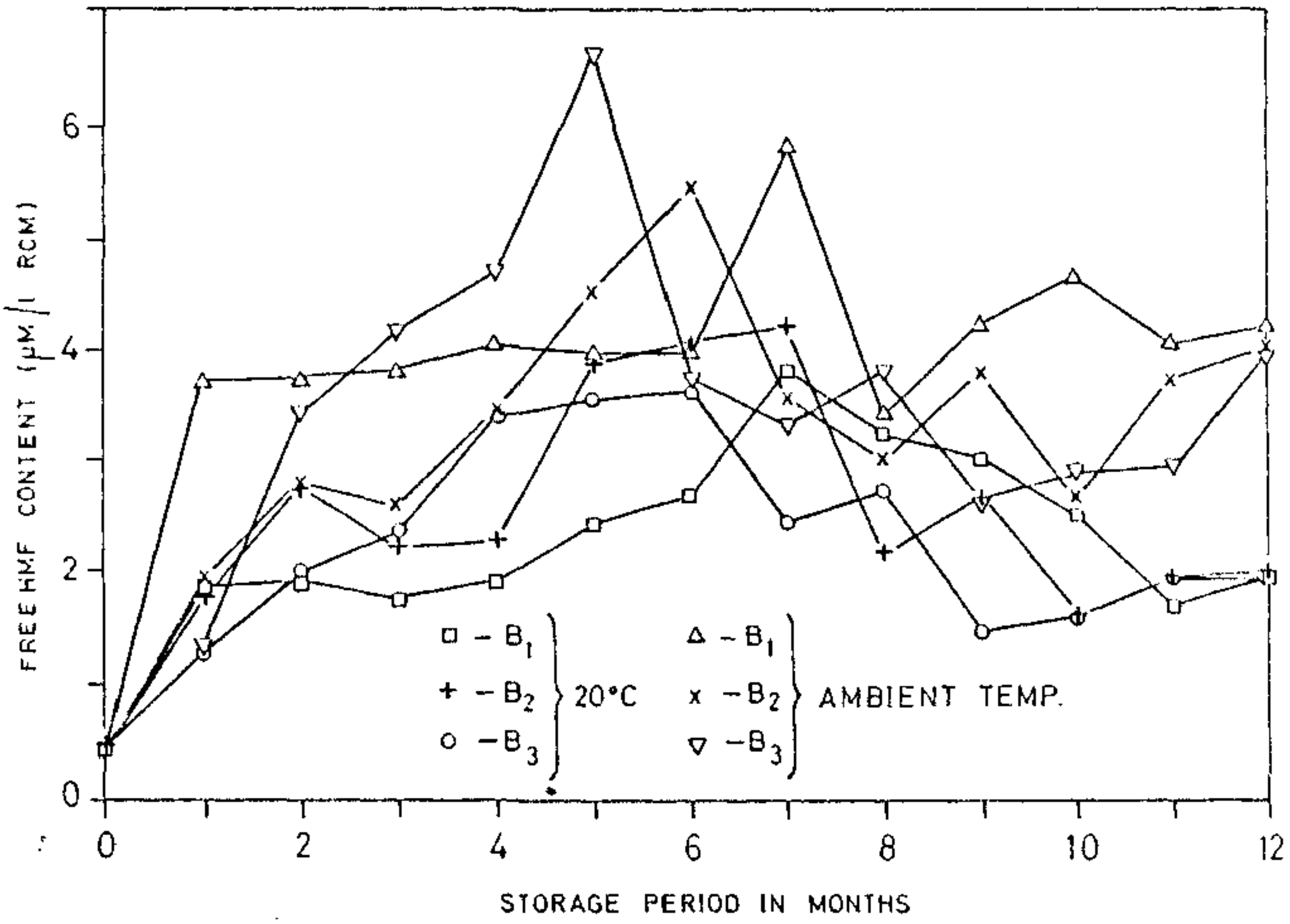

Fig. 21. CHANGES IN FREE HMF CONTENT OF WMP DURING STORAGE.

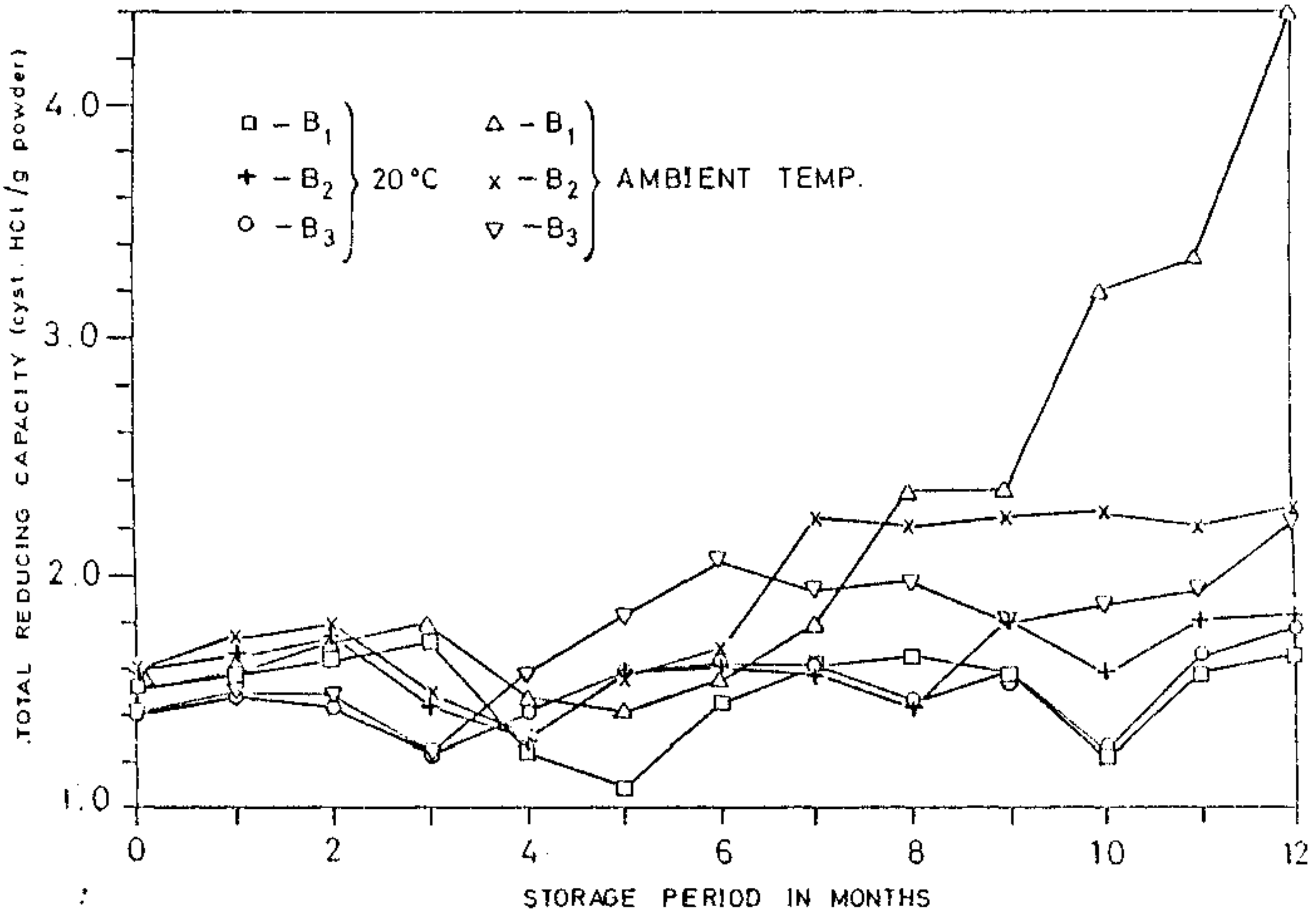

Fig. 22. CHANGES IN TOTAL REDUCING CAPACITY OF WMP DURING STORAGE.

reducing capacity were very small and the average net increase in 12 months was only 0.25. In the samples stored at ambient temperature, which remained organoleptically acceptable even at the end of 12 months of storage, the corresponding average net increase in total reducing capacity was slightly higher (0.74).

