SOME PHYSICAL AND CHEMICAL PROPERTIES OF PROCESSED MILK PRODUCTS

A Thesis submitted to the University of Glasgow for the Degree of Doctor of Philosophy in the Faculty of Science

by

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Synopsis

The experiments described in the thesis were mainly concerned with the nature and the prevention of deterioration in spray-dried, whole and separated milk. Part I describes the analytical methods and techniques used, Part II deals with the work on whole-milk powder and Part III with the work on separated-milk powder. Part IV describes an investigation to determine the validity of the methods which have been proposed to measure the solubility of milk powders.

Part I Methods

(a) In this section a brief description is given of the methods used in the routine analysis of powders, i.e. measurement of moisture, 'crude' protein, fat, lactose and ash.

(b) In the second section, the various physical and chemical methods used to measure deterioration and other miscellaneous analytical methods are described.

Part II The effect of a high pre-heating temperature with and without ethyl gallate on the storage life of whole-milk powder spray-dried on a Gray-Jensen drier

Two methods of improving the keeping quality of whole-milk powder prepared on a Gray-Jensen spray-drying plant have been tested separately and together. These methods are pre-heating the liquid milk to a high temperature (190°F. as compared with 160°F.) and the addition of an antioxidant (ethyl gallate) to the milk just before drying.

Some 500 gallons of milk were dried, samples of the various powders packed in small cans (plain and lacquered) and then stored in incubators at 47 and 37°C. and also at laboratory temperature (c.17°C.). Deterioration of the stored powders was measured by examination at intervals for palatability, absorption of oxygen and production of carbon dioxide, and apparent peroxide value.

It was found that the use of a high pre-heating temperature or the addition of ethyl gallate made the storage-life of the powders three times as long as it was when these modifications were not introduced. The use of both methods together extended the storage-life by about seven times that of the control powder at the same storage temperature. Lacquered cans improved storage-life by about 10% compared with plain tinplate cans. The results showed that the use of a high pre-heating temperature plus ethyl gallate would be a valuable alternative to gas-packing for moderately long storage periods.

Part III The deterioration on storage of spray-dried separated milk with special reference to the influence of moisture content

This work was initiated by reports that when a spray-dried whole-milk powder containing excessive moisture was stored, the ensuing deterioration involved protein (in addition to fat) and that when a separated-milk powder had been stored for some time under conditions which did not exclude moisture, the biological value of its proteins had decreased.

To investigate these deteriorative changes, 900 gallons of separated milk were spray-dried and three powders obtained, of low (2.9%), medium (4.7%) and high (7.3%) moisture content. The powders were stored in air and also in nitrogen at 37, 28.5 and 20°C. and examined at intervals for palatability, colour, pH, absorption of oxygen, production of carbon dioxide, solubility in water at 20 and 50°C. and moisture content. A study was also made of the relative amounts of α- and β- lactose present, the distribution of soluble 'nitrogen', the bacterial count and the composition of insoluble sediment.

After storage for more than three years, very little change had occurred in the powders with 2.9 and 4.7% moisture except for a decrease in palatability and some absorption of oxygen and production of carbon dioxide. On the other hand, the 'high moisture'

powder (7.3%) deteriorated very rapidly at 37°C. and more slowly at 28.5 and 20°C.; palatability decreased markedly, a brown discoloration developed, a decrease in the solubility of the protein and other important changes took place. Evidence was obtained which showed that a reaction had occurred between protein and lactose, and it was found by other collaborators that this reduced the biological value of the milk protein by rendering its lysine less available. However, the powder became unpalatable long before its nutritive value decreased.

It was concluded that a moisture content of 4% is a safe maximum for spray-dried separated milk during storage at normal temperatures but that the value should be kept as low as possible particularly if the powder is to be stored at high temperatures or in the tropics.

Part IV. The solubility of milk powders

To determine the sources of error in the methods which have been proposed to measure the solubility of milk powders, six whole-milk powders, two roller-dried and four spray-dried by different processes, were reconstituted at 20 and 50°C. and centrifuged. The distribution of the milk constituents in the three layers, top or fat, middle or liquid and bottom or sediment was then determined. It was found that the more insoluble a powder, the greater was the

amount of insoluble protein in the top (fat) layer and the greater the amount of fat in the sediment. The sediments were examined in greater detail by preparing a large bulk of them in a Sharples super-centrifuge. The washed and dried sediments consisted mainly of insoluble protein which appeared to be in the form of the calcium caseinate- tricalcium phosphate complex known to exist in liquid milk. The solubility methods were criticized in the light of these findings.

General Introduction

Types of milk powder

Milk is dried by two main processes, spray-drying and roller-drying. In spray-drying, the milk is condensed and projected as minute globules (formed either by pressure-spray or centrifugal spray) into a blast of hot air. In roller-drying, milk is dried as a thin film on the surfaces of heated rollers. Spray-process powder of good quality is very soluble even in cold water and although it may have a slightly 'cooked' flavour, closely resembles liquid milk when reconstituted with water. Roller-dried powder is subject to more intense heat during the drying process with the result that while it is fairly soluble in hot water, it has a poor solubility in cold and has a pronounced 'cooked' flavour.

Nutritive value

Much research has been done in the past two decades to improve the methods of dehydration so that the nutritive value of the dried product approaches as nearly as possible to that of the fresh milk from which it was produced, and in experiments published a few years ago by Henry, Houston, Kon & Osborne (1939) it was shown that when milk is dried by modern spray-drying methods, the only significant decrease in the nutritive value of the fresh powder is a decrease in the content of ascorbic acid. The partial destruction of this

Table 1. Average analytical figures for the composition and food value of dried whole and separated milk*

	Dried whole milk	Dried separated milk
Moisture (%)	2.3	3.0
Protein (%)	26.5	36.9
Fat (%)	26.7	0.9
Lactose (%)	38.5	51.0
Ash (%)	6.0	8.2
Calcium (%)	0.97	1.31
Phosphorus (%)	0.75	1.02
Vitamin A (I.U./100g.)	1092.	36.
Riboflavin (mg./100g.)	1.5	2.0
Thiamine (mg./100g.)	0.3	0.4
Niacin (mg./100g.)	0.7	0.9
Pantothenic acid (mg./100g.)	2.9	3.3
Pyridoxin (mg./100g.)	0.3	0.4
Biotin (mg./100g.)	0.04	0.04
Choline (mg./100g.)	88.	110.
Energy (kg.cal./100g.)	499.	360.

* Data taken from Production Trends, Dry Milk and Related Products, Bull.no.1147, p.14 (Nov., 1947). Chicago: Amer.Dry Milk Inst., Inc.

vitamin is, however, no disadvantage since liquid milk contains but little vitamin C and is not regarded as an important source of this vitamin. The high nutritive value of dried milk is characteristic not only of whole-milk powder but also of the separated product which is rich in protein and minerals. Typical figures for the more important constituents of dried whole and separated milk are shown in Table 1. Only typical approximate figures can be given because the analysis varies slightly according to the composition of the liquid milk from which the powder is made.

Keeping quality

Although some years ago spray-dried milk was shown to be highly nutritious when freshly made, it had the disadvantage that it did not keep long in good condition. Sometimes spray-dried whole-milk powder became unpalatable after only about 3 months, and obviously it was important that powder made from milk in the early summer when milk was plentiful should keep at least throughout the following winter when milk would be scarce. The prevention of the deterioration of whole- and separated-milk powders presents two different problems and they will be discussed separately.

(a) Whole-milk powder

The most important defect likely to occur in a whole-milk powder is a most objectionable and nauseating tallowy flavour. It is caused by the presence of

peroxides and their decomposition products (such as hydroxy-aldehydes, aldehydes and ketones) which are formed by the oxidation of the unsaturated fatty acid components of the milk triglycerides.

Since 1939, much research has been devoted to finding methods of preventing or inhibiting this oxidation of milk fat in spray-dried powders. For example, in 1943, Lea, Moran & Smith published the result of an extensive series of experiments which they made to determine to what extent the storage life of dried milk could be extended by packing the product in nitrogen instead of air. The results showed that by reducing the amount of oxygen in the atmosphere of a can of dried milk to below 3%, spray-dried whole-milk powders would keep in good condition for several years at ordinary temperatures. As a result of this work much of the powder produced during the recent war for the use of forces overseas was gas-packed in large 21 lb. cans. Gas-packing, however, has the disadvantage that special equipment and really gas-tight cans are required and on a large factory scale it is difficult to ensure that the process is done with 100% efficiency. Moreover, relatively slight damage to a gas-packed can in transit or during storage may cause a small leak which admits air and renders the gas-packing useless. Other methods of prolonging the storage-life of dried milk have therefore been sought.

Compressing the milk powder into blocks was investigated at the Hannah Institute some years ago.

By greatly reducing the amount of oxygen in contact with the powder this process lengthened its storage life, but it could not be adopted in practice because the blocks of powder had to be so hard that they required special machinery to reduce them to a powder again.

At the time when these matters were being considered it was known that the fat in roller-dried powder did not oxidize so readily as that in spray-dried powder, and it was believed that this might be due to traces of sulphydryl compounds formed from the milk protein as a result of the more intense heat treatment which the milk received in the roller-drying process. Two large scale collaborative investigations (Mattick, Hiscox, Crossley, Lea, Findlay, Smith, Thompson, Kon & Edgell, 1945; Findlay, Higginbottom, Smith & Lea, 1946) were therefore done to determine the effect of raising the temperature at which the liquid milk was heated before it was dried, on the keeping quality of the resulting powder. It was found that by using a pre-heating temperature of 190°F. instead of the more usual 160°F., the storage life of the powder made on two different types of spray-drier could be greatly extended. The two types of spray-drier used were the Kestner and the Krause (Scott, 1932) both of which make the spray by centrifugal force.

Another method by which the oxidation of fat in dried milk might be retarded was by the addition of suitable chemicals with antioxidant properties to the

milk before it was dried. When a number of possible antioxidants were tested by Findlay, Smith & Lea (1945), it was found that ethyl gallate was the most effective. It was therefore decided to investigate further the value of a high pre-heating temperature with and without the addition of ethyl gallate to milk when dried by the Gray-Jensen process in which the spray is made by forcing the milk through a small aperture under high pressure. This is the method by which spray-dried powder is manufactured in Scotland. A description of the work is given in Part II of this thesis.

(b) Separated-milk powder

Since the amount of fat present in separated-milk powder is so low (see Table 1), it is unlikely that oxidation of fat will be an important form of deterioration when this type of powder is stored, and certainly it has been found that the unpalatable flavours which develop in dried separated-milk cannot usually be described as tallowy but are of a type which can more accurately be called gluey or 'cardboardy'. It was found several years ago (Henry & Kon, 1945; Henry, Kon & Rowland, 1946) that on storing dried separated milk for some years its nutritive value decreased significantly, and it has since been found in an extensive investigation that the development of the 'gluey-cardboardy' flavours and the decrease in nutritive value are associated with the moisture content

of the powders, high moisture contents accelerating these types of deterioration. The present writer's part in the experiments done to investigate this problem in detail is described in Part III of this thesis.

Physical differences between the particles of roller- and spray-dried whole-milk powders

It has already been mentioned that the more severe heat treatment given the milk during the roller-drying process reduces the solubility of the protein but at the same time, probably through the influence of liberated sulphydryl compounds, confers resistance to oxidation on the fat. Another important factor when considering the relative keeping properties of roller and spray powders is the difference in the physical structure of the particles of the two types of dried milk.

The particles of roller-dried whole-milk powders are irregular platelets whose size depends on the degree of disintegration given the sheet of dry milk which leaves the rollers. Microscopical examination reveals that the particles have a wavy or 'ridge and valley' appearance with the fat present in fairly large fragments and much of the lactose crystalline. In contrast, the particles of spray powders consist of minute spheres usually with a core of trapped air. The fat is very finely subdivided and scattered uniformly throughout the mass of each particle and the lactose is in a wholly amorphous 'glass' form.

The finely divided fat of spray powders is

thus subject to oxidation both from the outside and the inside of the particles. Moreover, during spray-drying, the temperature of the milk globules or the dry particles probably never exceeds about 30°C. so that the extent of liberation of sulphydryl compounds will be less than in roller-drying and will depend wholly on the pre-heating of the liquid milk. Although the fat of roller powders is more resistant to oxidation, there is evidence (Washburn, 1922; King, 1948) that the protective protein membrane normally surrounding each fat globule in liquid milk is disrupted during the roller-drying process but only to a slight extent during spray-drying. The influence of this factor on the keeping quality of the fat is not known but that it does influence the appearance and behaviour of the constituents of reconstituted milk, especially when centrifuged to sediment insoluble material in solubility determinations, is shown in Part IV of the thesis.

PART I

Methods

A. Analysis of the Milk Powders

In this section, the methods used to measure the amount of moisture, 'crude' protein, fat, lactose and ash in the milk powders are described briefly.

Before proceeding with an analysis, the sample of powder was mixed thoroughly but with minimum exposure to the atmosphere.

(1) Moisture

The method was to weigh a small amount of powder into a metal dish and heat the powder in an oven at 100°C. The details are as follows:-

Dishes. Two types of dish were used: aluminium, 6 cm. in diameter and 1.5 cm. in depth, and stainless steel, 6.5 cm. in diameter and 2.5 cm. in depth. The dishes had close fitting but easily removable lids.

Procedure. The uncovered dish and lid were placed in an electrically heated oven maintained at a temperature of 100° ± 1°C. for 1 hr. The lid was replaced, the dish removed from the oven, allowed to cool for 30 min. in a desiccator containing P_2O_5 and weighed. Approximately 1 g. of the powder was transferred to the dish, the lid replaced and the dish weighed rapidly. The uncovered dish and lid were replaced in the oven for 3 hr. The dish was then covered and allowed to cool in the desiccator as before. Finally, the dish was weighed rapidly and the loss in weight expressed as grams of moisture per 100 g. of the original powder.

This method is very similar to that recommended in the Report of the Milk Products Sub-Committee of the Society of Public Analysts (1936). It requires no special apparatus and readily gives reproducible results. Two

other methods of determining the moisture content of milk powder have been described, the vacuum-oven method (Methods of Analysis of the Association of Official Agricultural Chemists, 1945) and the direct determination of water by distillation with toluene (Grading of Dry Whole Milk, 1947). The latter method especially has been thoroughly investigated in Great Britain and the U.S.A. but both procedures require special apparatus and have no real advantage over the simpler method described above.

(2) 'Crude' protein

The usual Kjeldahl procedure was adopted to obtain the content of total nitrogen in the powders. The amount of 'crude' protein was then obtained by multiplying the nitrogen value by 6.38, the factor generally accepted for milk products. The catalyst used for the Kjeldahl process consisted of K_2SO_4(40 parts), $CuSO_4.5H_2O$ (5 parts) and selenium powder (1 part) ground together in a mortar. It was used at the rate of 46 g. per 100 ml. of conc. H_2SO_4. The digestions were continued for 2 hr. after the contents of the Kjeldahl flasks had become 'clear'. This was found to be sufficient for dried milk; the 8 hr. digestion period recommended by Chibnall, Rees & Williams (1943) is only necessary for pure proteins. For the titrations, a modification of de Wesselow's mixed indicator (Cole, 1933) technique was used. It consisted of adding 3 drops of a saturated solution of methyl red in 95-96% (v/v) ethanol and 1 drop of a 1% solution of methylene

blue in 95-96% (v/v) ethanol.

(3) Fat

The method for estimating fat was that recommended in the Report of the Milk Products Sub-Committee of the Society of Public Analysts (1936). This procedure incorporates the advantages of the Röse-Gottlieb and Werner-Schmid methods. In principle, it consists of digesting the dried milk with hydrochloric acid and extracting the fat with ether and light petroleum in special apparatus and under strictly specified conditions.

(4) Lactose

A polarimetric method was used because this is probably the quickest and most accurate method of estimating lactose in milk products. The only drawback is that a correction has to be applied for the volume of the precipitated protein and fat. Vieth's method (Richmond, Elsdon & Walker, 1942) of estimating lactose in liquid milk was used with slight modifications.

Reagent. 5 ml. of mercury were dissolved in 96 ml. of conc.nitric acid and diluted with an equal volume of water.

Procedure. 9 g. of separated-or 12.5 g. of whole-milk powder were weighed into a 100 ml. volumetric flask (with the graduation mark low in the neck) and the powder dissolved in warm water (30-40°C.). After being cooled, the solution was made up to 100 ml. Then 3 ml. of acid mercuric nitrate solution were added and the flask shaken vigorously. Thereafter, the method was the same as for liquid milk and merely involved filtering the contents of the flask and finding the rotation of the clear filtrate in a polarimeter.

Correction for the volume of precipitate

(a) Protein. Vieth found that the volume of the

precipitated proteins from 100 ml. of milk averaged about 3 ml. His method, as described above, compensates for this average volume of protein. When, however, the protein content of the powder is known, an alternative and more precise procedure is to calculate the volume of the precipitated proteins. This can be done since the specific gravity of milk protein is known to be 1.346 (Davies, 1939; Richmond et al., 1942), corresponding to a specific volume of 0.734. When this method is adopted, the reconstituted powder can be made up to 100 ml. including the 3 ml. of reagent if more convenient.

(b) Fat. The average specific gravity of milk fat is about 0.94 (Davies, 1939) and hence its specific volume is 1.064. The volume of fat in 13.5 g. of whole-milk powder can therefore be calculated from the percentage of fat in the sample. For a detailed discussion of the effect of the volume of the precipitate on the accuracy of polarimetric determinations of lactose reference should be made to papers by Perkins (1920) and Garrison (1939).

It is fairly certain that in freshly prepared powder (both spray and roller) and in stored powder which has a low moisture content, the lactose is in the form of a non-crystalline, anhydrous 'glass' (Troy & Sharp, 1930; Schloemer & Catravas, 1940). The lactose content of a normal powder was therefore expressed as grams of anhydrous lactose per 100 g. of powder.

(5) Ash

The determination of mineral matter is probably the least precise of the elementary analyses. If the charred powder is heated to too high a temperature in the muffle furnace, some constituents of the ash may volatilize, or if heated insufficiently, combustion of carbon may not be complete. The following method has proved rapid and satisfactory:-

Procedure. A small silica crucible (5 cm. in diameter) was heated in a muffle furnace adjusted to a temperature of about 550°C. (checked by a thermocouple) for 1 hr. The crucible was removed, cooled for 30 min. in

a desiccator and about 5 g. of powder weighed into it. The milk powder was then charred slowly over a Bunsen flame until no more smoke was evolved. The crucible was placed in the furnace for 2 hr. It was then taken out, left to cool, and the ash moistened with a few drops of diluted nitric acid (1:1). The moist ash was dried over a small Bunsen flame or in an oven and replaced in the furnace for 1 hr. The crucible was then cooled and weighed as before and the heating continued for 1 hr. periods until the ash was constant in weight. The weight of the residual material was expressed as grams of ash per 100 g. of the original powder.

By the addition of a little diluted nitric acid, a white ash completely free from carbon, was obtained with a minimum amount of heating.

B. Special Analyses and Methods

The special methods common to Parts II and III of this thesis will be described first and then the procedures relevant to each part in turn. The reasons for using a particular method are given together with a brief outline of the experimental details.

Special methods and analyses common to Parts II and III

(1) Palatability

Although chemical tests can often give helpful information, one of the most important criteria of the suitability of a milk powder for human consumption is its palatability. The obvious way of finding whether a powder is palatable is to taste and smell the powder. But tasting the solid powder is of little use as the palate becomes 'clogged' and the concentration of lactose masks any 'off'-flavours. Another difficulty

is the different response of individuals to the same powder and also the variable sensitivity of one person at different times. The variability would probably be even more marked if assessment of smell were attempted. In the experiments described later, it was necessary to judge the palatability of powders which were being stored for 2-3 years and hence it was essential to maintain as consistent a standard of tasting as possible. The tasting technique has therefore been to reconstitute and code the powders, use a small but experienced tasting panel of five persons and have a simple system of scoring or grading. The size of the tasting panel may seem small but as will be shown later, good agreement was obtained with another panel tasting the same powders at another Research Institute, and moreover, Weaver (1939) has shown that valid scoring of the flavour of liquid milk can be obtained by a panel of seven even when two of them are inexperienced.

Procedure. The powder was mixed with slightly warm water (c.35°C.) using a powder/water ratio of 1/8 for whole-milk powder and 1/10 for separated-milk powder. (To have the same solids content as liquid milk, a whole-milk powder should be reconstituted with water in the ratio 1/7 but experience has shown that it is easier to detect minor variations in palatability with the more dilute reconstituted milk). A little water was first added to the powder and the mixture well stirred into a paste to eliminate the formation of lumps. The remainder of the water was then added and the 'milk' well shaken. The reconstituted milk was labelled in a code unknown to the tasters and tasted within 30-60 min. when it had cooled to only slightly above the laboratory temperature. A special form was provided for each taster to enter comments and a mark according to the scheme described below.

A powder similar to those being tasted and known to be of good quality was 'remade' and used as a control or standard. In earlier work in this laboratory (Lea et al., 1943), the control was not labelled as such but merely included in the coded series. In later experiments (Mattick et al., 1945; Findlay et al., 1945; Findlay et al., 1946) and in the present series, the tasters knew which bottle contained the control powder and they used it as a standard for comparison.

Before tasting the 'remade' milks, it was advisable to place any which seemed 'off' (either by smell or appearance) at the end of the tasting line. In this way the most objectionable flavours were encountered last and were not carried on to the next powder. To lessen the risk of carrying 'off'-flavours to the next powder, the tasters were asked to rinse their mouths with lukewarm water after each sample and in addition, if a pronounced 'off'-flavour had been encountered, to retaste the control.

The system of scoring was kept as simple as possible and the grades were defined as follows:- 0 = very good and like ordinary fresh milk, 1 = fairly good and quite palatable, 2 = slightly but definitely unpalatable due to the presence of slight 'off'-flavours, 3 = unpalatable due to the presence of pronounced 'off'-flavours, 4 = very unpalatable. The tasters were asked to award marks according to the general palatability, and degree and type of 'off'-flavour. The average of the 'off-flavour marks for each sample was then recorded

as the 'off'-flavour score of the powder. The significance of the 'off'-flavour scores will be discussed in the experimental sections.

(2) Solubility

At the moment of writing, work is being done to investigate insolubility in milk powders and with the knowledge gained, to improve the existing methods of measuring solubility or devise new ones. This work, which is described in Part IV, is a continuation of earlier investigations at the Hannah Institute (Wright, 1932; Howat & Wright, 1933, 1934; Howat, Smith, Waite & Wright, 1939). The solubility methods used in Parts II and III were as follows:-

(a) Measurement of soluble solids. Some years ago a relatively rapid method for determining solubility was worked out at the Hannah Institute by Howat et al. (1939). This method has proved satisfactory for highly soluble dried milk and has been shown (Findlay, 1944) to give results for spray-dried whole-milk powders very similar to those obtained by the more elaborate and exact method of Lampitt & Bushill (1931a). The procedure was as follows:-

> 1g. of powder was weighed into a 15 ml. centrifuge tube. About 2 ml. of water were added from a burette and the mixture stirred with a glass rod which had been previously wetted. When all the powder was thoroughly moistened, more water was added until a total of 9 ml. had been run in, the stirring rod being washed with the last few ml. of water. The tube was then stoppered, kept in a water-bath either at 20 or 50°C. for 5 min., and then shaken rapidly with 4-6 complete double excursions per sec. for 1 min. (When the solubility at 50°C. was desired, the tube was placed inside an insulated container while being shaken and afterwards cooled to 20°C.)

The tube was then centrifuged for 15 min. at 3,000 r.p.m. (corresponding to a gravitational force at the bottom of the tube of 1,800 x g). The supernatant layer was poured off as completely as possible (including the fat layer if the sample was a whole-milk powder) and its total solids content estimated by the rapid method of Golding (1934). When a fat layer was present in the 'poured-off' supernatant liquid, it was reincorporated by gentle heating and shaking and the homogeneous liquid cooled before using Golding's procedure. The ratio of the dissolved solids to the solids initially present (expressed as a percentage) was taken as an index of the solubility. The initial solids were corrected for the moisture content of the powder. Golding's method has been found very convenient and reliable provided P_2O_5 is used as a desiccant.

In the paper of Howat et al.(1939), it was pointed out that when this method was applied to very soluble spray-dried whole and separated milk, the solubility values often exceeded the theoretical maximum of 100% by 2-3 units. In a later paper by Lea et al. (1943), it was suggested that this anomaly was due to the fact that the method of drying a portion of the supernatant liquid to measure the amount of dissolved solids caused the lactose, which is normally almost entirely anhydrous in milk powders, to crystallize and be weighed as $C_{12}H_{22}O_{11}.1H_2O$. A small correction was therefore applied to allow for this as was done by Lea et al.

(b) Measurement of sediment volume. A simpler method of assessing solubility and one much used in industry was also adopted to obtain the relative solubility of the experimental milk powders. The principle of this method is to shake a certain amount of powder with water, centrifuge down the insoluble material, wash the sediment once and centrifuge again. The volume of insoluble

material is taken as the solubility index of the powder. Such a method has been recommended by the American Dry Milk Institute (Grading of Dry Whole Milk, 1947; Grading of Nonfat Dry Milk Solids, 1948) but as special apparatus which was unobtainable is prescribed, the procedure was modified slightly.

> Procedure. 20 g. of separated-or 25 g. of whole-milk powder were added to 200 ml. of water at 20°C. contained in a 500 ml. wide-mouthed bottle. Before adding the powder, the bottle was shaken to wet its sides and prevent the powder sticking to the bottle. The bottle was shaken for 30 sec., allowed to stand for 5 min., shaken again for 30 sec. and a graduated centrifuge tube of 50 ml. capacity filled to the mark with the 'milk'. The tube was then centrifuged for 15 min. at 1,000 r.p.m. (216 x g). The supernatant liquid was removed by suction, 25 ml. of water at 20°C. added and the tube shaken for 30 sec., taking care to dislodge all the sediment. The tube was filled to the mark with water at 20°C. and finally centrifuged for 15 min. as before. The volume of sediment thus obtained gave a measure of the solubility of the powder, the results being expressed as ml. of sediment.

(3) Absorption of oxygen and production of carbon dioxide

In the experiments described in Parts II and III, it was necessary to measure the uptake of oxygen and possible production of carbon dioxide by spray-dried whole- and separated-milk powders packed in air-tight cans.

The method used to obtain a sample of gas from the cans has been described in detail by Waite (1941a) so only a brief outline will be given here. A similar technique has also been used by Lea et al. (1943).

Procedure. A can was attached via a puncturing unit to a gas-sampling tube. The system was evacuated by an oil-pump and a hole pierced in the can. Some of the gas in the can immediately passed into the sampling-tube. About 9 ml. of the gas were then transferred to the burette (10 ml. capacity) of a Haldane gas analysis apparatus and the percentage by volume of O_2 and CO_2 obtained. The technique of the method has been described by Haldane & Graham (1935) and Peters & Van Slyke (1932). A 10% solution of KOH was used to absorb CO_2 and the alkaline pyrogallol solution recommended by Peters & Van Slyke was used to absorb O_2.

This procedure gave the percentage of O_2 and CO_2 in the atmosphere of the can, but in order to know the weight of oxygen absorbed or carbon dioxide produced per 100 g. of powder it was necessary to know the actual weight of O_2 and CO_2 present in the can originally and finally. To obtain this, a knowledge of the density of the air-free, whole-and separated-milk solids was required. One of the most recent measurements of these values is that of Lea & Gane (1946) being a continuation of earlier work by Lea et al. (1943). The true density of air-free whole-milk solids is accepted as 1.27 (Muers & Anderson, 1944; Lea & Gane, 1946), but for the purpose of the calculation in view, this figure must be increased to allow for the selective solution of oxygen in milk-fat and the space occupied by water vapour in a powder. The corrected value of 1.33(5) g./ml. recommended by Lea & Gane has therefore been used. For separated-milk solids, the complication caused by the solubility of gases in the milk fat does not arise, and the figure of 1.465 g./ml. has been used (Lea &

Gane, 1946). Further data required for the calculation were, the weight of the powder in the can, the volume of the can, and the barometric pressure and the temperature at the moment the can was sealed.

Some of the actual values from Part III will be used to illustrate the method of calculation:-

Volume of can = 315 ml.

Weight of powder = 150 g.

Apparent density of powder (separated-milk) = 1.465 g./ml.

Bar. pressure = 750 mm.of Hg) when
Temperature = 18°C.) the can was closed

Assumed composition of air:- (Haldane & Graham, 1935)

O_2 : 20.93%

CO_2 : 0.03%

N_2 : 79.04%

(a) The initial O_2 content of can

Volume of 150 g. powder = $\frac{150}{1.465}$ = 102.3 ml.

'Free space' in can or original volume of air in can = 315-102.3 = 212.7 ml.

Converted to N.T.P. = $212.7 \times \frac{273}{291} \times \frac{750}{760}$ = 196.9 ml.

Volume of O_2 = $196.9 \times \frac{20.93}{100}$ ml.

$\frac{\text{Wt. of } O_2/100\text{g.}}{\text{powder}} = 196.9 \times \frac{20.93}{100} \times \frac{32}{22.4} \times \frac{100}{150}$

= 39.24 mg.

(b) Conversion of percentage values to absolute units

Findlay (1944) used a method of calculation

which did not allow for any CO_2 produced. Any error would be extremely small as very little CO_2 is produced by milk powders under normal storage conditions. But the abnormal powders used in Part III produced an appreciable amount of CO_2. For this reason, a method of calculation which compensated for CO_2 had to be used (Lea, 1944) and it is best explained by working out an example.

Let us suppose that the composition of the gas in a can after a period of storage had altered from that of air, viz, 29.93% oxygen, 0.03% carbon dioxide and 79.04% nitrogen to 10.00% O_2, 0.50% CO_2 and 89.50% nitrogen. (It should be emphasized that both sets of figures show what fractions of the available or 'free space' in the can were occupied by the respective gases.) The latter figures cannot be compared directly with the former figures because at the time of the final analysis, the gas in the can would be at a different pressure (lower in this example) and possibly at a different temperature, than when the can was originally sealed. This difficulty was overcome by converting the figures for the final composition of the gas to what they would have been at the original temperature and pressure. The calculation was done by assuming that the weight of nitrogen in the can did not change during storage. This weight of nitrogen occupied 79.04% of the available or 'free space' in the can under the original conditions of packing and the same weight occupied 89.50% of the available space under the different conditions at the

final analysis. Thus the oxygen remaining in the can after storage and occupying 10.00% of the available space would occupy less, viz, $10.00 \times \frac{79.04}{89.50} = 8.83\%$, at the original temperature and pressure. Therefore, the oxygen absorbed during storage would occupy a volume equivalent to 20.93 - 8.83 = 12.10% of the 'free space' in the can under the original conditions. This 'free space' has been shown to be 212.7 ml. or 196.9 ml. at N.T.P. The volume of oxygen absorbed by the 150 g. of powder was therefore $\frac{196.9}{100} \times 12.10$ ml. at N.T.P. which weighs $\frac{196.9}{100} \times 12.10 \times \frac{32}{22.4}$ mg. The final result was expressed as mg. O_2 absorbed per 100g. of powder, i.e. $\frac{196.9}{100} \times 12.10 \times \frac{32}{22.4} \times \frac{100}{150}$ mg.

Similarly, the final percentage of carbon dioxide was converted to what it would have been at the original temperature and pressure, viz, $0.50 \times \frac{79.04}{89.50} = 0.44\%$. This value was corrected to allow for the small amount of CO_2 originally present in the air in the can (0.03%) and thus became 0.44 - 0.03 = 0.41%. The volume of carbon dioxide produced by the powder was therefore equivalent to 0.41% of the 'free space' in the can (under the original conditions) which equals $\frac{196.9}{100} \times 0.41$ ml. at N.T.P. This volume of CO_2 weighs $\frac{196.9}{100} \times 0.41 \times \frac{44}{22.4}$ mg. and the final result was expressed as mg. CO_2 produced per 100g. of powder, i.e. $\frac{196.9}{100} \times 0.41 \times \frac{44}{22.4} \times \frac{100}{150}$ mg.

Special methods and analyses used in Part II

(spray-dried whole-milk powders)

(1) Apparent peroxide value

An exhaustive review and study of the deterioration of fat and methods of measuring such deterioration has been made by Lea (1938). This work covers the literature up to 1938. The two most widely used methods for determining peroxide oxygen at that time were those of Wheeler (1932) and Lea (1938), the former in the U.S.A. and the latter in Great Britain. The principle of both methods is to dissolve the fat in a mixture of glacial acetic acid and chloroform, add potassium iodide and titrate the liberated iodine with sodium thiosulphate solution.

These methods usually cannot be applied directly to milk powders because the large amount of non-fatty substances present, especially protein, may combine with or adsorb a proportion of the liberated iodine. The milk fat must therefore be extracted from the powders. This is a troublesome procedure for two reasons. First, the most satisfactory fat solvent is peroxide-free ether but this is expensive, takes time to prepare and finally has to be evaporated from the fat. Secondly, the amount of fat which can be extracted directly from spray-dried whole-milk powder by either hot or cold extraction is only about 3-14% of that present, although almost all the fat can be extracted directly from roller-dried powder. It has been shown by Lampitt & Bushill (1931b) and pointed out by Smith (1939) that if the moisture content of a powder is raised to about

10% practically all the fat can be extracted by solvents. Presumably raising the moisture content of the powder causes the anhydrous lactose 'glass' to crystallize and disintegrate with the result that the solvent can readily penetrate to the fat.

In an attempt to get over these difficulties, Smith (1939) developed a method in which the powder was added directly to a glacial acetic acid-chloroform mixture without previously raising the moisture content of the powder. An objection to this method was that although the fat was completely extracted, some non-fatty material (which might vary in amount from powder to powder) was also extracted which diminished the apparent 'true' peroxide value by about 10%. All iodimetric procedures have been criticized on account of the slow liberation of iodine (Wheeler, 1932) and possible readsoption of iodine by fat or some of the other substances present. Moreover, Hollender & Tracy (1942) and Chapman & McFarlane (1943) came to the conclusion that Smith's method was not sensitive enough to detect incipient oxidative changes.

The latter authors therefore developed a method for estimating the peroxide value of the fat in milk powders (1943) which is based on the oxidation of ferrous to ferric iron by the peroxides and the subsequent colorimetric determination of the ferric iron as ferric thiocyanate. In this method, the moisture content of the powders was not raised but a very small sample (200 mg.) was refluxed with pure acetone for 20 min. Although all the fat was not extracted by

this treatment, Chapman & McFarlane obtained no increase in peroxide value by longer extraction even when this was continued for several hours and the fat almost completely removed from the powder. Findlay (1944) has used a modified version of this method to measure the peroxide value of the fat of milk powders and he showed that higher values were obtained if the moisture content of the powders was raised before the acetone extraction. He also showed that if the humidification of the powders was done in nitrogen at 2°C., lower peroxide values were obtained than when the powders were exposed to moist air at room temperature.

Thus it seemed from the work of previous investigators that to obtain as accurate and absolute peroxide values as possible, the ferrous salt method should be used with the following precautions:-

(a) The moisture content of the powder should be raised in an atmosphere of moist nitrogen at as low a temperature as possible. This procedure would minimize possible decomposition of peroxides by a prolonged extraction of fat and prevent oxidation during the humidification.

(b) If this procedure were used, a larger sample of powder could be taken (say 2g.) to minimize sampling errors.

(c) There should be minimum exposure of the solutions of fat to sunlight or strong electric light, especially during colour development (Chapman & McFarlane, 1943).

