THE EFFECT OF HEAT ON MILK

AND

MILK PRODUCTS.

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

by

Christopher James Jackson, M.Sc.

The Hannah Dairy Research Institute,

Kirkhill,

A Y R.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

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CONTENTS.

General Introduction.

The object of the work detailed in this thesis was to ascertain the causes of certain defects that occur in canned cream. These faults consisted of a corrosion of the metal of the tin-plate container and what was more serious of the deposition of a purple film on the surface of the cream. In severe cases discrete black particles were distributed throughout the body of the cream.

It was apparent in the early stages of this problem that the defects concerned were largely influenced by reactions which occurred in the milk constituents as the result of heat treatment.

It was therefore considered necessary to investigate, as a preliminary, the effect of heat on certain properties of milk, the most important of which appeared to be the changes occurring in the protein and hydrogen ion concentration.

The first section of this work has been devoted to a brief consideration of the effect of heat on the nitrogen distribution of milk and cream, on the denaturation of the protein and on the hydrogen ion concentration.

In the second section are reported the actual results of processing canned cream under carefully controlled conditions.

PART I.

THE EFFECT OF HEAT

ON

CERTAIN PROPERTIES OF MILK.

I. THE EFFECT OF HEAT ON THE NITROGEN DISTRIBUTION OF MILK AND CREAM.

(a) Introduction.

The effect of heat on milk and milk products has been widely studied from the nutritional aspect with particular reference to the stability of the vitamins and the deposition of minerals. From the latter viewpoint Stirling and Blackwood (1933) compiled a comprehensive review of existing knowledge.

With respect to nitrogen distribution Mattick and Hallett (1929) found no diminution in the amount of diffusible nitrogen-containing substances on heating milk for thirty minutes at temperatures from 40-90°C. Matsuo (1929) found a decrease in the total nitrogen of a milk which had been boiled for a short time, the albumin and globulin nitrogen were decreased while the casein nitrogen was increased. Kieferle and Gloetzl (1930) heated large quantities of milk at 63°, 85°, 100° and 115°C. for thirty minutes and reported the distribution of nitrogen in the milks before and after heating. They found a slight decrease in the total nitrogen content at all temperatures, as well as a diminution in the albumin and globulin fractions. There was no decrease in casein nitrogen after heating which is in agreement

with Wright (1924) who reported no change in the casein molecule on heating for thirty minutes at temperatures up to 120°C.

Wright (1932) and more recently Howat and Wright (1934, 1936) have demonstrated the changes in chemical composition, and physical properties of caseinogen when heated in solution. They found that dephosphorylation accompanied the heat coagulation of caseinogen and that a small part of the protein underwent degradation. The dephosphorised product underwent rapid coagulation on continued heating, due to its decreased heat-stability in comparison with the original caseinogen. There was also a concurrent liberation of calcium due to the fact that the base-binding capacity of the dephosphorised product was less than that of the caseinogen. They suggested that changes in pH of the protein solution and alterations in the degree of dispersion and the degree of hydration of the protein particles exerted considerable influence on the rate of heat coagulation.

Rowland (1937) has demonstrated the heat denaturation of albumin and globulin in milk at 80°-100°C. and concluded that the soluble protein fraction of normal fresh milk was composed of approximately 76 per cent. albumin and globulin and 24 per cent. proteose-peptone substances. He found no change in the non-protein nitrogen content

of milk on heating at temperatures up to 100°C., but that small amounts of proteose nitrogen were produced by hydrolysis of the protein on prolonged heating at 95° and 100°C. Through lack of suitable methods to separate the protein he was unable to state whether these small amounts of proteose nitrogen were the result of hydrolysis of the casein as well as of the denatured albumin and globulin. Heating at 115° and 120°C. for thirty minutes caused appreciable hydrolysis of the protein with resultant increases in the proteose and non-protein nitrogen contents

As may be seen, much of the investigation on the effect of heat on milk has been carried out for shorter periods of time and at lower temperatures than those which are associated with the manufacture and preservation of milk and milk products.

Similarly, while a great deal of work has been done on the effect of heat on the chemical composition and physical structure of pure proteins, less attention has been paid to the effect of heat on protein in solution in such complex colloidal systems as milk and cream. It is then not surprising that the changes, due to heat treatment, that take place in pure proteins isolated from milk are not exactly similar to those that take place when the proteins are present in milk; the presence in milk of various electrolytes and non-electrolytes and the fact

that the heat treatment of milk causes a significant decrease in pH makes this behaviour more understandable.