In the case of sample of batch No.1, stored at ambient temperature, the change in total reducing capacity was very small up to the end of 9th month of storage and thereafter it increased rapidly. The net change from 9th to 10th month was 0.84 and it reached a value of 4.4 at the end of 12 months of storage. The rapid change in reducing capacity corresponded with the development of off-flavour. Hence, a rapid increase in total reducing capacity is indicative of onset of spoilage. Kieseker and Clarke (1984) also observed that SMP kept at 10°C there was practically no change in total reducing capacity, but as the temperature of storage increased (at 20°, 30° and 40°C) the rate of increase in total reducing capacity was substantial.

During storage of powder, due to Maillard reactions, protein sugar complexes are formed and these are likely to be degraded simultaneously to a certain extent. Such degradation increases the reducing capacity of the powder (Lea, 1947). Coulter et al. (1948) found that moisture content of the powder was the primary factor in influencing the total reducing capacity during storage.

They found an increase in total reducing capacity as the moisture content increased (1.32 to 4.78% range). The content of free sulphydryl groups may show a decrease with increase in storage period (Rotkiewicz, 1979). Such decrease in free sulphydryls decreases the total reducing capacity of the powder. Thus, the net total reducing capacity of the powder depends on the rate at which these two opposing factors occur during storage. This explains the fluctuations observed in reducing capacity of the powder during storage.

5.2.12.4 FREE SULPHYDRYL GROUPS

The changes in free sulphydryl groups (SH- groups) in the WMP samples are shown in Fig.23. The initial concentration of free SH- groups of the freshly manufactured WMP were same in all the three batches. The initial concentration of free SH- groups indicate the condition of preheating used for the manufacturing of WMP (Baldwin *et al.*, 1981; Ipsen and Hansen, 1988). Initial free SH-groups content is an important factor to provide antioxidant effect to fat in WMP. A maximum amount of SH- groups in powder could be achieved by heating milk to 90 to 95°C before drying (Kirch Mayer *et al.*, 1984). Rotkiewicz (1979) reported that preheating of milk from 80 to 90°C for 16 s gave free SH-groups of 2.94 µM/g and 7.01 µM/g respectively.

1.5

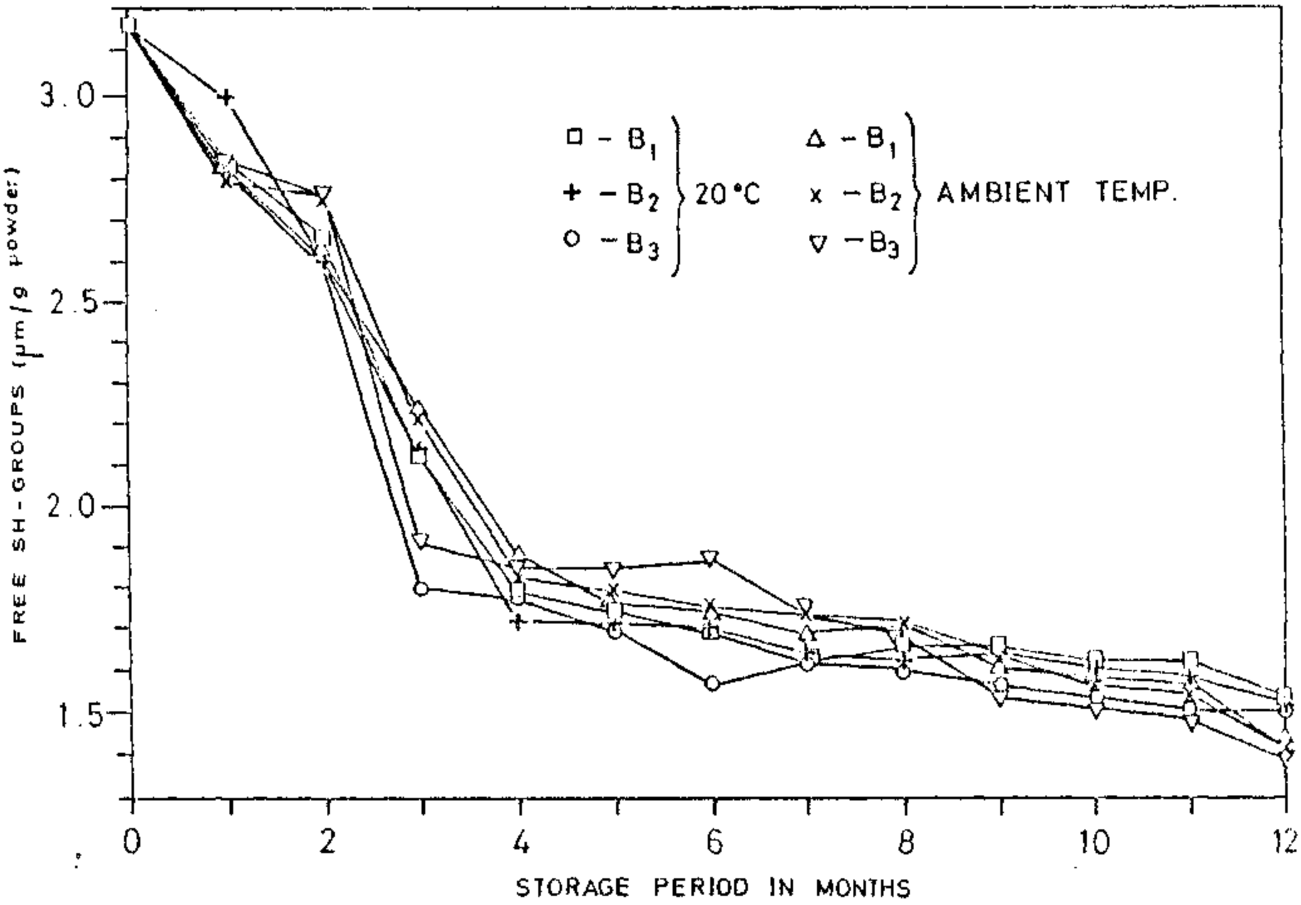

Fig. 23. CHANGES IN FREE SH-GROUPS OF WMP DURING STORAGE.

The free SH- groups of WMP decreased throughout the storage period at both the conditions of storage. The rate of decrease of free SH- groups was faster up to 3rd month of storage in all the samples and thereafter the decrease was slower. The net decrease in free SH- groups ranged from 1.62 to 1.77 after 12 months of storage. Temperature of storage had no significant effect on the rate of decrease of free SH- groups (Table-19). The change in free SH- groups did not correlate with changes in organoleptic scores. The decrease in the content of SH- groups is attributed to the absorption of oxygen during Storage (Coulter et al., 1951).