Table 2. Apparent peroxide values of two spray-dried whole-milk powders after humidification in air and nitrogen and extraction of fat by a long and short method

	Fresh powder				Deteriorated powder			
Humidifying atmosphere :	Air		Nitrogen		Air		Nitrogen	
Extraction method :	Long	Short	Long	Short	Long	Short	Long	Short
Apparent peroxide value (m. equiv. per kg. powder)	0.94	1.02	0.83	0.62	6.41	5.52	5.17	4.65

A few preliminary experiments showed that exposure to an atmosphere of 100% relative humidity for 24 hr. at laboratory temperature (it was not possible to use a low temperature as a routine procedure) was ample to raise the moisture content of the powders above 10%. In fact, after this period, the moisture contents of the powders used in Part II were 18.9-20.5%. So in practice, the powders were placed in the moist atmosphere in the evening and removed the next morning (i.e. after 16-18 hr.) just prior to extraction of the fat. It was also found that, instead of refluxing the 'humidified' powders for 20 min. the same amount of fat could be extracted if the powders were first shaken vigorously in cold acetone and the flask dipped in boiling water until the acetone began to boil, and then removed and well shaken. This operation was repeated 2 or 3 times and only took a few minutes. This technique was compared with the refluxing method and the same amount of fat (c. 91% of the total) was extracted. As a final check, the peroxide values of a fairly fresh and a deteriorated whole-milk powder were estimated by the method described below to compare humidification (in darkness) in air and nitrogen, and the long and short methods of extraction of fat. The results are shown in Table 2 and they indicate that the absence of air during humidification and the shorter extraction method both tend to lower the subsequent peroxide values of the fat. Although these modifications had lessened

oxidation of the fat during the procedure, there was no way of knowing whether some oxidation had in fact taken place, but if it had, the relative nature of the peroxide values obtained would not be significantly affected. The method finally adopted was as follows:-

Reagents. Anhydrous acetone: distilled twice from anhydrous $CaCl_2$.

Ferrous salt solution: 1 g. of NH_4CNS was dissolved in 7.5 ml. of water in a 250 ml. volumetric flask which was then almost filled with acetone. 0.25 g. of $FeSO_4 . (NH_4)_2 SO_4 . 6H_2O$ was added, followed by acetone to the 250 ml. mark, and the flask well shaken. The mixture was allowed to remain in the dark for 30-60 min. with occasional shaking, and was finally filtered through an acetone-washed filter paper (Whatman No. 42).

Procedure. 2 g. of milk powder were weighed into a small Petri dish (7 cm. in diameter) and exposed in the dark to a humid atmosphere of nitrogen (at room temperature) for about 18 hr. The powder was then transferred to a 100 ml. volumetric flask, about 50 ml. of acetone added and the flask shaken vigorously. The acetone was brought just to the boil 2 or 3 times, the mixture shaken well, cooled, and the volume made up to 100 ml. with acetone. The solution was filtered through a Whatman No. 42 filter paper (rejecting the first few ml.) and the acetone extract kept in the dark until required.

10 ml. of the ferrous salt solution and 2 ml. of the acetone extract were mixed in a tube and warmed in a water-bath at 70-80°C. until the first evolution of gas bubbles occurred, and then for 10 min. at 50°C. A tube containing 10 ml. of reagent and 2 ml. of acetone was similarly treated to provide a 'blank'. The acetone solution was cooled, an aliquot transferred to a 1 cm. cell and its extinction measured on a Spekker absorptiometer using the blue Spekker colour filter no.6. (A calibration curve had previously been prepared by adding 2 ml. aliquots of standard ferric chloride solutions to a series of tubes containing 10 ml. of the reagent.) By reference to this curve, the weight of ferric iron per ml. of the solution could be found.

The peroxide value in milligram-equivalents per kg. of powder was calculated thus:- Suppose that the acetone solution (12 ml.) contained A μg. (minus 'blank') of ferric iron per ml. The whole volume of 12 ml. which was equivalent to 2 ml. of the original acetone extract would contain 12 A μg. Fe^{+++} and therefore 100 ml. would contain 12A x 50 μg. Fe^{+++}. But 100 ml. of acetone extract (neglecting the small volume of the undissolved portion of the powder) contained the fat from 2 g. of powder, therefore the weight of ferric iron produced by the peroxides in the fat from 1 kg. of powder would be

$$12A \times 50 \times \frac{1000}{2} \mu g.$$

When converted to milligram-equivalents of ferric iron, the value would become

$$\frac{12 \times 50 \times 1000}{55.84 \times 2} A$$ (equivalent weight of iron is 55.84)

$$= \underline{\underline{5.38A}}$$ m.equiv. of ferric iron (or of peroxide) per kg. of powder

This value was called the 'apparent' peroxide value of the powder.

The following additional points should be noted:-

(a) All glassware was rinsed with nitric acid and glass-distilled water before use. (b) The advisability of regarding the results obtained by the above method as 'apparent' was confirmed by the later work of Lea (1945), who showed that this ferrous salt method is very sensitive to the presence of atmospheric oxygen, the values being reduced to about 1/4 when oxygen is rigidly excluded. (c) When the present work was almost completed, an improved ferric thiocyanate method was described by Hills & Thiel (1946). However, these authors made no attempt to exclude atmospheric

oxygen and it seems probable that results by their method will still have to be regarded as relative rather than absolute.

(2) Copper

As liquid milk contains, on the average, only about 0.12 p.p.m. of copper (Sylvester & Lampitt, 1935), uncontaminated dried milk will contain approximately 1 p.p.m. Because of the pro-oxidant activity of copper, it was necessary to know whether the whole-milk powders used in Part II contained excessive amounts of this metal. Numerous colorimetric methods for estimating such small quantities of copper have been published (Mellan, 1941; Sandell, 1944; Allport, 1945); most of them are based on the property which copper has of forming coloured complexes with diphenyl-thiocarbazone ('dithizone')*, or sodium diethyldithio-carbamate**. Several of them were examined to find a method which combined accuracy with rapidity, for in addition to the examination of the powders in Part II, other work was in progress which necessitated a large number of copper analyses. The method of Sylvester & Lampitt (1935) is well known but although it is accurate, it was found to be long and tedious. Eden & Green (1940) developed a method for the estimation of copper in which the whole process is done in one tube thus minimizing contamination. Their procedure is to digest the material in a 8 x 1" Pyrex tube with a

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* $S = C\begin{matrix}\diagup NH.NH.C_6H_5 \\ \diagdown N = N.C_6H_5\end{matrix}$

** $(C_2H_5)_2N - CS.SNa$

mixture of sulphuric, perchloric and nitric acids, deionize the iron present with citrate or pyrophosphate in strongly alkaline solution, add the 'carbamate' reagent and extract the coloured complex with amyl alcohol. This though otherwise good method, had the disadvantage that only a small sample could be used (c. 1 g. of dry material). Moreover, the digestion of whole-milk powder would be difficult because of its high fat content. Therefore, a method was evolved in which 5 g. of powder were ashed in a muffle-furnace, the ash dissolved in hydrochloric acid and the solution transferred to a stoppered, graduated cylinder. The copper complex was then formed and extracted in the cylinder. This method was very convenient and rapid but the sample had to be limited to about 5 g. to prevent the precipitation of $Ca_3(PO_4)_2$ when the ash solution was made alkaline.

About this time, several methods were published with the special object of determining copper in milk powders (Hetrick & Tracy, 1945; Boulet & McFarlane, 1945; Menefee, 1945). Of these, that of Hetrick & Tracy was very similar to the method already devised except that the ash solution was transferred to a separating-funnel, made alkaline to a definite pH and the copper complex extracted with carbon tetrachloride. The method finally adopted incorporated the advantages of both and was as follows:-

Reagents. Water: 'glass'-distilled water was used throughout.

Hydrochloric acid (6N): Conc. HCl was diluted to approx. 6N (the constant b.p. mixture) and distilled in an 'all-glass' apparatus.

Nitric acid (16N): conc. HNO_3 was 'glass'-distilled.

Citric acid solution (15%)

Ammonia solution (conc.): 1 litre of conc. ammonia solution (sp.gr. 0.88) was boiled for 3 hr. and the NH_3 absorbed in 600 ml. of water cooled in ice. After 3 hr. practically all the NH_3 was evolved and the volume of solution in the receiving flask was about 1 litre.

Cresol red solution: 0.02 g. of cresol red was dissolved in 100 ml. of water.

Sodium diethyldithiocarbamate solution: 1 g. of the reagent (recrystallized from alcohol and ether) was dissolved in water, diluted to 1 litre and stored in a brown bottle in a refrigerator.

Amyl alcohol: 'glass'-distilled, b.p. about 130°C.

Standard copper solution: 0.1000 g. of copper turnings was dissolved in 10-15 ml. of distilled HNO_3 and the solution warmed gently to expel the fumes. When cool, the solution was diluted to 500 ml. 5 ml. of this solution was then diluted to 1 litre to give a standard solution containing 1 μg. of Cu per ml.

Procedure. 5 g. of milk powder were weighed into a silica basin (8 cm. in diameter) and charred by gently heating it over a glass-tipped Bunsen burner until smoke ceased to be evolved. The basin was then placed in a muffle-furnace (c. 550°C.) for 2 hr., allowed to cool and the residue moistened with a few drops of 1:1 HNO_3. The ash was heated to dryness, replaced in the muffle-furnace for 1 hr and then allowed to cool. 5 ml. of 6N HCl solution were added to the ash and the mixture gently heated with stirring until the ash dissolved. The milk ash solution was transferred to a 100 ml. 'stoppered' graduated cylinder and the dish rinsed with water until the

volume in the cylinder was 40 ml. 10 ml. of citric acid solution and 5 drops of cresol red indicator were added. The pH was then adjusted to 8.5-9.0 by adding conc. ammonia solution. This operation required about 0.5 ml. of NH_4OH after the solution had reached the violet colour of cresol red (in all about 3.5 ml.). The volume was then made up to 55 ml. with water and 10 ml. of carbamate reagent added. 5 min. were allowed for the formation of the coloured complex and 15 ml. of amyl alcohol added. The cylinder was shaken vigorously for 2 min. and left in the dark for 30 min. to allow the amyl alcohol to separate. The supernatant amyl alcohol layer containing the coloured copper complex was pipetted off and filtered through a 12.5 cm. Whatman No.41 filter paper to remove the last traces of water by absorption on the paper. The first few ml. were rejected and a Spekker cell (1 cm.) filled with the remainder. The amount of copper present was then determined with the aid of the Spekker absorptiometer using the Spekker no.7 violet filter. A 'blank' analysis was also done to compensate for any copper in the reagents but this was usually very small. By reference to a calibration curve, the weight of copper in the solution (and hence in 5 g. of powder) was found and the result expressed as mg. of copper per kg. of powder, i.e. p.p.m.

(3) Sulphydryl compounds

The so-called sulphydryl or nitroprusside test can be used in certain circumstances to detect any type of denaturation of proteins which gives rise to the formation or unmasking of sulphydryl groups. The test is based on the reaction of sodium nitroprusside [$Na_2Fe(CN)_5.NO.2H_2O$] with R-SH compounds (or soluble sulphides) to give a pink colour. Jackson (1936) applied the test to fresh milk and found no free sulphydryl compounds present. But Josephson & Doan (1939) and Gould & Sommer (1939) found that when milk had been heated to a sufficiently high temperature, a positive sulphydryl test was obtained. Since in manufacturing

spray-dried whole-milk powder nowadays the liquid milk is usually pre-heated to 190°F., the dried product also normally shows the presence of sulphydryl compounds, and because of the possible effect of such compounds on the storage life of the powders, an indication of the amount present was necessary.

Diemair, Strohecker & Keller (1939), Townley & Gould (1943) and Lea (1946a) have used a somewhat elaborate method of estimating heat-labile sulphur in milk and milk powder. In this method, the volatile sulphur is carried into a zinc acetate solution by a stream of nitrogen and the sulphur finally measured colorimetrically as methylene blue. In the present work, however, the simple qualitative test of Josephson & Doan (1939) was sufficient to indicate whether detectable amounts of sulphydryl compounds were present.

Procedure. 1 g. of powder was weighed into a test-tube, 5 ml. of water added and the mixture thoroughly shaken for 1 min. to dissolve the powder. 5 g. of $(NH_4)_2SO_4$ were added and the tube shaken again. After being cooled in ice-water, the solution was shaken with a few drops of conc.ammonia solution (sp.gr.0.88). Finally, 5 drops of freshly made 5% sodium nitroprusside solution were added. The formation of a pink colour indicated the presence of free -SH groups, and in the absence of these groups, only a pale brown colour resulted. The ice-water delayed the fading of the colour for about 10 min. and the colour intensity could be measured in a Lovibond tintometer if necessary.

(4) Ethyl gallate

Small quantities of ethyl gallate* were present in some of the milk powders used in Part II, and it was necessary to have a method of estimating this compound. Two colorimetric methods of estimating ethyl gallate have been described by Mitchell (1923, 1924). In the first, a solution containing ferrous sulphate and sodium potassium tartrate, and in the second, osmic acid, was used to produce a coloured compound or complex with ethyl gallate. The former reagent was found satisfactory and the following modified method used:-

Reagents. Ferrous tartrate solution: 0.1 g. of $FeSO_4$ and 0.5 g. of sodium potassium tartrate ($4H_2O$) were dissolved in water in a volumetric flask and the volume made up to 100 ml.

Sodium bicarbonate solution (10%)

Trichloracetic acid solution (10%)

Procedure. Approximately 2 g. of milk powder were weighed accurately into a 100 ml. volumetric flask and shaken well with 50 ml. of water at 50°C. The mixture was left at room temperature for 30 min. with occasional shaking to assist complete extraction of the ethyl gallate.

Then 10 ml. of trichloracetic acid solution were added, the volume made up to 100 ml. with water, and the flask shaken vigorously. The flask was allowed to stand for a further 30 min. with occasional shaking and was then tiltered through a Whatman No.42 paper to give a clear filtrate. A 75 ml. aliquot was transferred to a 100 ml. volumetric flask

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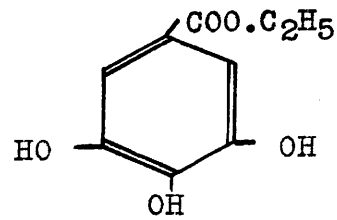
* 

and to it were added 5 ml. of sodium bi-carbonate solution. The flask was shaken gently and placed under vacuum for 10 min. to remove bubbles of CO_2. Then 5 ml. of ferrous reagent were added and the volume made up to 100 ml. After remaining for 1 hr. in the dark, the amount of ethyl gallate in the solution was determined with a Spekker absorptiometer using the green colour filter no.5.

To obtain a calibration curve, a filtrate was prepared as above from 10 g. of a similar powder containing no ethyl gallate. Different amounts of ethyl gallate were added to 75 ml. aliquots of this filtrate and the colour developed by addition of the $NaHCO_3$ solution and the ferrous reagent.

The following points should be noted:- (a) The violet colour formed in the presence of ethyl gallate developed only in alkaline solution and was more intense with increasing alkalinity. (b) If the bubbles of CO_2 were not removed by reducing the air-pressure, they tended to adhere to the sides of the Spekker cell and caused erroneous results. (c) Since the colour tended to deepen on standing, its density had to be measured after a definite time, preferably within 1 hr of adding the ferrous reagent. (d) Mattil & Filer (1944) have used a modification of Mitchell's ferrous tartrate method to determine gallic acid when used in fats and oils as an antioxidant. Their method is similar to that already described, the only difference being that the solution is buffered to pH 7 by addition of ammonium acetate solution.

(5) Readily extractable or 'free' fat

By the 'free' fat of a milk powder is meant the fat which can be extracted directly by solvents,

usually light petroleum or ether. It has already been pointed out (p.27) that the fat of roller-dried whole-milk powders is almost completely extractable in this way but that in spray-dried powders, only a fraction is 'free' unless the moisture content of the powder is first raised to about 10%. The 'free' fat was measured thus:-

Procedure. Approximately 1 g. of the milk powder was weighed into a Soxhlet extraction thimble. The powder was extracted with about 100 ml. of diethyl ether in the usual Soxhlet apparatus for 3 hr., the solvent evaporated and the residual fat weighed.

Special methods and analyses used in Part III
(spray-dried separated-milk powders)

(1) Colour

To measure changes in colour of stored powders, a small sample was first ground in a mortar. The powder was packed in a standardized manner into small white porcelain dishes and examined in a Lovibond tintometer under artificial light. Colour quality was matched by the standard yellow and red glass slides, while the relative brightness of the sample and of the comparison field could be equalized by use of the neutral tint slides.

(2) pH

Procedure. The powder was reconstituted in water at 20°C. to give a 9% solution of milk solids and the hydrogen ion concentration measured by glass electrode and a Cambridge pH meter.

(3) α- and β- lactose

Methods of detecting and estimating α- and β- lactose have been investigated by Hudson & Brown

(1908), Troy & Sharp (1930) and Sharp (1938). More recently Sharp & Doob (1941) have described a method for determining the relative amounts of α- and β-lactose in dried milk. The basis of their method is to obtain quickly a clarified solution of the milk powder, polarize the solution immediately and repolarize after the two forms of lactose have reached equilibrium. The relative amounts of α- and β- lactose can be calculated from the changes in rotation, and the total amount of lactose can be found from the final rotation. This method proved satisfactory and has been used in the present work.

Reagents. Oxalic acid solution: 0.9 g. of oxalic acid dihydrate was dissolved in water and the volume made up to 1 litre.

Alcoholic mercuric chloride solution: 264 g. of $HgCl_2$ were dissolved in 1 litre of 95% (v/v) ethanol and the solution filtered after standing overnight.

Procedure. 2.5 g. of milk powder were transferred to a dry porcelain mortar. (The sample should not contain less than 1 g. nor more than 2.5 g. of lactose.) The sample was ground for 1 min. in the mortar and 10 ml. of oxalic acid solution (at 17°C.) added to the powder. A stop-watch was then started and the powder gently ground at first to form a smooth thin paste which was then ground vigorously. More oxalic acid solution was added and the grinding continued until about 30 ml. had been added. The mixture was poured into a 100 ml. volumetric flask and the mortar rinsed with 3-4 ml. portions of the solvent until a total amount of 45 ml. at 17°C. had been used. The rinsing was completed with 15-25 ml. of water at 25°C. from a wash-bottle. The entire sample, with the lactose completely extracted and dissolved, had now been transferred to the volumetric flask. The mixture was swirled, care being taken to prevent foaming. 10 ml. of alcoholic $HgCl_2$ solution were added to the flask and the contents swirled for 30-60

sec. to mix thoroughly. The mixture was made up to the 100 ml. mark with water at 25°C. 2-3 min. should have elapsed by the time this stage was reached. The flask was now shaken vigorously for 30 sec. and the contents filtered through a Whatman No.42 filter paper into a polarimeter tube immersed in water at 25°C. The remainder of the filtrate was kept in a stoppered Erlenmeyer flask. Immediately the polarimeter tube was filled, a reading was taken at minute intervals until a total of 11 were made. Usually, about 10 min. had elapsed before the first reading was taken.

The 11 polarimeter readings were plotted against the time as indicated by the watch. The 'best' line was drawn through the points and extrapolated to zero time, i.e. when the water was added and the watch started. The extrapolated reading for zero time was taken as the initial rotation of the solution. The final rotation was obtained after the solution had stood for at least 8 hr. The most convenient method was to add a drop of toluene and allow the solution to stand overnight at room temperature. Ten readings of the final rotation were made and the average used in the equations below.

Calculation. The relative amounts of anhydrous α-lactose or of β-lactose were obtained by substituting the initial (I) and the final (F) rotations in one of the two equations whose derivation is described in detail by Sharp & Doob:

$$\% \text{ anhydrous } \alpha\text{-lactose} = \left[\frac{I}{F} - 0.635\right] 101.1$$

$$\% \text{ anhydrous } \beta\text{-lactose} = \left[1.624 - \frac{I}{F}\right] 101.1$$

If only the relative amounts of α- and β-lactose were required, it was not necessary to weigh exactly the sample taken. However, by taking an accurately weighed sample of 2.5 g., the total amount of lactose (calculated as anhydrous) could be found by multiplying the final rotation (F) by 36.3 (if a 2 dm. tube were used).

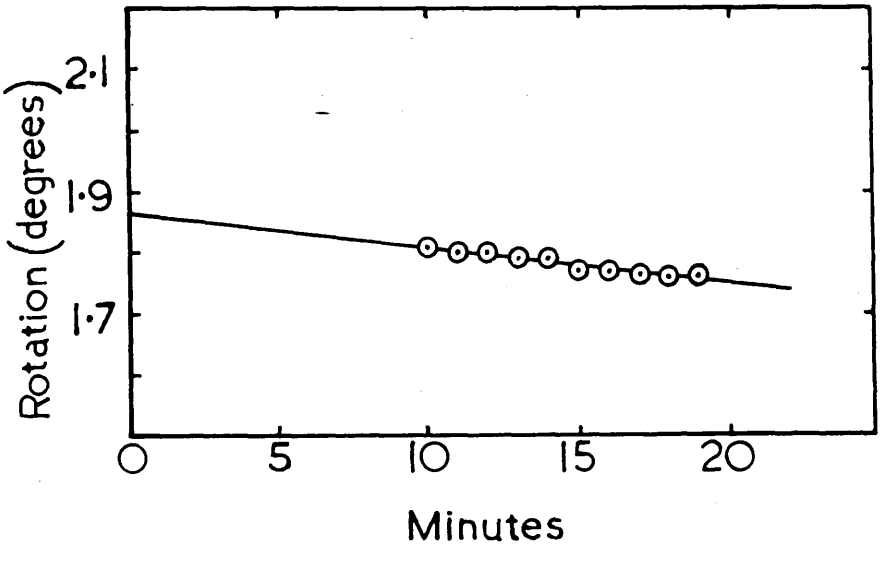


Fig. 1. Typical set of polarimeter readings with extrapolation to zero time for the measurement of the relative amounts of α- and β- lactose in separated-milk powder.

The following points should be noted:-
(a) Sharp & Doob state that the first polarimeter reading is usually obtained within 5 min. after the starting of the watch. The time taken depends largely on the rate of filtration of the solution and in these estimations about 10 min. were required before a reading could be made. A typical set of readings with extrapolation to zero time is shown in Fig.1. (b) The amount of solutions and temperatures used were worked out by the originators of the method so that the final mixture would have a temperature of 25°C. and all polarimeter readings were made at this temperature. A water-jacketed 4 dm. polarimeter tube was recommended but as none was available, an ordinary 2 dm. tube was used. The tube was immersed in a beaker of water at 25°C. before and during filling with a filtrate, and by taking the readings as quickly as possible, any decrease in temperature of the polarized solution was reduced to a minimum. (c) The use of 'Norrit' (a type of charcoal) was advocated by Sharp & Doob as a decolorizing agent when examining brown deteriorated samples, especially of dried whey, but this was not found necessary with milk powders.

(4) The nitrogen partition in the powder

For the work in Part III, it was necessary to have a method of measuring the amounts of the various proteins and other nitrogenous constituents in separated-milk powders, i.e. casein, lactalbumin, lactoglobulin, proteose-peptones and non-protein nitrogen. Methods have been devised for such an

analysis of liquid milk and they should be applicable to reconstituted dried milk. The analytical procedure can be divided into two parts: (a) the separation of the various nitrogenous fractions, and (b) their estimation. They will be discussed in that order.

(a) Fractionation of protein

One of the first thorough investigations into the conditions necessary for the separation of the various nitrogenous constituents of milk was that of Moir (1931). Subsequently, a very detailed study of the same subject was made by Rowland (1933, 1937, 1938) which culminated in the publication of a method for the determination of the nitrogen partition in milk, and of the results obtained when the method was applied to normal and abnormal milks. Rowland's fractionation procedure has been used in the present work. The separation procedure is outlined below:-

Reagents. Acetic acid solution (10%)

Sodium acetate solution (N)

Trichloracetic acid solution (15%)

Bromthymol blue indicator solution: solution as supplied by British Drug Houses, Ltd.

Sodium hydroxide solution (approx. 0.1N)

Magnesium sulphate crystals (powdered): $MgSO_4.7H_2O$ was ground in a mortar.

Magnesium sulphate solution (saturated): 400 g. of $MgSO_4.7H_2O$ were added to 500 ml. of water.

Procedure. A minimum of 35 ml. of reconstituted milk powder prepared as described on p.118 & p.121 was required for an analysis.

(1) Total nitrogen. 5 ml. of the reconstituted milk were pipetted into a weighed 100 ml. volumetric flask, the flask reweighed and the milk diluted with water to the mark. The diluted solution was used for the estimation of total nitrogen.

(2) Non-casein nitrogen. 10 ml. of the 'milk' were pipetted and weighed into a 100 ml. volumetric flask. 70-80 ml. of water at 40°C. and 1.0 ml. of 10% acetic acid solution were added and the flask swirled to mix the contents. After 10 min., 1.0 ml. of N sodium acetate solution was added and the contents mixed again. When the liquid had cooled to room temperature, water was added up to 100 ml., the flask inverted several times to ensure mixing and allowed to stand until the precipitate had settled. The solution was filtered through a dry pleated No.40, 11 cm. Whatman paper into a dry flask. This filtrate contained all the nitrogenous constituents of the original milk, except the casein, i.e. it contained lactalbumin, lactoglobulin, proteose-peptones and non-protein nitrogen.

(3) Non-protein nitrogen. 10 ml. of the 'milk' were pipetted and weighed into a 50 ml. volumetric flask. The 'milk' was diluted to the mark with 15% trichloracetic acid solution and the flask immediately shaken. When the precipitate had settled, the contents of the flask were filtered through a dry pleated No.40, 9 cm. Whatman paper into a dry flask. The final concentration of 12% trichloracetic acid precipitated casein, lactalbumin, lactoglobulin and proteose-peptones so that the filtrate contained only non-protein nitrogen which consists of traces of amino-acids, urea, creatine, creatinine, uric acid and ammonia.

(4) Proteose-peptone plus non-protein nitrogen. 10 ml. of the 'milk' were pipetted and weighed into a 100 ml. volumetric flask. The flask was immersed in water at 95°C. for about 15 min. and allowed to cool. From this point, the procedure was exactly the same as in (2) above. The lactalbumin and lactoglobulin which had been denatured by the heat treatment were thus precipitated with the casein. When the precipitate had settled, the contents of the flask were filtered through a dry pleated No.40, 11 cm. Whatman paper and the filtrate collected in a dry flask. The filtrate contained

proteose-peptone plus non-protein nitrogen.

(5) Lactoglobulin nitrogen. 20 ml. of the casein-free filtrate from (2) were pipetted into a 50 ml. beaker and a few drops of bromthymol blue solution added as an indicator. Then 0.1N sodium hydroxide solution was added until the colour denoted a pH within the range 6.8-7.2. For reconstituted fresh powders (and normal liquid milk), 2.0-2.5 ml. were required. Powdered magnesium sulphate was then added (9 g. per 10 ml. of solution), and the liquid gently heated to 25-30°C. with stirring to assist solution. The beaker was left for several hours (usually overnight) and its contents filtered through a finely pleated No.40, 5 cm. Whatman paper. The precipitate was transferred completely to the paper and washed with sat.magnesium sulphate solution.

(b) The estimation of nitrogen in the various filtrates and the precipitated lactoglobulin

To estimate the nitrogen in aliquots of the filtrates by a macro-Kjeldahl procedure would have been impracticable because of the small amount of nitrogen in some of the fractions and also the time required. The choice lay between a semi-micro or a micro-Kjeldahl procedure. Semi-micro methods have been used by Rowland (1938) and Menefee & Overman (1940) for similar work. In these procedures, the maximum amount of nitrogen estimated is not more than about 5 mg., steam distillation being used to distil the ammonia into the standard acid. Because of the number of analyses which had to be done, it was felt that a rapid micro-procedure would be more convenient although it would be advisable to use a semi-micro method to estimate the lactoglobulin nitrogen as a filter paper had to be digested. For micro-work, the sample for analysis should contain not more than about 1 mg. of nitrogen.

The method used was based on those of Ma & Zuazaga (1942) and Miller & Houghton (1945). A very similar method to that finally adopted has been used successfully by Klein (1947).

Special apparatus

A Parnas & Wagner micro-Kjeldahl distillation apparatus (with automatic discharge arrangement) (Parnas, 1938): The vacuum-jacketed distillation flask was slightly larger than usual so permitting the use of ample water for rinsing the digest into the flask and lessening the risk of frothing-over.

A micro-Kjeldahl digestion stand and digestion flasks (length 18 cm., total capacity 70 ml., bulb capacity 27 ml.).

Reagents. The catalyst mixture was the same as that used for the macro-Kjeldahl procedure (p. 14).

Boric acid solution (2% w/v): 20 g. of H_3BO_3 were dissolved in 1 litre of boiling water. The solution was cooled and stored in a Pyrex bottle as recommended by Eisner & Wagner (1934). 20 ml. of the mixed indicator described below were added per 1 litre of the boric acid solution.

Mixed indicator solution: 0.1% solutions of bromcresol green and methyl red were prepared in 95% (v/v). The solutions were mixed in the ratio of 5 pt. bromcresol green to 1 pt methyl red.

Hydrochloric acid (0.01N)

Sodium hydroxide solution (30%)

Procedure. (a) Digestion of sample. 3-10 ml. of the filtrate (containing not more than 1 mg. of N) were pipetted into a micro-Kjeldahl flask containing a small glass bead and about 0.9 g. of catalyst mixture. 2 ml. of conc. H_2SO_4 were added, the mixture digested on a small electric heater until 'clear' and the boiling continued for 15 min. After cooling, the sides of the flask were washed with about 5 ml. of water and the solution boiled until all the water was evaporated. About 10 ml. of water were added, a little vaseline smeared on the lip

of the flask (to prevent dribbling) and the diluted digest transferred to the distillation apparatus, the flask being rinsed 3 times using about 20 ml. of water in all. Finally 16 ml. of 30% sodium hydroxide solution were added and rinsed in with a little water.

(b) Distillation. The steam distillation was begun with the tip of the condenser immersed in 5 ml. of boric acid solution contained in a 100 ml. Erlenmeyer flask. When the first drop of distillate entered the boric acid solution, the bluish-purple colour changed to bluish-green. The distillation was continued for 3 min. and then the receiving flask was lowered until the tip of the condenser tube was 1 cm. above the level of the boric acid solution. The end of the condenser tube was washed and the distillation continued for 1 min. The distillate was then titrated with 0.01N hydrochloric acid until a faint pink colour was obtained. A 'blank' estimation was also done and titrated to the same end-point.

(c) Lactoglobulin nitrogen. The technique adopted by Rowland (1938) was used. The precipitate (and paper) was transferred to a 200 ml. Kjeldahl flask containing 2 glass beads and 2 g. of catalyst mixture. 5 ml. of conc. H_2SO_4 were added and the mixture digested on an electric heater until 'clear' and then for a further 20-30 min. When cool, 40 ml. of water and 25 ml. of 30% NaOH solution were added and the mixture steam-distilled directly from the flask. The ammonia was collected in 10 ml. of boric acid solution, the distillation being continued for 15-20 min. when about 150 ml. of distillate had come over. The ammonia was titrated with 0.01N hydrochloric acid.

Calculation. The weight of nitrogen in the aliquots of the filtrates (and lactoglobulin) was expressed as percentages of the original 'milk' and so the following nitrogen percentages were obtained:-

(1) Total N, i.e. casein + lactalbumin + lactoglobulin + proteose-peptone + non-protein nitrogen.

(2) Non-casein N, i.e. lactalbumin + lactoglobulin + proteose-peptone + non-protein nitrogen.

(3) Non-protein N.

(4) Proteose-peptone + non-protein N.

(5) Lactoglobulin N.

The total, lactoglobulin and non-protein N were thus determined directly and the casein, lactalbumin and proteose-peptone N obtained by difference: casein N = (1) minus (2), lactalbumin + lactoglobulin N = (2) minus (4), lactalbumin N = (2) minus [(4) + (5)] and proteose-peptone N = (4) minus (3). The method of expressing the results and how the method was applied to fresh and deteriorated powders will be described later (Part III, p. 114).

Special methods and analyses used in Part IV

The methods used to estimate water, protein, fat and ash in the various fractions of the centrifuged, reconstituted milk powders were the same in principle as those described in Section A, p. 13. For the determination of calcium in the ash solutions a semi-micro method was used involving the usual procedure of precipitation as oxalate followed by titration with permanganate (Peters & Van Slyke, 1932); the colorimetric method of Fiske & Subbarow (1925) was used to measure phosphorus.

PART II

The Effect of a High Pre-Heating Temperature with and without Ethyl Gallate on the Storage Life of Whole-Milk Powder Spray-Dried on a Gray-Jensen Drier

Introduction

The important general aspects of the production and methods of lengthening the storage life of spray-dried whole-milk powder have already been mentioned (p. 4). The work now to be described is concerned with two of these methods of improving keeping quality, namely, the use of a high pre-heating temperature and the addition of antioxidants.

The use of a high pre-heating temperature

One of the earliest investigations of the effect of different heat treatments of liquid milk on the keeping quality of the powder was that of Holm, Greenbank & Deysher (1926). These authors pasteurized or pre-heated milk for 30 min. at 145°F., 163°F., 181°F. and at 200°F. and found that with one exception, the higher the pre-heating temperature, the longer the powders (presumably spray-dried) kept. The exception was the 200°F. powder; its keeping quality lay between that of the 163° and 181°F. powders. The conclusion was that the use of a pre-heating temperature of about 181°F. for 30 min. would be an improvement over the existing normal practice of heating at 145°F. for 30 min. but that no advantage was to be gained by increasing the temperature further. Holm et al. also claimed that

the removal of cellular material by using a centrifugal clarifier, and the use of fresh rather than staler milk, improved the keeping quality of the powder.

In the next fifteen years or so, very little work was published on this method of increasing the storage life of milk powders, but indirect evidence accumulated which showed the beneficial effect of high pasteurizing temperatures on the keeping quality of milk and various milk products. For example, Kende (1932) showed that the oxidative or oily flavours which developed in some milks, especially after storage at low temperatures, could be prevented by heating the milk at 185°F. for 5 min. and also that reducing substances were found in milk so heated. Other investigators obtained similar results. Dahle, Lawthorn & Barnhart (1940) showed that creams of superior keeping quality were obtained by high temperature flash-pasteurization (170-190°F.) and that this treatment was much more efficacious than heating at 150°F. for as long as 30 min. Trout (1942) recommended that cream intended to be stored frozen, should be pasteurized at not less than 165°F. for 15 min. and preferably at 185°F. for 5 min. Cooked flavours of variable intensity were produced in the cream by this treatment but these were not objectionable. To produce butter which would be resistant to oxidation during storage, Scheib, Stark & Guthrie (1942) found that pasteurization of the cream at 165°F. for 30 min.

was necessary and that 15 min. was insufficient. Without exception, the results of these investigators show that with a variety of milk products, by increasing the temperature and duration of pasteurization, a product of enhanced keeping quality is obtained.

The beginning of the recent war gave an impetus to research on the application of these earlier findings to the production of dried milk. Hollender & Tracy (1942) showed that milk powder prepared on a small laboratory vacuum roller-drier kept better when the liquid milk was heated at 170°F. for 30 min. than when temperatures of 150 or 190°F. were used. Jack & Henderson (1942), using an atmospheric roller-drier, found that powder made from milk pre-heated at 142°F. for 30 min. kept for only 5 months but that by pre-heating at 175°F. for 15 min., the resulting powder kept for over 2 years. These authors also said that a spray-dried powder which had been pre-heated at 220°F. for 10 sec. kept for over 2 years but little data were supplied in support of the claim. As with cream, the high pre-heating temperatures produced a cooked flavour in the powder but this was never considered objectionable.

The war also stimulated research on the manufacture of butter, butter-oil and dry butter-fat, suitable for long storage at high temperatures. With these products, it was again found that high temperature treatment during their manufacture gave increased

keeping quality (Josephson, 1943; Ewbank & Gould, 1943; El-Rafey, Richardson & Henderson, 1944; Pont, 1945). The effect can probably be attributed to the formation of traces of decomposition products with antioxidant properties from the small amount of protein present in the butter.

It will be noticed that most of the work described so far has been American and this was reflected in the fact that at this time (1942), in America , milk powders were in commercial production for which a very long storage life in air was claimed. Thereupon, the Agricultural Research Council and the Food Investigation Organisation of the Department of Scientific and Industrial Research sponsored the first thorough British investigation into the effect of temperature of pre-heating, of clarification and of bacteriological quality of the raw milk on the keeping properties of spray-dried whole-milk powder. This experiment (Mattick et al., 1945) showed that for powder manufactured by the Kestner spray-process, increasing the pre-heating temperature from 165 to 190°F. (in both instances for 20 sec. plus 3-5 min. at a slightly lower temperature) greatly retarded the development of tallowy 'off'-flavours and that a powder with a storage life of about 2 years could thus be obtained. Removal of cellular debris by clarification did not affect the keeping properties of the powders, and improvement of the bacteriological quality of the

milk, while producing a definite improvement in the keeping quality of the low temperature powder, had little effect when the high pre-heating temperature was used. It was suggested that the protective effect of the high pre-heating temperature was due to the antioxidant properties of the sulphydryl compounds produced by the action of heat on the proteins and possibly also to the more complete destruction of oxidizing enzymes in the milk.