It has long been recognised that the physical condition of coagulation is preceded by "an irreversible chemical change in the protein known as denaturation", Hardy (1899). Denaturation followed under many conditions by coagulation is typical of the albumins and globulins. Jordan Lloyd (1926) observed that denaturation and coagulation were both influenced by temperature, time, the reaction of the solution, the presence of water, and by the nature and concentration of the electrolytes present; all of which are significant factors in the processing of milk products.

It was considered necessary, in view of the nature of this problem, to investigate the effect on the milk proteins of prolonged heating at high temperatures.

(b) The nitrogen distribution of heated milk.

Technique.

Heating Control. As the experiments were carried out at temperatures above 100°C. it was necessary to use closed tubes. It was found that Monax test tubes sealed off by flame withstood the pressures exerted at temperatures of 100-120°C. The tubes were heated in an open glycerol bath fitted with two stirrers working in opposite direc-

tions, the temperature being controlled by a thermionic valve relay in conjunction with a contact thermometer (Baily, Grundy and Barrett, Cambridge). The tubes were immersed in a container rack from which they could be easily and independently removed.

Experimental Procedure. Tubes, containing 10 ml. of milk were sealed off, immersed in the bath, and thoroughly shaken at 15 minute intervals. The tubes were removed from the bath at the required times, cooled rapidly in running water, and shaken vigorously prior to analysis.

Analysis. The following nitrogen determinations were made according to the micro-Kjeldahl method of Pregl (1930).

(i) Total nitrogen. 2 ml. of the milk were weighed into a 50 ml. volumetric flask and diluted to the mark with water. 2 ml. of the diluted milk were digested in a micro-Kjeldahl flask with 1 ml. of concentrated sulphuric acid and 1 drop of selenium oxychloride. $N/_{100}$ hydrochloric acid and sodium hydroxide were used for the steam distillation.

(ii) Soluble protein nitrogen and non-protein nitrogen. 5 ml. of the milk were weighed into a 50 ml. volumetric flask and made up to the mark with water. The flask was then placed in water at 40°C. and after 10 minutes 1 ml. of 1-10 acetic acid was added. After standing and cooling

the contents of the flask were well shaken and filtered through a dry paper into a dry flask. 2 ml. of the filtrate were digested as in (i).

(iii) Non-protein nitrogen. 10 ml. of the milk were weighed into a 50 ml. volumetric flask and diluted with a little water. 20 ml. of 20% trichloracetic acid were added and the contents made up to the mark. The contents of the flask were well shaken and filtered, and the nitrogen content of the filtrate determined as above.

In order to ascertain the accuracy of obtaining identical samples from tubes, a number of determinations were made in quadruplicate. Agreement was found to 1 mg.% which was considered satisfactory.

There was no change in the total nitrogen content of the samples as the result of heat treatment so this determination was only done on the unheated samples.

All nitrogen determinations were done in duplicate.

Hydrogen-ion Concentration. pH was determined potentiometrically, a quinhydrone electrode being used in combination with a saturated calomel half-cell.

Results.

In this present investigation, denaturation of the soluble proteins was invariably complete and in general

very rapid; it was therefore considered desirable to quote the nitrogen content of the filtrates. Thus the trichloracetic acid filtrate in the unheated sample will contain non-protein nitrogen, and in the heated sample non-protein nitrogen plus proteose-like substances (which are molecularly too small to be precipitated by the trichloracetic acid) produced by hydrolysis of the protein. The acetic acid filtrate in the unheated sample will contain soluble protein nitrogen plus non-protein nitrogen, and in the heated sample any soluble protein which has not been denatured plus non-protein nitrogen and proteose substances produced by hydrolysis of the protein.

Tables 1 - 5 report the nitrogen distribution of milks heated for various intervals at different temperatures. Table 6 records the nitrogen and phosphorus distribution of a heated milk.

TABLE 1.

Changes in the nitrogen distribution of a milk heated at 100° C.

Time of Heating mins.	Total	N. mgs. % T.C.A.	A.C.	pH
Unheated	496	31	146	6.87
15		31	61	6.78
30		31	63	6.75
45		31	65	6.70
60		32	68	6.68
75		34	71	6.60

TABLE 2.

Changes in the nitrogen distribution of a milk heated at 110°C.