5.2.12.5 COLOUR

The changes in colour of WMP during storage are shown in Fig.24. The initial colour index of the powder ranged from 0.134 to 0.141. The rate of change in colour was significantly higher in samples stored at ambient temperature than in those stored at 20°C (Table 19). The rate of change was gradual throughout the storage period in all the samples which remained organoleptically acceptable.

In the case of sample of batch No.1, stored at ambient temperature, the increase in colour was similar to that observed in other samples up to 9th month of storage and thereafter it increased suddenly. This rapid change in colour coincided with the development of off-flavour in

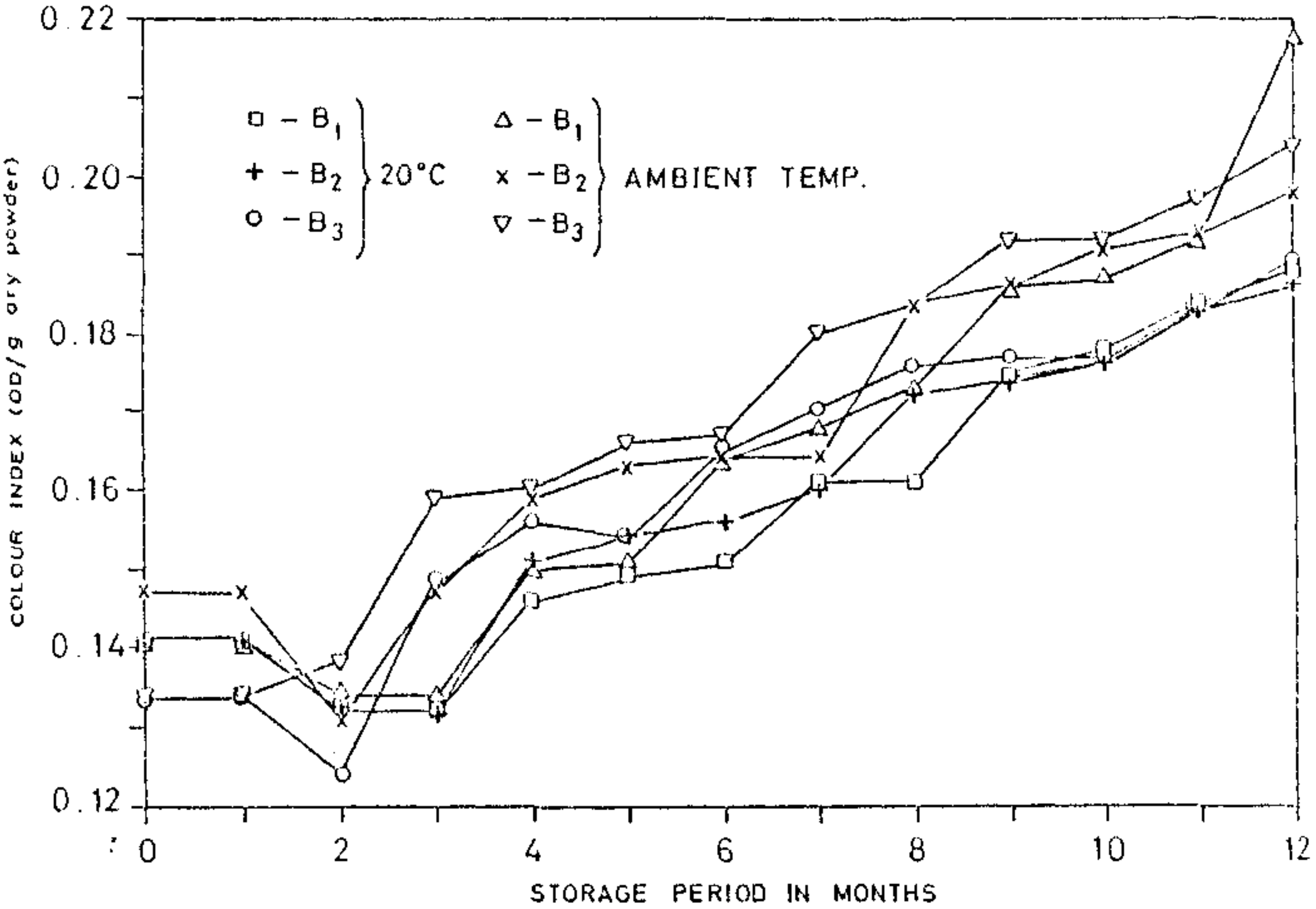

Fig. 24. CHANGES IN COLOUR INDEX OF WMP DURING STORAGE.

the powder thereby indicating that accelerated browning changes were accompanied by development of off-flavour. This rapid increase in colour is due to increase in moisture content of storage and ambient temperature of storage which was relatively high. Min _et al._ (1989) also reported that production of brown colour was related with the development of off-flavour in air packed WMP samples. Moisture content of the powder appears to have greater role in production of brown colour during storage. Henry _et al._ (1948) reported that dried milk with less than 5% moisture showed a very slight changes in colour even after two years of storage at 37°C.

Since milk powders contain high levels of lactose and proteins, non-enzymatic browning reactions (Maillard reaction) are important changes leading to development of off-flavour during storage (Coulter _et al._, 1948; Labuza, 1970; De Vilder, 1982; Kieseker and Clarke, 1984; Renner, 1988). The Maillard reaction can be conveniently divided into three stages. The initial reaction involves condensation of reducing sugar and amino compound to form schiff base followed by the formation of N-substituted glycosyl amine. The intermediate stage of the reaction is characterized by the formation of furfurals, reductones and also liberation of water. Among the furfurals, the estimation of HMF gives the extent of initial browning reaction. The reductones increase the reducing capacity of the powder during storage.

The third stage of the reaction involves the conversion of furfurals and reductones to high molecular weight melanoidins having brown colour. Although the compounds formed in the intermediate stage do not have brown colour yet they are prone to immediate formation of coloured compounds (Hodge, 1953; Nurstein, 1986).

5.3 PHYSICO-CHEMICAL PROPERTIES OF MARKET SAMPLES OF WMP

Different brands of WMP, packed in tins under nitrogen are sold in the market. This is an ideal conditions of packing of WMP. A knowledge of the type of changes taking place in WMP packed under such conditions and their relationship with the development of off-flavour is necessary in assessing the relative merits of bulk packing of WMP in HDPE bags in respect of storage stability.

Market samples of three different brand of WMP packed in 500 g tins with or without nitrogen were analysed for organoleptic quality and some of the selected physico-chemical changes which were considered important from the point of view of spoilage. The results of the study are presented in Table 20. It may be seen from the table that the initial properties of the different brands of the market sample varied widely and the variation were statistically highly significant.

The moisture, which was the most important factor influencing several other physico-chemical changes during

Table 20: Analysis of market milk powder samples gas packed in tins and kept at 30°C with and without gas.