The beneficial effect of the higher pre-heating temperature was so great that experiments were begun to see whether similar results could be obtained using other types of spray-driers and also to find the temperature which would give maximum storage life with a minimum of 'cooked' flavour. These later experiments involved the use of a Krause plant (Findlay et al.,1946). The effect on keeping quality of using the following pre-heating temperatures was investigated: 160, 170, 180, 190 and 200°F. The powder pre-heated at 180°F. had the best flavour and kept in good condition about 3 times as long as the 160 and 170°F. powders. The 190 and 200°F. powders had a definite but pleasant 'cooked' or 'boiled' flavour and both kept about 5 times as long as the 160 and 170°F. powders.

There is no doubt therefore that a pre-heating temperature of 190°F. greatly extends the storage life of dried milks made by the Kestner and Krause methods in which the milk is condensed in vacuo and converted

to spray by centrifugal force. In Scotland at the present time, all spray-dried milk is made by the Gray-Jensen process in which the milk is first concentrated by allowing it to shower through the hot exhaust air from the drying chamber and then sprayed or atomized by forcing it through a fine orifice under high pressure. It was important to determine, therefore, whether in this process also, a high pre-heating temperature would improve the keeping quality of the powder; this was investigated in the present work.

The use of antioxidants

Antioxidants are substances which have the property of retarding or inhibiting the oxidation of fats. Generally, only a very minute amount of the antioxidant is necessary. Its effect is usually to delay oxidation by lengthening the induction or lag period during which the fat is oxidized very slowly. The mechanism of this phenomenon is not yet understood although the published work on antioxidants is voluminous. This work has been reviewed critically by Lea (1938) and a short discussion of more recent investigations has been published by Longenecker & Daubert (1945). In the present instance, it will suffice to mention briefly the main types or classes of antioxidants available and some of their recent applications to the preservation of edible fats, especially milk fat.

(a) Natural antioxidants. Empirical use of anti-

oxidants has been made ever since 1843 when Deschamp discovered that gum benzoin improved the keeping quality of lard. However, it was not until the 1920's that thorough scientific investigation began. These researches were initiated by the observation that naturally occurring oils and fats, such as those present in ripening seeds and nuts or in plant and animal tissues, do not become 'rancid'. But when these oils and fats were purified or refined, they became very much less resistant to oxidation. The obvious conclusion was that the process of refining had extracted or destroyed some constituent which prevented oxidation. This led to a search for the naturally occurring antioxidants. Their precise chemical nature is not yet fully known but concentrates of some of these substances have been prepared from the unsaponifiable fractions of several vegetable oils. These fractions are uncrystallizable oils and give tests for hydroxyl groups and double bonds (Olcott & Mattill, 1936). The active substances in some of these vegetable concentrates are now thought to be tocopherols* (Olcott & Emmerson, 1937).

Since these discoveries, vegetable oil concentrates have found many applications in the

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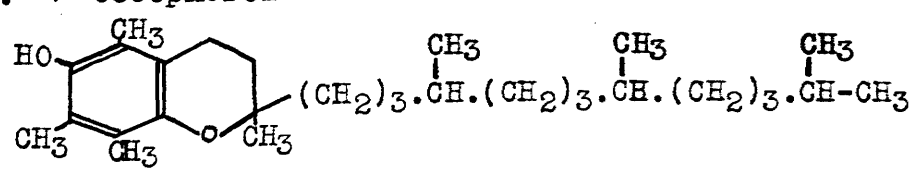
* e.g. α-tocopherol



stabilizing of edible fats, e.g., the addition of 5-10% of sesame-oil to lard and other fats (Grettie, 1933), and of crude cottonseed-oil to refined cottonseed-oil or lard (Grettie & Newton, 1934). Another method has been to treat the fatty foodstuff with a finely divided vegetable material such as oat flour (Musher, 1935) either by direct incorporation or by sprinkling or dusting the flour on the surface of the fatty material. These examples, by no means the most recent, illustrate the use of these natural antioxidants.

(b) Hydroxy-aromatic derivatives. In addition to these natural antioxidants, the early work of Moureau & Dufraisse (1922) showed that many substances could act as inhibitors of the oxidation of fat but outstanding among them were substances containing phenolic groups. Since then, the antioxidant properties of many natural and synthetic compounds have been extensively examined. Several workers showed that pyrogallol, hydroquinone and catechol were among the most potent. From about 1930 onwards, Mattill and co-workers in America published many papers on the relative antioxidant activity of hydroxy-aromatic compounds. Their conclusion was that such a compound will only be an antioxidant if it contains hydroxyl groups directly substituted in an aromatic nucleus. Phenol itself is not an antioxidant but the substitution of another hydroxyl group in the ortho- or para-position (i.e. giving catechol and hydroquinone (quinol) respectively)

gives strong antioxidant properties, while the introduction of two hydroxyl groups in the 2, 3 or 2, 4 positions (i.e. giving pyrogallol and hydroxyhydroquinone respectively) still further increases the effect.

Carotene, lecithin, gum guaiac and the organic acids, maleic, malic and citric also have antioxidant properties.

New antioxidants and some recent applications to milk and milk products. Nordihydroguaiaretic acid* which was synthesized as long ago as 1918, has been shown by Lundberg, Halverson & Burr (1944) to have powerful antioxidant activity for lard, especially when used in combination with ascorbic acid. Riemenschneider, Turer, Wells & Ault (1944) have found that the fatty acid mono-esters of L-ascorbic acid and D-isoascorbic acid are also good antioxidants for lard. Riemenschneider et al. (1944) noticed that combinations of antioxidants were sometimes better than individual additions and also that when an antioxidant was added to a vegetable oil containing a natural antioxidant, the same synergistic effect was obtained. The kinetics of the antioxygenic synergism of quinones with ascorbic acid in fat systems have been investigated by Calkins & Mattill (1944). This synergistic effect has been utilized by Lips &

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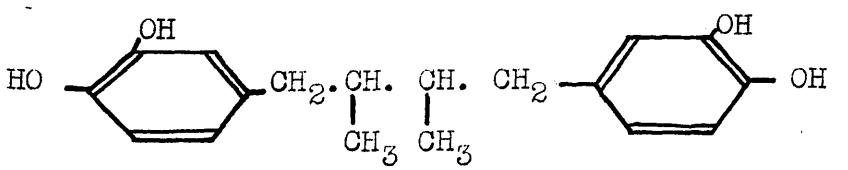
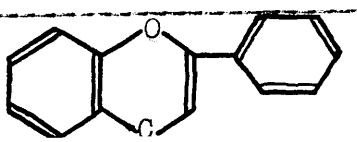
* 

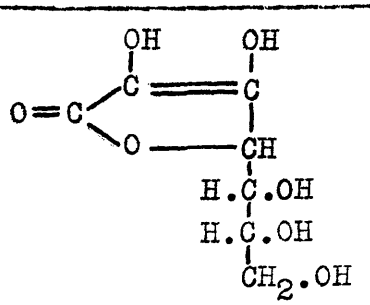
McFarlane (1943) who have developed an antioxidant for lard and shortening which consists of wheat germ oil fortified with 0.5 - 2% of citric acid. Among other compounds, Lea (1944a) has used ethyl gallate as an antioxidant for dried pork. The work of Boehm & Williams (1943) and Mattil, Filer & Longenecker (1944) with propyl gallate also demonstrated the antioxidant power of the lower esters of gallic acid. Ethyl gallate has even been used to preserve fish (Tarr, 1944). These few examples show the range of antioxidants now available.

Most of the work utilizing antioxidants for the preservation of milk and milk products has been done in America. The use of finely milled oat flour has been quoted by Mueller & Mack (1939) who have themselves tested the value of oat and other cereal flours in milk and ice-cream. Cereal antioxidants for dairy products have also been investigated by Garrett (1940), Dahle & Nelson (1941) and Corbett, Tracy & Hansen (1941). Doan & Miller (1940) have suggested that trypsin can be used as an antioxidant for milk. More recently, Richardson, El-Rafey & Long (1947) have shown that flavones* and flavone derivatives can be used as antioxidants for milk fat. In Great Britain, much work on the subject has been done by Lea, and in one of his recent investigations (1944b) many phenolic compounds were tested for their antioxidant properties

* Flavone: 

when present in butter-fat, and be concluded that although not the most powerful, ethyl gallate had much to recommend it.

From 1939 onwards, a number of experiments were done to see whether antioxidants were of use in lengthening the storage life of whole-milk powder. The method was to incorporate a very small amount of antioxidant in the liquid milk just before drying. One of the earliest investigations was that of Gray & Stone (1939) who used D-glucoascorbic acid* at concentrations of 0.03 and 0.01% in the dry milk, the effect of the latter concentration proving very good. Two years later, Waite (1941b) showed that when 0.25% of oat flour was added to liquid milk before drying, the storage life of the resulting powder was increased by about 4 months compared with a control powder. Hollender & Tracy (1942) investigated the effect of several antioxidants on the development of tallowy flavours in vacuum-roller whole-milk powders. The greatest preventive effect was exerted by gum guaiac (5 p.p.m. in liquid milk), followed by hydroquinone (10 p.p.m.), ascorbic acid (0.01%) and sodium citrate (0.20%). Many papers were published about this time



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and the subject up to 1944 has been reviewed by Coulter (1944). Since then, the use of wheat germ oil in dried milk has been studied by Tracy, Hoskisson & Trimble (1944) and by Chapman & McFarlane (1946) with some success. In Great Britain, Lea's work (1946b) with antioxidants for butter-fat led Findlay et al.(1945) to test a similar series in dried milk. This experiment showed that ascorbic acid and ethyl gallate were the most promising, and of these two substances ethyl gallate was the more powerful. At a concentration of ethyl gallate of only 0.07%, the storage life of a spray-dried whole-milk powder was increased 2½-3 fold.

Objects of the present work

This summary of past work on the use of high pre-heating temperatures and antioxidants to increase the storage life of whole-milk powder showed some gaps in the existing knowledge. First, it was not known with certainty what was the effect of a high pre-heating temperature on powder manufactured by the important Gray-Jensen process. Secondly, as work in Great Britain pointed to ethyl gallate as being one of the best antioxidants, it was desirable to test this compound on a commercial scale in greater detail than had so far been possible. It was also important to determine whether the protective effects of using a high pre-heating temperature and of adding ethyl gallate were additive. To settle these problems, it was decided that the two methods of improving keeping

quality should be examined and compared under adequately controlled conditions for the same batch of liquid milk dried on the same plant. The opportunity was also taken of comparing the storage lives of the experimental powders in lacquered as well as in plain tinplate cans since earlier experiments (Hollender & Tracy, 1942; Findlay, 1944; Findlay et al., 1946) indicated that whole-milk powders stored in the former type of can kept better than in plain cans.

The present experiments were therefore planned with the following objects in view:-

1. To find the effect of a high pre-heating temperature on the storage life of a whole-milk powder spray-dried on a Gray-Jensen drier.

2(a) To find the effect of ethyl gallate on the storage life of a spray-dried whole-milk powder pre-heated at low and high temperatures.

(b) To find if the use of a low pre-heating temperature in conjunction with ethyl gallate would give a whole-milk powder of good keeping quality but without a 'cooked' flavour.

3. To determine whether Gray-Jensen powders kept better in lacquered than in plain tinplate cans.

EXPERIMENTAL

Preparation of the powders

To satisfy the objects of the experiment, it was necessary to prepare four powders, namely, (1) a low temperature control, (2) a low temperature powder containing ethyl gallate, (3) a high temperature control, and (4) a high temperature powder containing ethyl gallate. It was decided that the low temperature pre-heating treatment would be approximately 30 min. at 160°F. and the high temperature treatment as short a time as possible at 190°F. In view of the work of Findlay et al. (1945) on the use of ethyl gallate, a concentration of 0.07% in the powders was aimed at.

Through the courtesy of Mr W.B. Barbour, the Managing Director of the Scottish Milk Powder Co.Ltd, the Company's Gray-Jensen plants at Kircudbright and Maychline were made available for the preparation of the powders used both in this experiment and also in the experiment described in Part III. Thanks are due to Mr M. Neilson and Mr R.C. Hallett, the managers of the factories, for their very helpful cooperation.

The details of the Gray-Jensen system of drying have been described by Hunziker (1946) and Scott (1932). The procedure is frequently as follows. The liquid milk is circulated from a storage tank through a tubular heater to a processing tank and then back to the storage tank until the desired pre-heating temperature is reached. The milk is maintained at

this temperature for 30 min. From the processing tank, the milk is pumped into the concentrator or 'liquid collector' via a second tubular heater. In the concentrator, the milk showers through the exhaust air (c.160°F.) from the drying chamber. The partly concentrated milk circulates through the second heater and the concentrator (along with the incoming pre-heated milk) until the whole bulk of milk has a solids content of about 30%. The second heater maintains the milk at a temperature of 165°F. while it is being condensed. From the concentrator, the condensed milk passes to a three-throw ram type pump and finally reaches the spray-nozzle at a temperature of about 130°F. and a pressure of about 3000 lb./sq.in. The milk spray meets the incoming hot air which is at a temperature of about 240°F. and the dehydrated milk falls to the bottom of the conical drying chamber as a powder and is removed by suction.

Because only a relatively small amount of milk had to be dried in the present experiment, the pre-condensing stage had to be omitted, but this was unlikely to render the general conclusions inapplicable to the normal Gray-Jensen process. The milk was dried in this way:- A total of 500 gal. of raw whole milk was run into a storage tank. From this tank, it was circulated through a tubular heater at 160°F. into another tank and thence back to the first tank. The milk was thus circulated until the whole bulk was at a temperature of 160°F. In this system of pre-

heating, it was difficult to know the exact 'time-temperature' treatment given to the milk but an endeavour was made to ensure that it was as near as possible to 30 min. at 160°F. Heating was then stopped and 150 gal. of the milk were transferred to a glass-lined tank fitted with a stirrer from which it could be pumped through the spray-nozzle into the drying chamber. A sample of the milk was taken at this stage. Then 50 gal. of the milk were dried and the powder discarded. The next 50 gal. were dried and the powder collected to serve as the low temperature control sample. To the remaining 50 gal. of milk, 25 g. of ethyl gallate dissolved in 500 ml. of hot water were added and the mixture well stirred. The third batch of 50 gal. was then dried, only the product emerging from the drier towards the middle of the brief drying period being collected as the low temperature ethyl gallate sample.

The remaining 350 gal. of milk were circulated through the heater at 190°F. until the whole volume had been maintained at that temperature for 5 min. when the temperature was allowed to fall. Five minutes is a longer period of heating than was used in experiments on the Anestner and Krause plants (Mattick et al., 1945; Findlay et al., 1946), but it was adopted in the present experiment in order to make sure, with the particular type of heater available, that all the milk had attained a temperature of 190°F. 150 gal. of the heated milk were transferred to the clean glass-lined tank and dried as before in three batches of 50 gal. each. A sample

Table 3. Composition of the low-temperature control powder

	%
Moisture	1.1
Fat	30.8
'Crude' protein (N x 6.38)	27.0
Ash	6.3
Lactose, etc., (by difference)	34.8

of the heated milk was again taken from the tank.

The four samples of dried milk were collected directly at the bottom of the drying chamber in clean bags, each thoroughly mixed in a wooden vat and immediately transferred to 21 lb. cans.

Analysis and properties of the fresh powders

The low temperature control powder was analysed by the methods already described. The results are shown in Table 3 and they are quite typical for a whole-milk powder.

Ethyl gallate content. The amount of ethyl gallate in the powders was determined by the method already described (p. 38). In the low temperature ethyl gallate powder, the ethyl gallate content was 0.08% and in the corresponding high temperature powder, 0.06%. No gallate was present in either the low or the high temperature control powders.

Moisture content. The moisture content of the powders as measured by the air-oven method are recorded in Table 4. Since none of the values exceeded 1.2%, it was to be expected that as storage of the powders progressed, the predominating type of spoilage would be oxidation of fat rather than the protein-lactose type of deterioration which is very marked in powders of high moisture content (Findlay et al., 1945; Henry, Kon, Lea, Smith & White, 1946; Henry, Kon, Lea & White, 1948), and which is referred to in detail in Part III.

Table 4. Properties of the fresh milk powders

Powder	Pre-heating temp. (°F.)	Ethyl gallate content (%)	Moisture content (air-oven) (%)	Copper content (p.p.m.)	'Free' fat (p. 39) (%)	Sulphydryl test	Solubility				Biological value* of the proteins	True digestibility* of the proteins
							Solubility index at		Sediment volume at (p. 21)			
							20°C.	50°C.	20°C.	50°C. (ml.)		
1.	160	none	1.1	1.4	14.6	Negative	89.7	97.7	4.2	0.2	89.0	91.8
2.	160	0.08	1.2	2.3	-	Negative	89.0	95.8	4.3	0.7	-	-
3.	190	none	0.8	2.1	11.9	Negative	87.7	90.0	4.5	2.3	87.7	90.3
4.	190	0.06	1.1	3.8	-	Negative	89.6	93.1	4.2	1.8	-	-

* Measured by Henry & Kon (1947); the differences are not significant.

Copper content. The copper contents of the powders are recorded in Table 4 and they show that the low temperature control powder had a minimal copper content of 1.4 p.p.m. In the low temperature ethyl gallate and the high temperature control powders, the values were but little higher and almost equal (approx. 2 p.p.m.). In the fourth powder, however, in whose preparation a high pre-heating temperature had been used and ethyl gallate added, the value was 3.8 p.p.m. Copper tends to decrease the storage life of a whole-milk powder. It follows therefore that since the low temperature control powder had the lowest copper content, the improvement in keeping quality which resulted in the present work from the addition of ethyl gallate and from the use of a high pre-heating temperature or to both, would have been still greater if the copper content of all four powders had been equal.

Readily extractable or 'free' fat. In previous experiments (Findlay, 1944; Findlay et al., 1946), the keeping quality of milk powders tended to be better in lacquered than in plain tinplate cans. It was thought that the degree to which the fat was readily extractable might have some bearing on this finding, and in the present work, values for the 'free' fat were determined by extraction with ether (Table 4). It can be seen that the powder which had the high pre-heating treatment had slightly less 'free' fat than

the low temperature powder but this small difference would be unlikely to have any detectable influence on the keeping properties of the powders.

Sulphydryl compounds. It has already been explained (p. 36) that the nitroprusside test for sulphydryl compounds can be used in certain circumstances to detect protein denaturation of different degrees caused by heat or other means. In previous work with milk powder (Mattick et al., 1945; Findlay et al., 1946), the extended storage life of high temperature powders was associated with the presence of these sulphydryl groups or compounds. In these experiments, the presence of volatile sulphur compounds was also shown by more quantitative methods (Gould & Sommer, 1939; Townley & Gould, 1943; Lea, 1946a). In the present investigation, the liquid milk pre-heated at 160°F. gave a negative sulphydryl test and the milk heated at 190°F. a positive test, as was expected, But with the high temperature powder, the test was negative, the addition of the nitroprusside producing only a slight brown coloration typical of a negative test and similar to the result with the low temperature powder. Findlay (1944) made a similar observation when he applied the nitroprusside test to Gray-Jensen powder.

It may be that under the influence of the high pressure 'atomizer', the particular sulphur compounds

which are responsible for the pink coloration are volatilized and driven off in the current of hot air during the drying process. However true this suggestion may be, the fact remains that not withstanding the absence of the characteristic pink colour in the sulphydryl test, the high temperature powders still showed the greatly improved keeping quality which can now be regarded as typical of such powders.

Solubility. The solubility of the powders was determined by the two methods already described. The resulting values recorded in Table 4 show that the powders were not so soluble as those normally obtained from a Gray-Jensen plant. This almost certainly resulted from the fact that an unusually small amount of milk was dried and that slight but unavoidable changes were made in the drying procedure.

Flavour. The flavour of all the powders when reconstituted after manufacture was satisfactory. As usual, the high pre-heating temperature had imparted a slight but not unpleasant 'cooked' flavour to the product. For this reason, there was a tendency for the low temperature powders to be preferred at the beginning of the storage period, but subsequently they deteriorated much more rapidly than the high temperature powders. The ethyl gallate was not found to cause any degree of unpalatability in the powders.

Biological value of the proteins. It has been shown that the biological value of the proteins of milk is not affected by pasteurization (Henry, Kon & Watson, 1937), by evaporation and condensation (Henry, Houston, Kon & Thompson, 1944) or by spray- or roller-drying (Henry, Houston, Kon & Osborne, 1939). On the other hand, sterilization (Henry & Kon, 1938) causes a statistically significant decrease of 6% in the biological value, no doubt due to the fact that this process involves heating at a high temperature for much longer than in any of the other processes.

Because of these findings, it was of interest to know whether the high temperature pre-heating treatment would have any effect on the biological value of the proteins of the powders. Samples of the low and high temperature control powders were sent to Drs Henry and Kon at the National Institute for Research in Dairying, Reading for examination. Their results have been published (Henry & Kon, 1947) and it was concluded that the biological value and true digestibility of the proteins of milk powder pre-heated at 190°F. are not inferior to those of powder pre-heated at the lower temperature of 160°F. (see Table 4).

Packing the powders and storage tests

The plan of the experiment was to pack the powders in air-tight cans, store them at different temperatures and examine them at intervals.

Both plain tinplate and lacquered cans of the type known as 'sanitary' or 'open-top' were used. Two types of lacquered cans were available, one coated with 'meat' lacquer (a phenol-formaldehyde thermo-setting resin) and the other with 'fruit' lacquer (a natural oleo-resin). The 'meat' lacquer type was chosen. So that one can would give sufficient powder for all the tests, a 6 oz. size was used.

The cans and lids were thoroughly wiped. 75 g. of powder were packed into each can and after removing any powder on the rims of the cans, the lids were sealed on by machine. During this process, several readings of the barometric pressure and temperature were taken. As the volume of this size of can is 186 ml., the packing density was 0.403 g. of powder per ml. of can space. Assuming an apparent density of 1.33 for the milk solids, the free oxygen content of the can was calculated (p. 24) to be 48.3 mg. of oxygen per 100 g. of powder. The seams of the cans were then given two coats of bitumen* to ensure gas-tightness, and when this had hardened, the cans were placed in water-jacketed incubators at 47 and 37°C. and some were left at room temperature (c. 17°C.). In addition to giving an early indication of the results to be expected at ordinary temperatures, the use of these high storage temperatures indicated how the powders

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* The bitumen was type L.459, supplied by British Bitumen Emulsions, Ltd.

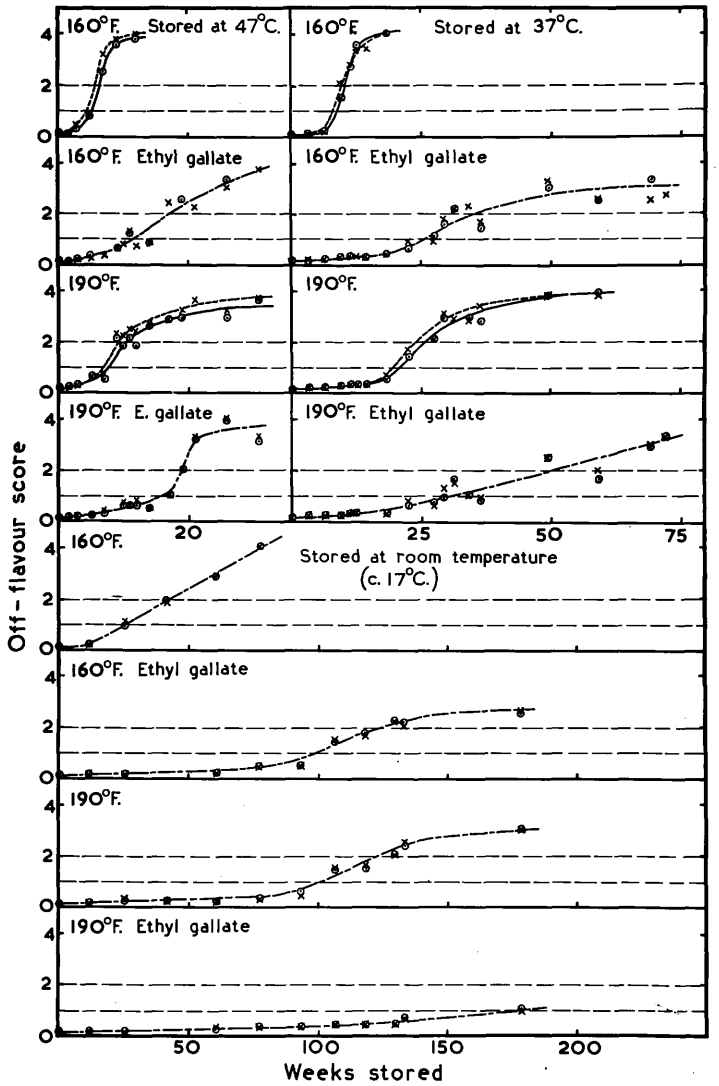

Fig. 2. Changes in the palatability of whole-milk powders prepared from milk pre-heated at 160 and 190°F., with and without ethyl gallate, during storage at 47, 37°C. and room temperature (c. 17°C.).

x - - - x = plain tinplate can.

⊙———⊙ = lacquered tinplate can.

Table 5.

Table 5. Keeping properties of the milk powders as measured by palatability

Pre-heating temp. (°F.)	Ethyl gallate (%)	Weeks to deteriorate to 'off'-flavour score 1.0						Weeks to deteriorate to 'off'-flavour score 2.0						Lacquered / plain ratio						
		Lacquered cans			Plain cans			Lacquered cans			Plain cans			At 'off'-flavour score 1.0			At 'off'-flavour score 2.0			Averages
		47°C.	37°C.	Room temp.	47°C.	37°C.	Room temp.	47°C.	37°C.	Room temp.	47°C.	37°C.	Room temp.	47°C.	37°C.	Room temp.	47°C.	37°C.	Room temp.	
160	none	5.4	8.7	28.0	5.1	7.6	26.0	6.7	10.3	45.5	6.0	9.3	45.0	1.1	1.1	1.1	1.1	1.1	1.0	1.1
160	0.08	12.6	25.7	102.5	12.1	24.6	101.0	17.6	31.1	128.0	17.6	30.6	129.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
190	none	8.4	20.7	102.5	8.0	19.7	103.0	10.6	26.9	129.5	9.3	25.0	126.0	1.1	1.1	1.0	1.1	1.1	1.0	1.1
190	0.06	17.3	32.4	c.180	17.1	30.0	c.185	19.4	40.0	-	19.3	39.4	-	1.0	1.1	1.0	1.0	1.0	-	1.0
		Relative storage life as measured by palatability												Averages						
160	none	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0				
160	0.08	2.3	3.0	3.7	2.4	3.2	3.9	2.6	3.0	2.8	2.9	3.3	2.9	2.6	3.1	3.3				
190	none	1.6	2.4	3.7	1.6	2.6	4.0	1.6	2.6	2.8	1.6	2.7	2.8	1.6	2.6	3.3				
190	0.06	3.2	3.7	c.6.4	3.3	3.9	c.7.1	2.9	3.9	-	3.2	4.2	-	3.2	3.9	6.8				

would keep in tropical climates. A number of cans, both air-packed and nitrogen-packed, were kept in a refrigerator to act as controls.

Examination of the stored powders

At intervals, the powders were examined for palatability, absorption of oxygen, production of carbon dioxide and apparent peroxide value by the methods described in Part I.

Palatability. The average of the 'off'-flavour marks awarded by the five tasters (p. 14) for each sample at each tasting was plotted against the storage time to give the curves shown in Fig. 2. When the curves so obtained passed an 'off'-flavour score of 1.0, the trained panel was beginning to detect the development of slight 'off'-flavours. When score 2.0 was reached, the 'off'-flavours were believed to be sufficiently obvious to be noticed and objected to by the ordinary consumer. However, for powders intended for human consumption it would probably be safe to regard the time taken by a powder to reach an 'off'-flavour score of 1.0 as its effective storage life. Powders with 'off'-flavour scores of up to 2.0 might be used for cooking or baking. The times required for 'off'-flavour marks of 1.0 and 2.0 to be reached and the calculated relative storage lives of the powders have been recorded in Table 5.

Absorption of oxygen and production of carbon dioxide. At intervals cans were removed from the

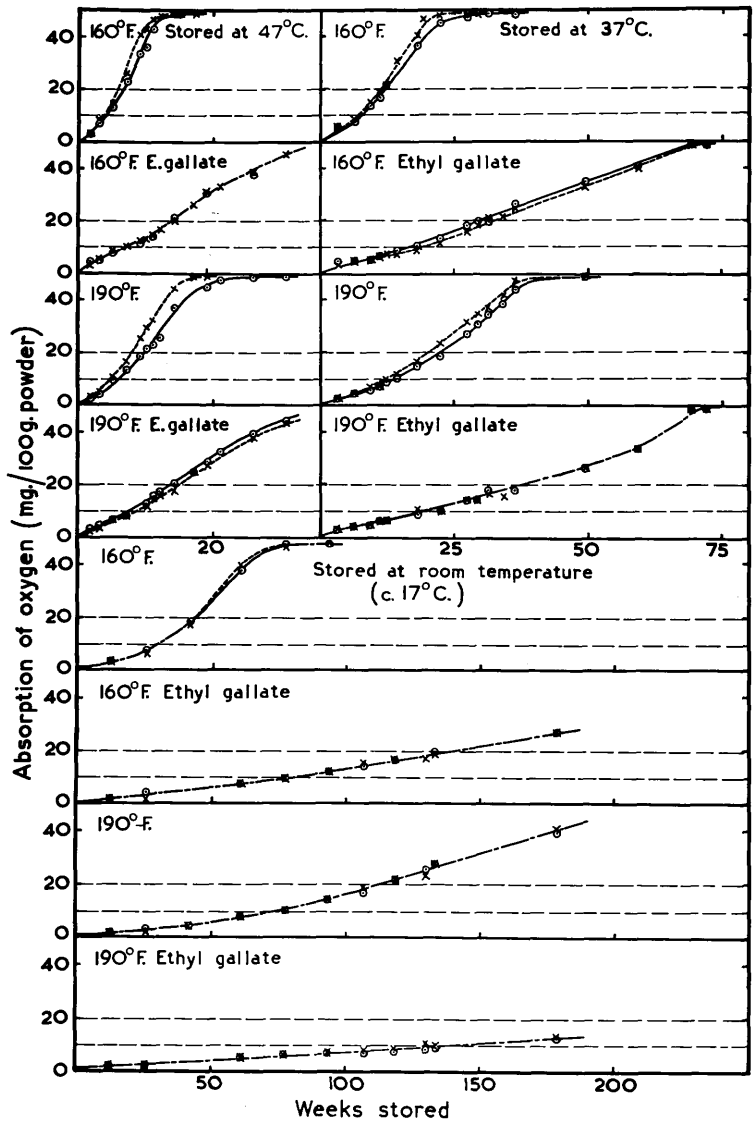

Fig. 3. Absorption of oxygen by whole-milk powders prepared from milk pre-heated at 160 and 190°F., with and without ethyl gallate, during storage at 47, 37°C. and room temperature (c. 17°C.).

x - - - x = plain tinplate can.

⊙———⊙ = lacquered tinplate can.

Table 6.

Table 6. Rates of absorption of oxygen by the milk powders

Pre-heating temp. (°F.)	Ethyl gallate (%)	Weeks to absorb 10 mg./100g. powder						Weeks to absorb 20mg./100g. powder						Lacquered / plain ratio						Averages
		Lacquered cans			Plain cans			Lacquered cans			Plain cans			At 10 mg.			At 20 mg.			
		47°C.	37°C.	Room temp.	47°C.	37°C.	Room temp.	47°C.	37°C.	Room temp.	47°C.	37°C.	Room temp.	47°C.	37°C.	Room temp.	47°C.	37°C.	Room temp.	
160	none	4.1	7.4	30.5	3.7	6.9	32.0	6.7	12.7	43.3	6.1	12.0	43.7	1.1	1.1	1.0	1.1	1.1	1.0	1.1
160	0.08	7.7	16.4	82.0	6.6	18.7	82.0	14.1	30.9	138.0	14.0	32.4	142.0	1.2	0.9	1.0	1.0	1.0	1.0	1.0
190	none	5.3	13.7	75.0	4.9	12.7	77.0	9.9	22.7	115.0	7.9	20.3	114.0	1.1	1.1	1.0	1.3	1.1	1.0	1.1
190	0.06	7.7	20.7	145.9	8.3	20.0	130.0	14.1	38.4	c.260	15.0	38.6	c.260	0.9	1.0	1.1	0.9	1.0	-	1.0
		Relative storage life as measured by oxygen absorption												Averages						
160	none	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0				
160	0.08	1.9	2.2	2.7	1.8	2.7	2.6	2.1	2.4	3.2	2.3	2.7	3.3	2.0	2.5	3.0				
190	none	1.3	1.9	2.5	1.3	1.8	2.4	1.5	1.8	2.7	1.3	1.7	2.6	1.4	1.8	2.6				
190	0.06	1.9	2.8	4.8	2.2	2.9	4.1	2.1	3.0	c.6.0	2.5	3.2	c.6.0	2.2	3.0	5.2				

incubators, allowed to cool to room temperature and the headspace gas analysed by the method described on p.23. The amount of oxygen absorbed by the various powders has been shown graphically in Fig. 3. From these curves, the times taken by the powders to absorb 10 and 20 mg. of oxygen per 100 g. of powder were found and they have been used as a basis on which to compare the rates at which the powders deteriorated. These times and relative storage lives are given in Table 6.

The production of carbon dioxide was small and rather erratic, the latter effect probably being due in part to the solubility of carbon dioxide in the fat of the powder. The results are shown in Fig. 4 as percentages of CO_2 in the headspace gas and have not been converted to absolute units.

Apparent peroxide value. The apparent peroxide values of the powders, measured by the method described on p. 31 , are shown diagramatically in Fig. 5.

RESULTS

Palatability

Fig. 2 shows clearly that at all storage temperatures, the low temperature control powder deteriorated in flavour most rapidly and that the high temperature powder plus ethyl gallate kept longest. Between these two extremes were the low temperature plus ethyl gallate and the high temperature control powders. When stored at room temperature (c. 17°C.), the 160°F. ethyl gallate and the 190°F. control powder

remained palatable for practically the same period but at the two higher storage temperatures, the 160°F. ethyl gallate powder kept slightly longer than the 190°F. control powder. The relative storage lives of the powders, calculated from the times required to deteriorate to 'off'-flavour scores 1.0 and 2.0, are given in Table 5. Raising the pre-heating temperature of the liquid milk from 160 to 190°F. or the addition of ethyl gallate to the milk before drying, extended the storage life of the control powder by a factor of the order of 1½ - 2½ at 47°C., 2½ - 3 at 37°C. and just over 3 at room temperature (c. 17°C.).

The results for the 190°F. ethyl gallate powder are incomplete, as even now, after about 3½ years storage at room temperature, the 'off'-flavour score for this powder is only about 1.0. However, it can be said that increasing the pre-heating temperature and incorporating ethyl gallate in the milk extended the storage life by factors of about 3 at 47°C., 4 at 37°C. and at least 7 at room temperature.