Time of Heating mins.	Total	N. mgs. % T.C.A.	A.C.	pH
Unheated	495	32	129	6.70
15		32	64	6.65
30		34	75	6.61
45		37	77	6.55
60		37	78	6.49
75		39	80	6.43

TABLE 3.

Changes in the nitrogen distribution of a milk heated at 115°C.

Time of Heating mins.	Total	N. mgs. % T.C.A.	A.C.	pH
Unheated	480	30	125	6.87
15		31	62	6.76
30		33	70	6.69
45		35	75	6.60
60		37	79	6.51
75		40	89	6.49

TABLE 4.

Changes in the nitrogen distribution of milks heated at 120°C.

	Time of Heating mins.	Total N. mgs. %	T.C.A.	A.C.	pH
	Unheated	502	28	116	6.64
	15		30	68	6.50
Milk A	30		34	75	6.47
	45		39	108	6.32
	60		43	112	6.26
	Unheated	477	24	108	6.54
	30		31	73	6.27
Milk B	60		39	84	6.09
	90		44	100	5.91
	Unheated	507	27	127	6.78
Milk C	30		28	120	6.50
	60		40	127	6.25

TABLE 5.

Changes in the nitrogen distribution of a milk heated for 30 mins. at 100-120°C.

Temperature of Heating	Total	N. mgs. % T.C.A.	A.C.	pH
Unheated	425	33	111	6.68
100°		33	69	6.66
105°		33	71	6.62
110°		35	75	6.46
115°		37	77	6.42
120°		39	81	6.25

TABLE 6.

Changes in the nitrogen and phosphorus distribution of a milk heated at 120°C.

Time of Heating mins.	Total	N. mgs. % T.C.A.	A.C.	P. mgs. % (A.C. filtrate)
Unheated	505	27	113	65
30		35	75	73
60		39	90	76

Discussion.

Since in all instances there was no difference in the total nitrogen content of the heated and the unheated milks, only the original total nitrogen content of each milk has been reported.

Table 1 shows that at 100°C. there was no change in the non-protein nitrogen content, as shown by the T.C.A. filtrate, until after 45 minutes when there was a slight increase. The sudden drop in the A.C. filtrate due to denaturation was followed by a subsequent rise due to appreciable hydrolysis of the protein. A further increase in temperature, as illustrated in Tables 2 and 3, caused a more pronounced hydrolysis starting after shorter periods of heating. It is evident from Table 4 that at 120°C. hydrolysis was very severe. In milk C at 60 minutes a value for the A.C. filtrate was obtained equal to that recorded for the unheated sample. Observations for continued periods of heating, at this temperature, were prohibited due to the coagulation of the milk. This is not surprising in view of the severity of the heat treatment with the consequent marked increase in pH. The stability of the milk proteins will, of course, be largely dependant upon the stage of lactation, but with the exception of one milk all the samples used were known to be more than six weeks post-partum, of normal appearance,