Parameter Studied		Initial	After three months		After nine months	
			Gas packed	Non gas	Gas packed	Non gas
Moisture	1.	3.01	3.01	3.01	3.02	3.03
	2.	3.79	3.81	3.81	3.81	3.81
	3.	3.14	3.15	3.17	3.15	3.18
Acidity (% LA)	1.	0.70	0.73	0.75	0.78	0.81
	2.	0.99	1.06	1.06	1.09	1.10
	3.	1.06	1.06	1.06	1.11	1.13
Solubility index (ml)	1.	0.10	0.13	0.13	0.31	0.35
	2.	0.14	0.18	0.20	0.32	0.40
	3.	0.10	0.14	0.15	0.37	0.37
Heat stability (at 130°C,min)	1.	45	37	35	31	30
	2.	40	33	29	32	28
	3.	33	27	27	6	6
Total HMF	1.	12.59	18.11	19.25	22.31	23.71
	2.	11.81	17.94	18.56	21.61	22.06
	3.	7.60	15.05	15.83	18.30	18.60
Colour	1.	0.151	0.163	0.163	0.167	0.169
	2.	0.151	0.161	0.166	0.178	0.182
	3.	0.168	0.177	0.179	0.192	0.194
TRC mg cysteine HCl/g powder	1.	1.36	1.90	2.04	2.38	2.51
	2.	1.80	2.14	2.26	2.51	2.62
	3.	1.58	1.78	1.80	2.18	2.24
Free fat (% total fat)	1.	23.4	24.0	24.0	24.8	25.6
	2.	26.8	27.2	27.5	29.1	29.5
	3.	4.0	4.4	4.6	6.1	6.0
TBA value (A 530)	1.	0.022	0.028	0.030	0.042	0.048
	2.	0.011	0.021	0.024	0.043	0.048
	3.	0.021	0.034	0.040	0.045	0.046
Peroxide value (nm O2/kg fat)	1.	0	0.7	0.7	1.3	1.5
	2.	0	0.9	0.9	1.2	1.4
	3.	0	0.8	0.8	1.1	1.0
OD 280 nm	1.	0.070	0.080	0.080	0.093	0.093
	2.	0.080	0.091	0.094	0.108	0.112
	3.	0.070	0.084	0.082	0.099	0.101
Organoleptic scores	1.	8.9	7.1	7.1	6.0	6.0
	2.	8.0	7.5	7.5	5.7	5.7
	3.	8.3	7.6	7.6	6.2	6.2

storage, had not increased in tins. Thus the tins acted as perfect barrier against the moisture absorption. Under these conditions, in which moisture remained thus constant, changes in all other parameters were noticed. Among these changes titratable acidity, solubility index, total HMF, colour, total reducing capacity, free fat, TBA value, peroxide value and OD at 280 nm showed progressively increasing trends while heat stability and organoleptic scores showed decreasing trends. This indicated that most of these physico-chemical changes cannot be prevented even by keeping the moisture at constant level, but they can only be controlled during storage. These physico-chemical changes can be related to decrease in organoleptic scores. The net change observed at the end of nine months of storage was not sufficient to contribute to any detectable off-flavour. It has already been shown that rapid increase in total HMF, TBA value and OD at 280 nm and decrease in heat stability when crossed certain limits indicated the onset of spoilage. However, in none of the market samples studied here, the change in respect of the above mentioned parameters did not cross these limits even at the end of 9th month of storage at 30°C. From the data it may be concluded that market WMP samples showed reasonably good storage stability and this was attributed to the controlled level of moisture in them.

5.3.1 EFFECT OF GAS PACKING

The major physico-chemical changes studied during storage indicated no significant difference between gas packed and non gas packed powder (Table 20a,b). This can be attributed to the type of WMP produced in the country. The storage stability of WMP was directly correlated with heat treatment given to milk prior to spray drying (Jensen and Hansen, 1974; Rotkiewicz, 1979; Baldwin et al., 1981; Kieseker et al., 1984; Ipsen and Hansen, 1989). The whey protein nitrogen index (WPNI) of the powder studied ranged from (1.9 to 2.5 mg nitrogen/g powder) indicating that these powder fall under the category of medium heat type (cf. 2.3.1.1). Since the higher preheat treatment is expected to produce more antioxygenic substances, specially the free SH- groups, the medium heated powder will have better oxidative stability during storage.

Tamsma et al. (1962) reported that a heat treatment of 74°C for 30 min, 77°C for 6 min and 90.5°C for 15 s effectively stabilized the flavour of air packed samples stored for 6 months at 27°C. Further, they observed that gas packing had no advantage in respect to oxidative stability of such high heated powder. Baldwin et al. (1981) found that storage stability of WMP depended on its heat classification. Thus they suggested that low-heat powder could be kept for 4 to 6 months, medium-heat for 6 to 8 months and high-heat for more than 12 months. The commercial samples examined in the present study also

Table 20(a): ANOVA for market milk powder samples

Source of variation	d.f	Mean sum of squares				
		Moisture	Acidity	S.I	Heat stability	Free fat
Between replicate	2	1.06**	2.04×10^{-1} **	2.84×10^{-3} *	3.78×10^{2} **	9.11×10^{-2} **
Between temperature (A)	1	2.72×10^{-3}	3.54×10^{-4}	1.25×10^{-3}	6.72	1.44×10^{-1}
Between storage period (B)	2	1.52×10^{-3}	1.14×10^{-2} **	9.86×10^{-2} **	4.42×10^{2} **	7.13 **
Interaction (A X B)	4	4.17×10^{-5}	7.18×10^{-4} **	5.22×10^{-4}	6.36×10^{1} **	9.59×10^{-2} **
Error	8	5.97×10^{-5}	5.65×10^{-5}	3.75×10^{-4}	1.47	4.36×10^{-2}

* Significant at 5.0 per cent level
** Significant at 1.0 per cent level

Table 26(b): ANOVA for market milk powder samples

Source of variation	d.f	Mean sum of squares					
		Organoleptic scores	EMF	Colour	Reducing capacity	TBA	O.D 280 nm
Between replicate	2	1.55×10^{-1} **	3.04×10^{1} **	5.98×10^{-4} **	1.81×10^{-1} **	1.09×10^{-4} **	2.81×10^{-4} **
Between temperature (A)	1	0.00	6.22×10^{-1}	9.25×10^{-6}	6.68×10^{-2}	2.7×10^{-6}	2.7×10^{-6}
Between storage period (B)	2	8.92 **	1.67×10^{2} **	8.40×10^{-4}	1.02	1.02×10^{-3} **	1.16×10^{-3} **
Interaction (A X B)	4	2.61×10^{-3} **	4.46×10^{-1}	2.60×10^{-5} **	3.25×10^{-2} **	2.62×10^{-5} **	8.3×10^{-6} *
Error	8	0.00	1.21×10^{-1}	1.75×10^{-6}	1.85×10^{-3}	6.51×10^{-7}	1.7×10^{-6}

* Significant at 5.0 per cent level

** Significant at 1.0 per cent level

showed a shelf-life of about 9 months because they were also found to be medium heat type. Since, the difference between the changes in gas packed and non-gas packed tins are very small in respect of changes in all the parameters throughout the storage period, avoiding gas packing will not substantially affect the shelf-life of WMP.

5.4 COMPARISON OF CHANGES TAKING PLACE IN WMP PACKED IN TINS AND 25 kg BAGS

The various physico-chemical changes determined at the end of 3, 6 and 9 months of storage in all the three batches of WMP packed in 500 g tins and stored at ambient temperature and 20°C were compared with corresponding changes observed in WMP bulk packed in HDPE bags. The data pertaining to powders packed in HDPE bags are already presented in earlier chapters. These comparisons are given in Tables 21, 22, and 23.