Absorption of oxygen and production of carbon dioxide

Oxygen absorption data (Fig. 3 and Table 6) confirmed the beneficial effects of high temperature pre-heating and of the incorporation of ethyl gallate, as well as the further increase in stability obtained when both means were employed together. Protection factors too were progressively higher as the temperature of storage decreased from 47°C., through 37° to 17°C.,

Table 7. Relation between absorption of oxygen and deterioration in palatability of the milk powders

Storage temp. (°C.)	Pre-heating temp. (°F.)	Ethyl gallate (%)	mg. oxygen absorbed /100 g. powder for deterioration to 'off'-flavour scores 1.0 and 2.0					
			Lacquered cans			Plain cans		
			1.0	2.0	Difference	1.0	2.0	Difference
47	160	none	15	20	5	16	20	4
	160	0.08	17	27	10	17	27	10
	190	none	16	22	6	21	26	5
	190	0.06	25	29	4	24	28	4
37	160	none	12	15	3	11	15	4
	160	0.08	16	20	4	14	19	5
	190	none	18	26	8	19	27	8
	190	0.06	16	21	5	15	20	5
c.17 (room temper-ature)	160	none	9	22	13	7	21	14
	160	0.08	13	18	5	13	18	5
	190	none	16	25	9	17	24	7
	190	0.06	13	-	-	14	-	-

as in the palatability tests. As can be seen by comparing Tables 5 and 6, the improvement in keeping properties when absorption of oxygen was used as a criterion was slightly smaller than when the powders were graded by tasting panel. For example, Table 6 shows that raising the pre-heating temperature from 160 to 190°F. or the addition of ethyl gallate extended storage life by a factor of $1\frac{1}{2}$ - 2 at 47°C., 2 - $2\frac{1}{2}$ at 37°C. and $2\frac{1}{2}$ - 3 at room temperature whereas by tasting tests the corresponding factors were $1\frac{1}{2}$ - $2\frac{1}{2}$, $2\frac{1}{2}$ - 3 and just over 3.

While it was obvious from a comparison of Figs. 2 and 3 that there was a general parallelism between absorption of oxygen and development of 'off'-flavour, the ratio of the quantity of oxygen absorbed to the degree of 'off'-flavour produced was not constant. The values given in Table 7 show the magnitude of the variation. It can be seen that at 47, 37 and 17°C. the least stable powder, i.e. the 160°F. powder, generally required the least oxygen for it to deteriorate to 'off'-flavour scores of 1.0 and 2.0. The converse was not always true but there was a tendency for the more stable powders to require more oxygen to deteriorate to a given 'off'-flavour score. It is interesting to note that at 47°C. the 160°F. ethyl gallate powder required about twice as much oxygen as the other powders for a further increase in 'off'-flavour score from 1.0 to 2.0, but that at 37°C. the same phenomenon was obtained with the 190°F. powder. Generally, slightly more oxygen was

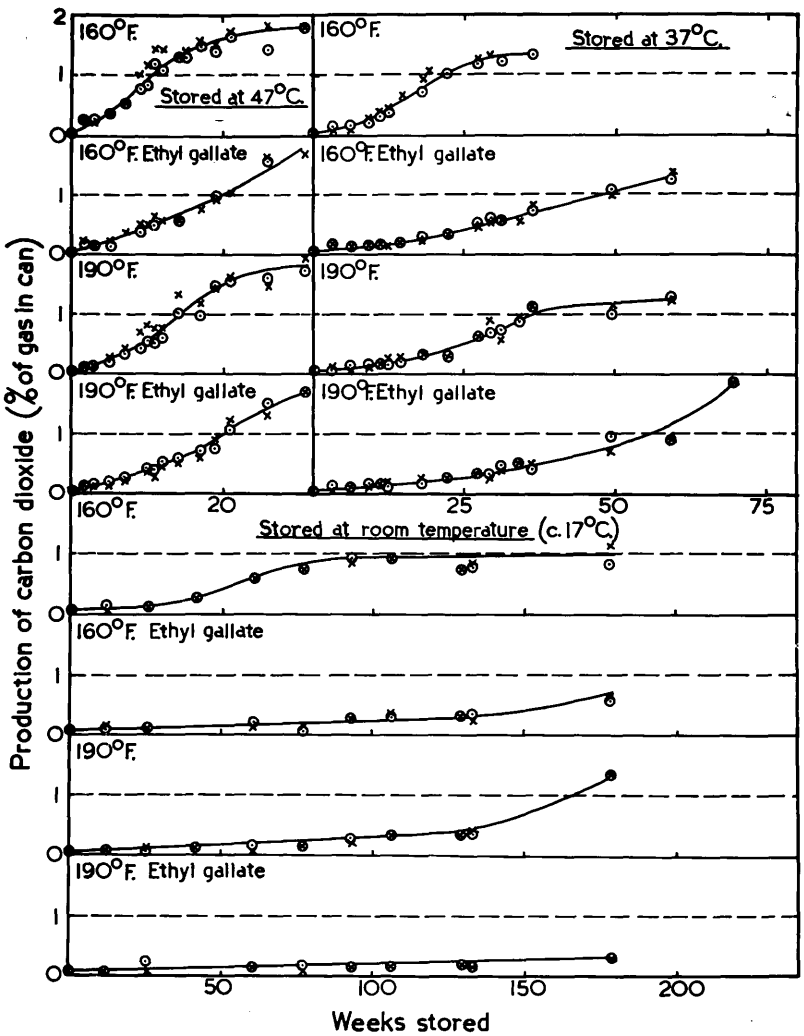

Fig. 4. Production of carbon dioxide by whole-milk powders prepared from milk pre-heated at 160 and 190°F., with and without ethyl gallate, during storage at 47, 37°C. and room temperature (c. 17°C.).

x = plain tinplate can. 0 = lacquered tinplate can.

required at 47°C than at 37°C. and considerably more at 37° than at 17°C. to deteriorate to the specified 'off'-flavour scores. To sum up, high temperature pre-heating, incorporation of ethyl gallate and high storage temperatures all tended to increase the amount of oxygen required to produce a given degree of 'off'-flavour.

As Fig.4 shows, the rate of production of carbon dioxide was an indication of the keeping quality of the powder, the powder with the shortest storage life producing carbon dioxide most rapidly and vice versa.

Apparent peroxide value

The relative rates at which peroxides developed in each of the four powders confirmed the main conclusions drawn from the palatability and oxygen absorption tests. The peroxide values given in Fig. 5 show that the fat in the control powder made from milk pre-heated at 190°F. was more resistant to oxidation than the fat of the corresponding powder for which the pre-heating temperature was 160°F. They also show that the addition of ethyl gallate increased still further the resistance of the fat to oxidation.

The curves showing the apparent peroxide values of the ethyl gallate-free powders are quite typical and similar in shape to these obtained by Lea et al. (1943). It is interesting to note the influence of the presence of free oxygen in the cans on the peroxide values of these powders. By comparing the oxygen absorption curves and the peroxide values,

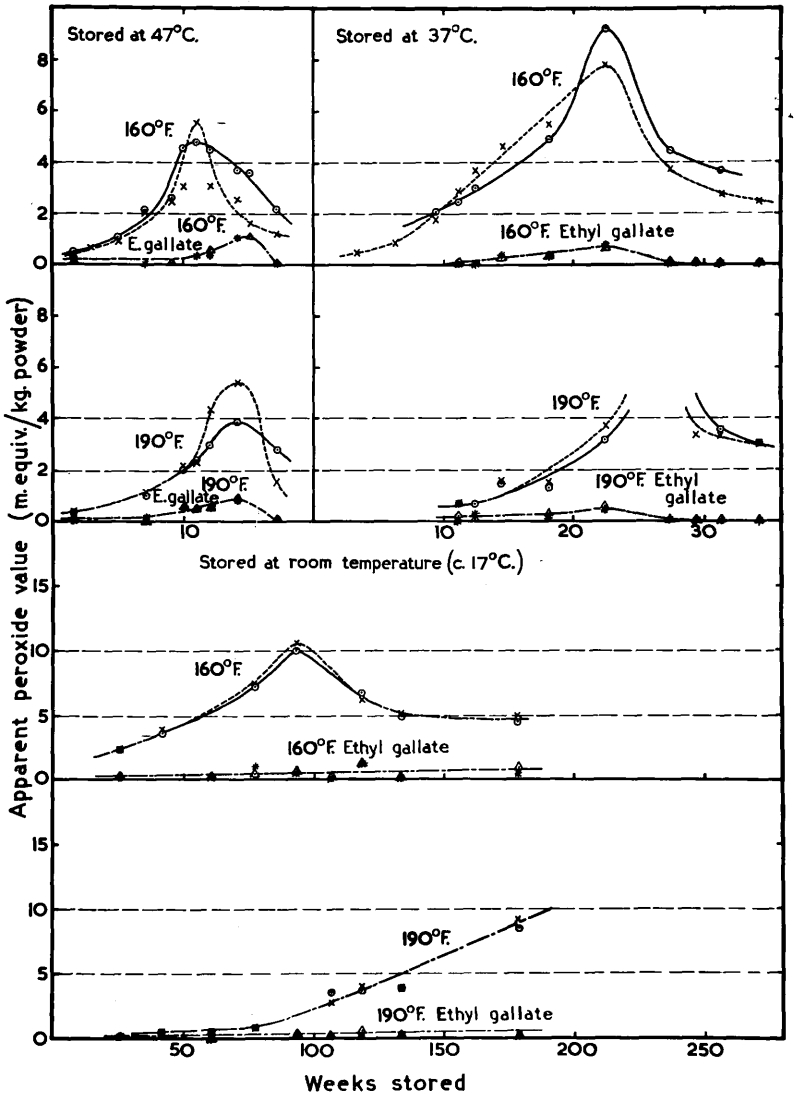

Fig. 5. Peroxide values of whole-milk powders prepared from milk pre-heated at 160 and 190°F., with and without ethyl gallate, during storage at 47, 37°C. and room temperature (c. 17°C.).

x - - - x) = plain tinplate can.
* - - - *)

⊙———⊙) = lacquered tinplate can.
△———△)

it can be seen that the peroxide values reached a maximum just before all the oxygen had been consumed and then they decreased fairly rapidly. The conclusion is that the peroxides, or the compounds responsible for the oxidation of ferrous iron, are intermediate and not ultimate products of the oxidation of milk fat; and further, the peroxides are decomposed simultaneously with their production and it is only in the presence of free oxygen that the amount of peroxide accumulates. When all the oxygen has been used, the decomposing reaction proceeds 'unopposed' and a rapid decrease occurs.

Effect of the container on keeping properties

All the data cited (Tables 5 and 6) show that the storage life of the two control powders was about 10% longer in lacquered than in plain tinplate cans but the difference was small compared with that recorded in earlier work with powder made on a Krause plant (Findlay et al., 1946) and with that in a previous unpublished experiment with Gray-Jensen powder (Findlay, 1944). The powders containing ethyl gallate showed practically no container effect, the pro-oxidant action of the tinplate surface presumably being neutralized by the presence of the fat-soluble antioxidant. The effect of tinplate and of lacquered surfaces on the oxidative deterioration of butterfat has been discussed by Lea (1946b).

Temperature coefficients

The relation between the rates of deterioration at the various storage temperatures is shown in Table 8.

Table 8. Temperature coefficients* for deterioration in palatability and absorption of oxygen of the milk powders

Pre-heating temp. (°F.)	Ethyl gallate (%)	Measured at 'off'-flavour score 1.0				Measured at 'off'-flavour score 2.0				Averages	
		Lacquered cans		Plain cans		Lacquered cans		plain cans			
		47-37°C.	37-17°C.**	47-37°C.	37-17°C.	47-37°C.	37-17°C.	47-37°C.	37-17°C.	47-37°C.	37-17°C.
160	none	1.6	1.8	1.5	1.8	1.5	2.1	1.6	2.2	1.6	2.0
160	0.08	2.0	2.0	2.0	2.0	1.8	2.0	1.7	2.1	1.9	2.0
190	none	2.5	2.2	2.5	2.3	2.5	2.2	2.7	2.2	2.6	2.2
190	0.06	1.9	2.4	1.8	2.5	2.1	-	2.0	-	2.0	c.2.5
		Measured at 10 mg. O_2/100g. powder				Measured at 20 mg. O_2/100g. powder					
160	none	1.8	2.0	1.8	2.2	1.9	1.8	2.0	1.8	1.9	2.0
160	0.08	2.1	2.2	2.8	2.1	2.2	1.6	2.3	1.6	2.4	1.9
190	none	2.6	2.3	2.6	2.5	2.3	1.8	2.6	1.9	2.5	2.1
190	0.06	2.7	2.6	2.4	2.5	2.7	2.6	2.6	2.6	2.6	2.6

* Defined as the ratio of the times required to produce the specified change at two temperatures 10°C. apart.

** The average room temperature approximated to 17°C.

As in earlier work with Krause powder (Findlay et al., 1946), there was a tendency for the temperature coefficients, whether measured from deterioration in palatability or from absorption of oxygen, to be lower for the low temperature powders than for the high temperature powders. The temperature coefficients agree well with those found for similar powders in previous work (Mattick et al., 1945; Findlay et al., 1946).

Estimation of ethyl gallate during storage

It will be recollected that the low temperature ethyl gallate powder contained 0.08% of gallate and that the high temperature ethyl gallate powder contained 0.06% of gallate. Findlay et al. (1945) found that, with whole-milk powders containing about the same amount of this antioxidant, even after storage for 80 weeks at 47°C., there was apparently no decrease in the amount of ethyl gallate present. To find whether similar results would be obtained, the amount of antioxidant in the two powders containing ethyl gallate was measured at the end of the period of storage at the high temperature, i.e. after 216 days at 47°C. and 505 days at 37°C.; a powder which had been stored at 0-4°C. in which the ethyl gallate would not be expected to have altered was included in the examination. In all the powders, the same amount of ethyl gallate as that originally present was found, thus confirming the earlier report.

Relation between absorption of oxygen and deterioration in flavour

It has already been pointed out that there was a general parallelism between oxygen absorption and development of 'off'-flavour (p. 45) although the ratio of the quantity of oxygen absorbed to the degree of 'off'-flavour produced was not constant.

Because of this obvious relation, it is of interest to note the finding of Pearce (1945) that no chemical or physical tests on milk powders were related to palatability. This author examined powders from several sources manufactured over a period of three months and stored at different periods at temperatures ranging from 0 to 60°C. in air and in inert gas and also in darkness and in light. He investigated a large number of physical and chemical tests, and with the possible exception of titratable acidity, none of them gave any significant correlation with the quality of the powders as measured by a tasting panel.

In contradiction to this conclusion, Findlay et al. (1946) in their experiment with Krause powder showed that there was very good correlation between palatability on the one hand and absorption of oxygen and apparent peroxide value on the other. It was therefore thought worthwhile in the present work to calculate the correlation coefficients between absorption of oxygen and palatability for the four powders at each storage temperature. The results are shown in Table 9

Table 9. Correlation and regression of 'off'-flavour score (y) on oxygen absorption (x); results for the four powders, in plain and lacquered cans, at each storage temperature

Storage temp. (°C.)	No. of pairs of observations	Correlation coefficient	Regression coefficient yx
47	77	+ 0.93***	+ 0.08 (3)
37	97	+ 0.90***	+ 0.07 (9)
c.17	70	+ 0.93***	+ 0.08 (0)

*** This c.c. is significant when $P < 0.001$

and they indicate that there was a highly significant correlation between these two criteria. The interesting point is that the close relationship held even at the high storage temperatures and this is another indication of the value of using these elevated storage temperatures and of the reliability of the results obtained.

By calculating the regression coefficients, it can be shown that for every increase in absorption of oxygen of 1 mg. per 100 g. of powder there was on the average an increase of 0.08 in the 'off'-flavour score. However, it would be unwise to apply statistical treatment of these results too far because of the great number of factors which could influence both the objective and subjective tests used. It is well known that the relation between palatability and chemical criteria can be altered to a considerable degree by variations in the conditions of preparation and storage of the powder. Other factors bound to have an effect are moisture and copper content, exposure to light and possibly others yet unknown.

It is probable that Pearce's failure to find any correlation was due to the diversity of conditions under which his tests were done for there is no doubt that under more restricted conditions of experiment, chemical determinations can provide very useful confirmation of results obtained by the tasting method.

DISCUSSION

At all stages in the deterioration of the powders it was quite easy to place the 160°F. powder as the worst and the 190°F. plus ethyl gallate powder as the best in palatability. But one of the most difficult problems was the assessment of the relative merits of high temperature pre-heating without ethyl gallate and of low temperature pre-heating with ethyl gallate and on this question no very definite conclusion was reached. Both the oxygen-absorption and the tasting-panel methods agreed in favouring the gallate-treated powder when the temperature of storage was high but at room temperature the differences were small.

It has already been noted that the protection factors obtained from the rates of absorption of oxygen by the powders were smaller than these obtained from the tasting tests. The interpretation of this fact is difficult due to the complexity of the chemical system undergoing oxidation and the variety of the compounds which will make up the taste or flavour of a milk powder. But at the moment, palatability tests are the most accurate measure of the keeping quality of milk powders and it seems most unlikely that a chemical test will supercede taste as the final criterion.

Little mention has been made of the absolute storage lives of the powders because the opinion of a small panel of tasters, while very useful for estimating the relative merits of powders, can only be taken as a rough

measure of the absolute storage life. Nevertheless, a conservative estimate of the storage lives of the powders, measured by the time taken to reach 'off'-flavour score 1.0 at room temperature, would be 7 months for the 160°F. powder, about 2 years for the 160°F. powder containing ethyl gallate and also for the 190°F. powder, and slightly over 3 years for the 190°F. gallate powder.

The main objects of this experiment have thus been answered:-

1. As with whole-milk powders manufactured by the Kestner and Krause spray-methods, the effect of using a high pre-heating temperature in the preparation of powder on a Gray-Jensen plant, was to increase the storage life of the powder by a factor of about 2.5.

2. The incorporation of ethyl gallate (c.0.07%) in a low temperature, pre-heated powder increased its keeping quality by a factor of about 3 but although this powder had no 'cooked' taste, the flavour of the high temperature powder was preferred. When the anti-oxidant was incorporated in the high temperature powder, the protection factor was increased to 3-4 at 47°C. and 37°C., and to as high as 7 at room temperature. A possibility would therefore seem to exist of using high temperature pre-heating <u>plus</u> ethyl gallate as an alternative to high temperature pre-heating plus gas-packing for moderate periods of storage. For storage up to 1-2 years at ordinary temperatures, a high pre-heating temperature above would be sufficient safeguard.

It only remains now to consider the effect of using lacquered cans and to endeavour to explain the mechanism of the stabilizing effect of high pre-heating temperatures and also of ethyl gallate.

Effect of type of can

It has already been pointed out (p. 44) that with the powders containing ethyl gallate no increase in keeping quality was obtained by using a lacquered can. With the other powders, the storage life was about 10% longer in the lacquered cans. In other experiments (Findlay, 1944; Findlay et al., 1946), a slightly greater 'container effect' was obtained. The explanation seems to be that the bare metallic surface of the can in contact with the powder catalyses the oxidation of the fat. Experiments by Lea (1946b) with films of milk fat on glass, as well as on lacquered and plain tinplate, suggest that the differences in keeping quality due to the container are caused by the acceleration of oxidation by the metallic surface rather than to a protective effect by the lacquer. No tests were made to discover whether oxidation of fat was greater in the layer of powder in contact with an uncoated can; the fat in this layer would presumably be oxidized first but since fat oxidation is autocatalytic further oxidation throughout the mass of powder would probably be accelerated.

Protective effect of high pre-heating temperatures

The stabilizing effect of high temperature pre-heating in almost any milk product has been emphasized in the introduction to this investigation. Now, inseparable with the use of high pre-heating temperatures is the production of what is called a 'cooked' flavour in the milk or milk product. Two very interesting papers published by Gould & Sommer (1939) and Josephson & Doan (1939) showed that there was a close relationship between 'cooked' flavour and the production or liberation of sulphides or sulphydryl compounds in liquid milk. Moreover, these sulphydryl compounds lowered the oxidation-reduction potential of heated milk and appeared to be active antioxidants. Consequently, when later work showed that high temperature pre-heating had a great stabilizing effect on the fat of milk powder and that sulphydryl compounds were undoubtedly present, this protective influence was attributed to the so-called antioxidant effect of these sulphydryl compounds. It was also suggested that another cause of the lengthening of storage life might be the more complete destruction of oxidizing enzymes but this explanation, if true, would almost certainly be of minor importance compared with the formation of antioxidants. Even at low pre-heating temperatures most enzymes would be destroyed.

The sulphur compounds produced by high pre-heating temperatures must be derived from the milk

Protein which contains about 1 g. of sulphur per 16 g. of nitrogen (calc. from Block & Bolling, 1945) and more especially from the lactalbumin fraction which contains a higher percentage of sulphur than the main protein, casein. The protein constituent of the fat-globule membrane may be an additional source of these sulphur compounds. The sulphur in milk protein (and in proteins generally) constitutes part of the molecules of the amino-acids methionine, cysteine and cystine*:-

Methionine	Cysteine	Cystine
$S-CH_3$	SH	S —— S
CH_2	CH_2	CH_2 CH_2
CH_2	$CH(NH_2)$	$CH(NH_2)$ $CH(NH_2)$
$CH(NH_2)$	$COOH$	$COOH$ $COOH$
$COOH$		

According to the generally accepted theory of peptide linkage in proteins, these amino-acids are attached to other amino-acids in the protein molecule by their α-amino groups, thus leaving the sulphur portion of their molecules apparently 'free' or 'available'. However, native proteins, with the exception of muscle protein and thymus histone (Schmidt, 1945) contain no free or reactive -SH or potentially active -SS- groups, or at most only a trace. But when a protein has been

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* It is possible that both cysteine and cystine are present in the protein molecules although after the acid-hydrolysis procedure for the analysis of proteins, usually only cystine can be detected. This is because two molecules of cysteine can be easily oxidized to form one molecule of cystine.

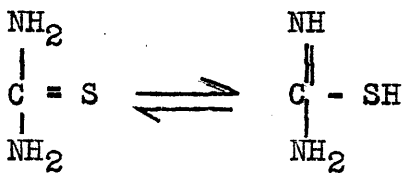
denatured by heat or other means, the -SH and -SS- groups become free or unmasked and can readily be detected by the nitroprusside test (-SS- only after reduction).

Thus it would appear that the first effect of a high pre-heating temperature on milk proteins, especially the lactalbumin, is to unmask at least a proportion of these sulphur atoms. The denaturing influence of heat does not stop at this stage however. It has been shown by several workers that volatile sulphur compounds can be obtained from heated milk by bubbling a stream of nitrogen through it, and the obvious conclusion is that after the initial freeing of the sulphur atoms, a further reaction takes place at the exposed 'sulphur-ends' of the amino-acid residues with the liberation of a trace of H_2S. It would seem probable therefore that the positive nitroprusside test obtained with heated milk and high temperature pre-heated milk powder is due mainly to the presence of H_2S derived from cysteine or cystine, and possibly also to methyl mercaptan ($CH_3.SH$) which could be formed by the decomposition of methionine. The free -SH groups of cysteine residues attached to the protein molecule may also contribute to a positive test.

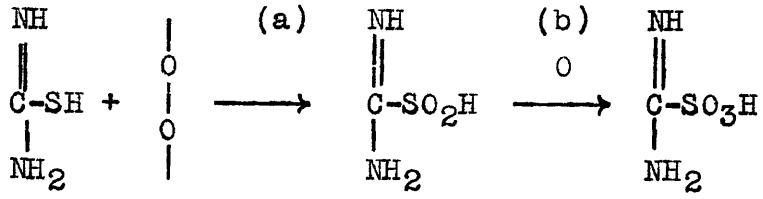
If sulphydryl compounds are directly responsible for inhibiting the oxidation of milk fat, the question remains - what is the mechanism of the antioxidant effect of these groups whether they are

simply the -SH groups of sulphuretted hydrogen or of cysteine or methyl mercaptan? This is no easy question to answer considering that the mechanism of antioxidant activity in general is not understood.

An interesting theory relevant to the present discussion has been proposed by Williamson (1944) to explain the apparent antioxidant effect of thiourea in milk powder. First, it is known that <u>in the presence of water</u>, thiourea tautomerizes to the thiol form:-



Secondly, Kitamura & Suzuki (1938) have shown that thiourea, presumably in the thiol form, reacts with peroxides to form a sulphinic acid which in turn may be oxidized to a sulphonic acid:-



Williamson was of the opinion that the 2% or so of moisture in the milk powder he used was sufficient to enable the tautomeric reaction to proceed and then as soon as peroxide was produced, the second reaction would commence and peroxides would not accumulate.

At first sight this seems an attractive theory to explain the antioxidant effect of sulphydryl compounds but there are several objections. First, H_2S, which appears to form the bulk of the -SH compounds in heated milk, would be ruled out as an antioxidant because the formation of $H\text{-}SO_2H$ (an unknown compound) would be involved, though the formation of a sulphinic acid with a cysteine residue does seem within the bounds of possibility. Secondly, there is the fact that a high temperature pre-heated milk powder absorbs oxygen more slowly than a low temperature powder (see Fig. 3). A mechanism such as that just described means that oxygen would be consumed to form peroxides without any hindrance, the so-called antioxidant only serving to decompose the peroxides once formed but without the reliberation of molecular oxygen. However, it is possible that because peroxides do not accumulate in this system, the absence of their own catalytic effect on further oxidation slows the oxidation of the fat with a resulting slow absorption of oxygen.

However true or false these theories may be, there is one fact which suggests that sulphydryl compounds per se are not antioxidants in milk powder. It will be recalled (p. 68) that although the liquid milk pre-heated at 190°F. gave a positive -SH test before drying, the powder made from this milk gave a negative sulphydryl test, and yet the powder had the typically enhanced keeping quality. This result was not

fortuitous or an isolated instance for Findlay (1944) in unpublished work also got a negative sulphydryl test with high temperature powder prepared on a Gray-Jensen plant, although again the pre-heated liquid milk gave the usual positive test.

The following theory is proposed to explain these facts, the basis of it being that the sulphydryl compounds act as indirect antioxidants by immobilizing the traces of pro-oxidants copper and iron or other similar pro-oxidants which may be present by forming insoluble sulphides. Thus when milk is heated, the liberated -SH groups, whether those of H_2S or of cysteine residues or both, react with the pro-oxidants, the positive sulphydryl test obtained in some spray-dried powders pre-heated at a high temperature being due to the excess of H_2S which is not blown off in the drying process. The Gray-Jensen process differs from the Kestner and Krause processes in that the liquid milk is condensed by allowing it to shower through the hot exhaust air from the drying chamber, the concentrated milk then being atomized by high-pressure spraying. It is suggested that in these final stages of the drying process, the excess of free H_2S or other volatile sulphur compounds is volatilized, leaving only the insoluble metallic sulphides in the dry powder. In this way, the active pro-oxidant catalysts, copper and iron (and possibly other metals) are inactivated and cannot accelerate the oxidation of the fat. Support for this

theory is given in the work of Gould & Sommer (1939) and Josephson & Doan (1939) where it was shown that the addition of copper sulphate to heated milk minimized the 'cooked' flavour, the inference being that the sulphydryl compounds had reacted with the copper. No doubt when traces of H_2S remain in milk powder, the lowered oxidation-reduction potential will still further retard the onset of fat oxidation.

Protective effect of ethyl gallate

The incorporation of ethyl gallate in a whole-milk powder slows the absorption of oxygen and prevents the accumulation of peroxides while the ethyl gallate appears to remain unaltered. Because of this fact, it is interesting to note that Filer, Mattil & Longenecker (1944) found that gallic acid, used as an antioxidant in an oil substrate, had completely disappeared at the end of the induction period of the oxidation of the oil; it appeared that the gallic acid was preferentially oxidized. It is difficult to reconcile these two observations and they show the complexity and difficulty of explaining the mode of action of inhibitors of oxidation. But because ethyl gallate can form salts or complexes with iron and copper in aqueous solution, it seems not unreasonable to suppose that this triphenolic compound can also inactivate the traces of pro-oxidant metals in milk. This theory can obviously only be a partial explanation because ethyl gallate in some way prevents the accumulation of peroxides and also

gives additional storage life to a high temperature pre-heated powder in which presumably, the catalytic metals are already inactivated by the -SH compounds. A canned whole-milk powder containing ethyl gallate does eventually absorb all the available oxygen yet very little peroxide can be detected. The work described in Part III of this thesis shows that under the conditions of the experiment now under discussion, the oxygen would only be utilized by the fat in the powders. It seems therefore that peroxides must be formed but that a function of ethyl gallate is to decompose the peroxides as quickly as they are formed and so prevent the catalytic oxidative power of these compounds from coming into operation.

Ethyl gallate could readily be used in commercial practice as it is not expensive and is usually considered to be non-toxic. As a matter of fact, gallic acid and derivatives constitute part of the tannins which are in many vegetable foods and especially in tea. Hilditch (1944) quotes tests done by Professor J.A. Gunn in which ethyl gallate was fed to or injected into mice in excessive amounts without any apparent ill-effects, and the use of gallic acid and its esters as antioxidants in foodstuffs has been patented by Sabalitschka & Böhm (1942). In Britain however, the Public Health (Preservatives, etc., in Food) Regulations do not at present permit the addition of ethyl gallate to milk before it is dried.

Summary and Conclusions to Part II

1. Storage tests have been made on four samples of spray-dried whole-milk powder prepared on a Gray-Jensen plant from one batch of milk using pre-heating temperatures of 160 and 190°F. with and without the addition of 0.06 - 0.08% of ethyl gallate as an anti-oxidant. The powders were packed in lacquered and in plain tinplate cans and stored at 47 and 37°C. and at room temperature (c. 17°C.). Deterioration was followed by measuring palatability, absorption of oxygen and apparent peroxide value.

2. Raising the pre-heating temperature from 160 to 190°F. or the addition of ethyl gallate to the milk improved the keeping quality of the resulting powder, as measured by taste, by a factor of the order of 1½ - 2½ at 47°C., 2½ - 3 at 37°C. and just over 3 at room temperature (c. 17°C.).

3. Increasing the pre-heating temperature and incorporating ethyl gallate in the milk extended the storage life of the powder by a factor of the order of 3 at 47°C., 4 at 37°C. and 7 at room temperature (c.17°C.). These two methods used together would seem to be an alternative to gas-packing for reasonably long periods of storage.

4. For an equivalent loss of palatability, powder from high temperature pre-heated milk absorbed appreciably more oxygen than the corresponding powder

from low temperature pre-heated milk, and the gallate-treated powders slightly more than the corresponding control powders.

5. The temperature coefficients for deterioration were slightly higher for the high temperature powders than for the corresponding low temperature powders.

6. With the two control powders, storage in lacquered tinplate resulted in a small improvement, of the order of 10%, in keeping properties as compared with storage in plain tinplate. With the powders containing ethyl gallate, no advantage resulted from the use of lacquered tinplate.

7. A theory has been proposed to explain the 'antioxidant' effect of sulphydryl compounds and also in part, that of ethyl gallate.

PART III

The Deterioration on Storage of Dried Separated Milk

Introduction

From time to time, observations have been made that high moisture content induced insolubility and browning during storage in whole- and separated-milk powders. Lea et al. (1943) noticed this deleterious effect of high moisture content on roller-powders. Later, Findlay et al. (1945) observed that, with whole-milk powder, there was a critical moisture content below which fat deterioration was the first noticeable effect of storage, but above which severe non-fatty deterioration was the first to occur on keeping. These changes occurred very slowly at room-temperature but much more rapidly at 37 and 47°C. The non-fatty deterioration resulted in unpleasant 'cardboard' or 'gluey' flavours, marked darkening of the powder and a great decrease in the solubility of the protein.

Later, Henry & Kon (1945) and Henry, Kon & Rowland (1946) found, by the methods of H.H. Mitchell (1924) and Mitchell & Carman (1926) that the biological value of the proteins of a bulk of dried separated milk stored at room-temperature under conditions which did not exclude atmospheric moisture, gradually deteriorated from 88.5 when the powder was a year and a half old to 71.1 three years later. The original moisture content

of the powder was not known but at the end of the storage period it was about 7%.

It was realized that these two observations, viz the effects of high moisture content and the decrease in biological value, were probably closely related and a large-scale experiment was arranged to enquire fully into the deterioration on storage of separated-milk powder with special reference to the influence of moisture. The experiment was a cooperative one and was done at the Hannah Dairy Research Institute, Ayr, the National Institute for Research in Dairying, Reading and the Low Temperature Station for Research in Biochemistry and Biophysics, Cambridge. The collaborators at these centres were J.C.D.White (Ayr), K.M.Henry and S.K.Kon (Reading) and C.H.Lea (Cambridge).

For the purpose of this large-scale experiment, a quantity of dried separated milk was prepared and samples of low, medium and high moisture content, packed in air and nitrogen, were stored at three temperatures as described below. At frequent intervals, cans were removed from storage and the powder examined by taste and by various physical and chemical methods (Lea & White). On the basis of these tests, a smaller number of samples of the stored powders were selected for the determination of their nutritive value (Henry & Kon) and for the microbiological assay of some of the 'essential' amino-acids. A preliminary account of this work was published some time ago (Henry et al., 1946) and the complete results are also now available (Henry et al., 1948).

EXPERIMENTAL

Preparation, packing and storage of the powders

It was necessary to prepare three batches of spray-dried separated milk of at least one cwt. each and with moisture contents of the order of 2, 5 and 7%. A Gray-Jensen plant was used (p.55).

Bulk whole milk (late April, 1945) was separated and then dried in three, 300 gal. batches. It was decided to use a pre-heating temperature of 165°F. with as short a holding time as possible. (The details of the pre-heating system in this type of drier have been described on p.63.) In the present experiment, the first 300 gal. of separated milk were circulated from the storage tank through a tubular heater and back to the storage tank until the desired temperature of 165°F. was attained. This operation took about 20 min. 200 gal. were then pumped to the 'concentrator' or 'liquid collector' and thence to the drying chamber. In the meantime, the remaining 100 gal., which subsequently passed to the concentrator within 15-20 min., were held at about 160°F. The whole batch was dried in 45-60 min. Thus the milk which was dried towards the end of the 'run' was heated for a longer period than that at the beginning, but the average duration of the 165°F. pre-heating was about 30 min. The other two batches were treated in a similar way.

It was thought that powders of the requisite

moisture contents could be obtained by making very small alterations in the drying process but when the drying of the powder was begun, it was found that the lowest moisture content conveniently obtainable was about 2.8% and the highest just under 5%. One batch of milk was dried to give powder of the 'low' moisture content, about 180 lb. being collected. The powder was well mixed and packed into cans holding 21 lb. The other two batches of separated milk were then dried to give powders containing about 4.7% of moisture. These two latter powders were mixed together very thoroughly in a stainless steel cheese-vat to get a homogeneous powder with a uniform moisture content. Half of the mixture was packed into 21 lb. cans and the moisture content of the other half was raised at the Hannah Institute from 4.7 to 7-8% in the following way.

About 190 lb. of the powder were spread out on two curd-cooling troughs made of tinned steel and having a depth of 9 in. and a combined surface area of about 33 sq.ft. The air of the room was kept humid by an open vat of boiling water. Every 10-15 min., the powder was thoroughly mixed and built into ridges to increase the surface exposed. On the first day, the powder was exposed for 12 hr., the relative humidity being about 75% for most of the time but rising to 85% towards the end of the period. Representative samples were taken at intervals for the determination of moisture content. The powder was then packed into cans for the

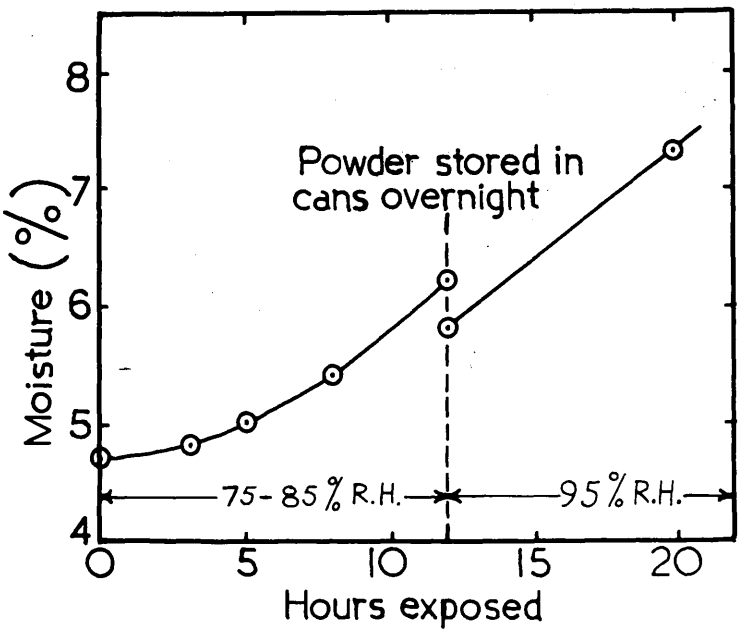


Fig. 6. Uptake of moisture by separated-milk powder of 4.7% moisture content during conversion to powder of 7.3% moisture content.