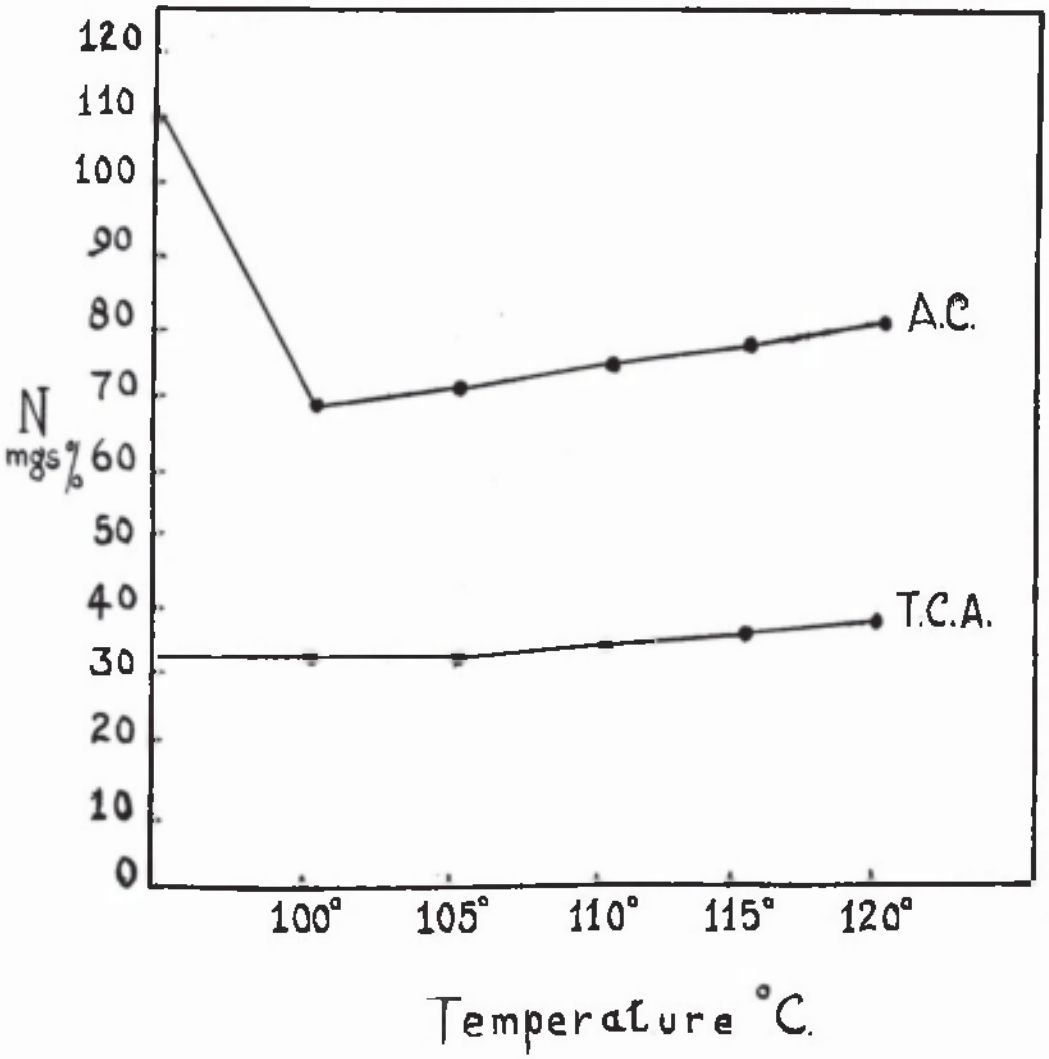
and obtained from the same source.

The comparative changes in nitrogen distribution due to heat treatment at different temperatures for a constant period of time are illustrated in Table 5, and graphically on page 14. It will be seen that the changes that occurred are in close agreement with the other individual experiments.

In reporting very slight increases of proteose nitrogen in similar experiments at lower temperatures Rowlands (1937) stated that owing to lack of suitable methods of precipitation he was unable to determine whether these increases were produced by hydrolysis of the denatured albumin or globulin or by hydrolysis of casein. A method of determining whether the caseinogen was partially hydrolysed was suggested by the work of Howat and Wright (1934) who demonstrated the rapid cleavage of phosphorus from the protein molecule when pure solutions of sodium and calcium caseinogenate were heated at 120°C. They also demonstrated an accompanying but slower dissociation of nitrogen. In order to demonstrate, therefore, whether caseinogen was being hydrolysed a milk was heated at 120°C. in the usual manner and phosphorus was estimated, by the method of Fiske and Subbarow (1925), in the A.C. filtrates of the unheated and heated samples. Any increase of phosphorus in the heated samples would

Fig. 1.

The nitrogen distribution of a milk heated for 30 mins. at temperatures 100°-120°C.



thus demonstrate the hydrolysis of caseinogen. From Table 6 it may be seen that there was a distinct increase in phosphorus in the A.C. filtrate which must have been due to hydrolysis of the caseinogen.

The results reported in this section are in general agreement with those obtained by other workers who, however, in most instances used lower temperatures for shorter periods of heating. Rowland's (1937) observation that there was no change in the total nitrogen content of milk when heated was confirmed, though this disagrees with the findings of Kieferle and Gloetzl (1930) and Matsuo (1929) who report slight changes.

Hydrolysis of the soluble proteins and caseinogen has been found. The degree of hydrolysis, as might be expected, was closely correlated with the temperature and duration of the heat treatment.

Addendum on the effect of heat on milk proteins in the process of manufacture.

An interesting observation on the effect of heat on milk proteins in the process of manufacture is illustrated in Table 7. A number of the milks used in the experiments reported were evaporated in a vacuum pan condenser. Nitrogen determinations were made on these condensed milks, but since in condensing the total solid contents will, of course, be increased, an adjustment has had to be made

in the observed values. The total solid contents of the condensed milks were divided by the total solid contents of the original milk giving a factor by which the nitrogen values found for the condensed milk must be divided to give comparable results.