The changes in moisture content during storage was significant in the case of powder bulk packed in HDPE bags and the rate of change was higher at ambient temperature than at 20°C (Table 24). On the other hand, the level of moisture remained practically constant in WMP packed in tins. This is the only major difference between these two types of packings. The other parameters changed significantly in both the packings throughout the storage period. The differences between tins and bags in respect of changes in all the other parameters were similar throughout the storage period in powder stored at 20°C. But in

Table 21: Physico-chemical changes in WMP packed in 500g tins and 25 kg bags during storage

BATCH NO. 1

Parameters Studied	Initial	3 months				6 months				10 months			
		20°C		Ambient		20°C		Ambient		20°C		Ambient	
		TIN	BAG	TIN	BAG	TIN	BAG	TIN	BAG	TIN	BAG	TIN	BAG
Moisture	3.73	3.74	3.76	3.74	3.77	3.74	3.87	3.75	3.82	3.75	4.19	3.75	4.99
Solubility	0.10	0.10	0.11	0.10	0.13	0.15	0.14	0.24	0.28	0.16	0.15	0.34	0.41
Heat Stability	31	24	23	24	23	19	19	17	17	16	16	14	14
HMF	3.46	7.78	9.28	11.02	11.72	8.82	12.46	14.17	20.74	12.03	13.61	17.25	21.26
Colour	0.141	0.144	0.146	0.147	0.150	0.150	0.151	0.162	0.164	0.170	0.175	0.180	0.186
TBC	1.53	1.72	1.72	1.77	1.80	1.43	1.46	1.56	1.56	1.58	1.58	2.18	2.36
% Free fat	3.48	4.21	4.21	4.36	4.22	4.36	4.38	4.51	4.54	5.04	5.04	5.09	5.11
TBA value	0.002	0.012	0.015	0.019	0.022	0.025	0.032	0.030	0.030	0.032	0.040	0.044	0.058
CD 280 nm	0.067	0.069	0.069	0.069	0.069	0.075	0.075	0.077	0.078	0.075	0.075	0.089	0.092
Organoleptic scores	8.7	8.4	8.3	8.1	8.1	7.9	7.9	7.5	7.4	7.0	7.0	6.6	6.5

Table 22: Physico-chemical changes in WMP packed in 500g tins and 25 kg bags during storage

BATCH NO. 2

Parameters Studied	Initial	3 months				6 months				10 months			
		20°C		Ambient		20°C		Ambient		20°C		Ambient	
		TIN	BAG	TIN	BAG	TIN	BAG	TIN	BAG	TIN	BAG	TIN	BAG
Moisture	2.62	2.64	2.72	2.64	3.04	2.64	2.87	2.64	3.44	2.65	3.19	2.65	3.80
Solubility	0.10	0.12	0.12	0.12	0.14	0.13	0.13	0.23	0.26	0.13	0.14	0.29	0.29
Heat Stability	31	25	25	25	25	22	22	21	22	17	17	15	15
HMF	3.15	7.49	9.01	11.08	11.26	8.96	12.69	13.64	15.84	12.16	13.96	18.15	20.49
Colour	0.147	0.149	0.151	0.154	0.159	0.156	0.157	0.164	0.164	0.173	0.176	0.184	0.191
TRC	1.60	1.64	1.44	1.50	1.50	1.62	1.46	1.70	1.70	1.82	1.82	2.24	2.24
% Free fat	2.82	3.39	3.38	3.32	3.46	3.36	3.77	3.59	3.89	4.11	4.21	4.31	4.32
TBA value	0.008	0.018	0.019	0.019	0.023	0.021	0.023	0.025	0.025	0.031	0.037	0.039	0.042
Peroxide value	0.0	0.5	0.5	0.07	0.6	0.5	0.6	0.7	0.7	0.8	0.8	0.8	0.9
OD 280 nm	0.070	0.073	0.073	0.074	0.073	0.079	0.078	0.082	0.084	0.079	0.079	0.094	0.093
Organoleptic scores	8.8	8.5	8.4	8.4	8.4	8.1	8.0	7.6	7.6	7.0	7.1	6.7	6.8

Table 23: Physico-chemical changes in WMP packed in 500g tins and 25 kg bags during storage

BATCH NO. 3

Parameters Studied	Initial	3 months				6 months				10 months			
		20°C		Ambient		20°C		Ambient		20°C		Ambient	
		TIN	BAG	TIN	BAG	TIN	BAG	TIN	BAG	TIN	BAG	TIN	BAG
Moisture	3.33	3.34	3.35	3.34	3.38	3.34	3.37	3.34	3.81	3.35	3.82	3.35	3.96
Solubility	0.3	0.31	0.33	0.38	0.40	0.33	0.33	0.52	0.54	0.35	0.35	0.71	0.72
Heat Stability	30	23	22	23	22	20	20	17	17	17	17	14	14
HMF	3.15	7.14	11.92	11.76	11.78	10.24	13.48	15.76	18.14	13.73	14.31	17.61	19.34
Colour	0.134	0.142	0.148	0.157	0.159	0.164	0.165	0.167	0.167	0.170	0.177	0.192	0.192
TRC	1.42	1.24	1.24	1.24	1.24	1.72	1.72	1.89	1.89	1.58	1.58	1.88	1.88
% Free fat	5.61	6.44	6.43	6.53	6.48	6.77	6.79	7.49	7.10	7.33	7.44	7.99	7.38
TBA value	0.004	0.019	0.021	0.019	0.022	0.022	0.029	0.031	0.028	0.026	0.034	0.038	0.039
Peroxide value	0.0	0.7	0.4	0.7	0.4	0.7	0.7	0.7	0.8	0.8	0.9	1.3	0.9
O.D 280 nm	0.064	0.069	0.069	0.071	0.072	0.076	0.073	0.081	0.080	0.081	0.081	0.092	0.094
Organoleptic scores	8.5	8.1	8.1	8.0	8.0	7.8	7.7	7.5	7.5	6.9	6.9	6.6	6.8

Table 24: ANOVA for effect of packing in 500 g tins and 25 kg bags on different physico-chemical properties

Source of variation	d.f	Mean sum of squares					
		Organoleptic scores	Moisture	Heat stability	HMF	TBA	OD at 280 nm
Between replicate	2	2.02×10^{-1} **	4.16 **	1.54×10^{1} **	1.09	7.6×10^{-6}	6.09×10^{-5} *
Between packings	1	4.08×10^{-2}	7.62×10^{-1} **	1.88×10^{-1}	3.26×10^{1} **	6.53×10^{-1} *	3.3×10^{-7}
Between temperature	1	7.5×10^{-1} **	9.99×10^{-2} **	1.30×10^{1} **	1.51×10^{2} **	2.25×10^{-4} **	2.90×10^{-4} **
Between storage period	3	8.39 **	2.56×10^{-1} **	5.05×10^{2} **	3.80×10^{2} **	2.37×10^{-3} **	7.94×10^{-4} **
Error	40	2.02×10^{-2}	3.08×10^{-2}	9.79×10^{-1}	2.63	1.72×10^{-5}	1.19×10^{-5}

* Significant at 5.0 per cent level
** Significant at 1.0 per cent level

the case of powder stored at ambient temperature the difference between tins and bags was revealed only at the end of 9 months of storage in respect of HMF, TBA and OD at 280 nm. Whereas the other parameters viz., solubility index, heat stability, total reducing capacity, colour, free fat and organoleptic scores, the differences were negligible. In general, the rate of changes in respect of all the parameters were slightly higher in powder packed in bags than in tins. This may be attributed to gradual absorption of moisture by the powder in the bags during storage. Thus, it can be concluded that the powder packed in HDPE bags remained as good as that packed in tins even when stored at ambient temperature for 9 months, which may be considered as reasonably good period.