Table 10. Bacterial counts of the fresh separated-milk powders after packing

Powder	Plate count / g. of powder	
	3 days at 37°C.	5 days at 30°C.
High moisture (7.3%)	132,500	239,500
Medium moisture (4.7%)	180,000	281,000
Low moisture (2.9%)	186,000	290,000

night. Next morning, the apparent moisture content of the powder had fallen from 6.2 to 5.8% despite the fact that it had been in tightly closed cans in the interval. (This decrease may have been due to crystallization of a small proportion of the anhydrous lactose.) On the second day, rain fell heavily and the relative humidity remained fairly constant at 95%. After the powder had been exposed for 8 hr. with frequent thorough mixing as before, the moisture content had risen to just over 7%. The powder was then mixed thoroughly and packed into 21 lb. cans. Fig. 6 illustrates the rate of increase in moisture content.

This procedure made available three batches of separated-milk powder of low, medium and high moisture contents, which will be denoted by the letters 'L', 'M' and 'H' respectively. 'L' was a type of powder which can readily be produced on a normal spray-drying plant when the necessary precautions are taken for obtaining a powder of reasonably low moisture content; 'M' was a powder of a kind frequently obtained under normal factory conditions; while 'H' had a moisture content which is encountered when powder has been stored under bad conditions.

Bacteriological examination of the powders by Dr C. Higginbottom of the Hannah Institute, by the method usually used by her (Higginbottom, 1945) indicated that no measurable increase in bacterial count occurred during the raising of the moisture content (Table 10).

Table 11. Composition of the fresh separated-milk powders

Constituent	Powder (%)			Moisture-free basis (%)		
	H	M	L	H	M	L
Moisture	7.3	4.7	2.9	0.0	0.0	0.0
Fat	1.6	1.5	0.8	1.7	1.6	0.8
'Crude' protein (N x 6.38)	32.0	32.7	34.2	34.5	34.3	35.2
Ash	8.2	8.2	7.9	8.9	8.6	8.1
Lactose (by difference:	50.9	52.9	54.2	54.9	55.5	55.9
Lactose (measured polarimetrically:	50.6	52.8	53.6	54.6	55.4	55.2

Composition and moisture content. The powders were analysed for fat, protein, lactose and ash by the methods already described. The analyses, which are given in Table 11, are typical of normal dried separated milks except of course for the unusually high moisture content of the H powder.

Moisture contents, determined by the air-oven method, are also given in Table 11. It is known, however, that the apparent moisture content of milk powder varies in some degree with the conditions of the determination. This variation will be particularly marked when any appreciable proportion of hydrated lactose is present because α- lactose monohydrate, which loses practically none of its 5% of water of crystallization during 3 hr. at 100°C. in the air-oven, is dehydrated completely in 20 hr. at 100°C. and 5 cm. pressure (Hart, 1941). Moisture contents were therefore determined by Dr Lea on several samples of the powders by heating in a vacuum-oven at 100°C. for periods up to 20 hr. Although the results did not exclude the possibility that a small proportion of the lactose had crystallized during the preparation of the powder, the greater part of the lactose must have been in the form of a non-crystalline, super-cooled 'glass' such as is usually present in spray-dried milk or whey (Troy & Sharp, 1930). There was a suggestion of slight heterogeneity in the moisture figures for the H powder but the variation was remarkably small considering the

quantity of powder which was humidified. Supplee (1926) and Lampitt & Bushill (1931a) have previously stressed the difficulty of raising uniformly the moisture content of a bulk of milk powder.

Packing in air and nitrogen

Part of each of the three powders was packed at the Hannah Institute in Al (315 ml.) tinplate 'open-top' cans, 150 g. per can, and sealed in air. Another portion of each powder was weighed into similar cans and gas-packed at Cambridge, using oxygen-free nitrogen and a cabinet of the type previously described (Lea et al., 1943). The remainder of the powders, also gas-packed, was held at -20°C. in reserve. The seams of all cans were treated with bitumen to ensure gas tightness. The residual oxygen in the nitrogen-packed cans ranged from 0.0 to 0.3% of the atmosphere in the cans, with an average of 0.15%.

Conditions of storage

Air- and gas-packed cans of all three powders were stored at 20, 28.5 and 37°C. in thermostatically controlled incubators, the temperatures of which were checked daily. The powders were examined at intervals as described below.

Physical and Chemical Changes in the Stored Powders

The stored powders were examined for palatability, colour, pH, absorption of oxygen and production of carbon dioxide, solubility (two methods), moisture

content, relative amounts of α- and β- lactose, soluble lactose, distribution of soluble 'nitrogen', bacterial count and composition of insoluble material.

Changes in palatability

The technique of tasting and scoring used at the Hannah Institute has already been described (p. 14) and this system, devised originally for whole-milk powder, was extended to separated powder. It may be emphasized that when a powder passed 'off'-flavour score 1.0, the trained panel was beginning to detect very slight or incipient 'off'-flavours. When score 2.0 was reached, the 'off'-flavour would probably have been noticed and objected to by the ordinary consumer.

The tasting panel experienced some initial difficulty in grading the powders as its members were more accustomed to assessing the tallowy 'off'-flavour of whole-milk powder. Nevertheless, the panel was able to detect slight but definite differences in the palatability of the fresh powders, the initial 'off'-flavour scores being 0.4, 0.2 and 0.1 for the H, M and L powders respectively. It was obvious that deterioration of the H powder had commenced already in the short interval of about 10 days between the preparation of the powders and the first tasting. Thereafter, only M and L powders, stored at 0-4°C., were used as tasting controls.

The changes in palatability of the powders are

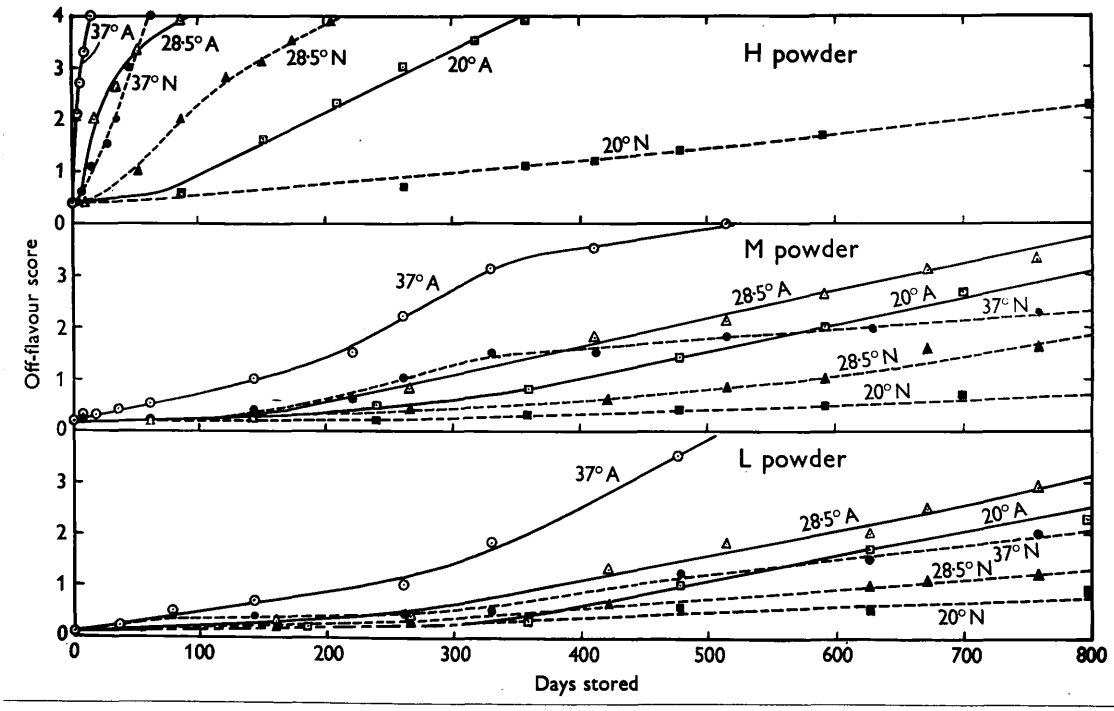


Fig. 7. Changes in palatability of the air-packed and nitrogen-packed separated-milk powders during storage at 37, 28.5 and 20°C.

Table 12. Deterioration in palatability of the separated-milk powders during storage

Powder	Storage temp. (°C.)	Days to deteriorate to 'off'-flavour scores of 1.0, 2.0 and 3.0					
		Air-pack			Nitrogen-pack		
		1.0	2.0	3.0	1.0	2.0	3.0
H	37	2	4	8	18	34	49
	28.5	12	21	44	51	91	140
	20	110	180	270	340	700	880
M	37	140	250	320	260	630	1000
	28.5	290	480	670	600	850	>1095
	20	410	590	800	900	>1095*	>1095
L	37	240	350	430	450	780	>1095
	28.5	370	590	800	650	1080	>1095
	20	500	720	1090	1000	>1095	>1095

* i.e. > 3 years

Table 13. Effect of moisture content on the storage life of the separated-milk powders as measured by palatability

Powder	Relative storage life as measured by palatability											
	At 'off'-flavour score 1.0			At 'off'-flavour score 2.0			At 'off'-flavour score 3.0			Average ratios		
	Storage temp.(°C.)			Storage temp.(°C.)			Storage temp.(°C.)			Storage temp.(°C.)		
	37	28.5	20	37	28.5	20	37	28.5	20	37	28.5	20
					Air-pack							
H	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
M	70	24	3.7	63	23	3.3	40	15	3.0	58	21	3.3
L	120	31	4.5	88	28	4.0	54	18	4.0	87	26	4.2
					Nitrogen-pack							
H	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
M	14	12	2.6	19	9.3	> 1.6	20	> 7.8	> 1.2	18	> 9.7	> 1.8
L	25	13	2.9	23	12	> 1.6	> 22	> 7.8	> 1.2	> 23	> 11	> 1.9

shown in Fig. 7 and in Tables 12-16 and they are summarized below as follows:-

Effect of moisture content. The most noteworthy feature of the results was the great rapidity of the deterioration in palatability of the H powder (7.3% moisture) as compared with the M (4.7% moisture) and L (2.9% moisture) powders. The H powder, packed in air and stored at 37°C., passed 'off'-flavour score 1.0 after only 2 days and reached score 4.0 after 15 days. The M and L air-packed powders, in contrast, took about 140 and 240 days to reach score 1.0 at 37°C. and both powders took 500 days to reach score 4.0. The maximum storage life (time to reach 'off'-flavour score 1.0) of the H powder under the best conditions, viz packed in nitrogen and stored at 20°C., was about 1 year whereas both the M and L powders under these conditions of storage were still palatable after 3 years and will probably remain in good condition for a much longer period.

The higher the storage temperature, the greater was the deleterious effect on palatability of high moisture content. In Table 13 it is shown that in the air-stored series, the ratios of the storage lives of the H, M and L powders were roughly 1:58:87 at 37°C., 1:21:26 at 28.5°C. and 1:3.3:4.2 at 20°C. In the nitrogen-packed series, the results at 20°C. are not yet quite complete but the corresponding ratios were approximately 1:18:23 at 37°C., 1:9.7:11 at 28.5°C. and 1:1.8:1.9 at 20°C. They indicate that the effect of

Table 14. Effect of nitrogen-packing on the storage life of the separated-milk powders as measured by palatability

Powder	Storage temp. (°C.)	Nitrogen/air ratio of days to reach 'off'-flavour scores 1.0, 2.0 and 3.0			
		1.0	2.0	3.0	Average
H	37	9.0	2.0	6.1	7.9
	28.5	4.3	4.3	3.2	3.9
	20	3.1	3.9	3.3	3.4
M	37	1.9	2.5	3.1	2.5
	28.5	2.1	1.8	> 1.6	> 1.8
	20	2.2	> 1.9	> 1.4	> 1.8
L	37	1.9	2.2	> 2.5	> 2.2
	28.5	1.8	1.8	> 1.4	> 1.7
	20	2.0	> 1.5	> 1.0	> 1.5

Table 15. Combined effect of lower moisture content and nitrogen-packing on the storage life of the separated-milk powders as measured by palatability

Powder	Ratio of times taken by the nitrogen-packed powders to reach 'off'-flavour scores of 1.0, 2.0 and 3.0 at each storage temperature to the times taken by the H air-packed powder to reach the same scores at the same temperatures											
	At 'off'-flavour score 1.0			At 'off'-flavour score 2.0			At 'off'-flavour score 3.0			Average		
	Storage temp. (°C.)			Storage temp. (°C.)			Storage temp. (°C.)			Storage temp. (°C.)		
	37	28.5	20	37	28.5	20	37	28.5	20	37	28.5	20
H	9.0	4.3	3.1	8.5	4.3	3.9	6.1	3.2	3.3	7.9	3.9	3.4
M	130	50	8.2	158	40	> 6.1	125	>25	>4.1	138	>38	> 6.1
L	225	54	9.1	195	51	> 6.1	>137	>25	>4.1	>186	>43	> 6.4

moisture content was not so marked as with the air-packed powders, but again the ratios decreased as the storage temperature decreased.

Effect of nitrogen-packing. The beneficial effect of nitrogen-packing is shown very clearly in Fig.7 and in Table 14. Nitrogen-packing approximately doubled the storage life of the M and L powders at all three storage temperatures. With the H powder, gas-packing extended the storage life about 3-4 times at 20°C. and 28.5°C., but at 37°C. the storage life was increased about eight-fold. The advantages of nitrogen-packing suggest that in powders of all moisture contents, part at least of the 'off'-flavours produced must result directly or indirectly from reactions involving atmospheric oxygen. This point is discussed later on p. 105 and p.133.

Combined effect of lower moisture content and nitrogen-packing. Table 15 shows the relative storage lives of the nitrogen-packed powders when compared with the H air-packed powder at the same storage temperature. When stored at 37°C., the nitrogen-packed M powder kept about 138 times, and the L powder more than 186 times longer than the H air-packed powder at the same storage temperature. The combined beneficial effect of lower moisture content and nitrogen-packing was less at the lower storage temperatures.

Temperature coefficients. The rates of decrease in palatability at the various storage temperatures are

Table 16. Effect of temperature of storage on the rate of deterioration of the separated-milk powders as measured by palatability

Powder	Pack	Temperature coefficients* (Q) for deterioration in palatability									
		Measured at 'off'-flavour score 1.0		Measured at 'off'-flavour score 2.0		Measured at 'off'-flavour score 3.0		Average $Q_{8.5}$		Calculated** average for a range of 10°C.	
		Temp.range (°C.)		Temp.range (°C.)		Temp.range (°C.)		Temp.range (°C.)			
		37-28.5	28.5-20	37-28.5	28.5-20	37-28.5	28.5-20	37-28.5	28.5-20		
H	Air	6.0	9.2	5.3	8.6	5.5	6.1	5.6	8.0	6.0	8.6
	Nitrogen	2.8	6.7	2.7	7.7	2.9	6.3	2.8	6.9	3.0	7.4
M	Air	2.1	1.4	1.9	1.2	2.1	1.2	2.0	1.3	2.2	1.4
	Nitrogen	2.3	1.5	1.3	> 1.3	> 1.1	-	> 1.6	> 1.4	> 1.7	> 1.5
L	Air	1.5	1.4	1.7	1.2	1.9	1.4	1.7	1.3	1.8	1.4
	Nitrogen	1.4	1.5	1.4	> 1.0	-	-	1.4	> 1.3	1.5	> 1.4

* The ratio of the times required to produce the specified change at two temperatures 8.5°C. apart.

** $Q_{10} = Q_{8.5} \times \frac{\log 10}{\log 8.5} = Q_{8.5} \times 1.076$

summarized in Table 16. The temperature coefficients for the deterioration of the H powder were much higher than those usually encountered in palatability measurements. The H powder, air-packed, had temperature coefficients of approximately 5.6 and 8.0 for the two ranges of storage temperature 37-28.5°C. and 28.5-20°C. respectively. The nitrogen-packed H powder showed a similar temperature coefficient between 28.5 and 20°C. but only a value of about 2.8 between 37 and 28.5°C. These results for the H powder led to two conclusions, (a) the increase in rate of 'off'-flavour production caused by raising the storage temperature from 20 to 28.5°C. was much greater than that obtained by further raising the storage temperature to 37°C., and (b), above 28.5°C., the absence of oxygen slowed the rate of deterioration in palatability.

The effect of temperature on the deterioration of the M and L powders was very much less marked. The M and L powders, whether air- or nitrogen-packed, had much lower and similar temperature coefficients over both ranges, these being about 1.3-2.0 for the M powder and 1.3-1.7 for the L powder. Therefore, the rates of the reactions responsible for flavour deterioration were not greatly influenced by an increase in moisture content from 2.9 to 4.7% but the further increment of 2.6 units to 7.3% moisture caused a decided increase in the rate of deterioration. It may be concluded that in powder of high moisture content, and perhaps even to some extent in powder of medium moisture content at high storage

temperatures, changes resulting in spoilage of flavour occur which do not take place to any appreciable extent in powders of low moisture content even during storage at 37°C.

Types of 'off'-flavour. Two main types of 'off'-flavour were detected. In the air-packed series, particularly at the higher moisture contents, unpalatability commenced by the development of a stale or 'cardboard' flavour which gradually intensified to a characteristic nauseating and very unpleasant 'gluey' taste. In air-packed powders of low moisture content, 'off'-flavours developed much less rapidly and were less obviously 'glue-like' in character. Since a recognizably tallowy flavour has previously been observed to develop in air-packed separated-milk powder containing 2.4% of fat (Lea et al., 1943), it is possible that oxidation of the rather smaller proportions of residual fat in these powders (see Table 11) may have made a significant contribution to deterioration over long periods of storage, although a tallowy flavour could not be identified specifically.

The nitrogen-packed powders did not develop the same type or degree of unpalatability during storage. Their typical 'off'-flavour was a 'heated', 'cooked', 'caramelized' or slightly 'stale' flavour suggestive of evaporative milk. A long stored, gas-packed, high moisture powder might have a very poor solubility and a brownish colour, yet, its predominantly 'caramelized' flavour, though far removed from that of fresh milk, could not be considered really unpleasant. Gas-packed

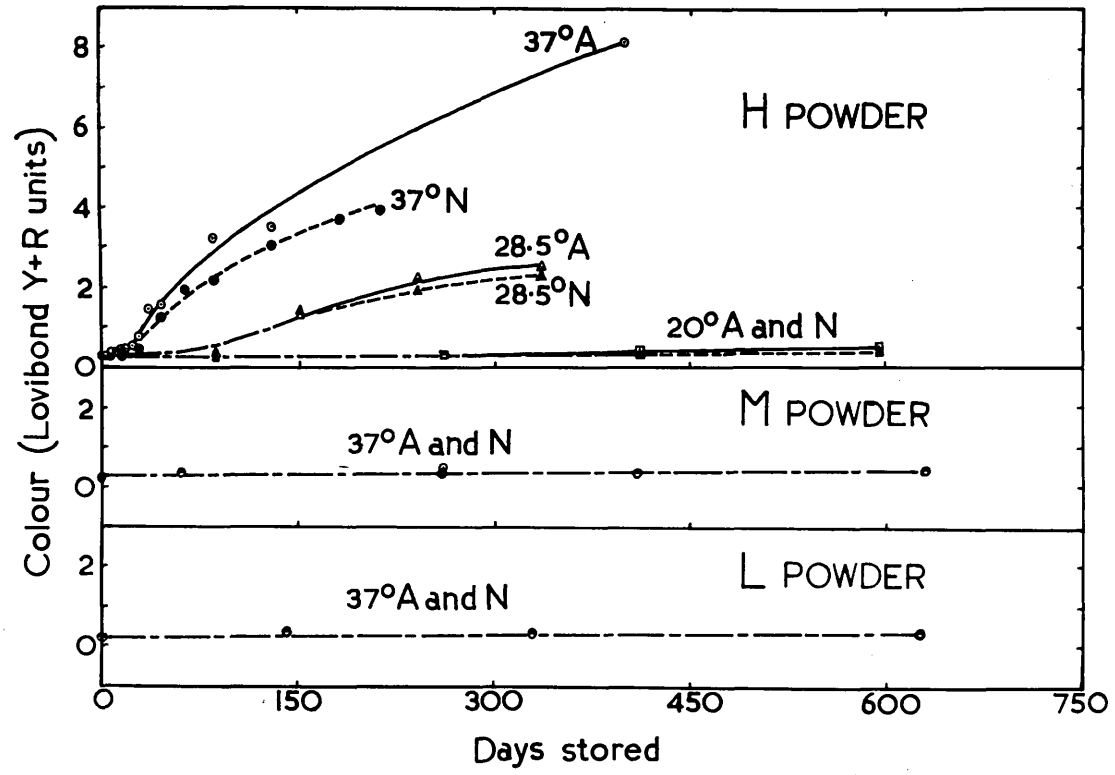


Fig. 8. Development of discoloration of the separated-milk powders during storage.

Table 17. Rates of discoloration of the separated-milk powders during storage

Powder	Storage temp. (°C.)	Increase of discoloration (Lovibond units (R+Y) /100 days)		Relative rates of discoloration		
		Air-pack	Nitrogen-pack	Air-pack	Nitrogen-pack	Average
H	37	2.9	1.9	58	63	61
	28.5	0.9	0.8	18	27	23
	20	0.0(5)	0.0(3)	1	1	1
M	37	0.0(3)	0.0(3)			
L	37	0.0(2)	0.0(2)			

powders of low moisture content showed very little deterioration in flavour during the period of the experiment. The advantage of nitrogen-packing, especially for the H powder, was explained by the failure of the gas-packed powders to develop the 'cardboard' flavour and suggested that this particular flavour may have been caused directly or indirectly by an oxidative reaction, the rate of which was increased by the presence of excess moisture.

Changes in colour

The method of measuring the colour of the powders with a Lovibond Tintometer has been described on p. 40. In practice, it was found that little use of the neutral tint slides was necessary over the limited range of brightness encountered in the storage experiments and, for simplicity, colours have been recorded simply as the sum of the yellow and red units used. Reproducibility in the measurement of colour by this means was not of a high order and different operators were liable to obtain appreciably different results. The figures obtained, however, indicate sufficiently clearly the order of magnitude of the effects of the various factors under investigation on the rate of discoloration of the powders (Fig.8, Table 17).

As with the majority of the other characteristics measured, high moisture content and high storage temperature were the factors chiefly concerned in causing spoilage. The progress of the brownish discoloration

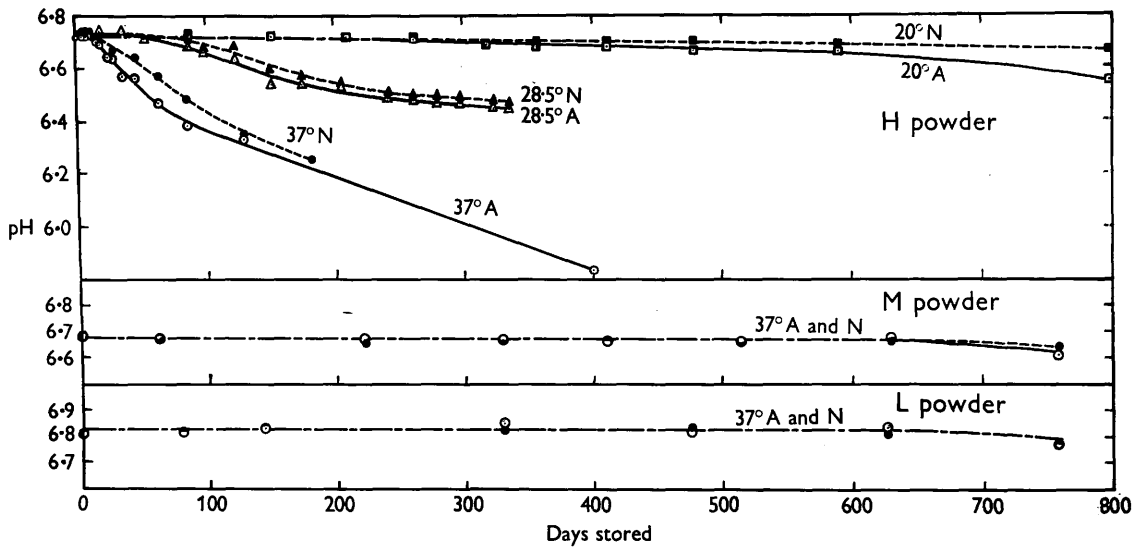


Fig. 9. Changes in the pH value of the separated-milk powders during storage.

was very roughly linear, the air-packed powders changing just perceptibly more rapidly than those in nitrogen. Relative rates of discoloration of the H powder at 20, 28.5 and 37°C. were approximately 1:23:61 (Table 17). Changes in the colour of the M and L powders, even after 3 years at 37°C., were very slight.

Changes in pH

The method of measuring the pH values of the stored powders has been described on p. 40 . The results (Fig.9) show that, as with the development of colour, high moisture content and high storage temperature were the factors mainly concerned in producing a change in pH: after 400 days at 37°C., the pH of the reconstituted H powder had decreased from 6.27 to 5.83. Packing in nitrogen slightly retarded but did not prevent the decrease. The M and L powders retained their initial pH values almost unchanged, even after storage for 3 years at 37°C. Possible reasons for the decrease in pH of the H powder will be discussed later (p. 135).

Absorption of oxygen and production of carbon dioxide

As previously stated, the powders were packed in gas-tight cans of 315 ml. capacity, each can receiving 150 g. of powder. Under the conditions of sealing, the free oxygen content of the air- and gas-packed cans was 39.2 and 0.3 mg. per 100 g. of powder respectively. To calculate these figures, the density of the air-free separated-milk powder was assumed to be 1.465 (see p. 23). Before opening the cans to examine the powders, a sample

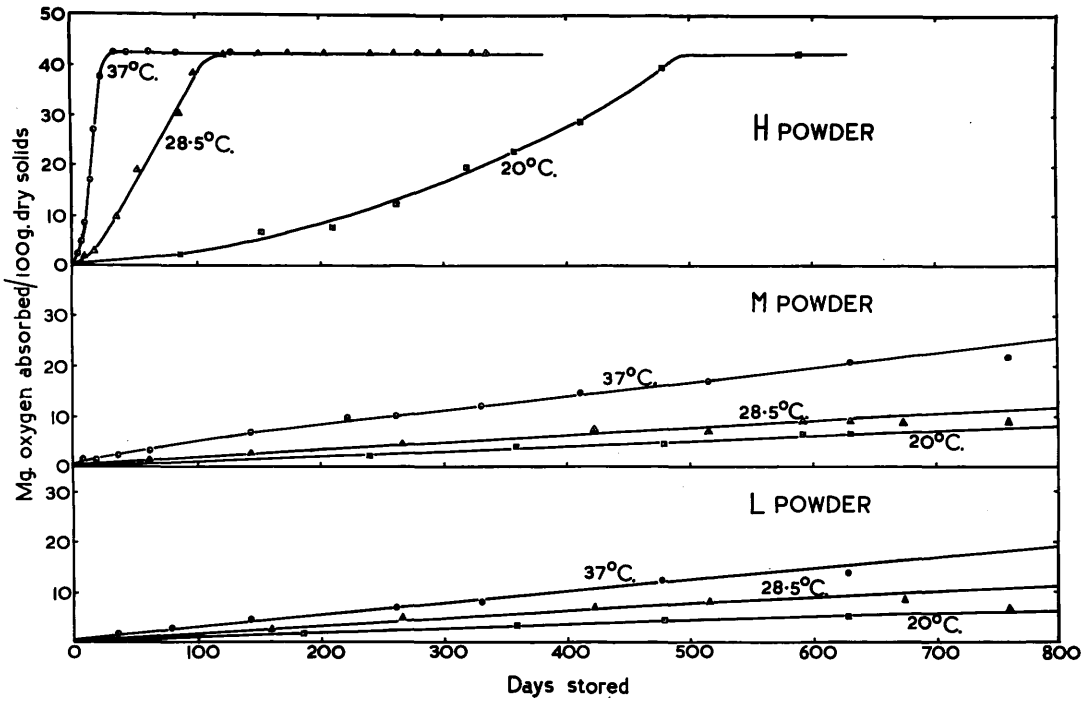


Fig. 10. Absorption of oxygen by the separated-milk powders during storage.

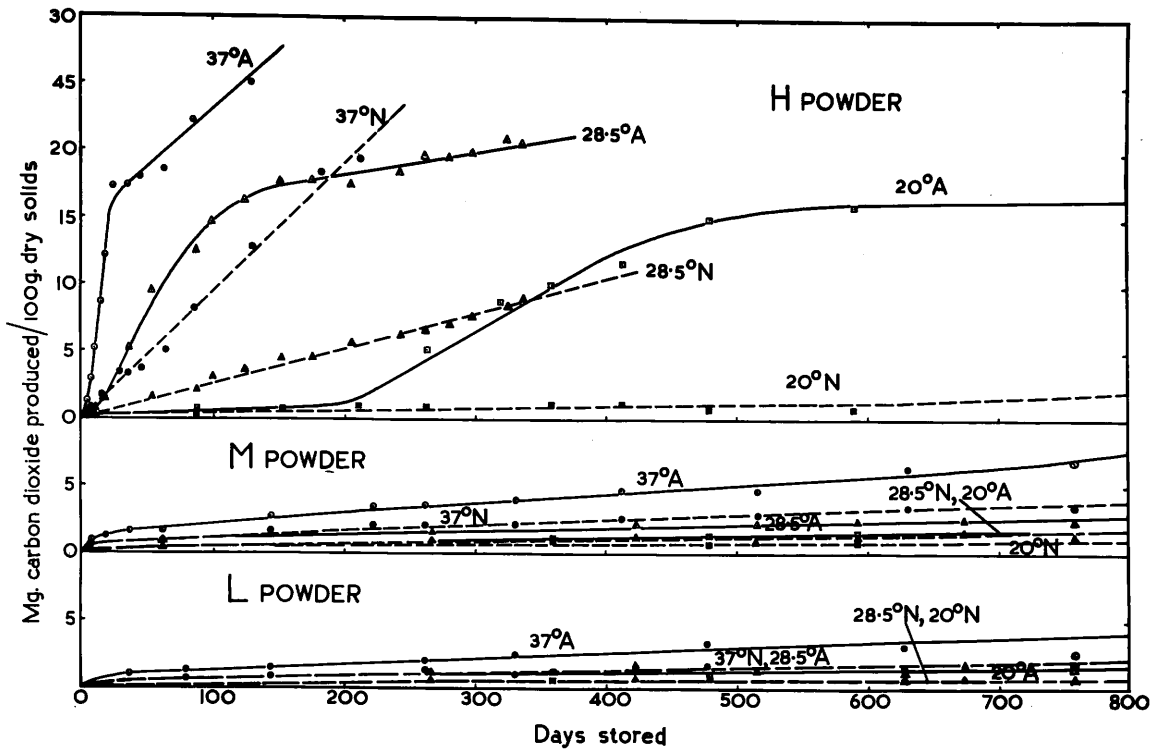


Fig. 11. Production of carbon dioxide by the separated-milk powders during storage.

Table 18. Keeping quality of the separated-milk powders as measured by absorption of oxygen

Powder	Days to absorb, per 100 g. of milk solids,											
	5 mg. O_2, at			10 mg. O_2, at			20 mg. O_2, at			30 mg. O_2, at		
	37°C.	28.5°	20°	37°C.	28.5°	20°	37°C.	28.5°	20°	37°C.	28.5°	20°
H	8	23	150	12	38	220	16	60	330	20	84	420
M	105	300	490	260	730	1050	620	>1095	>1095	900	>1095	>1095
L	190	320	660	400	840	>1095	900	>1095	>1095	>1095	>1095	>1095

	Relative storage life as measured by absorption of oxygen												Average at		
													37°C.	28.5°	20°
H	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
M	13	13	3.3	22	19	4.8	39	>18	>3.3	45	>13	>2.6	30	>16	>3.5
L	24	14	4.4	33	22	>5.0	56	>18	>3.3	>55	>13	>2.6	>42	>17	>3.8

Table 19. Rates of absorption of oxygen and production of carbon dioxide by the separated-milk powders

Absorption of oxygen or production of carbon dioxide (mg./100 g. milk solids / 100 days)									
Powder :	H			M			L		
Storage temperature (°C.):	37	28.5	20	37	28.5	20	37	28.5	20
Oxygen absorption in air-pack: Main reaction	250	45	12	3.1	1.1	1.0	2.1	0.9	0.6
Carbon dioxide production in air-pack:									
Main reaction	100	18	6	0.7	0.3	0.1	0.2	0.1(7)	<0.1
After disappearance of free oxygen	9	1.9	0.3	-	-	-	-	-	-
Carbon dioxide production in nitrogen-pack:									
Main reaction	9.5	2.7	0.6	0.4	0.1	<0.1	0.1(7)	<0.1	<0.1

of the head-space gas was withdrawn from each can and analysed for oxygen and carbon dioxide. The details of the analytical procedure and the method of calculation have been described in Part II. The results, converted to absolute units, are shown in Figs. 10 and 11 and in Tables 18 - 20.

In both the absorption of oxygen and especially in the production of carbon dioxide by the H powder, there were definite induction periods. These were of the order of 2, 20 and 200 days at 37, 28.5 and 20°C. Thereafter, the rate of gas exchange was rapid, production of carbon dioxide being about ten times more rapid by air-packed than by gas-packed powder. When all free oxygen in the container had been exhausted, the rate of carbon dioxide production was reduced and became approximately equal for both air-packed and nitrogen-packed powders. The H air-packed powder at 37°C. absorbed all the available oxygen in 24 days. At 28.5°C. the time taken was 108 days and at 20°C. it was 496 days.

Absorption of oxygen and production of carbon dioxide by the M and L powders were very much slower and the 'curves' were almost linear except for a small, initial, rapid production of carbon dioxide which was possibly due to equilibration of gas within and between the particles or to decomposition of some minor labile constituent. The gradients of these lives are given in Table 19 together with approximate estimates of the corresponding values for the H powder. As with palatability, the increase in rate of gas exchange in

Table 20. Effect of temperature of storage on the rate of absorption of oxygen by the separated-milk powders

Powder	Temperature coefficients* (Q) for absorption of oxygen, measured at										
	5mg. O_2 absorbed per 100 g. milk solids		10mg. O_2 absorbed per 100 g. milk solids		20mg. O_2 absorbed per 100 g. milk solids		30mg. O_2 absorbed per 100 g. milk solids		Average $Q_{8.5}$		Calculated** average for a range of 10°C.
	Temp. range (°C.)		Temp. range (°C.)		Temp. range (°C.)		Temp. range (°C.)		Temp. range (°C.)		
	37-28.5	28.5-20	37-28.5	28.5-20	37-28.5	28.5-20	37-28.5	28.5-20	37-28.5	28.5-20	
H	2.9	6.5	3.2	5.8	3.8	5.5	4.2	5.0	3.5	5.7	3.8 6.1
M	2.9	1.6	2.8	1.4	> 1.8	-	> 1.2	-	> 2.2	1.5	>2.4 1.6
L	1.7	2.1	2.1	> 1.3	> 1.2	-	-	-	> 1.7	> 1.7	>1.8 >1.8

* The ratio of the times required to produce the specified change at two temperatures 8.5°C. apart.

** $Q_{10} = Q_{8.5} \times \frac{\log 10}{\log 8.5} = Q_{8.5} \times 1.076$

passing from 2.9 or 4.7 to 7.3% of moisture was very great and was greater the higher the storage temperature.

The relative storage lives of the air-packed powders as measured by absorption of oxygen (Table 18) were smaller than those obtained from the palatability results (Table 13). This finding was similar to that obtained with the whole-milk powders (p. 45). The temperature coefficients for absorption of oxygen are given in Table 20. The relationship between these values was on the whole similar to that between the temperature coefficients for decrease in palatability (Table 16).