TABLE 7.

The comparative values for the nitrogen distribution of milk before and after evaporation.

Batch		Temperature of Sterilization °C.	Time of Sterilization mins.	Original Milk N. mgs. % T.C.A.	A.C.	Condensed Milk N. mgs. % T.C.A.	A.C.
1	A	117-118	18	28	116	38	60
	B	117-118	18			33	53
	C	117-118	15			43	72
2	A	117-118	18	33	111	36	57
	C	115-116	15			36	56
3	A	117-118	18	29	118	34	56
	C	115-116	15			37	56
4	A	115-116	18	31	146	36	57
	B	115-116	18			36	52
	C	115-116	15			38	59
5	B	115-116	15	32	129	37	56
6	A	115-116	15			33	55
	B	115-116	15			34	58
	C	115-116	15	29	120	33	51

In spite of the fact that the methods of manufacture differed slightly in these batches, the changes in the nitrogen distribution are surprisingly uniform. There was obvious denaturation of the soluble proteins with a very slight hydrolysis and consequent increase in proteose nitrogen.

(c) The nitrogen distribution of heated cream.

The experimental procedure and technique for analysis were the same as those employed in the previous section.

It is necessary at this point to realise that cream is obtained by centrifuging milk, and that it consists essentially of milk fat together with a portion of the other solids and water of milk. According to Rogers (1928) "It has been repeatedly demonstrated that the ratio of water to solids-not-fat in cream is the same as that in the milk from which the cream has been produced". Creams with a butterfat of approximately 25% have been used in the following experiments, since they approximate most nearly to those used in the manufacture of canned cream. As far as can be ascertained from the literature no work has previously been done on the effect of heat on the nitrogen distribution of cream.

Results.

TABLE 8.

Changes in the nitrogen distribution of a cream heated at 100°C.

Time of Heating mins.	Total	mgs. N % T.C.A.	A.C.	pH
Unheated	367	25	104	6.89
15		25	83	6.68
30		28	65	6.67
45		28	68	6.62
60		28	81	6.60
75		29	83	6.55

TABLE 9.

Changes in the nitrogen distribution of a cream heated at 110°C.

Time of Heating mins.	Total	mgs. N % T.C.A.	A.C.	pH
Unheated	346	24	85	6.74
15		24	58	6.66
30		25	60	6.54
45		26	60	6.53
60		27	67	6.46
75		28	69	6.34

TABLE 10.

Changes in the nitrogen distribution of a cream heated at 115°C.

	Time of Heating mins.	Total	mgs. N % T.C.A.	A.C.	pH
Cream A	Unheated	384	23	109	6.80
	15		24	49	6.74
	30		24	58	6.54
	45		24	58	6.51
	60		28	66	6.48
	75		28	66	6.39
Cream B	Unheated	388	23	103	6.85
	30		24	66	6.68
	60		27	66	6.57
	90		29	66	6.42
	120		31	66	6.30
	150		37	75	6.17

TABLE 11.

Changes in the nitrogen distribution of a cream heated at 120°C.

Time of Heating mins.	Total	mgs. N % T.C.A.	A.C.	pH
Unheated	366	28	104	6.69
15		28	65	6.67
30		29	69	6.52
45		31	71	6.38
60		32	75	6.16
75		37	90	6.13
90		40	95	6.10

TABLE 12.

Changes in the nitrogen distribution of a cream heated for 60 mins. at temperatures 100°-120°C.

Temperature of Heating °C.	Total	mgs. N % T.C.A.	A.C.	pH
Unheated	370	22	96	6.69
100°		22	59	6.60
105°		26	66	6.54
110°		28	68	6.50
115°		30	79	6.44
120°		38	82	6.26

TABLE 13.

Changes in the nitrogen and phosphorus distribution of a cream heated for 60 minutes at 120°C.

Time of Heating mins.	Total	N. mgs. % T.C.A.	A.C.	P. mgs. % A.C. filtrate
Unheated	385	27	112	39
60		39	97	44

? Total P
? P in T.C.A filt.

Discussion.