5.5 EFFECT OF TEMPERATURE AND GAS PACKING ON THE STORAGE STABILITY OF WMP PACKED IN TINS

Whole milk powder packed in tins, with and without nitrogen, was stored at three different temperatures (20°, 30° and 34°C) and the changes taking place in various physico-chemical parameters after a storage period of 3 and 9 months are given in Table 25. The moisture content of the powder remained constant during storage. The other parameters showed tendency to changes during storage. The temperature of storage had significant effect on the rate of changes (Table 25a). The rate of changes due to gas packing and non gas packing was not significantly different and temperature of storage had no significant effect on this difference (Table 25a).

Table 25: Physico-chemical changes in WMP packed in tins with and without gas during storage at 20°, 30° and 34°C

Parameter Studied	Initial	20°C				30°C				34°C			
		GAS		NON GAS		GAS		NON GAS		GAS		NON GAS	
		3m	9m	3m	9m	3m	9m	3m	9m	3m	9m	3m	9m
Moisture	3.14	3.14	3.16	3.14	3.16	3.15	3.15	3.17	3.18	3.14	3.16	3.19	3.19
Solubility index	0.1	0.12	0.14	0.12	0.14	0.15	0.37	0.15	0.37	0.19	0.41	0.19	0.41
Heat stability	33	28	10	28	10	27	6	27	6	25	2	25	2
HMF (Total)	7.69	8.14	10.41	8.76	10.67	15.[illegible]5	18.31	15.83	18.6	16.19	19.50	16.28	19.50
Colour	0.168	0.171	0.177	0.171	0.173	0.177	0.192	0.179	0.191	0.182	0.194	0.185	0.190
TRC	1.58	1.58	1.59	1.58	1.59	1.78	2.18	1.80	2.18	2.20	2.40	2.20	2.40
Free fat (%)	4.03	4.45	5.21	4.48	5.21	4.45	6.11	4.64	6.01	4.72	6.54	4.76	6.76
TBA value	0.021	0.025	0.036	0.025	0.037	0.034	0.045	0.040	0.046	0.039	0.048	0.042	0.048
OD 280 nm	0.070	0.074	0.087	0.074	0.090	0.080	0.093	0.080	0.100	0.085	0.100	0.090	0.108
Organoleptic scores	8.5	8.0	7.1	8.0	7.0	7.9	6.3	7.9	6.3	7.9	6.2	7.9	6.2

Table 25(a): ANOVA for effect of temperature and gas packing on different physico-chemical properties

Source of Variation	d.f	Mean sum of squares					
		Organoleptic scores	Solubility index	Heat stability	HMF	TBA	O.D 280 nm
Between gas packing	1	5.56×10^{-4}	0.00	0.00	1.31×10^{-1}	6.72×10^{-6}	1.25×10^{-5}
Between temperature (A)	2	1.82×10^{-1} **	2.11×10^{-2} **	2.02×10^{1} **	5.62×10^{1} **	1.34×10^{-4} **	1.32×10^{-4} **
Between storage period (B)	2	2.26 **	6.91×10^{-2} **	1.19×10^{3}	1.12×10^{2} **	7.56×10^{-4} **	1.11×10^{-3} **
Interaction (A X B)	4	1.28×10^{-1} **	1.19×10^{-2} **	8.22 **	1.42×10^{1} **	3.57×10^{-5} **	3.67×10^{-5} **
Error	8	5.56×10^{-4}	0.00	0.00	4.31×10^{-2}	2.09×10^{-6}	2.90×10^{-6}

** Significant at 1.0 per cent level

Whole milk powder packed in tins gradually deteriorated in quality during storage, as shown by changes in flavour scores. This was due to both oxidative changes, as reflected by TBA value and OD at 280 nm, and browning changes as reflected by HMF, colour and total reducing capacity. The oxidative changes are mainly controlled by the antioxidant effect of high levels of free SH- groups expected to be present in the WMP of medium-heated type (WPNI = 2.0 mg nitrogen/g powder). The browning changes were controlled because the moisture content remained constant in the powder during storage.

Whole milk powders gas packed under nitrogen as practiced commercially may still have a head space residual oxygen up to 2 to 3% (Coulter et al., 1951). Tamsma et al. (1961) found that this level of residual oxygen was ineffective to provide an inert atmosphere to prevent autoxidation. They demonstrated that it was necessary to reduce head space oxygen level to less than 0.01% to prevent oxidative spoilage in WMP.

5.6 MICROBIOLOGICAL QUALITY

Total bacterial counts and coliform counts of the three different batches of WMP are shown in Table 26. The initial bacterial counts of the three batches of WMP ranged from 4,500 to 10,700/g which is lower than the maximum of 50,000/g permitted by BIS, indicating that the powder samples were of standard quality. As expected, the

Table 26: Microbiological quality of WMP during storage at 20°C and ambient temperature

Microbiological analysis	Batch No.	Storage period (in months)						
		0	2	4	6	8	10	12
					20°C Storage			
Total plate count/g	1.	10,500	7,000	4,500	4,000	4,000	4,000	3,500
	2.	10,700	7,200	4,500	4,000	4,000	4,000	3,500
	3.	4,500	3,000	1,300	1,200	1,000	1,000	1,000
Coliform count/g	1.	NIL	NIL	NIL	NIL	NIL	NIL	NIL
	2.	NIL	NIL	NIL	NIL	NIL	NIL	NIL
	3.	NIL	NIL	NIL	NIL	NIL	NIL	NIL
					Ambient temperature storage			
Total plate count/g	1.	10,500	7,000	4,700	4,200	3,800	3,700	3,500
	2.	10,700	7,200	4,700	4,100	3,800	3,700	3,400
	3.	4,500	3,000	1,400	1,100	1,000	1,000	1,000
Coliform count/g	1.	NIL	NIL	NIL	NIL	NIL	NIL	NIL
	2.	NIL	NIL	NIL	NIL	NIL	NIL	NIL
	3.	NIL	NIL	NIL	NIL	NIL	NIL	NIL

total bacterial count decreased during storage, the decrease was slightly higher at ambient temperature than at 20°C. The coliform count was nil initially as well as during storage.

From the above results it may be concluded that WMP during storage is highly dynamic in respect to chemical changes, but static in respect of bacterial activity. The chemical changes are mainly due to autoxidation and browning reactions. These changes, when they crossed certain limits, were responsible for the development of off-flavours. Moisture content of the powder is the most important factor influencing the rate of chemical changes. HDPE bags gave reasonably good protection against moisture absorption by the powder. The WMP manufactured in the country is of good quality and showed satisfactory storage stability when packed in HDPE bags and stored at ambient temperature and humidity. As our powders are of medium-heated type, it does not need gas packing.

SUMMARY

6.0 SUMMARY

1. Three batches of WMP manufactured from buffalo milk at different periods in a year at a commercial Dairy in Dharwar, Karnataka, were packed in HDPE bags encased in hessian laminated kraft paper bags and stored at ambient temperature and RH conditions of Dharwar and also at controlled temperature of 20°C for a period of 12 months. The stored powders were examined for chemical, bacteriological and organoleptical changes during storage at monthly intervals. The ambient temperature at Dharwar ranged from 28-38°C and RH from 18 to 100 %.