Changes in solubility

The solubility of the stored powders was measured at intervals by two methods, first, that of Howat et al. (1939) (p. 20) and that of the American Dry Milk Institute, Inc. (Grading of Nonfat Dry Milk Solids, 1948); the latter method was modified slightly (p. 22).

Measurement of soluble solids. The method of Howat et al. was used to measure the solubility of the powders at 20 and 50°C. At these temperatures, the solubility of the fresh powders in water approximated to 99 and 100% respectively. The results obtained after storage of the three powders in air and nitrogen at 37, 28.5 and 20 C. are shown graphically in Fig.12 and the times taken by the powders to reach various stages of insolubility are listed in Table 21. The data show that the moisture

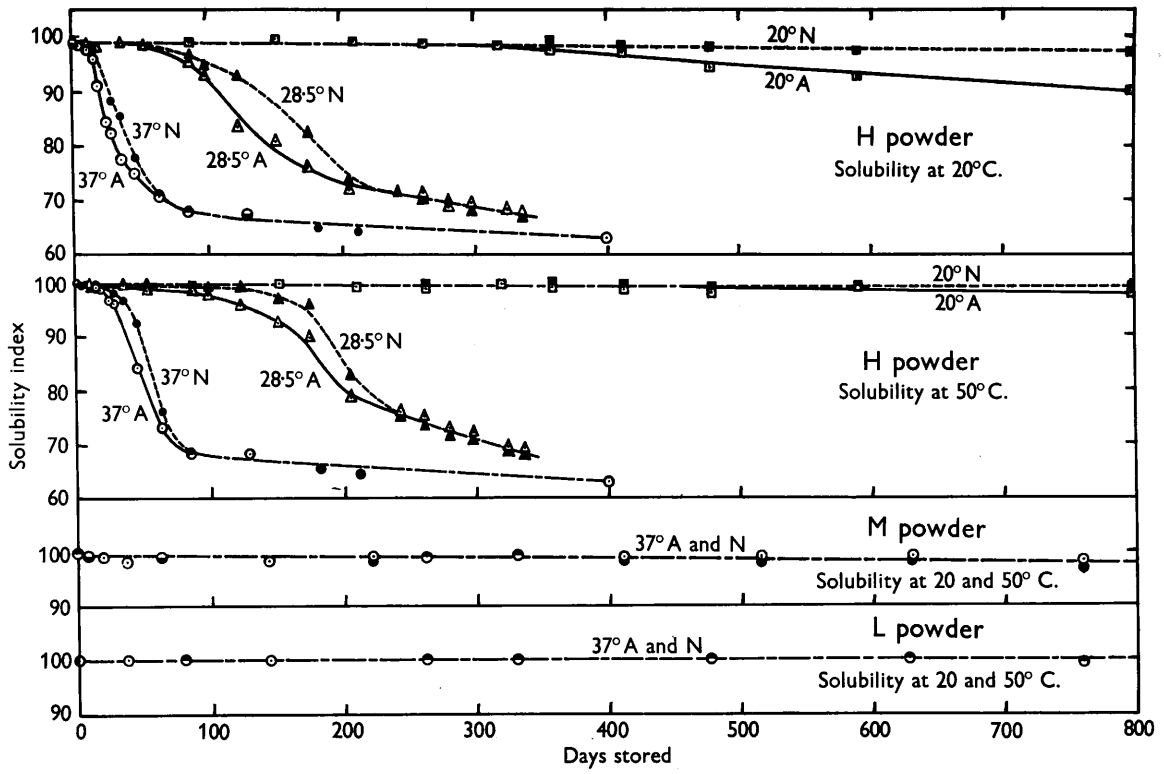


Fig. 12. Decrease in solubility of the separated-milk powders during storage, as determined after reconstitution in water at 20 and 50°C.

Table 21. Loss of solubility of the H powder during storage

(Initial values 99% at 20°C.; 100% at 50°C.)

Storage temp. (°C.)	Pack	Time taken to deteriorate to the stated percentage solubility (days)							
		In water at 20°C.				In water at 50°C.			
		95%	90%	80%	70%	95%	90%	80%	70%
37	Air	16	19	30	68	28	36	52	74
	Nitrogen	20	28	42	70	39	48	58	78
28.5	Air	88	110	150	260	130	165	200	310
	Nitrogen	100	135	180	270	170	190	220	310
20	Air	500	780	>1095	>1095	960	>1095	>1095	>1095
	Nitrogen	>1095	>1095	>1095	>1095	>1095	>1095	>1095	>1095

Table 22. Effect of temperature of storage on the rate of loss of solubility of the H powder

Temperature coefficients* (Q) for loss of solubility												
Temp. range (°C.)	Measured at percentage solubility of:	In water at 20°C.				In water at 50°C.				Average $Q_{8.5}$		Calculated** average Q_{10}	
		95	90	80	70	95	90	80	70	At 20°C.	At 50°C.	At 20°C.	At 50°C.
37-28.5	Air-pack	5.5	5.8	5.0	3.8	4.6	4.6	3.8	4.2	5.0	4.3	5.4	4.6
	Nitrogen-pack	5.0	4.8	4.3	3.9	4.4	4.0	3.8	4.0	4.5	4.1	4.8	4.4
28.5-20	Air-pack	5.7	7.1	>7.3	>4.2	7.4	>6.6	>5.5	>3.5	>6.1	>5.8	>6.6	>6.2
	Nitrogen-pack	>11	>8.1	>6.1	>4.1	>6.4	>5.8	>5.0	>3.5	>7.3	>5.2	>7.9	>5.6

* The ratio of the times required to produce the specified change at two temperatures 8.5°C. apart

** $Q_{10} = Q_{8.5} \times \frac{\log 10}{\log 8.5} = Q_{8.5} \times 1.076$

content of the powders exercised a decisive influence on the development of insolubility; the solubility of the H powder decreased considerably after storage for only one week at 37°C. whereas after 3 years storage at the same temperature, the M powder showed only a small decrease and the L powder no decrease in solubility.

The temperature coefficient for this form of deterioration was again high, an average factor of 4-5 being observed for the temperature difference 37-28.5°C., with a factor of 5-7 between 28.5 and 20°C. (Table 22). As in the development of other types of deterioration, the decrease in solubility of the H powder was preceded by induction periods of the order of 5-10 days at 37°, 40-80 days at 28.5° and 300 days or longer at 20°C. A possible explanation of these induction periods and others mentioned previously will be given later (p.140).

Insolubility in water at 50°C. developed much later than insolubility in water at 20°C. (cf. Fig.12, Table 21) and can be considered as representing a more advanced stage of deterioration. Partly deteriorated samples which, although of poor solubility in cold water, still remained quite soluble in hot, thus displayed an interesting similarity to roller-dried powder in which some protein insolubility is caused by the rather severe heat treatment. Wright (1932) has shown that the difference in solubility of roller-dried powders when estimated at 20 and 50°C. is a measure of the amount of protein rendered insoluble by the dry heat. Whether or not the mechanism of deterioration by excessive heat

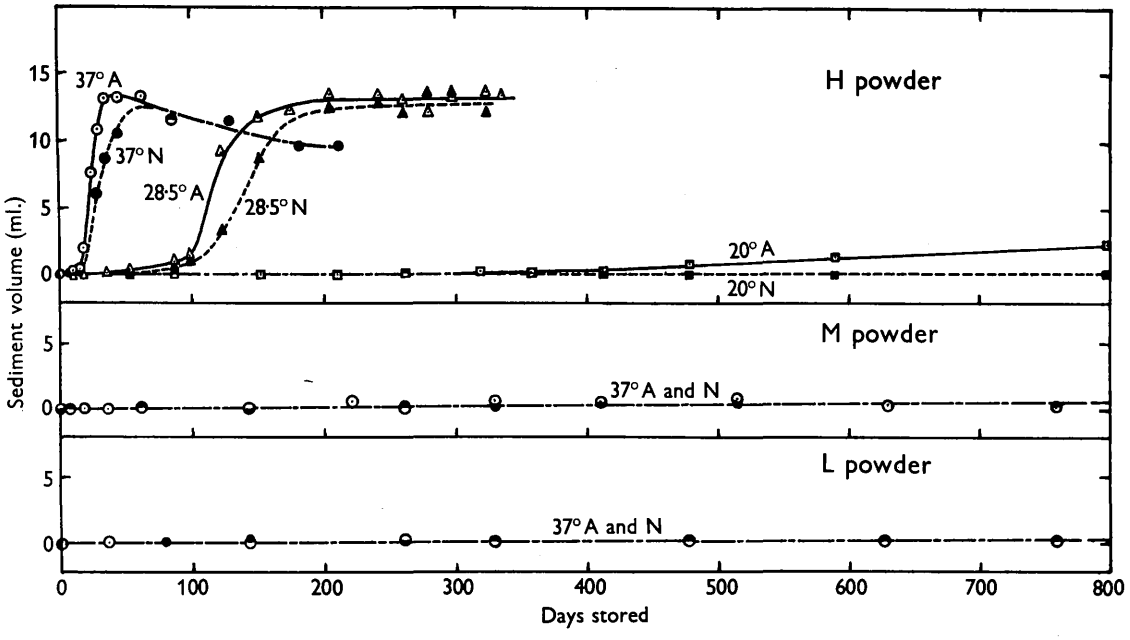


Fig. 13. Decrease in solubility of the separated-milk powders as measured by increase in sediment volume after reconstitution in water at 20°C.

during and immediately after removal of most of the water in the roller-drying process is the same as that produced by storage of powder at too high a moisture content and storage temperature, is not yet known and requires investigation.

The gas-stored samples developed insolubility more slowly than the air-stored samples but the differences were never very great and can be accounted for, in part at least, by the slightly delayed crystallization of lactose (with its accompanying increase in equilibrium relative humidity) in the gas-stored powders as explained on p. 140.

Measurement of sediment volume. The solubility results obtained by the sediment method of the American Dry Milk Institute are shown in Fig.13 and they confirm the conclusions obtained by the quantitative method. First, an increase in the sediment volume of the H powder occurred only after the same induction periods as found with the other solubility method. Secondly, the higher the storage temperature, the greater was the rate of increase of sediment volume, the increase obtained by raising the storage temperature from 20 to 28.5°C. being specially pronounced. Thirdly, the sediment volume of the gas-packed powders increased at a slower rate than the air-packed powders. After 3 years at 37°C., the sediment volumes of the M and L powders had not altered.

It was found for the H powder at 37°C. that

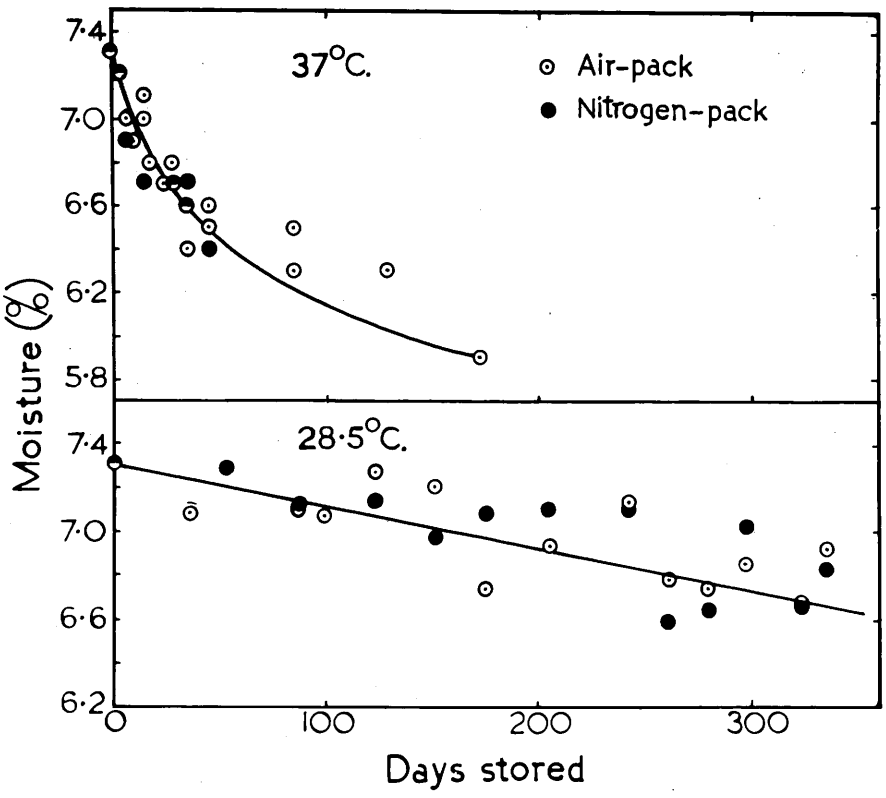


Fig. 14. Decrease in moisture content of the H powder (initially containing 7.3% moisture) during storage at 37 and 28.5°C.

after minimum solubility was reached, the sediment volume, which was greatest at that point, did not remain constant but tended to decrease as the storage period lengthened. This decrease may have been due to some physical change in the coagulated protein particles which enabled them to 'pack' more closely when centrifuged. It was also noticed with severely deteriorated H powder that some insoluble material was present on the surface of the supernatant liquid after centrifuging. When this layer was broken up and the suspension recentrifuged, the amount of floating material was reduced but could not be removed completely. The trace of fat present may have carried up some insoluble protein which would otherwise have sedimented and this may possibly be a partial explanation of the decrease in sediment volume.

Changes in moisture content*; 'caking' of the powder

When the moisture content of a sample of H powder which had been stored at 37°C. for some time was redetermined, it was found to be lower than the original value. Other samples of H powder stored at 37 and 28.5°C. were therefore examined. The results, which are shown in Fig.14, indicate that as storage progressed, the amount of water which could be removed by heating for

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* In the present work, the 'moisture content' of a powder is regarded as the amount of water which is removed (per 100g. of powder) by heating at 100°C. for 3 hr. in an air-oven; 'water content' is regarded as the total amount of water in a powder.

3 hr. at 100°C. diminished. These observations, coupled with the fact that the H powder at 37°C. set to a solid mass in the cans after a few days, led to the obvious conclusion that the lactose was crystallizing. Microscopical examination of fresh and stored H powders showed that the deteriorated samples contained crystalline material while a fresh powder was wholly amorphous in appearance. These findings are compatible with previous work for it is known that in fresh spray-dried powder, the lactose exists in an amorphous or 'glass' form composed of α- and β- sugars in the ratio of about 1:1.5 which is stable for long periods at normal temperatures and low relative humidities. But if the moisture content is sufficiently high, or if a dry powder absorbs sufficient water vapour from the atmosphere, α- lactose monohydrate containing 5.0% of water crystallizes during storage. The result is that the powder 'cakes' and forms a mass which gradually gets harder, the change being greatly accelerated by high storage temperatures.

Both the amonphous forms of lactose can become crystalline but only α- lactose takes up water of crystallization. Thus, although it would seem possible that the crystalline material in a deteriorated powder could consist of both α- and β- lactose, it has been shown in the present work that the crystalline α- form predominated. If all the α- lactose originally present in the 'glass' form crystallized to the hydrate, only

about 1.1% of the 7.3% of the moisture originally present in the H powder would be 'bound'. The lowest moisture content found for the H powder was 5.9%, this being only slightly less than the expected figure if all the lactose had crystallized.

In another section of this large-scale collaborative investigation, Dr Lea showed that the equilibrium relative humidities of the fresh powders were, H powder, 7.3% moisture, 41-43% R.H.; M powder, 4.7% moisture, 29% R.H.; L powder, 2.9% moisture, 17.5% R.H. The M and L powders retained their initial equilibrium relative humidities throughout storage but the H powder, after different periods of storage at the three storage temperatures, reached an equilibrium R.H. of about 55%. This increase in equilibrium R.H., which was delayed slightly in nitrogen as compared with air, meant that the 'activity' of the water in the powder had increased. This was almost certainly caused by the crystallization of the α- lactose and is compatible with an apparent decrease in the water content of the powder as will be explained below (p. 138). The rate of decrease of moisture content was not directly proportional to the rate of crystallization of the α-lactose as measured by changes in equilibrium relative humidity. It is probable that the hygroscopic nature of the protein or other reactions involving water, also had some influence on the apparent loss of water.

Conversion of β - lactose to α - lactose hydrate and apparent decrease in total lactose

In addition to the crystallization of α - lactose, it was considered that another change in the lactose of the H powder was possible. Troy & Sharp (1930) observed a considerable change of β - lactose to α - lactose hydrate in milk products stored at laboratory temperature and 70% relative humidity for 5 months. Since the initial high equilibrium relative humidity (c.42%) of the H powder gradually increased to about 55% as the anhydrous α - lactose crystallized, it was thought probable that, in this powder at least, some of the β - lactose would be converted to α - hydrate, especially at the higher storage temperatures. Samples of the stored powders were therefore examined to find the relative amounts of α - and β - lactose present.

A method of doing this has already been described (p.41). To gain experience of the method, it was applied to fresh milk. The proportions of α - and β - lactose found were 38.5% α and 61.5% β at 25°C. and were very close to the β/α equilibrium ratio of 1.58/1 (corresponding to 38.7% α, 61.3% β) generally accepted for that temperature. Examination of the fresh H, M and L powders showed that 41-43% of the lactose was in the α - form. As Troy & Sharp (1930) pointed out, this slightly higher percentage of α - lactose in the powders is to be expected since the temperature of the milk during dehydration would

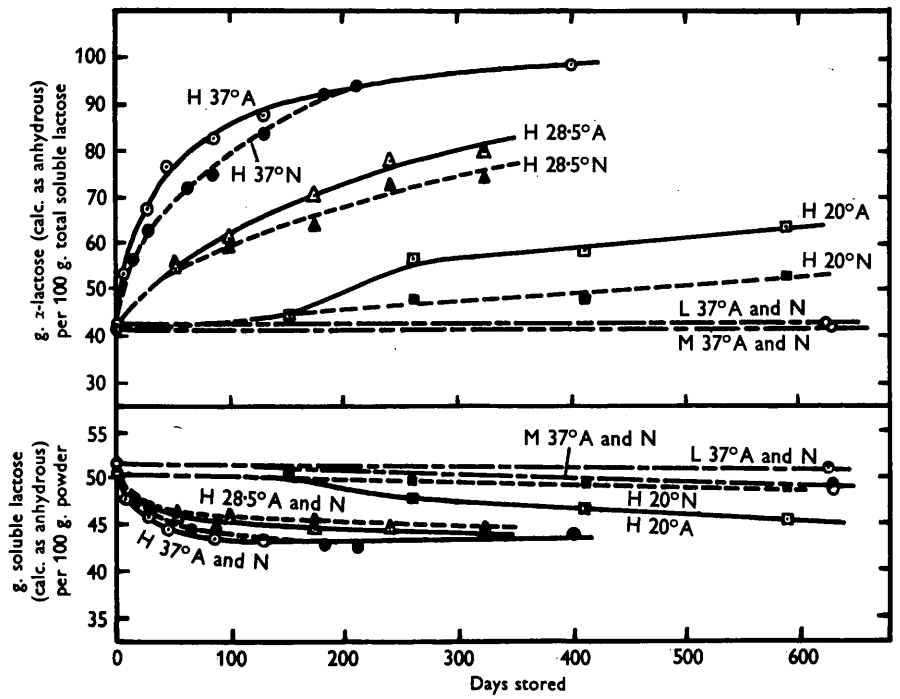

Fig. 15. Conversion of β - lactose to α- lactose hydrate, and decrease in total soluble lactose during storage of the separated-milk powders.

probably exceed 25°C. and so cause a lowering of the β/α ratio.*

Air- and nitrogen-packed samples of each powder were examined after different periods of storage. The results (Fig.15) show that in the air- and gas-packed M and L powders the proportions of α- and β-lactose were unchanged even after more than 600 days at 37°C. In the H powder, however, β- lactose was converted to α- lactose, presumably the hydrated form, fairly rapidly at 37°C. and more slowly at 28.5 and 20°C. until after 400 days at 37°C., 98.5% of the lactose was in the α- form.

The H samples were examined after they had been taken from the storage incubators and kept in bottles at 0-4°C. for some time, so that any differences in the rate of conversion of β- to α- lactose due (indirectly) to gas-packing may have been minimized. Nevertheless, as in the crystallization of the anhydrous α- lactose, packing in nitrogen appeared to retard the change, but this effect was probably due simply to the slower rise in the equilibrium relative humidity of the gas-packed powders.

Apparent decrease in total lactose. The method of estimating α- and β- lactose also measured total lactose,

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* The β/α lactose ratio of a milk powder would seem to indicate the temperature attained during the drying process just before the milk becomes anhydrous. But in spray-drying, the rate of dehydration is so high that the lactose would probably not have time to equilibrate at the temperature of the almost dry particles.

but it has been shown by the originators of the procedure that the protein precipitant used, an alcoholic solution of mercuric chloride, causes a slightly low value for total lactose. Nevertheless, it was obvious that during storage of the H powder there was a progressive decrease in the amount of total soluble lactose (lower half of Fig. 15). After 400 days at 37°C., the percentage of total lactose, as measured by this method, had decreased from 50.3 to about 44%. To make sure that this result was not an artefact caused by the alcoholic mercuric chloride solution, three other protein precipitants were used (acid mercuric nitrate, cadmium hydroxide and zinc ferrocyanide) and the total soluble lactose averaged 45.3%. The reason for this decrease in soluble lactose will be discussed later (p.128 and p.136).

Changes in the distribution of the soluble 'nitrogen'

It has been shown that the solubility of the M and L powders remained almost unaltered even after storage for 3 years at 37°C. The H powder, however, although showing little change in solubility when stored at 20°C., rapidly became insoluble when stored at 28.5 and 37°C. whether packed in air or nitrogen. Since the loss of solubility of a milk powder is largely due to a decrease in the solubility of the proteins, it was decided to determine the distribution of the soluble 'nitrogen' and thus indirectly find which fractions of the protein were becoming insoluble and also whether soluble nitrogenous decomposition products were being

formed. Such an examination would show whether the deterioration involved only the casein or whether lactalbumin and lactoglobulin were also involved.

Examination of the fresh powders. For the determination of the initial nitrogen partitions, the fresh powders were reconstituted with water at 20°C. in the manner prescribed for the measurement of solubility (p. *20*) and the 'milk' made up to a known volume. Since the fresh powders were practically 100% soluble, aliquots for analysis could be pipetted easily and accurately. The various nitrogen fractions were separated and measured by the methods already described (p. *44*).

It should be pointed out that the methods used for the fractionation of 'nitrogen' were developed for normal liquid milk in which the proteins are in their natural state. In milk or milk products which have had a certain degree of heat treatment, it is to be expected that some or all of the heat-sensitive proteins, viz lactalbumin and lactoglobulin, will be denatured and thus be precipitated with casein at pH 4.6. This means that under these circumstances the 'casein' fraction will include denatured lactalbumin and lactoglobulin; it follows that the fractions reported as 'lactalbumin' and 'lactoglobulin' will consist of the undenatured fractions of these proteins.

The nitrogen partitions found for the fresh H, M and L powders are given in Table 23. Rowland's (1938) average values for the nitrogen partition of

Table 23. Distribution of nitrogen in the fresh separated-milk powders as compared with that found by others for spray-dried separated milk and liquid milk

Constituent	mg. N per g. of milk solids					Percentages of total nitrogen						
	Powder				Average values for 32 samples of spray-dried separated-milk (1)	Powder				Average values for 32 samples of spray-dried separated-milk (1)	Average values for liquid milk (2)	Ranges for liquid milk (2)
	H	M	L	Average		H	M	L	Average			
Total N	54.1	53.8	55.2	54.5	58.13	100.0	100.0	100.0	100.0	100.0	100.0	-
'Casein' N	44.1	44.1	46.0	44.7	52.80	81.5	81.9	83.3	82.2	90.75	78.5	77.7 - 78.9
Non-casein N	10.0	9.7	9.2	9.6	5.50	18.5	18.1	16.7	17.8	9.25	21.5	21.1 - 22.3
Lactalbumin + lacto-globulin N	3.4	3.3	2.3	3.0	0.23	6.2	6.2	4.1	5.5	0.40	12.5*	10.3 - 14.1
Proteose-peptone N	3.4	3.1	3.9	3.5	2.30	6.2	5.8	7.0	6.3	3.95	4.0	2.8 - 5.3
Non-protein N	3.3	3.3	3.1	3.2	3.06	6.1	6.1	5.6	5.9	5.26	5.0	3.7 - 6.4

(1) Ashworth & Van Orden (1943).
(2) Rowland (1938).

* Consisting of 9.2% lactalbumin and 3.3% lactoglobulin; it now appears that the latter figure for lactoglobulin is about twice its true value (Rowland, 1948).

fresh liquid milk and Ashworth & Van Orden's (1943) data for spray-dried separated milk are included in the Table. The values for the three powders have been averaged to facilitate comparison with the data for fresh milk and the other separated-milk powders. It will be noticed that the figures for the H and M powders are very similar; this was to be expected because the H powder was prepared by raising the moisture content of a quantity of the M powder. Comparison of these average values for the three powders with those for fresh milk, shows that the percentage of the total nitrogen in the form of lactalbumin and lactoglobulin was only 5.5 as compared with 12.5 for fresh milk, i.e. an apparent decrease of 7.0%. This difference was compensated by an apparent increase of 3.7% in 'casein' nitrogen and increases of 2.3 and 0.9%* in proteose-peptone and non-protein nitrogen respectively, i.e. an apparent combined increase of 6.9%. Thus it would appear that in the preparation of the powders, part of the lactalbumin plus lactoglobulin fraction was denatured (and so was precipitated with the casein) and a smaller proportion broken down to form proteose-peptones. Ashworth & Van Orden's (1943) data for other spray-dried separated milks also show a deviation from the nitrogen partition of fresh milk. Their results differ slightly from these reported here in that almost

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* This apparent increase in non-protein nitrogen is probably not significant since all the N.P.N. values are lower than the maximum normal limit for N.P.N. in fresh milk (see last column in Table 23).

the whole of the lactalbumin plus lactoglobulin appeared to be denatured, thus causing a corresponding apparent increase in the casein fraction. They did not detect an increase in the proteose-peptone fraction.

Since the milk was dried by the Gray-Jensen spray-process, the milk or powder would not be subjected to high temperatures during the actual dehydration (Allen, 1932; Scott, 1932; Hunziker, 1946). The deviation of the nitrogen distributions of the fresh powders from that of normal liquid milk would thus depend largely on the temperature (165°F.) and duration (c. 30 min.) of the pre-heating treatment. Rowland (1933, 1937) has examined the effect of various 'temperature-time' treatments on the distribution of nitrogen in milk. He states that lactalbumin and lactoglobulin are rapidly denatured at temperatures of 75°C. (167°F.) and above, and that there is no change in non-protein nitrogen content on heating at temperatures up to 100°C. On continued heating (30 min.) at 95 and 100°C. very small amounts of proteose-peptone substances are produced. Menefee, Overman & Tracy (1941) found that in the preparation of evaporated and condensed milk, pasteurization at 145°F. (62.8°C.) for 30 min. produced no significant differences in nitrogen distribution. On the other hand, fore-warming to 150°F. (65.6°C.) caused slight 'coagulation' of lactalbumin whereas pasteurization at 190°F. (87.8°C.) for 30 min. and fore-warming to 203°F. (95°C.) both caused complete 'coagulation' of lactalbumin and probably of some lactoglobulin.

Examination of the stored powders. Because the M and L powders at all storage temperatures, and the H powder at 20°C., altered very little in solubility, examination for changes in the distribution of nitrogen was restricted to the H powder, air- and gas-packed, stored at 37 and 28.5°C. It was desirable to make the method of reconstitution as similar as possible to the method used in the determination of solubility so that the measurement of total soluble nitrogen (which gives an estimate of the solubility of the proteins) could be compared with the solubility values based on the solubility of the total solids of the powders. The presence of increasing amounts of insoluble material complicated the analytical procedure; the method finally adopted was as follows:-

> 5 g. of powder (ground in a mortar if 'caked') were weighed into a 250 ml. Erlenmeyer flask and 45 ml. of water at 20°C. added. The flask was shaken for 1 min. and the 'milk' centrifuged, both operations being made as similar as possible to those used in the determination of solubility. After carefully removing any insoluble material on the surface of the liquid (cf. p. 112), the supernatant layer was poured off and used for nitrogen analyses by the methods already described.

The nitrogenous fractions were expressed firstly as mg. of nitrogen per 100 g. of the supernatant liquid. To get a truer picture of the changes in the distribution of the soluble nitrogen, the results were expressed as percentages of the initial values for each fraction, percentages of total nitrogen, percentages of initial total nitrogen and also as mg. of soluble nitrogen per 1 g. of dry milk solids. The last method

of expressing the results gives more information than the others and it has been used to construct the graphs mentioned below. For this method, it was necessary to calculate the weight of supernatant liquid and it was possible to do this by knowing the percentage solubility of the milk solids and the moisture content of the powder. The calculation was as follows:-

The H powder contained 7.3% of moisture and so the composition of an uncentrifuged solution of powder was :-

45 g. water plus 5 g. powder (0.37g. moisture plus 4.63g. separated-milk solids)

If the solubility index of the powder was S, then of the 4.63g. of milk solids, $\frac{4.63S}{100}$ g. were soluble and $\frac{4.63(100-S)}{100}$ g. were insoluble. When the insoluble solids were removed by centrifuging, the weight of the supernatant liquid containing the soluble protein of the powder would be $45 + 0.37 + \frac{4.63S}{100}$ g. Let us take a particular sample of H powder whose solubility index (i.e. the percentage solubility of its dry milk solids) was 90. Then the weight of the supernatant liquid would be $45 + 0.37 + \frac{4.63 \times 90}{100}$ g. = 49.54 g., i.e. the soluble solids of 5 g. of powder (or 4.63g. of milk solids) were contained in 49.54 g. of solution.

Thus, by first finding the weight of nitrogen per 100 g. of the supernatant liquid it was possible to express the various nitrogenous fractions as mg. of soluble nitrogen per 1 g. of dry milk solids. Figs.16 and 17 illustrate the changes in the distribution of

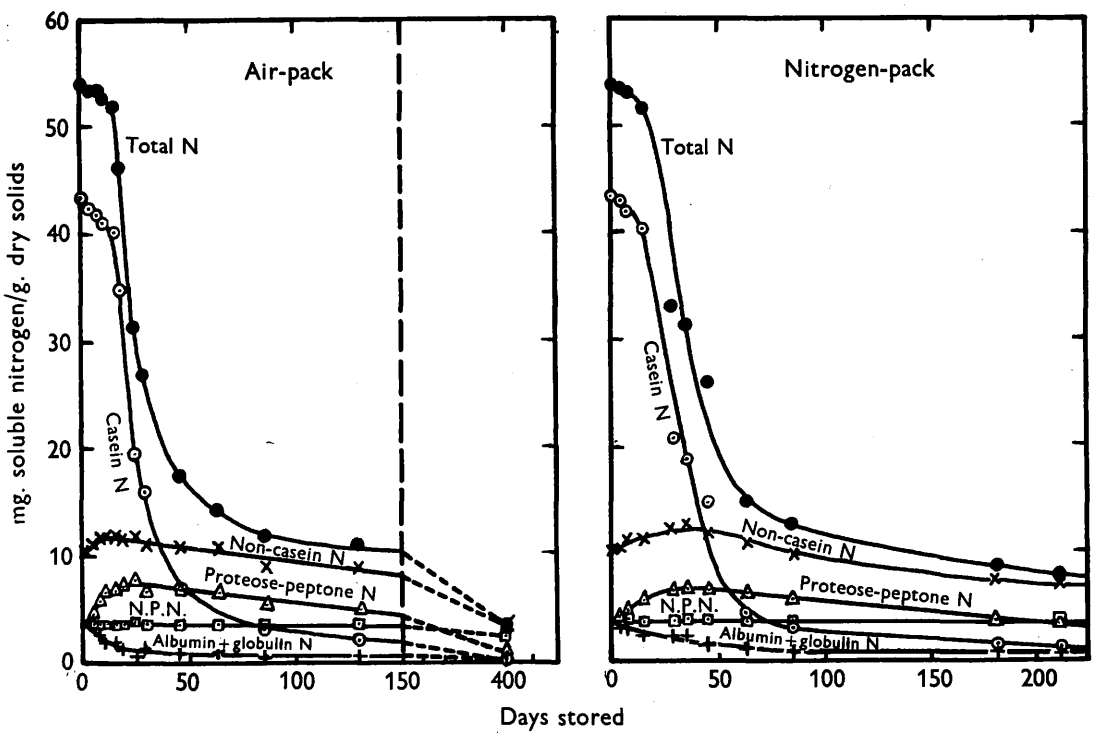

Fig. 16. Changes in the distribution of the soluble nitrogen of the H powder during storage at 37°C.

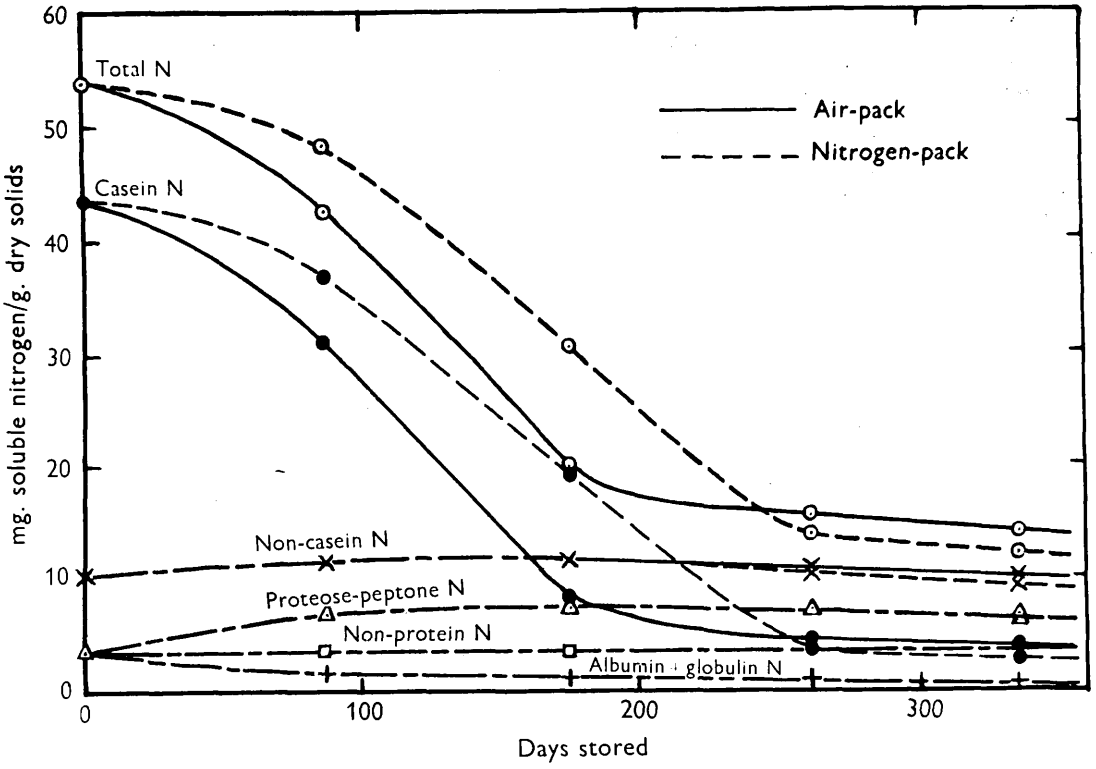

Fig. 17. Changes in the distribution of the soluble nitrogen of the H powder during storage at 28.5° C.

the soluble nitrogen during storage of the H powder at 37 and 28.5°C. These changes may be summarized as follows :-

(1) The same changes took place at both storage temperatures although at a much slower rate at 28.5°C.; the changes were less rapid in nitrogen compared with air. As would be expected, they ran concurrently with the decrease in solubility of the powder.

(2) As storage progressed, the casein rapidly became insoluble and was completely insoluble after 400 days at 37°C. Since the casein nitrogen constituted about 81% of the total nitrogen, the decrease in soluble casein was closely paralleled by a decrease in total soluble nitrogen.

(3) The proteose-peptone fraction increased to about twice its initial value after about 24 days at 37°C. and also after about 90 days at 28.5°C. and then decreased slowly until after 400 days at 37°C., only about one quarter of the initial amount remained in solution.