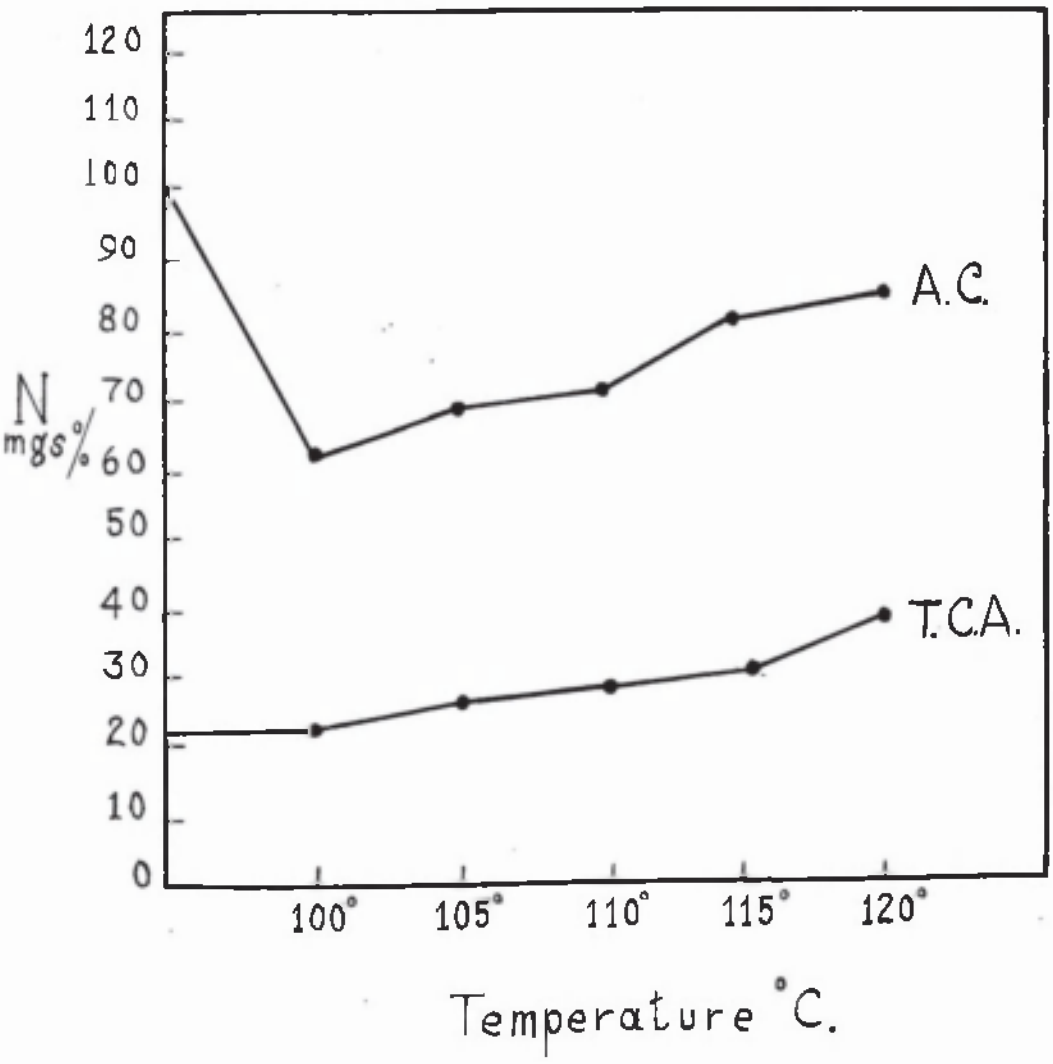
From Table 8 it may be seen that the increase in the non-protein nitrogen was very small, and that denaturation of the albumin and globulin was not complete until after 15 minutes, since a minimum in the A.C. filtrate was not reached till the 30 minute period. At 110°C. there was still only a small increase in the non-protein nitrogen, but the denaturation of the albumin and globulin was completed in under fifteen minutes. A rise in 5°C. caused a further slight increase in the non-protein fraction, which was more evident in the second cream in Table 10 which was heated for a longer period.

At 120°C. there was a marked increase in non-protein nitrogen associated with severe hydrolysis of the protein. The comparative changes in nitrogen distribution due to heat treatment at different temperatures for a constant period of time is illustrated in Table 12 and graphically on page 22. It will be seen that the changes that occur are in close agreement with the other individual experiments.

To ascertain whether hydrolysis of the caseinogen took place in heated cream, a phosphorus determination was made on the A.C. filtrate of an unheated sample and a sample which had been heated for 60 minutes at 120°C.

Fig. 2.

The nitrogen distribution of a cream heated for 60 mins. at temperatures 100°-120°C.