2. The chemical changes examined were moisture, fat, titratable acidity, pH, solubility index, lactic acid, free fatty acids, viscosity, whey protein nitrogen index, heat stability, lactones, diene conjugation, peroxide value, TBA value, total carbonyl value, steam volatile monocarbonyls, UV absorption at 280 nm, fatty acid profile, free fat, free and total HMF, colour index, ferricyanide reducing value and free sulphydryl groups. The bacteriological examination consisted of determining standard plate count and coliform count. The organoleptic examination was made on the powder as well as on reconstituted milk (RCM) by a panel of judges on the basis of 9 point hedonic scale. The quality of all the samples in respect of chemical, bacteriological and organoleptic properties conformed to Indian standards.

3. The organoleptic scores of freshly made powders ranged from 8.5 to 8.8 indicating that all the three batches of WMP used in the study were of good quality and highly acceptable. During storage the organoleptic score decreased gradually in all the samples. The powders stored at 20°C remained acceptable (scores of 6.6 to 6.7) even at the end of 12 months of storage. The samples stored at ambient temperature showed tendency to become unacceptable after 9th month of storage and the sample of batch No.1 developed off-flavour and became organoleptically unacceptable after 9th month of storage. Judging of RCM was more sensitive than the dry powder for organoleptic evaluation.

4. The initial moisture content of the three batches of WMP ranged from 2.62 to 3.73%. The initial period during which powder showed resistance to absorption of moisture was inversely related with the initial moisture content of the powder. The average rate of increase of moisture was 0.06% at 20°C and 0.09% at ambient temperature. There was no definite trend between the rate of moisture absorption and RH of the place which even touched 100% during certain months. Thus, HDPE possessed sufficiently good moisture barrier properties even under high humid conditions. The WMP of batch No.1 stored at ambient temperature developed sudden off-flavour after 9th month of storage and this coincided with the level of moisture becoming more than 4%. Thus a moisture level of

around 4% appears to be very critical in accelerating the development of off-flavour. On the basis of initial moisture content and the rate of absorption of moisture, guide lines were suggested to predict the shelf-life of the powder in HDPE bags.

Initial moisture	Period of storage	
	Ambient	20°C
4.0 %	cannot be stored	Cannot be stored
3.5 %	up to 5 months	up to 12 months
3.0 %	up to 10 months	more than 10 months

5. The initial titratable acidity (TA) of all the batches of WMP ranged between 0.94 to 0.98. The average increase in TA per month was 0.019 and 0.035% in sample stored at 20°C and ambient temperature respectively. The increase in TA was attributed to browning reactions. Since, the increase in TA is very small during storage the possibility of TA exceeding the maximum limits of 1.2% (IS: 1165-1986) is very less. Hence, increase in TA of WMP during storage is not of much importance.

6. The initial solubility index (SI) of all the three batches of WMP ranged from 0.1 to 0.3 ml which indicated that powder possessed very high solubility (99.9 to 99.7% solubility). The SI increased gradually and slowly in all the samples during storage as long as the powder remained organoleptically acceptable and showed no development of off-flavour. But in the powder, which developed off-

flavour and became unacceptable, the SI increased suddenly and rapidly coinciding with the stage at which the development off-flavour started as a result of the level of moisture in the powder exceeding 4%. The change in SI was attributed to browning reactions.

7. The initial free fatty acid (FFA) content ranged from 0.53 to 0.94 mM/100 g fat. The FFA content increased during storage and the rate of increase was significantly influenced by the temperature of storage. In the samples which remained organoleptically acceptable till the end of storage study of 12 months, the maximum increase of FFA was 1.78 mM/100 g fat. However, in the sample which developed off-flavour the increase in FFA was sudden and rapid and reached a value of 3.15 mM/100 g fat. The rapid increase in FFA coincided with the high increase in the level of moisture as well as development of off-flavour in the sample.

8. The initial lactic acid (LA) content ranged from 1.54 to 2.88 mg/100 g powder and this did not affect the flavour quality of the powder. The LA content of WMP showed practically no increase during storage. Determination of LA by ferric chloride method of Ling (1951) gave more consistent value than p-hydroxydiphenyl method of Davidson(1949)

9. The initial pH values of all the samples were similar. The pH decreased gradually in all the samples

which remained organoleptically acceptable even at the end of 12 months of storage and the net change ranged from 0.08 to 0.12. The temperature of storage had no effect on the rate of change of pH. Development of off-flavour in the powder coincided with a sudden and rapid decrease in pH.

10. The initial heat stability of the powders was 30 to 31 min at 130°C. The heat stability decreased considerably at a steady rate in all the samples which remained organoleptically acceptable even at the end of 12 months. The rate of decrease was significantly higher at ambient temperature than at 20°C. A highly significant correlation between changes in pH and heat stability was found (r=0.97). Appearance of off-flavour in the powder was accompanied by a sudden decrease in heat stability.

11. The initial viscosity of RCM made from WMP samples ranged from 1.344 to 1.356 centipoise. The net average increase in the acceptable samples was only 0.29 after 12 months of storage. The temperature of storage had no significant effect. In the case of spoiled sample the viscosity changed suddenly after 9th month of storage coinciding with the development of off-flavour and it reached a value of as high as 1.702. An important conclusion made was that when the sample remained good during storage the viscosity of the RCM showed values which were comparable to those of normal cow or buffalo milk.

12. The lactones isolated from freshly made powder showed 44 components when separated by GLC. Of these, 20 peaks were characterized as delta- C_8-C_{16}, C_{18} and gamma-C_6-C_{12}, C_{15}, C_{16}, C_{18} lactones, 4 peaks were tentatively identified as delta-C_6, C_7 and gamma-C_{13}, C_{14} lactones. During storage there was no significant change in the composition of lactones either qualitatively or quantitatively.

13. The autoxidative changes occurring in WMP during storage were determined by estimating diene conjugation, peroxide value, TBA value, total carbonyl value and UV absorption at 280 nm.

14. The initial diene conjugation ranged from 0.705 to 0.894 %. The diene conjugation increased gradually and reached a maximum value at the end of about 7 months of storage in all the samples. Since, the change in diene conjugation was not significant in the initial period of storage, its determination did not serve to predict the onset of oxidative changes as indicated by development of peroxide value or TBA value.

15. Peroxide development was detected in WMP at the end of 2 to 3 months of storage and at the end of 12 months of storage the values reached were 0.9 to 1.3 mM/kg in samples stored at 20°C and 1.2 to 1.5 mM/kg in those stored at ambient temperature. The sample which developed off-flavour at the end of 10th month of storage, showed only a

peroxide value of 1.2. The fat extracted from WMP contained certain substances which interfered with peroxide estimation by iodometric method.

16. The peroxide value of WMP as determined by colorimetric method during storage was erratic and inconsistent. This was attributed to certain reducing substances coming into the fat during extraction of fat from WMP. Therefore, the estimation of peroxide value by colorimetric method is not dependable.

17. The initial TBA value of three batches of WMP ranged from 0.002 to 0.008. The rate of increase was significantly higher in samples stored at ambient temperature than in those stored at 20°C. In all the organoleptically acceptable samples the TBA values were less than 0.045 even at the end of 12 months of storage. In the case of spoiled sample the TBA value was 0.068 at the end of 10th month when the sample had just started showing off-flavour. Therefore, TBA value of 0.05 in WMP can be taken as limit to which powder can be stored without the development of detectable oxidized flavour.