(4) The amount of non-protein nitrogen did not increase showing that although the physical state of the protein was being considerably changed it was not being broken down to simple water soluble compounds to any significant extent. There was a marked decrease in the N.P.N. of the powder stored in air for 400 days at 37°C.

(5) The lactalbumin plus lactoglobulin fraction slowly decreased in solubility and eventually became almost entirely insoluble (see data for H powder stored for 400 days at 37°C., Fig.16).

(6) There was a small initial increase in non-casein nitrogen. Thereafter, the amount of non-casein nitrogen decreased slowly.

It is obvious from Figs. 16 and 17 that loss of solubility of the casein was mainly responsible for the decreased solubility of the powders. This was emphasized by the fact that after a period of storage when the soluble casein had fallen to a very low level, the sum of the other nitrogenous fractions, i.e. the non-casein nitrogen, had decreased only slightly and indeed had shown a small initial increase. The small increases in non-casein nitrogen seemed real since they were detected in air- and nitrogen-packed powders at both 28.5 and 37°C.; the increases result from an over-compensation of the loss of soluble lactalbumin-lactoglobulin by the production of additional proteose-peptone. The fact that the non-casein nitrogen increased meant that at least a part of the additional proteose was produced by the breakdown of casein but the analytical data so far available do not show whether the remainder was also derived from casein or from the lactalbumin-lactoglobulin fraction or both. It will be remembered that even the fresh powders contained an excessive amount of proteose-peptone compounds and it may be that the high moisture content and high storage temperature caused a decomposition reaction, started at some stage in the preparation of the powders, to continue during storage.

To find what was happening to the lactalbumin and lactoglobulin fractions individually, direct

estimations of lactoglobulin were made as described on p. 46. In the fresh powders, the amount of apparent lactoglobulin was more than twice the expected value with the result that the amount of lactalbumin (obtained by difference) was unduly small. As storage progressed, the apparent lactoglobulin remained practically at its initial high value while lactalbumin plus lactoglobulin decreased; thus the amount of lactalbumin appeared to diminish and become increasingly negative. It was apparent that saturation of the filtrate containing non-casein nitrogen with magnesium sulphate caused the precipitation of more than just the lactoglobulin fraction. Ashworth & Van Orden (1943) and Menefee et al. (1941) also found this discrepancy when estimating lactoglobulin in heated milk and milk powders.

An explanation of the results described above has been given recently by Rowland (1948). It appears that magnesium sulphate precipitates some of the proteose-peptone fraction of milk protein (probably the proteose moiety) in addition to lactoglobulin and that for this reason, the figures normally given for the lactoglobulin content of unheated milks are about twice their true value. However, the data obtained from the present work show that only a very small proportion of the additional proteose-peptone produced during storage was precipitated with the lactoglobulin. The error in the lactoglobulin results does not affect the lactalbumin plus lactoglobulin values shown in Figs. 16 and 17.

Lampitt & Bushill (1931a) have also investigated the distribution of the different protein constituents between the soluble and insoluble fractions of milk powders of high moisture content, stored at 30°C. They state, contrary to the findings of Supplee & Bellis (1925), that a proportion of the casein always remained soluble after storage, although in one instance the casein decreased from 83.1% of the total soluble protein to only 2.4%. It has been shown in the present work that the casein of a milk powder with a high moisture content can become completely insoluble after prolonged storage at 37°C. Moreover, Lampitt & Bushill (1931a) say that a relatively large proportion of the lactalbumin and lactoglobulin fractions remained soluble. But the technique of their analyses did not differentiate between proteose-peptones and the 'heat-sensitive' proteins and thus soluble proteose-peptones would be reported as lactalbumin plus lactoglobulin.

Bacteriological examination of the stored powders

It has been shown by Higginbottom (1944) and is now generally recognized that the plate counts of both roller- and spray-dried milk powders tend to decrease during storage. Many samples, however, show little or no change in bacterial count after six months or longer. Since the present experiment made available three separated-milk powders of different moisture contents which had been kept in air and in nitrogen at three different temperatures, the opportunity was taken

Table 24. Bacterial counts of the separated-milk powders after storage for 600 days

(The counts of the fresh powders are included for comparison)

Powder	Storage temp. (°C.)	Pack	Plate count / g. of powder	
			3 days at 37°C.	5 days at 30°C.
H	20	Air	104,000	224,000
		Nitrogen	264,000	-
Fresh	-	-	132,500	239,500
M	37	Air	171,000	75,000
		Nitrogen	89,000	98,000
	28.5	Air	94,000	79,000
		Nitrogen	148,000	172,000
	20	Air	122,000	151,000
		Nitrogen	232,000	244,000
Fresh	-	-	180,000	281,000
L	37	Air	179,000	187,000
		Nitrogen	246,000	249,000
	28.5	Air	232,500	246,000
		Nitrogen	274,000	249,000
	20	Air	255,000	238,000
		Nitrogen	228,500	256,500
Fresh	-	-	186,000	290,000

to make a bacteriological examination of the powders after storage for 600 days. Of special interest was the possible influence of the different equilibrium relative humidities of the powders on the survival of the bacteria. It has already been mentioned that the L and M powders remained at their initial equilibrium relative humidities of 17.5 and 29% respectively throughout storage but that the equilibrium R.H. of the H powder increased from about 42 to about 55% after different periods which depended on the storage temperature and the atmosphere in the can.

The H powder stored at 28.5 and 37°C. was not examined since the complete insolubility of the protein made a reliable plate count difficult to obtain. The plate counts were made by Dr C. Higginbottom by the same technique as for the fresh powders (p. 98). The results are recorded in Table 24. Many of the apparent differences in the plate counts shown in the Table are within the experimental error usually recognized in this type of work, but it may be concluded that the abnormally high moisture content of the H powder did not lead to bacterial multiplication during storage at 20°C. There may have been a tendency for a greater survival rate in nitrogen than in air (cf. Nichols, 1939) but the difference was probably very slight.

Examination of the insoluble fraction of a deteriorated H powder

It will be recollected (p. 116 and Fig.15) that the amount of lactose in the H powder apparently

decreased during storage and that the decrease was more rapid at the higher storage temperatures, and more rapid in air than it was in nitrogen. At the same time, the powder gradually became brown in colour. Such discoloration in concentrated milk products has been attributed to caramelization of lactose (Wright, 1924; Kass & Palmer, 1940), but it now appears that it is due in part at least, to a reaction between lactose and protein, probably involving the free amino-groups of the protein and the potential aldehyde group of the lactose (Ramsay, Tracy & Ruehe, 1933; Webb, 1935; Gould, 1945). There were thus two possible explanations for the decrease in soluble lactose, but since it was known that the free amino-groups of the protein of the H powder decreased during storage (p. 131), it was decided to concentrate on the 'protein-lactose' theory. If it could be shown that the insoluble fraction of a deteriorated H powder contained 'combined' lactose, this would be strong evidence of a protein-lactose reaction. Consequently, some of the insoluble material from a badly deteriorated H powder (stored at 37°C. in air) was isolated and analysed.

From Fig. 15, it can be seen that after almost 100 days at 37°C. the soluble lactose of the H powder, whether air- or nitrogen-packed, reached a minimum. So, an H powder which had been stored in air at 37°C. for an arbitrary period of 173 days was used for the preparation of the insoluble material. This deteriorated powder was brown and 'caked'. Its moisture content had decreased from 7.3 to 5.9%, its solubility index at

both 20 and 50°C. was 66 (indicating severe protein insolubility) and its apparent lactose content was 44.8% (calc.as anhydrous) whereas in the fresh powder, the lactose content was 50.6%, i.e. 5.8 g. of anhydrous lactose per 100 g. of powder were unaccounted for. The insoluble material from this powder was obtained in the following way :-

> 150 g. of the powder were ground in a mortar and added to 1 litre of water at 20°C. The mixture was well shaken, centrifuged and the supernatant layer decanted. 1 litre of water was added to the sediment and the mixture shaken and centrifuged as before. Finally, 1 litre of water was added to the remaining sediment and the mixture shaken again; after remaining overnight at 0-4°C., the mixture was re-shaken and centrifuged and the sediment washed 3 times with alcohol, once with ether and placed under continuous vacuum for 30 min. The sediment, a fine brown powder, was dried over P_2O_5. With this treatment, it was probable that the sediment would be contaminated with little, if any, 'soluble' lactose.

The dry sediment was analysed for moisture, protein, ash (including Ca and P) and 'combined' lactose. It was considered that a method of hydrolysis suitable for converting starch to glucose would be sufficient to detach lactose from protein and also to hydrolyse the freed lactose to glucose and galactose. The following method was therefore used (Methods of Analysis of the Ass.off.agric.Chem., 1945) :-

> 3 g. of sediment were boiled for 2½ hr. in 200 ml. of water plus 20 ml. of hydrochloric acid (sp.gr. 1.125, 25% w/w HCl) in a flask fitted with a reflux condenser. The hydrolysate was cooled, nearly neutralized with sodium hydroxide solution, and its volume made up to 250 ml. The solution was then filtered and the amount of reducing sugar in

Table 25. Composition of the insoluble fraction of a deteriorated H Powder (air-pack, stored for 173 days at 37°C.)

Constituent	%	Moisture-free %
Moisture	5.1	0.0
Protein (N x 6.38)	77.4	81.5
Ash	7.0	7.4
Lactose (by difference)	10.5	11.1
Lactose (determined, calc. as hydrated)	8.1	8.5
Calcium	2.17	2.29
Phosphorus	1.29	1.36
Ca/P ratio	1.68	

50 ml. aliquots measured by the Munson-Walker gravimetric method (loc.cit.) which involves the reduction of Fehling's solution or Soxhlet's modified solution and the weighing of the precipitated Cu_2O. It was obvious that a fairly substantial amount of reducing sugar was present in the hydrolysate.

On the assumption that the reducing sugars present were glucose and galactose in equimolecular amounts, the percentage of combined lactose (calc. as hydrated) in the insoluble sediment was found to be 8.1, and on a moisture-free basis, 8.5. The composition of the insoluble material is given in Table 25. It will be noted that the lactose values obtained by difference were higher than those obtained by direct estimation. Reasons for this may be that the combined lactose was not completely detached from protein by the acid hydrolysis and possibly some sugar was decomposed during the reaction. In addition, the gravimetric method of estimating reducing sugars is not capable of the highest accuracy in the presence of extraneous substances such as were present in the hydrolysate. Therefore, the lactose value obtained by difference will be regarded as the true percentage of 'combined' lactose in the insoluble sediment. The significance of the calcium and phosphorus values and the Ca/P ratio will be mentioned in Part IV (p. 165).

Taking the lactose content of the moisture-free insoluble material to be 11.1%, and knowing the solubility index of the powder to be 66 (i.e. 34 g. of insoluble solids per 100 g. of milk solids), it was calculated that in 100 g. of the '173 days' H Powder,

approximately 3.5 g. (3.3 g. as anhydrous) of the total lactose were combined with protein in the insoluble fraction of the powder. But it has already been shown that the lactose content of this powder apparently decreased from 50.6 to 44.8% which meant that 5.8-3.3 = 2.5 g. of lactose per 100 g. of powder were still unaccounted for. Two explanations can be put forward: first, since it seems that the protein-lactose complex is soluble when first formed (Henry et al., 1948) and becomes insoluble due to secondary reactions, a fraction of the protein-lactose complex may have been still soluble in the '173 days' H powder and would be dissolved and discarded during the isolation of the insoluble material; secondly, as suggested above, some of the lactose may have been caramelized or otherwise partially decomposed during storage at 37°C. with the formation of traces of formic and lactic acids. Thus, although the decrease in the lactose content of the H powder was not explained quantitatively, the object of this experiment was attained, viz to show the presence of lactose in the washed insoluble fraction of the powder.

Work of the collaborators

The results of an examination of the equilibrium relative humidities of the powders have already been mentioned. Dr Lea also made a more detailed examination of the protein-sugar reaction and showed that there was a decraase in the free amino-nitrogen of the protein and an increase in the amount of combined lactose

as storage progressed. The reducing power of the powders on potassium ferricyanide and their changes in base-binding capacity and formal titration were also investigated.

Rat-feeding tests conducted by Drs Henry and Kon showed that the biological value of the proteins of the H powder decreased during storage and that this was due mainly to a loss, or more correctly 'inactivation' of lysine, as the decrease in free amino-nitrogen suggested. Further proof that lysine was the amino-acid chiefly involved in the protein-lactose reaction was obtained by microbiological assay of the 'essential' amino-acids in stored powders; it is probable that some loss or inactivation of histidine also occurred. From a practical point of view, it should be noted that the H powder was unpalatable long before its decreased nutritive value became apparent.

DISCUSSION

One of the most outstanding findings in this experiment was the extremely rapid deterioration of the H powder at the two higher storage temperatures compared with the same powder at 20°C. and the M and L powders at the three storage temperatures. After more than three years storage, the only important change in the M and L powders was decreased palatability accompanied by some absorption of oxygen and production of carbon dioxide. In contrast, the extra 2.6% of moisture in

the H Powder caused general chemical and physical deterioration, especially at the higher storage temperatures.

Absorption of oxygen, production of carbondioxide and palatability

It has been emphasized that two types of 'off'-flavour were detected depending on whether the powders were packed in air or in nitrogen. In air, the 'off'-flavour was described as 'cardboardy' or 'gluey', and in nitrogen, as 'heated' or 'cooked', the latter flavours being less unpleasant than the former. The protective effect of gas-packing for all the powders at the three storage temperatures suggested that an oxidative reaction took place in the air-packed cans and that this reaction was mainly responsible for the 'cardboard' flavour and hence for the more rapid decline in palatability of the powders. The H powder absorbed oxygen very rapidly at the higher storage temperatures and simultaneously produced carbon dioxide with the result that the oxidative defect was especially pronounced in this powder. Fig. 11 shows that carbon dioxide was also produced in the nitrogen-packed H powders and that production of carbon dioxide was even detectable in the M and L nitrogen-packed series. It is interesting to note that after all the oxygen was absorbed in the air-packed H powder, carbon dioxide was still produced but at the less rapid rate of the nitrogen-packed powder.

These facts indicate that probably two

deteriorative reactions took place in the powders depending on the atmosphere in the cans, viz an oxidative reaction(s) accompanied by liberation of carbon dioxide and an 'anaerobic' decomposition(s) with production of carbon dioxide. In the air-packed powder, both reactions were obviously occurring simultaneously but in the absence of oxygen, only the 'anaerobic' reaction could take place. The data for gas exchange in the air-packed powders showed that the carbon dioxide evolved did not compensate for the oxygen absorbed. For example, when the H powder (at 37°C.) had absorbed 42.4 mg. of O_2 per 100 g. of milk solids, the evolved CO_2 only accounted for 10.5 mg. of O_2; the remainder of the oxygen must have gone to the formation of oxidative products. However, the quantities of oxygen and carbon dioxide involved in these reactions were extremely small in proportion to the amount of possible reactants present (protein, lactose and trace of fat).

The evidence now available (Henry et al., 1948) suggests that both the absorption of oxygen and the production of carbon dioxide were related to the decomposition of a protein-lactose complex. The thermal or spontaneous decomposition of this complex was probably the source of the carbon dioxide in the gas-packed cans. In the oxygen-packed cans, there was probably oxidation of the decomposing protein-sugar compound with evolution of much carbon dioxide. In addition, some of the oxygen may have reacted with

protein or the trace of fat in the powders and this usage of oxygen may explain the relatively too rapid absorption of oxygen (with little production of carbon dioxide) by the M and L powders in which the protein-lactose reaction was very slow. There is also the possibility that some of the carbon dioxide was produced during a protein-catalysed caramelization of lactose.

The 'stale and glue-like' 'off'-flavours developed by the air-packed powders were most likely caused by the products of oxidation and decomposition of the protein-sugar compound, the protein-sugar compound itself and possibly caramelized lactose, in this order of importance. The more pleasant 'off'-flavours which were produced in the gas-packed powders were probably compounded of the flavours of the protein-lactose compound, its non-oxidative decomposition products and caramelized lactose. It was therefore the products of the oxidative reaction(s) which caused the 'off'-flavours of an air-packed powder to be more distasteful than those of a corresponding gas-packed powder. There is also the possibility that other reactions involving protein contributed to the 'off'-flavours of the powders.

Colour and pH

The brown discoloration and decrease in pH of the H powder undoubtedly involved lactose directly and indirectly. The discoloration of evaporated milk during sterilization has been considered due to a

protein-lactose reaction (Ramsey, Tracy & Ruehe, 1933; Webb, 1935) and perhaps to a protein catalysed caramelization of lactose (Wright, 1924; Kass & Palmer, 1940). In this experiment with milk powder, it has been shown (Henry et al., 1948) that $\frac{1}{2}$ - $\frac{3}{4}$ of the total free amino-nitrogen can combine with lactose before any appreciable change in colour occurs. It appears that the protein-lactose complex first formed is not coloured but becomes so on degradation. This reaction, possibly in conjunction with caramelization of lactose, was probably responsible for the 'non-enzymatic' browning.

The fact that a protein-lactose complex or compound is formed involving the blocking of the basic, free amino-groups of lysine residues, would itself cause the pH of the powder to decrease. But it has been shown recently that appreciable amounts of formic and lactic acids are produced, presumably from lactose, when milk is heated under sterilizing conditions (Gould, 1945). The caramelization of pure sugar is known to cause a fall in pH. To sum up, the decrease in pH of the H powder, as in strongly heated liquid milk (Gould & Frantz, 1945), was probably due to decomposition of lactose with the formation of volatile organic acids and to the blocking of free amino-groups by the protein-lactose reaction. The fact that the amount of combined lactose in deteriorated H powder did not account for the decrease in soluble lactose (p. /3/) suggests that a proportion of the lactose did decompose.

Solubility and partition of soluble 'nitrogen'

It has already been pointed out (p. 119) that the fresh powders differed appreciably from fresh milk in possessing a relatively lower lactalbumin plus lactoglobulin, and a slightly higher apparent casein and proteose-peptone content, these changes presumably being a result of the pre-heating of the liquid milk. As deterioration of the H powder progressed during storage, most of the casein and lactalbumin plus lactoglobulin became insoluble. A small amount of proteose-peptone was produced, of which part at least arose from decomposition of casein, but the absence of any increase in non-protein nitrogen indicated that no drastic fragmentation of the protein molecules had occurred. In fact, in a very badly deteriorated H powder (stored for 400 days in air at 37°C.), the amount of soluble non-protein nitrogenous compounds had decreased by about one third probably because some of the constituents of the N.P.N. fraction had reacted in such a way as to form part of the insoluble material. During the experiment, it was also found that the method of estimating lactoglobulin was not satisfactory and that it became less so the more deteriorated was the milk product analysed. It seems probable, as suggested by Rowland (1948), that some proteose-peptone is precipitated with lactoglobulin in the salting-out procedure although very little of the new proteose-peptone material produced during storage was so precipitated.

The similarity of the curves showing the

Table 26. Relationship between solubility of the protein and total solids of the air-packed H powder stored at 37°C.

Days stored	g. per 100 g. of reconstituted, centrifuged milk		Protein N as % of initial protein N	Total solids as % of initial total solids
	Total N minus N.P.N. (i.e. soluble protein N)	Total solids		
Uncentri-fuged	0.470	9.27	100	100
0	0.466	9.16	99.2	98.8
4	0.464	9.12	98.7	98.4
7	0.463	9.12	98.5	98.4
10	0.455	9.05	96.8	97.6
15	0.449	8.87	95.5	95.7
18	0.399	8.42	84.9	90.8
24	0.258	7.81	54.8	84.3
28	0.219	7.62	46.6	82.2
45	0.132	6.94	28.1	74.9
63	0.100	6.55	21.3	70.7
85	0.079	6.30	16.8	67.9
129	0.070	6.27	14.9	67.6
400	0.009	5.81	1.9	62.7

decrease in solubility of the H powder (Fig.12) and the decrease in solubility of the casein (Figs. 16 & 17) indicates that increasing insolubility of this protein was mainly responsible for the loss of solubility of the powder. The data collected in Table 26 show the relationship between solubility of the protein and the total solids of the H powder stored at 37°C. If only protein became insoluble, the lowest value for the solubility of the milk solids would be 100 - 34.5* = 65.5%. But it has already been shown(Table 25) that lactose and ash constitute part of the insoluble material and thus the seriously deteriorated '400 days' powder had a solubility of less than 65.5%, namely 62.7%.

It is not yet clear how far the protein-lactose reaction influences solubility but it seems that a considerable proportion of the free amino-groups of the protein molecule can combine with lactose without appreciably affecting the solubility of the protein (Henry et al., 1948). It is possible that insolubility is a result of an induced denaturation of the protein molecule, or of degradation of the protein-sugar complex by secondary reactions between the now adjacent protein molecules and carboydrate chains.

Interrelation of moisture content and crystallization of lactose

It has been shown that physical and chemical changes involving lactose, which constitutes over 50%

* The H powder contained 34.5 g. of protein per 100 g. of milk solids (Table 11).

of a separated-milk powder, were the principal causes of the deterioration of the H powder. The protein-lactose reaction has already been mentioned but it remains to discuss the phenomenon whereby the crystallization of lactose, by increasing the activity of the water or moisture in the H powder, aggravated the deleterious effect of an already high moisture content. Supplee (1926) has also noted this effect in milk powders; he showed that when a milk powder was exposed to an atmosphere of 50% relative humidity, moisture was first absorbed and then given up. Troy & Sharp (1930) confirmed this observation and they found that by the time the moisture had been 'freed', the powder had 'caked'. These authors have given a reasonable explanation of these facts. They say that in normal spray-dried milk powder, the anhydrous lactose is present as a very concentrated syrup or 'glass' and can be regarded as a solution with a very high osmotic pressure and a very low vapour pressure. (The M and L powders had the low equilibrium relative humidities of 29 and 17.5% respectively.) Such a 'solution' of lactose is so concentrated that the lactose cannot crystallize and will not do so if the moisture content is kept low. But this concentrated 'syrup' is very hygroscopic and when a powder is exposed to a moist atmosphere (as in the preparation of the H powder), water is absorbed which dilutes the lactose solution and tends to make the powder particles stick

to each other. Thereafter, the α- lactose (c.42% of the total lactose) crystallizes at a rate which increases with the temperature of storage.

Although when the crystals of the α- hydrate form (so binding or caking the powder) some water is bound as water of crystallization, the separation of the solid phase causes a dilution of the syrup with the result that its osmotic pressure decreases and its vapour pressure increases. The net result is a decrease in moisture* content and a rise in equilibrium relative humidity as was found for the H powder. Dr Lea showed that lactose commenced to crystallize in the H powder only after definite 'induction periods' of about 1 day at 37°, 10 days at 28.5° and 100 days at 20°C.; further, for some unknown reason, crystallization was slightly delayed in nitrogen compared with air (Henry et al., 1948). The resulting delays in the rise of equilibrium relative humidity at each storage temperature and the differences between air- and gas-packed cans were probably partly responsible for the induction periods observed in absorption of oxygen and production of carbon dioxide, decrease in solubility and pH, and conversion of β- lactose to α- lactose.

Such a mutarotational change as the conversion of β- lactose to α- lactose is known to be catalysed by

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* Moisture is regarded as adsorbed water only in contradistinction to the 'bound' water of crystallization or other bound water.

moisture and heat but this change was much slower than the crystallization of the α- lactose to the hydrate. It would seem practically certain that the α- lactose formed from the β- lactose would also take up water of crystallization yet is has been shown that the H powder reached a stable equilibrium relative humidity of 55% long before all the β- lactose was converted (Henry et al., 1948). It may be that the fall in concentration of 'free' water in the powder and possibly the hygroscopic nature of the protein prevented any further rise in equilibrium relative humidity.

It is thus apparent that by all criteria, the moisture content of the powders was the most important factor in determining storage life, the L (2.9%) and M (4.7%) powders being relatively stable with a definite advantage in favour of the former, while the H (7.3%) powder was very unstable. Part of the large difference in behaviour between the H powder and the others can be attributed to the fact that the deleterious effect of an already excessive moisture content was aggravated by the increased 'activity' of the water caused by crystallization of the lactose. It is not yet possible to explain fully why the presence of extra water initiated some of the physico-chemical changes in the high moisture powder; it may be simply that the solvent action of the water allowed reactive groups to come into contact.

SUMMARY AND CONCLUSIONS

1. Three spray-dried separated-milk powders with moisture contents of 2.9, 4.7 and 7.3% were packed in air and in almost pure nitrogen, in gas-tight cans, and stored at 20, 28.5 and 37°C. for over 3 years.

2. The powders were examined at intervals for palatability, colour, pH, absorption of oxygen, production of carbon dioxide, solubility in water at 20° (two methods) and 50°C., moisture content, relative amounts of α- and β- lactose, soluble lactose, distribution of soluble 'nitrogen', bacterial content and composition of insoluble material.

3. Little change was observed in the powders of low and medium moisture content except in palatability and gas exchange at the higher temperatures. The powder of the highest moisture content, particularly at the higher storage temperatures, rapidly became unpalatable, discoloured and insoluble.

4. The pH and soluble lactose content of the high moisture powder fell and evidence of a reaction between protein and lactose was obtained. The α- lactose crystallized causing the powder to 'cake', and its moisture content to decrease. There was a slow conversion of β- lactose to α- lactose. Oxygen was absorbed and carbon dioxide produced.

5. The loss of solubility of the high moisture powder was largely caused by a decrease in the solubility of the casein. The lactalbumin plus lactoglobulin fraction gradually became insoluble and proteose-peptones were produced.

6. The temperature coefficient (for a range of 8.5°C.) for the deteriorative reactions was high and approximated to 6 for those responsible for deterioration in palatability. Thus moisture contents which can be tolerated under temperate conditions for long periods will be unsatisfactory at high storage temperatures.

7. With the exception of the changes in palatability, the rate of deteriorative reactions was influenced only slightly by the nature of the atmosphere in the container.

8. It is probable that the protein-lactose reaction was responsible for the production of 'off'-flavours described as mainly 'cooked' or 'caramelized' in the case of nitrogen-packed powders and, in conjunction with oxidative reactions, for the 'stale' and 'gluey' 'off'-flavours in air-packed powders.

9. An oxidative reaction (or reactions) was probably responsible for the 'off'-flavours produced in the low and medium moisture powders after long periods of storage. It is possible that the small amount of fat present was involved.

10. Nitrogen-packing was decidedly advantageous in

delaying the development of 'off'-flavours in all the powders at the three storage temperatures.

11. A moisture content of about 4% would seem to be a safe maximum for separated-milk powder stored under temperate conditions.

PART IV

The Solubility of Milk Powders

Composition of the soluble and insoluble portions of 'reconstituted' milk powders

Introduction

It is important that milk powder should be capable of being easily dissolved or dispersed in water without leaving an insoluble sediment. Many methods of measuring this property, usually termed the 'solubility' of the milk powder, have been proposed. In all of them the powder is shaken or stirred with water and the reconstituted 'milk' centrifuged to sediment the insoluble material; thereafter the methods differ considerably but most fall into one of two groups. Methods in the first group depend on the measurement of the soluble total solids or of protein in the dispersed phase. These methods include those of Supplee & Bellis (1925), Hunziker (1926), Wright (1932), Howat et al. (1939) and Parsons (1947)*. The second group consists of methods which determine the weight or the volume of the insoluble, or more properly, non-dispersed sediment. Of this group, the methods of Marquardt (1920), Lampitt & Hughes (1924), Lampitt & Bushill (1931a), Cone & Ashworth (1947) and that advocated by the American Dry Milk Institute (Grading of Nonfat Dry Milk Solids, 1948) should be mentioned.

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* This method has not yet been published. It is described briefly on p.151.

But different results are often obtained when the various methods are used to measure the solubility of a milk powder and although arguments can be propounded in favour of the methods in each of the two groups mentioned above, it appeared that a better understanding of the way in which the major constituents of milk powders behaved when 'reconstituted' and centrifuged was essential to a correct evaluation of the merits of the available methods. It was with this object in view that the present work was done.

Six whole-milk powders, two manufactured by the roller process and four by different methods of spray drying, were reconstituted under standard conditions and separation of the fat and insoluble material accelerated by centrifuging. (These samples have been taken to represent some of the different types of powder which are at present manufactured, but they are not necessarily typical of the particular type of plant on which they were prepared.) When these powders were reconstituted and centrifuged, three layers were obtained, a top or fat layer, a middle or liquid layer and a bottom or sediment layer. These layers were analysed for the major milk constituents. Because one aim of good manufacture is to prepare as soluble a powder as possible, it was obviously important to examine the sediment fraction in greater detail. To do this, five of the whole-milk powders and five separated-milk powders were used and a sufficient bulk of sediment obtained by passing large

Table 27. Composition of the experimental whole- and separated-milk powders

Powder	g. per 100 g. of powder							g. per 100 g. of milk solids					
	Moisture	Protein	Fat	Ash	Lactose	Ca	P	Protein	Fat	Ash	Lactose	Ca	P
Whole-milk													
Roller A	2.5	25.6	28.4	6.7	36.8	0.97	0.76	26.3	29.1	6.9	37.7	1.00	0.78
Roller B	2.5	26.7	28.2	7.2	35.4	0.93	0.78	27.4	28.9	7.4	36.3	0.95	0.80
Kestner	2.2	32.3	27.1	6.4	32.0	0.92	0.76	33.1	27.7	6.5	32.7	0.94	0.78
'Klim'	2.1	26.3	28.0	7.1	36.5	0.91	0.74	26.9	28.6	7.2	37.3	0.93	0.76
Krause	2.1	26.9	28.3	6.3	36.4	0.93	0.77	27.5	28.9	6.4	37.2	0.95	0.79
Milkal	1.6	27.9	26.4	6.7	37.4	0.96	0.77	28.4	26.8	6.8	38.0	0.98	0.78
Separated-milk													
Roller	2.9	35.0	1.4	8.0	52.7	1.26	0.96	36.0	1.5	8.2	54.3	1.30	0.99
Gray-Jensen	2.1	32.7	2.0	7.7	55.5	1.27	0.96	33.4	2.0	7.9	56.7	1.30	0.98
Kestner	3.9	34.8	1.2	7.5	52.6	1.26	1.00	36.2	1.3	7.8	54.7	1.31	1.04
Krause	2.9	35.5	1.4	8.5	51.7	1.26	1.00	36.5	1.4	8.8	53.3	1.30	1.03
Milkal	3.1	36.6	0.8	8.1	51.4	1.29	1.01	37.6	0.8	8.3	53.3	1.33	1.04

volumes of the reconstituted milks through a Sharples super-centrifuge (14,500 x g). The sediments were washed, dried and analysed. The 'solubility' of each of the powders was measured by methods representative of the two groups and the validity of the results assessed.

EXPERIMENTAL

Each powder was first analysed for moisture, protein (total nitrogen x 6.38), fat and ash. The sum of these in percentage deducted from 100 was assumed to be lactose. The amount of calcium and phosphorus in the powders was also determined. There were no appreciable differences in the composition of the whole-milk powders or in that of the separated powders; they were all normal (Table 27).

(a) Analysis of fat and liquid layers. The powder (4 g.) was weighed into a 50 ml. centrifuge-tube of known weight and reconstituted with 36 ml. of distilled water. In one series of experiments, the tube was held at 20°C. and in a second series at 50°C. for 5 min. It was then shaken for 1 min., cooled where necessary to 20°C. and centrifuged at 3,000 r.p.m. (1,800 x g) for 15 min. This method of reconstitution, using 1 g. samples, has already been described in detail (Howat et al., 1939). After centrifuging, the tube was allowed to stand for 1 hr

at 4°C. to harden the top layer which was then removed as completely as possible and weighed. The liquid layer was siphoned off and its weight found. Any residual fat on the walls of the tube was removed on a tared filter paper and its weight added to that of the top layer. The tube was weighed again and the weight of sediment found. In order to provide sufficient material for analysis, eight tubes of the same powder were used in each determination and the fat and liquid layers bulked separately. Water, total nitrogen, fat and ash were determined on each of the bulked layers and lactose calculated by difference. The ash of each layer was analysed for calcium and phosphorus. The composition of the sediments, in view of the very small amounts obtainable from spray-dried powders by this method of reconstitution, was calculated from the composition of the fat and liquid layers and of the original powders. Sufficient sediment for analysis was obtained from the roller-dried powders and the results agreed well with the data obtained by calculation.

(b) Analysis of sediments. 1 kg. of each powder, reconstituted at 20°C. in the same proportions and in a similar way to the smaller samples, was run through the clarifier bowl of the Sharples super-centrifuge. This exposed the reconstituted milk to a field of 14,500 x g for about 5 min. and gave a highly compressed sediment. The sediment was removed, mixed with 200

ml. of distilled water at 20°C., shaken for about 1 min. and spun for 30 min. in the batch bowl of the Sharples. The mixing and washing was repeated twice again. The sediment was removed from the bowl and dehydrated by successive washings with 30, 50, 80 and 100% (v/v) ethanol. It was spun for 15 min. with each concentration of alcohol. Finally the sediment was dried *in vacuo* at room temperature over phosphorus pentoxide, allowed to reach moisture equilibrium by exposure to the laboratory air for two days and analysed for moisture, total nitrogen, fat, ash, calcium and phosphorus. Even with this treatment some small amount of occluded middle layer may have remained in the sediment and any difference between the percentage sum of the constituents determined and 100 has been reported as lactose.

(c) *Solubility of the powders.* The solubility of the six whole-milk powders in water at 20 and 50°C. was measured by four methods, namely those of Howat *et al.* (1939), Lampitt & Hughes (1924), Parsons (1947) and the American Dry Milk Institute (Grading of Nonfat Dry Milk Solids, 1948). The methods of Howat *et al.* and Parsons belong to the group in which the soluble or dispersible solids of the powder are measured and the methods of Lampitt & Hughes and the American Dry Milk Institute are representative of the other type of method in which the weight or the volume of the insoluble fraction of the powder is determined.

The method of the American Dry Milk Institute measures the volume of sedimented material and thus can only give a comparative index of solubility. The other three methods enable the percentage solubility of the milk solids to be calculated. Each of the four methods specifies different methods of reconstituting and centrifuging powders but in order to compare the methods, it was essential to use the same experimental conditions for each. This was not possible for the American Dry Milk Institute's method and the modification of this method already described in detail in Part I, p.22 was used. In it the volume of the once-washed sediment from 20 g. of powder is measured. For the other methods the experimental conditions specified by Howat et al. (1939) were adopted, i.e. a powder to water ratio of 1 to 9 was used, the powder was shaken with the water for 1 min. by hand and the reconstituted milk was centrifuged for 15 min. at 3000 r.p.m. (1800 x g).

The method of Howat et al. (1939) is fully described in Part I, p. 20 . To recapitulate briefly, the procedure in this method is that after the reconstituted milk is centrifuged, the top and middle layers are poured off and the solids content of the combined layers measured after redispersing the top (fat) layer. The ratio of this value, i.e. the amount of soluble solids, to the total dry solids initially present in the tube, expressed as a percentage, is taken as the solubility of the milk

Table 28. Weight and composition of the top (fat) layers obtained by reconstituting and centrifuging 100 g. of the milk powders in 900 ml. of water at 20 and 50°C.

(The values were calculated from those obtained by reconstituting 4 g. powder in 36 ml. water.)