A definite increase in the phosphorus content of the A.C. filtrate indicated, as in milk, that there had been hydrolysis of the casein.

The changes in nitrogen distribution of heated cream were similar to those occurring in heated milk, but in general of a smaller order since cream has a uniformly smaller protein content than milk.

In view of certain findings detailed in the second part of this thesis the effect of metals on the nitrogen distribution of heated cream was studied. About 1 g. each of pure copper, tin and iron was introduced into tubes of cream which were then heated in the usual manner. There was no difference in nitrogen distribution between a control cream and those containing metals.

II. DENATURATION AND THE CLEAVAGE OF THE SULPHUR LINKAGE.

It is known that in some cases denaturation is accompanied by a change in the sulphur grouping in the molecule. Harris (1923) demonstrated the presence of free sulphydryl groups in egg albumin which had been coagulated by heat; raw egg-white did not exhibit this phenomenon. Hopkins (1930) demonstrating the denaturation of proteins by urea and allied substances made use of the presence of the sulphydryl group, detected by

the nitroprusside test, as the criterion of denaturation.

Severe heat treatment, as described earlier, causes rapid denaturation of the soluble proteins in milk and cream. This is evident from the fact that raw milk or cream gives no nitroprusside test but when the milk or cream is heated the test becomes positive, showing the presence of free sulphydryl groups. Jackson (1936) in commenting on the absence of the sulphydryl compounds in milk, suggested that the positive nitroprusside test obtained in milk treated with sodium cyanide was traceable to the cystine in the protein complex.

It has been noticed that the nitroprusside test which is very marked in heated milks and creams becomes less evident on continued heating and eventually disappears. A cream that has been heated for a few minutes at a temperature of, for example, 90°C. gives a positive nitroprusside test; but on continued heating for over 60 minutes the nitroprusside test becomes negative. This is suggestive of the fact that there is concurrent with denaturation a reduction of the cystine in the protein complex, whereby the sulphur linkage is split from the R-S-S-R form to give the active group R-SH which then undergoes an oxidation to revert to an inactive form. From this point of view it is interesting that the sulphydryl group is the only sulphur group which is

readily oxidised in animal metabolism.

Since the isolation of glutathione by Hopkins (1921) a great deal of work has been done to explain the part played by thiol compounds in the oxidation-reduction system of muscle metabolism. Hopkins and Dixon (1922) demonstrated the uptake of oxygen by washed muscle in the presence of glutathione, which was confirmed by Meyerhof (1923) who, however, used thioglycollic acid. Warburg and Sakuma (1923) showed that the S-H groups of thiol compounds were not strictly autoxidisable but that they were oxidised by molecular oxygen only in the presence of iron. The application of this finding to glutathione was confirmed by Harrison (1924). Abderhalden and Wertheimer (1933) showed that egg albumin which had been coagulated by heat could form with cystine a system which took up appreciable amounts of oxygen.

Hopkins (1924) investigated the oxidation of unsaturated fatty acids and proteins by glutathione. He found that in acid systems (pH 3.0-4.5) reduced glutathione promotes oxidation of certain unsaturated fatty acids and of lecithin. In neutral or alkaline systems (pH 7.4-7.6) the process was slightly different, during oxidation of the S-H groups the fatty acids were simultaneously oxidised in such a way that an equipartition of oxygen occurred. Reduced and oxidised glutathione promoted oxidation of

certain pure proteins only when and while the protein itself displayed an S-H group. Allott (1926) reinvestigated the role of glutathione in the oxidation of fats, reported similar findings to those of Hopkins (1924) but in addition found that the behaviour of the fatty acids and their glycerides varied with their age. Some samples of linolenic acid and linseed oil were found to have a definite uptake of oxygen without the addition of glutathione. More recently Meldrum and Tarr (1935) have demonstrated that glutathione is reduced both aerobically and anaerobically by the Warburg-Christian enzyme-coenzyme system in the presence of hexosemonophosphoric acid. In view of these findings on the oxidation of the unsaturated fatty acids it appeared that some similar reaction might take place in milk and cream; a postulation strongly suggested by the fact that cream, with its much larger content of butter fat, exhibited the oxidation of the sulphydryl group more quickly than milk.

Accordingly simple experiments were conducted to find out whether the unsaturated fatty acids of butter fat were oxidised under particular conditions; and whether an unsaturated fatty acid, such as oleic acid, would be oxidised in the presence of cysteine.