18. The initial total carbonyl (TC) value ranged from 6.66 to 8.01 mM/g fat and this represents the carbonyl which are naturally present in WMP. During storage the TC value increased gradually and the rate of increase was markedly higher in samples stored at ambient temperature than in those stored at 20°C. Since, the initial TC

values varied widely its determination was not helpful in knowing the degree of autoxidation in WMP, especially when the initial value is not known.

19. The initial monocarbonyl contents varied widely from 0.45 to 0.61 mM/g fat. It increased during storage, the increase being significantly higher at ambient temperature than at 20°C. Development of off-flavour was not accompanied by any marked change in monocarbonyl content.

20. The initial steam volatile monocarbonyl (SVMC) content ranged from 1.21 to 1.49 µM/100 g of RCM. The SVMC content increased gradually and the rate of increase was higher at ambient temperature than at 20°C. Since the difference in the SVMC content between organoleptically acceptable and spoiled one was very small, the determination of SVMC did not help in identifying the powder which had started developing off-flavours. The level of saturated monocarbonyls (n-alkanals) and unsaturated carbonyls (alk-2-enals) was distinctly higher in spoiled than in organoleptically acceptable samples. Determination of steam volatile n-alkanals and unsaturated carbonyls help in identifying the onset of spoilage in WMP during storage.

21. The initial UV absorption values (at 280 nm) of steam distillate of fresh WMP ranged from 0.064 to 0.070 and these values increased gradually during storage, the

rate of change being significantly higher at ambient temperature than at 20°C. In all the samples which remained organoleptically acceptable, the increase was only 0.03 at the end of 12 months of storage whereas the powder which developed off-flavour after 9th month of storage showed an increase of 0.074 at the end of 10th month and 0.110 at the end 12 months. A highly significant correlationship was found between OD at 280 nm and organoleptic scores ($r = -0.96$). It is suggested that WMP which shows more than 0.050 increase in this value or reach a value of 0.150 or more may not be suitable for further storage. The increase in UV absorption during storage was attributed to increase in n-alkanals and alka-2,4,dienals. This method is very promising in judging the storage stability of WMP.

22. The initial free fat content of WMP ranged from 2.62 to 5.61. The free fat content increased gradually, and the rate of increase was significantly higher at ambient temperature than at 20°C. In the samples which remained acceptable even at the end of 12 months of storage the average net increase was 2.14%. But in the sample which developed off-flavour after 9th month, there was sudden increase in free fat content to 81.54% coinciding with the development of off-flavour. Therefore, increase in free fat content is a good index to judge the spoilage of WMP.

23. The free fat showed slightly higher rate of autoxidation as compared to total fat. This was attributed to the presence of higher proportion of unsaturated fatty acid in free fat than in total fat as determined by GLC analysis. An important observation made was that determination of peroxide value on free fat gave consistent and reproducible values unlike the values obtained when the test was made on total fat. This difference was attributed to absence of any interfering reducing substances coming into free fat as it was extractable by non polar solvents.

24. Browning changes in dried milks are important in the development in off-flavour during storage. Some of the manifestation of browning are increase of HMF, increase in reducing capacity, reduction of sulphydryl groups and development of colour.

25. The total HMF content of fresh WMP samples ranged from 3.15 to 3.46 µM/l and it increased considerably during storage. The average total HMF contents reached a value of 14.68 and 20.74 at the end of 12 months in samples stored at 20°C and ambient temperature respectively. Development of off-flavour coincided with a sudden increase in HMF content. This was attributed to the level of moisture becoming more than 4% at that stage. The changes in HMF showed a highly significant inverse relationship with organoleptic scores during storage of

WMP (r= - 0.91). Hence, determination of HMF can form an important test.

26. The changes in free HMF was very small during storage and its determination was not helpful in knowing the extent of browning.

27. The total reducing capacity ranged from 1.42 to 1.60 mg cysteine HCl/g powder. The change in reducing capacity was very small in powder stored at 20°C and it was only slightly higher in powder stored at ambient temperature. However, development of off-flavour coincided with a sudden increase in total reducing value.

28. The presence of free sulphydryl (SH-) in WMP depended on the heat treatment involved during the manufacture of powder. The SH-groups show good autoxidative property. During storage the SH- groups decreased considerably and this was attributed to the absorption of oxygen by the powder. The changes in SH-groups did not correlate with those of flavour scores.

29. The initial colour index ranged from 0.134 to 0.141. The average colour index at the end of 12 months was 0.188 at 20°C and 0.207 at ambient temperature. The colour index was slightly higher than the value given above in the sample which started developing off-flavour.

30. A survey of the quality of WMP manufactured in the country by different manufacturers was made and their

storage stability when kept at 30°C with or without nitrogen packing was studied. The market samples (three brands) although showed wide variation in their initial physico-chemical characteristics yet they were highly acceptable as judged by organoleptic examination and they conformed to specifications of BIS (IS: 1165-1986). All market samples showed reasonably good storage stability when stored for 9 months at 30°C.

31. The differences between gas packed and non gas packed tins were very small in respect of changes in all the parameters studied throughout the storage period. Thus, gas packing had practically no advantage. This was attributed to the fact that market WMP were of medium-heated type as classified on the basis of their whey protein nitrogen index (WPNI).

32. A comparison of physico-chemical changes taking place in WMP packed in 500 g tins and 25 kg bags, stored at ambient temperature and 20°C for a period of 9 months, was made. The moisture content was constant in tins but it slowly increased in the case of powder packed in bags as mentioned earlier (cf. 6.3). This was the only major difference between these two types of packings. The other parameter changed at almost similar rates in both the packings throughout the storage period in the powder kept at 20°C. But in the case of powder stored at ambient temperature the difference between tins and bags was revealed at the end of 9th month of storage only in

respect of HMF, TBA and OD at 280 nm. It was concluded that powder packed in HDPE bags remained as good as that packed in tins for nine months even when stored at ambient temperature.

33. WMP packed in tins with and without nitrogen was stored at three different temperatures of 20°, 30° and 34°C for nine months. The temperature of storage had significant effect on the rates of change in all the parameters. The difference in rates of change between gas packing and non gas packing was not significant and the temperature of storage had no significant effect on this difference.

34. The initial bacterial count (spc/g) of the three batches of WMP ranged from 4,800 to 10,700 which is well within the permitted standards and during storage the counts showed a decrease. The coliform count was nil initially as well as during storage period.

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7.0 BIBLIOGRAPHY

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APPENDIX - I

ORGANOLEPTIC EVALUATION CARD FOR HEDONIC RATING TEST OF RECONSTITUTED MILK AND WHOLE MILK POWDER

NAME: DATE:

TIME:

	RECONSTITUTED PRODUCT			DRY PRODUCT		
	I	II	III	I	II	III
1. Appearance	____	____	____	____	____	____
2. Flavour/Taste	____	____	____	____	____	____
3. Body/Texture	____	____	____	____	____	____
4. Overall Acceptability	____	____	____	____	____	____

SCALE FOR EVALUATION

Like extremely	- 9	Like slightly	- 6	Dislike moderately	- 3
Like very much	- 8	Neither like/ Nor dislike	- 5	Dislike very much	- 2
Like moderately	- 7	Dislike slightly	- 4	Dislike extremely	- 1

Comments (If any) : ____________________

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Signature of the Judge.