Powder :	Roller A			Roller B			Kestner			'Klim'			Krause			Milkal		
Constituent	Actual composition (g.)	% comp.	% comp. (dry wt)	Actual composition (g.)	% comp.	% comp. (dry wt)	Actual composition (g.)	% comp.	% comp. (dry wt)	Actual composition (g.)	% comp.	% comp. (dry wt)	Actual composition (g.)	% comp.	% comp. (dry wt)	Actual composition (g.)	% comp.	% comp. (dry wt)
At 20°C.	63	100	100	33	100	100	26	100	100	19	100	100	24	100	100	25	100	100
Water	37.2	59.1	-	8.7	26.4	-	17.2	66.1	-	9.2	48.6	-	11.3	47.0	-	12.0	48.1	-
Protein (Nx6.38) *	4.3 (0.31)	6.8	16.6	0.7 (0.21)	2.0	2.7	2.1 (0.41)	8.1	23.9	0.9 (0.25)	4.6	8.9	0.9 (0.32)	3.9	7.4	0.9 (0.36)	3.5	6.7
Fat *	19.5 (0.08)	30.9	75.6	23.5 (0.04)	71.1	96.7	5.5 (0.32)	21.2	62.6	8.6 (0.20)	45.2	87.9	11.3 (0.21)	47.0	88.7	11.9 (0.20)	47.5	91.5
Ash *	0.6 (0.20)	0.97	2.4	0.1 (0.06)	0.38	0.5	0.2 (0.09)	0.88	2.6	0.1 (0.07)	0.75	1.6	0.1 (0.07)	0.49	0.9	0.1 (0.09)	0.51	1.0
Lactose(diff.) *	1.4 (0.15)	2.2	5.4	0.0 (0.34)	0.1	0.1	1.0 (0.77)	3.7	10.9	0.2 (0.39)	0.8	1.6	0.4 (0.47)	1.6	3.0	0.1 (0.51)	0.4	0.8
At 50°C.	25	100	100	31	100	100	11	100	100	25	100	100	27	100	100	23	100	100
Water	7.6	30.4	-	6.4	20.8	-	5.3	48.4	-	8.3	33.3	-	11.9	44.1	-	8.2	35.5	-
Protein (Nx6.38) *	0.5 (0.13)	2.2	3.1	0.3 (0.18)	0.8	1.0	0.5 (0.16)	4.8	9.3	0.7 (0.25)	3.0	4.5	1.1 (0.34)	3.9	7.0	0.6 (0.24)	2.7	4.2
Fat *	16.7 (0.04)	66.9	96.2	24.2 (0.03)	78.2	98.8	4.9 (0.14)	44.3	85.9	15.4 (0.10)	61.5	92.2	13.6 (0.20)	50.4	90.2	13.8 (0.12)	60.0	93.0
Ash *	0.1 (0.05)	0.34	0.4	0.1 (0.05)	0.19	0.2	0.1 (0.04)	0.70	1.3	0.1 (0.06)	0.32	0.5	0.1 (0.09)	0.56	1.1	0.1 (0.06)	0.37	0.6
Lactose(diff.) *	0.1 (0.30)	0.2	0.3	0.0 (0.26)	0.0	0.0	0.2 (0.21)	1.8	3.5	0.5 (0.36)	1.9	2.8	0.3 (0.48)	1.0	1.7	0.3 (0.35)	1.4	2.2

* Figures in brackets are amounts contributed by entrained middle layer.

Table 29. Weight and composition of the sediment layers obtained by reconstituting and centrifuging 100 g. of the milk powders in 900 ml. of water at 20 and 50°C.

(The values were calculated from those obtained by reconstituting 4 g. powder in 36 ml. water.)

Powder	Roller A						Roller B		
Temperature of reconstitution :	20°C.			50°C.			20°C.		
Constituent	Actual composition (g.)	% comp.	% comp. (dry wt)	Actual composition (g.)	% comp.	% comp. (dry wt)	Actual composition (g.)	% comp.	% comp. (dry wt)
	200	100	100	169	100	100	192	100	100
Water	168.3	84.1	-	140.5	83.1	-	172.8	90.0	-
Protein (Nx6,38)	15.4 (1.4)*	7.7	48.4	12.2 (2.4)*	7.2	42.6	9.0 (4.1)*	4.7	47.0
Fat	7.4 (0.4)	3.7	23.3	7.6 (0.8)	4.5	26.6	1.6 (0.7)	0.8	8.0
Ash	2.4 (0.9)	1.2	7.5	1.8 (0.9)	1.1	6.5	1.8 (1.3)	0.9	9.0
Lactose (diff.)	6.5 (6.9)	3.3	20.8	6.9 (5.6)	4.1	24.3	6.8 (6.7)	3.6	36.0

* Figures in brackets are amounts contributed by entrained middle layer.

powder solids. In the method of Lampitt & Hughes (1924), the reconstituted milk is centrifuged, the top layer discarded and the middle layer poured off and its solids content found. The weight of the wet sediment in the tube is obtained by difference and then the sediment is dried to obtain the weight of solids and water in it. From the weight of the dry sediment is subtracted the weight of soluble solids contributed to the sediment by the permeating middle layer. Thus the weight of insoluble material derived from 100 g. of powder and hence the percentage solubility of the powder or milk solids can be calculated. In Parsons' (1947) method, the reconstituted milk is centrifuged, the top layer discarded, the sediment redispersed into the middle layer and the percentage of solids (a%) in the now homogeneous liquid measured, i.e. soluble plus insoluble solids. The 'milk' is centrifuged again to re-sediment the insoluble material and the solids content (b%) of the supernatant liquid layer measured, i.e. soluble solids. The solubility of the milk powder solids is calculated by expressing (b) as a percentage of (a), i.e. $\frac{100 \times b}{a}$.

RESULTS

Tables 28 and 29 give the weight and composition of the top layers and of the sediment layers when sufficient of the latter was obtained for analysis. Table 30 shows the distribution of the major constituents of the powders among the three

layers. In this Table the amount of each constituent appearing in each of the three layers is expressed as a percentage of the total amount in the reconstituted milk, and since the figures for the sediments were obtained by difference, each horizontal line totals 100. Negative values shown for some of the sediment constituents at 50°C. give an indication of the experimental errors inherent in the technique employed; these errors only became apparent when very small sediments were obtained. The partition of calcium and phosphorus between the layers is also given in Table 30, with figures in brackets to indicate the percentage of each contributed by the liquid entrained in the top and sediment layers, assuming such liquid to have the same composition as that of the middle layer. Thus the top layer of the reconstituted Roller-Dried Powder A contained 14.4% of the total calcium, but because 1.4% was contributed by the entrained liquid, the true proportion of calcium in the top layer was 13.0% of the total.

Table 31 shows the composition (as percentages) of the dry sediment produced in the Sharples super-centrifuge from five of the same whole-milk powders as reported in Table 30 and also from five separated-milk powders, four of which were manufactured in the same type of plant as four of the whole-milk powders. The calcium and phosphorus content of the ash of the original powders is included for comparison with that of the washed sediment ash.

Table 32 records the values for solubility of the six whole-milk powders as determined by four methods representing the two groups previously mentioned; the volume of unwashed sediment obtained in the method of Howat et al. (1939) is included for comparison.

DISCUSSION

General considerations. When whole-milk powder is reconstituted in water a number of physical changes take place concurrently. Lactose and the soluble salts go into true solution, fat is liberated from the particles and the protein which has not been denatured and made insoluble dissolves or becomes redispersed into a stable suspension. Where protein deterioration has taken place, as a result either of overheating during manufacture or bad storage conditions, the affected protein does not remain in suspension but slowly sediments. This sedimentation of protein is noticeable with almost all roller-dried powders but only to a much lesser extent with spray powders.

Another point of difference between roller- and spray-dried powders is in the behaviour of the fat when the two types of powder are reconstituted in warm water. The fat globules from roller powder are much larger than those from spray-dried powder and they rise to the surface at a much greater rate, joining with others either on the way or at the

surface to form an obvious layer of liquid fat. Any tendency which the fat in a reconstituted spray powder has to rise to the surface is more akin to the creaming of fresh milk than to fat separation per se. It would appear that the protein membrane normally surrounding the fat globules in liquid milk is disrupted to a much greater extent in roller-drying than in spray-drying. King (1948) has suggested that during roller-drying heat denaturation of the protein membrane surrounding each fat globule and also possibly mechanical fracture of the membrane by minute lactose crystals allow the globules to coalesce when the powder is reconstituted and so prevent the formation of a fine emulsion. In the spray-drying process there is less likelihood of the membrane being denatured by heat and it is well known that in properly made and stored spray powder the lactose exists as an amorphous 'glass' whereas in roller-dried powders it is mainly crystalline.

When reconstituted milk is centrifuged, the fat and non-dispersed material separate quickly and three fractions can be easily distinguished; the top containing a high proportion of fat, the sediment with a high proportion of protein and a liquid middle layer. In practice, the middle layer permeates the top and bottom layers so that soluble constituents are present in both. Moreover, the 'insolubility' of the reconstituted powder, or more properly, the amount of non-dispersible protein which may vary physically from

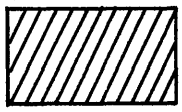
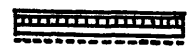
Powder	Temp. of reconstitution (°C.)	Thickness (mm.)	Appearance (actual size)	Description
Roller A	20	13	(1) 	Homogeneous but coarse in 'grain'; white in colour, no visible 'free' fat.
	50	2	(2)	Similar to top no.(1); two very thin layers underneath, the top one cream coloured & the bottom one white.
Roller B	20	5	(3) 	Three bands: Top, similar in appearance to no.(1); middle, clear yellow mobile fat; bottom, compounded of two very thin layers as described in (2)
	50	4	(4) 	Top band similar to no.(1); which merges into yellow fat (not clear & free from protein as at 20°C.)
Kestner	20	6	(5) 	Bulk similar to top no.(1); very thin 'skin' on top of white protein-material; trace of loose white material underneath.
	50	2	(6)	Thin wafer of white protein-like material on surface; remainder like cream.
'Klim'	20	2	(7) 	Top wafer of white protein-like material; bulk like cream; indefinite yellow creamy band underneath.
	50	2	(8)	Similar to top no.(6).
Krause	20	3	(9) 	White protein-like material on top; yellow cream layer merging into another layer of white material with a layer of loose cream-like material underneath.
	50	2.5	(10)	Similar to top no. (6).
Milkal	20	3	(11) 	Similar to top no. (9).
	50	2.5	(12)	Similar to top no. (6).

Fig. 18. Appearance of cross-section of the top layers after centrifuging 4 g. of the powders in 36 ml. water at 1,800 x g in tubes 23 mm. in diameter.

an almost granular to a flocculent form, considerably affects the composition of the fat and sediment layers. If the powder is relatively 'insoluble' and contains a fair proportion of denatured protein, there is an increased chance that as the fat rises to the surface it will carry with it some insoluble protein. Conversely, fat globules may be trapped by the large bulk of insoluble protein as it sediments. If at the same time the fat globules are large and the degree of protein denaturation high, as in some roller powders, both types of entrainment will have a greater chance of occurring. In addition, some fat may never escape from particles of powder because of a covering of insoluble protein. These undissolved particles will probably sediment if they contain more protein than fat and rise to form part of the top layer if they contain more fat than protein.

Milk powder is usually reconstituted by some form of shaking or mechanical agitation and often a considerable amount of froth is produced which may consist mainly of partially denatured protein (analogous to the process of 'whipping'). When such a liquid with supernatant froth is centrifuged, the froth collapses and some non-fatty material may remain on the surface layer. In Fig.18 an attempt has been made to depict the appearance in cross-section of the top layers of the centrifuged, reconstituted powders. The top layers of the spray-dried powders were all covered with a thin paper-like layer of collapsed

foam whereas the upper portion of the roller-dried top layers was a white, homogeneous, grainy plug. It is of interest to record that during reconstitution the spray-dried powders frothed much more than the roller-dried powders. It should be mentioned that the top layers from powders reconstituted at 20°C. often possessed a diffuse base of cream-like consistency whereas at 50°C., the demarcation of the top layer from the liquid or middle layer was sharp. The Krause and Milkal powders reconstituted at 20°C. appeared to have a top layer comprised of four parts, with a second protein-like layer interposed between two cream layers.

To summarize therefore, both the top 'fat' layer and the sediment layer of centrifuged, reconstituted whole-milk powder may be expected to contain fat, lactose, soluble salts and protein, in amounts governed primarily by the manufacturing process and also by the methods employed in reconstituting and centrifuging.

The nature of the layers obtained on centrifuging the reconstituted powders. It will be seen from Table 32 that the six powders varied from a rather insoluble atmospheric roller-dried product through a very soluble roller powder, a low and an average solubility spray-dried powder to two highly soluble spray-dried powders. The considerable differences in the analytical figures (Table 30) for the three layers

Table 30. Percentage distribution of certain constituents of the reconstituted whole-milk powders after centrifuging, and the weights of the top, middle and sediment fractions

Powder:	Roller A			Roller B			Kestner			'Klim'			Krause			Milkal		
Layer :	Top	Middle	Sediment	Top	Middle	Sediment	Top	Middle	Sediment	Top	Middle	Sediment	Top	Middle	Sediment	Top	Middle	Sediment
							Reconstitution at 20°C.											
Water	4.1	77.2	18.7	1.0	79.9	19.1	1.9	91.9	6.2	1.0	94.7	4.3	1.3	96.7	2.0	1.3	96.5	2.2
Protein(Nx6.38)	16.8	23.1	60.1	2.5	63.8	33.7	6.5	61.6	31.9	3.3	89.4	7.3	3.5	92.5	4.0	3.1	92.5	4.4
Fat	68.7	5.3	26.0	83.2	11.0	5.8	20.3	56.8	22.9	30.7	67.2	2.1	40.0	57.2	2.8	45.1	54.2	0.7
Ash	9.1	59.4	31.5	1.8	73.2	25.0	3.6	73.6	22.8	2.0	92.5	5.5	1.9	97.1	1.0	1.9	92.5	5.6
Ca	14.4	25.8	59.8	2.4	61.7	35.9	6.5	70.7	22.8	2.2	85.7	12.1	2.2	93.5	4.3	3.1	94.9	2.0
*	(1.4)		(6.3)	(0.7)		(14.7)	(1.5)		(4.8)	(0.9)		(3.9)	(1.2)		(1.9)	(1.3)		(2.1)
P	13.2	34.2	52.6	3.7	62.6	33.7	6.6	81.6	11.8	2.7	83.8	13.5	2.6	97.4	0.0	2.6	94.9	2.5
*	(1.8)		(8.2)	(0.8)		(15.0)	(1.7)		(5.5)	(0.9)		(3.8)	(1.3)		(2.0)	(1.3)		(2.2)
Wt of each layer from 100g. powder in 900 ml. water (g.)	63	737	200	33	775	192	26	906	68	19	940	41	24	956	20	25	954	21
Solubility (Howat et al., 1939)		76			96			93			97			100			100	
							Reconstitution at 50°C.											
Water	0.8	83.6	15.6	0.7	97.9	1.4	0.6	98.3	1.1	0.9	98.0	1.1	1.3	97.9	0.8	0.9	98.4	0.7
Protein(Nx6.38)	2.1	50.4	47.5	0.9	92.9	6.2	1.6	82.7	15.6	2.9	98.4	-1.3	3.9	93.2	2.9	2.2	93.9	3.9
Fat	58.9	14.2	26.9	85.9	13.6	0.5	18.0	83.1	-1.1	54.9	37.9	7.2	48.1	51.1	0.8	52.3	46.3	1.4
Ash	1.4	72.2	26.4	0.8	98.1	1.1	1.1	99.4	-0.5	1.1	91.1	7.8	2.4	101.1	-3.5	1.3	98.6	0.1
Ca	1.7	49.1	49.2	0.7	96.4	2.9	1.3	99.0	-0.3	2.1	99.8	-1.9	3.4	96.5	0.1	1.8	98.0	0.2
*	(0.5)		(9.2)	(0.7)		(1.1)	(0.5)		(1.1)	(1.0)		(1.1)	(1.3)		(0.8)	(0.9)		(0.7)
P	3.3	55.1	41.6	1.5	94.1	4.4	2.5	92.8	4.7	4.1	97.9	-2.0	5.6	95.2	-0.8	3.9	95.9	0.2
*	(0.5)		(10.3)	(0.6)		(1.3)	(0.5)		(1.1)	(0.9)		(1.2)	(1.5)		(0.8)	(0.9)		(0.6)
Wt of each layer from 100g.powder in 900 ml.water(g.)	25	806	169	31	954	15	11	979	10	25	966	9	27	965	8	23	971	6
Solubility (Howat et al., 1939)		91			98			100			100			100			100	

* Data in brackets represent amounts of Ca and P contributed by permeating middle layer.

The experimental errors in the technique were revealed when very small sediments were obtained: hence the small negative values for some sediment constituents at 50°C.

of these powders affords some indication of the variation in distribution of milk constituents which can occur when whole-milk powders of different solubility are reconstituted and centrifuged. The composition of the top layers, and of the sediment layers which were obtained in sufficient bulk for analysis, is given in Tables 28 and 29 respectively, but for the following discussion a better overall picture of the distribution of the milk powder constituents will be obtained by referring to Table 30.

Comparing first the top layers of the powders reconstituted at 20°C., those from the roller powders weighed most and contained by far the most fat, although they differed considerably from each other. The protein content of the top layers was inversely related to the solubility of the powder, the highly soluble Roller Powder B containing less than the Kestner spray material. The amount of ash in each top layer was almost directly proportional to both the water and protein content of the layer.

The sediment layers grouped themselves somewhat similarly, those from the roller powders being by far the greatest in weight and containing larger fractions of the total protein. As with the top layers, the sediment layers of Roller Powder A and Roller Powder B differed considerably in composition. The weights of sediment and sediment protein from the spray powders varied inversely with their solubilities. The ash in these sediments appeared to depend more on the amount

of protein in the sediment than on the amount of entrained liquor. The fat content of the sediments varied with the degree of solubility in much the same way as the protein content of the top layers, i.e. the more soluble the powder the less fat in the sediment.

The distribution of constituents in the middle layers was naturally dependent on that in the other two layers. Where much protein was insoluble, as in Roller Powder A and to a lesser extent in both Roller Powder B and the Kestner spray powder, the proportion of protein in the liquid was low; the composition of the middle layers from the three more soluble spray powders was very similar, the high proportion of all the constituents remaining in the liquid layers indicating stable suspensions.

The rather insoluble Roller-Dried Powder A and to a lesser extent the Kestner spray powder afforded good examples of the mutual trapping of fat and insoluble protein, resulting in a high proportion of fat in the sediment and of protein in the top layer. It seems reasonable to conclude that the very high proportion of fat in the top layer of Roller-Dried Powder B shows that so long as the powder has a high solubility, i.e. little denatured, insoluble protein, the quick rise of the large globules of non-emulsified fat and the gravitation of only a small amount of protein prevent the fat being carried into the sediment.

Before turning to the data obtained by

reconstituting the powders at 50°C. the mechanism of reconstitution in cold and hot water should first be considered in the light of earlier investigations and the present results obtained at 20°C. Wright (1932) suggested that milk powder insolubility can be considered as the result of two types of protein change during the drying process, that due to wet heat causing irreversible insolubility, and that due to dry heat which can be overcome by reconstituting at temperatures of 50 to 60°C. Hence the insoluble protein in the top and sediment layers at 20°C. represents the sum of these two types of insolubility. If one now considers what is likely to be the result of reconstituting the powders at a higher temperature, it might be expected, if Wright's (1932) theory is correct, that protein made insoluble by dry heat would be redispersed, giving less in both the top and bottom layers, and that the smaller amount of sediment would entrap less fat. The corresponding increase of protein in the liquid layer might then have the power to stabilize more fat, i.e. keep the fat in suspension. If the amount of protein dispersed in the middle layer at the higher temperature were large, it might stabilize fat which at a lower temperature would either be trapped in the larger sediment or appear in the top layer. The net result would then be a diminution of fat in the sediment and the top layer, and a corresponding increase of fat in the liquid middle fraction.

To come to the results obtained in the present work at 50°C., Roller-Dried Powder A was more soluble at this temperature than at 20°C. but the amount of fat in the sediment remained unchanged despite the decrease in the quantity of sediment protein. One explanation of this may be that the fat was associated with, or trapped within, some of the irreversibly insoluble protein and was never liberated during reconstitution. If this were so, then reversibly insoluble material would contribute little or no fat to the sediment. This view is borne out by the behaviour of Roller Powder B, in which reconstitution at the higher temperature reduced the amount of protein in the sediment from 33.7 to 6.2% of the total, showing that most of the sediment protein at 20°C. was of the reversibly insoluble type. At 20°C. as little as 5.8% of the total fat appeared in the sediment and at 50°C., 0.5%, indicating that at the lower temperature only 5.3% of the total fat in the reconstituted milk was trapped by the reversibly soluble protein. The same type of mechanism was apparently operative in the Kestner powder, although it is clear that protein can become irreversibly insoluble without necessarily enclosing fat. In the two roller-dried powders and the Kestner spray-dried powder, the smaller sedimentation of protein at 50°C. as compared with that at 20°C. enriched the liquid layer with that constituent and also stabilized more fat in this fraction. The reverse

occurred in the three more soluble spray powders where at 50°C. a little more fat passed from the liquid layer to the top layer than at 20°C., possibly as a result of the mobility of the liquid fat and the decreased viscosity of the liquor.

To attribute all the difference in solubility at 50°C. to redispersion of 'reversibly' denatured, insoluble protein may be to neglect the probably greater accessibility of the protein to water at the higher temperature. Indeed, it was found that soaking Roller-Dried Powder A in water at 20°C. for 16 hr. prior to reconstitution at the same temperature raised its solubility from 76 to 86% whereas the solubility of the powder at 50°C. was 91%. (These values were obtained by the method of Howat et al., 1949). Thus the increase in solubility at 50°C., which is regarded by Wright (1932) as being due solely to redispersion of 'reversibly' insoluble protein, may in fact be partly caused by more intimate contact and mixing of water with protein, probably aided by the liquefaction of the fat. This in turn would result in redispersion of some protein which although apparently non-dispersable at 20°C. would have been dispersed had the water reached it.

To summarize therefore, it would appear that the greater the insolubility of any milk powder, the greater the likelihood of finding appreciable amounts of fat in the sediment and of protein in the top layer. As the temperature of reconstitution is raised, or the

Table 31. Analysis of sediments obtained by centrifuging the reconstituted (20°C.) milk powders in the Sharples super-centrifuge (g. per 100 g. dry sediment)

Powder :	Roller		Kestner		'Klim'	Krause		Milkal		Gray-Jensen
	Whole A	Separated	Whole	Separated	Whole	Whole	Separated	Whole	Separated	Separated
Protein (N x 6.38)	76.0	79.7	86.8	86.6	83.9	86.3	87.0	83.7	85.4	84.5
Fat	10.7	1.8	2.4	0.3	5.8	1.2	0.3	2.4	0.2	0.7
Ash	9.3	8.6	9.1	9.3	8.9	9.8	10.2	9.2	9.6	10.1
Lactose (by diff.)	4.0	9.9	1.7	3.8	1.4	2.7	2.5	4.7	4.8	4.7
Ca	3.07	2.82	3.07	3.02	3.10	3.33	3.36	3.14	3.17	3.41
P	1.85	1.54	1.81	1.59	1.77	1.94	1.97	1.75	1.46	1.83
Ca/P ratio	1.66	1.83	1.70	1.90	1.75	1.72	1.71	1.79	2.17	1.86
Ca (g./100 g. ash)	33.0	32.6	33.7	32.3	34.8	34.0	33.0	34.1	33.1	33.7
P (g./100 g. ash)	19.9	17.4	19.9	17.2	19.9	19.8	19.4	19.0	15.3	17.8
Ash(g./100g. protein)	12.2	10.8	10.5	10.7	10.6	11.4	11.7	11.0	11.2	11.9
Ca (g./100g. protein)	4.08	3.51	3.57	3.47	3.70	3.83	3.91	3.70	3.75	4.03
P (g./100g. protein)	2.50	1.88	2.07	1.85	2.15	2.20	2.30	2.15	1.76	2.13
Wt of dry sediment obtained per 100 g. dry powder (g.)	6.8	16.0	0.9	1.2	1.4	0.7	0.9	1.2	1.0	3.0
Solubility of the powders (by the method of Howat et al., 1939)	76	81	93	100	97	100	100	100	99	98
Powder analysis										
Ca (g./100 g. ash)	14.5	15.9	14.5	16.7	12.9	14.8	14.8	14.4	15.7	16.5
P (g./100 g. ash)	11.3	12.2	12.0	12.8	10.6	12.3	11.4	11.5	12.1	12.7
Ca/P ratio	1.28	1.31	1.21	1.26	1.23	1.20	1.26	1.26	1.28	1.33

amount of soaking prior to reconstitution increased, the solids of the top layer will become more wholly fat and of the sediment more wholly protein, the amount of fat remaining in the sediment at 50°C. being closely associated with the quantity of irreversibly insoluble protein, or in other words, that protein which remains insoluble at 50°C.

The composition of the washed sediments. The analytical values given in Table 31 for the washed sediments obtained in a high gravitational field (14,500 x g) from whole- and separated-milk powders reconstituted at 20°C. are very similar, particularly when allowance is made for the fat content of the former. As would be expected, the main constituent of the sediment was protein. Washing did not remove all the fat from the sediments and a considerable amount remained in the sediment from Roller-Dried Powder A. No doubt this residual fat was mainly that associated with the irreversibly insoluble protein. It can also be seen from Table 31 that when a powder has a high solubility, as in spray-dried material, the washed sediment (whose volume is used as an index of solubility in the American Dry Milk Institute's method) will consist very largely of protein and ash. In all the sediments, the protein and ash were in fairly constant proportion. The significance of this relationship will be discussed in the next section.

It seems possible that the lactose apparently

present in these washed sediments may not be derived entirely from occluded residues of middle layers but that some of the lactose is actually bound to the insoluble protein as was found in the insoluble material isolated from a deteriorated separated-milk powder (Table 25). Although Henry et al. (1948) have shown that the dialysed protein of fresh liquid milk contains some 'bound' sugar they have also found that the dialysed protein of fresh separated-milk powders contains a greater proportion of sugar. This fact suggests that the protein-lactose reaction described in Part III may take place to a small extent during the process of milk-drying but it is unlikely that the primary stage of this reaction per se will affect the solubility of the milk protein (cf. p.138); heat denaturation of protein, which is no doubt the principal cause of insolubility in dried milk, may be a necessary precursor of the protein-lactose reaction.

The distribution of calcium and phosphorus. Because a fairly large proportion of the calcium and phosphorus in milk is so closely associated with the casein molecule it is probably important to consider these elements in some detail when discussing the solubility of milk powder.

It can be seen from Table 30 that the 'true' calcium and phosphorus content of the top and sediment layers was roughly proportional to the amount of protein in each, at both 20 and 50°C. Furthermore, in the washed sediments obtained by means of the Sharples super-centrifuge, and which consisted mainly of protein, there was great similarity between the ratio of calcium to phosphorus from powders of different manufacture

(Table 31), the average ratios for the sediments of the whole-milk powders being 1.72 and of the separated-milk powders 1.89. In the original milk powders these average ratios were much less, 1.24 and 1.30 respectively, indicating roughly a 40% increase of calcium relative to phosphorus in the washed sediments. Moreover, in the sediments the proportion of both calcium and phosphorus to other constituents was considerably greater than in the original powders. In fact, by expressing the calcium and phosphorus as a percentage of the ash it will be seen that the calcium content of the sediment ash was approximately double that of the ash of the original powder and that the phosphorus content was about 50% greater. In addition, the amounts of ash, calcium and phosphorus per 100 g. of protein in the dry sediments were remarkably similar for all powders (Table 31). The explanation would appear to be that the composition of these washed sediments was approaching that of true denatured protein, in all probability mainly casein; thus most of the calcium and phosphorus could be attributed to calcium caseinate and its associated tricalcium phosphate.

A summary of Ling's investigations (1936, 1937) into the state and distribution of the calcium and phosphorus in milk will help to clarify the present findings. His work led to the conclusion that (a) approximately one third (c.33%) of the calcium and

phosphorus is present in the form of soluble monocalcium hydrogen phosphate, $CaHPO_4$ (also called dicalcium phosphate), (b) 20% of the calcium and 19% of the phosphorus are constituents of the calcium caseinate, (c) the calcium caseinate has associated with it colloidal tricalcium phosphate [$Ca_3(PO_4)_2$] which accounts for 47% of the calcium and 30% of the phosphorus, and (d) the remaining phosphorus (18%) occurs mainly in the ester form. Other workers, Pyne (1934) and Eilers, Saal & van der Waarden (1947), are also of the opinion that the earlier concept of all the phosphate being present as monocalcium hydrogen phosphate was wrong, and that the colloidal phosphate probably exists as tricalcium phosphate bound in some way to the calcium caseinate. The analysis by Ramsdell & Whittier (1944) of the sediment obtained by subjecting fresh liquid milk to a very high centrifugal force supports the view that a calcium caseinate - tricalcium phosphate complex (consisting of 4.8% of tricalcium phosphate and 95.2% of calcium caseinate) is present in milk. In such a complex, the ratio of calcium to phosphorus is 1.76* which is similar to the

* It will be recollected (p.130, Table 25) that the Ca/P ratio in the insoluble fraction of a deteriorated separated-milk powder was 1.68 which suggests that most of the insoluble protein was probably in the form of the calcium caseinate-tricalcium phosphate complex. However, the Sharples sediments contained relatively more calcium and phosphorus and the amount of 'associated' $Ca_3(PO_4)_2$ was calculated to be 6.4 to 8.0 g. per 100g. of protein. This range is higher than the values given by others for $Ca_3(PO_4)_2$ associated with calcium caseinate in fresh milk, e.g. 5.85g. per 100g. protein (Ling, 1937) and 5.10g. (Ramsdell & Whittier, 1944). It would appear that some $Ca_3(PO_4)_2$ had become dissociated from calcium caseinate during denaturation and was sedimented along with the insoluble protein-phosphate complex. Such a dissociation is known to occur during the heat coagulation of milk and even during the storage of evaporated and condensed milks at ordinary temperatures (Leighton & Deysher, 1923; Deysher & Webb, 1948).

Table 32. Solubility of the whole-milk powders as determined by various methods

Powder :	Roller A		Roller B		Kestner		'Klim'		Krause		Milkal	
	Reconstituted at											
	20°C.	50°C.	20°C.	50°C.	20°C.	50°C.	20°C.	50°C.	20°C.	50°C.	20°C.	50°C.
Method	Solubility of the milk solids (%)											
Howat et al.(1939)	76	91	96	98	93	100	97	100	100	100	100	100
Lampitt & Hughes (1924)	75	80	95	100	93	100	97	100	100	100	100	100
Parsons (1947)	70	81	96	99	91	99	94	98	98	99	98	99
	Sediment volume (ml.)											
American Dry Milk Institute*	10.0	(11.5)**	(4.0)	0.4	0.6	<0.1	0.3	<0.1	0.1	<0.1	0.1	< 0.1
Howat et al. (1939)	1.6	(2.4)	(3.0)	0.1	0.5	<0.1	<0.1	0.05	<0.1	<0.05	<0.1	< 0.1

* (Grading of Nonfat Dry Milk Solids, 1948)

** Brackets indicate that the demarcation line between the middle layer and the sediment was indistinct.

values of 1.72 and 1.89 obtained in the present work for the washed sediments from whole- and separated-milk powders respectively. Moreover, when the amount of 'associated' calcium and phosphorus per 100 g. of sediment protein was calculated by allowing for the calcium and phosphorus of calcium caseinate (Söldner, 1888; Lehman, 1894; Ling, 1937; Ramsdell & Whittier, 1944), the 'associated' Ca/P ratios were found to be close to the Ca/P ratio in $Ca_3(PO_4)_2$, namely 1.94. Thus it would appear that most of the insoluble protein in these sediments was in the form of the casein-phosphate complex; the present work also supports the view that the colloidal phosphate associated with calcium caseinate is the tricalcium compound.

Methods of measuring solubility. Of the various methods of measuring solubility already mentioned, most give a reasonably accurate result with powders of high solubility such as a good spray-dried powder, but when applied to roller powders or to spray-dried powders of low solubility none of the methods give accurate results. The results in Table 32 may be critically considered in light of the knowledge of the distribution of constituents among the three layers.

The method of Howat et al. (1939) includes the top layer in the soluble portion whereas, in fact, up to 7% of this layer may consist of insoluble protein and so the solubility results may be too high.

In this method the top layer is removed, the liquid decanted and total solids measured in the mixed top and liquid layers, but as can be seen, the sediment often contains considerable quantities of liquid, which the method does not take into consideration. Thus for this reason also, when the sediment is large the solubility calculated from the percentage of solids in the combined top and middle layers will be erroneously high. This error will be compensated to some extent by the fact that the sediment contains some fat for which no allowance is made.*

The methods of Lampitt & Hughes (1924) and Lampitt & Bushill (1931a) measure the weight of solids in the sediment layer. In both methods allowance is made for the effect of the middle layer permeating the sediment and in the second method allowance is also made for entrapped fat, but in both methods some insoluble protein will be lost in the discarded top layer resulting in too high a solubility value. The fact that in the method of Lampitt & Hughes no allowance is made for fat trapped in the sediment may help to counterbalance the loss of insoluble protein in the top layer.*

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* It should be remembered that at 50°C. (for roller powders at least), the fat in the sediment - apart from that contributed by permeating middle layer - appears to be closely associated with the irreversibly protein. Whether this entrapped fat should be regarded as 'soluble' or 'insoluble' is a moot point.

Results obtained by the method of Cone & Ashworth (1947), in which the weight of so-called 'insoluble' material in the combined sediment and top layers is measured, will be too low because the fat in both of these layers is regarded as insoluble. Moreover, Cone & Ashworth assume that a single washing is sufficient to free the sediment of material from the permeating middle layer, whereas it has been shown (Howat et al., 1939) that at least three and possibly more washings are necessary.

In Parsons' method (1947), the top layer is discarded and the solubility expressed as the percentage ratio of the solids in the middle layer (i.e. soluble solids) to the total solids of the middle plus sediment layers, (i.e. soluble plus insoluble solids). In this procedure the errors compensate each other to some extent. For instance, by discarding the top layer, the percentage of solids in the combined middle and sediment layers will be lower than it should be because of the loss of the 'soluble' fat and insoluble protein which were in the top layer; again, when the insoluble material is re-sedimented, the solids content of the supernatant liquid will also be low because of the same loss of 'soluble' fat plus the loss of the fat entrapped in the sediment. Thus unless the sediment contains much fat when the top layer contains little protein or vice versa, results by this method should be very near the true solubility of the powder.

The inadequacy of the sediment volume method of measuring milk powder solubility is well demonstrated in Roller Powder A where the sediment volume at 20°C. by both the American Dry Milk Institute's method (Grading of Nonfat Dry Milk Solids, 1948) and that recorded during the solubility determination by the method of Howat et al., (1939) was less than at 50°C. The sediment at 20°C. differed markedly from that at 50°C. in being denser and more compact; moreover the top layer at 20°C. contained an appreciable amount of insoluble protein which, had it not been carried up by the fat, would probably have appeared in the sediment. It has often been noticed that sediments from reconstituted powders may differ in density and mode of packing even when other experimental conditions are kept constant. It would appear that the sediment volume method is best suited for powders in which the amount of sediment is normally small, as in most spray-dried powders. Under these circumstances it can afford a sensitive index of manufacturing conditions.

The sources of errors which have been mentioned and which can be seen to be common to the methods considered are minimized when the powder is reconstituted at 50-60°C. and unless a measure of the reversibly insoluble protein is required, solubility determinations at 20°C. even with soaking prior to reconstitution should be avoided, particularly for roller-dried powders. If

a fairly rapid and accurate method is required which is equally applicable to both whole and separated, roller- and spray-dried powders that of Parsons (1947) would appear to be the most suitable.

SUMMARY

1. The mode of distribution of the constituents of centrifuged reconstituted whole-milk powder manufactured by different processes has been determined at 20 and 50°C.

2. Where considerable 'insolubility' exists, protein is carried into the 'fat' layer and fat into the sediment layer in quantities directly related to the degree of 'insolubility'. The fat in the sediment of roller powders appears to be associated almost entirely with the protein which remains insoluble at 50°C.

3. The sediments from reconstituted whole- and separated- milk powders made by different manufacturing processes have been obtained in bulk by vigorous centrifuging (14,500 x g). Analysis of these sediments reveals considerable similarity between them and it is concluded that a well-washed sediment consists mainly of denatured calcium caseinate together with calcium and phosphorus in the same proportions as in tricalcium phosphate, $Ca_3(PO_4)_2$; the calcium caseinate and most

of the calcium phosphate probably exists as a casein-phosphate complex.

4. The accuracy of some published methods of measuring milk powder solubility has been considered and the inaccuracies arising from the lack of homogeneity of the 'fat' and sediment layers are stressed.

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