Tubes containing about 10 ml. of cream or oleic acid were sealed off and heated for 60 mins. at 120°C. Tubes,

as above, to which had been added 10 mg. of cysteine hydrochloride in aqueous suspension were similarly treated. Iodine values (Rosenmund and Kuhnhenn (1923)) were then determined on the fat from the unheated cream and the oleic acid and on the fat from the heated cream and oleic acid with and without the addition of cysteine hydrochloride. The fat was extracted from the cream in the following manner. About 5 ml. of cream were introduced, with vigorous shaking, into 100 ml. of equal parts of ether and ethyl alcohol. This was then filtered and the filtrate reduced in vacuo to a small volume. It was finally re-extracted with petroleum-ether and taken to dryness.

TABLE 14.

	Iodine Value
Unheated Cream	12.0, 12.0
Heated Cream	11.4, 12.0
Unheated Cream	37.9, 37.4
Heated Cream	38.1, 38.5
Heated Cream + Cysteine hydrochloride	38.6, 38.8
Unheated Oleic Acid	9.3, 9.6
Heated Oleic Acid	10.0, 9.9
Heated Oleic Acid + Cysteine hydrochloride	9.7, 9.8

The above table shows that the iodine value of the fat from a raw and a heated cream showed no appreciable difference; nor was there any difference in this respect between a heated cream and the same cream heated with an addition of cysteine hydrochloride. Similarly there was no difference in iodine value between heated and unheated oleic acid, or between heated oleic acid with and without cysteine hydrochloride. Thus while it seems that no direct evidence substantiates the theory of autoxidation of the sulphydryl group in heated cream it is possible that the reaction in cream is too small to be measured by iodine value. It must be borne in mind that substances other than the unsaturated fatty acids may very well be responsible for the reaction.

The fact that the cystine-cysteine system is not a truly reversible oxidation-reduction system is well known. Indeed it is probable that oxidation takes place in two stages. This is well demonstrated by the fact that very small quantities of cystine, when introduced into oxidation-reduction systems having a negative Eh with a large capacity, remains oxidised. Milk when held at 37°C. for a number of hours develops a negative potential of the value of Eh - 0.2 volts but will not reduce traces of cystine though the Eh of a 0.0001 N solution of cysteine at pH 7.4 and 38°C. is -0.21.

The disappearance of the active sulphur group on the prolonged heating of milk and cream is obviously a subject of considerable interest and importance and would appear to warrant some further investigation.

III. THE EFFECT OF HEAT ON THE HYDROGEN ION CONCENTRATION OF MILK AND CREAM.

Whittier and Benton (1926, 1927) investigated the change in titratable acidity and hydrogen ion concentration of milks heated at temperatures from 95°-120°C. Since the pH continued to become greater after the coagulation of the protein they ascribed the increase in acid to some constituent of the whey, which they concluded to be lactose. They observed that coagulation began at temperatures of 100° and 120°C. when the pH was approximately 5.60, which is in close agreement with the figure of pH 5.35 reported by Cosmovici (1925) for the coagulation induced by the addition of acids at ordinary temperatures. The sensitivity of caseinogen, in the presence of electrolytes, to heat coagulation increases towards its isoelectric point, and it was found in the previous experiments that coagulation in normal milk would take place within narrow limits on either side of pH 6.0. It was considered desirable to observe the changes in pH of milks and creams heated for periods at different temperatures.

The technique employed for the heating of the milk and cream and for the estimation of pH was the same as that used in the previous experiments.

Table 15 illustrates the changes in pH caused by heating a milk at 100° and 120°C. It will be seen that at 120°C. the pH had increased slightly more in 30 minutes than in 150 minutes at 100°C.

TABLE 15.

The effect of heat on the pH of a milk heated at 100° and 120°C.

Time of Heating mins.	pH 100°C.	120°C.
Unheated	6.72	6.72
30	6.65	6.42
60	6.63	6.28
90	6.57	6.19
120	6.48	6.00
150	6.46	5.90

A milk was heated at 120°C. for a long period, as shown in Table 16, coagulation occurred between pH 6.11 and 5.82.

TABLE 16.

The pH of a milk heated at 120°C.

Time of Heating hours	pH
Unheated	6.66
½	6.40
1	6.11
1½	5.82
2	5.74
2½	5.63
3	5.49
3½	5.37
4	5.35
4½	5.22

The effect of heat on the pH of a milk heated at different temperatures is reported in Table 17 and graphically on page 32.

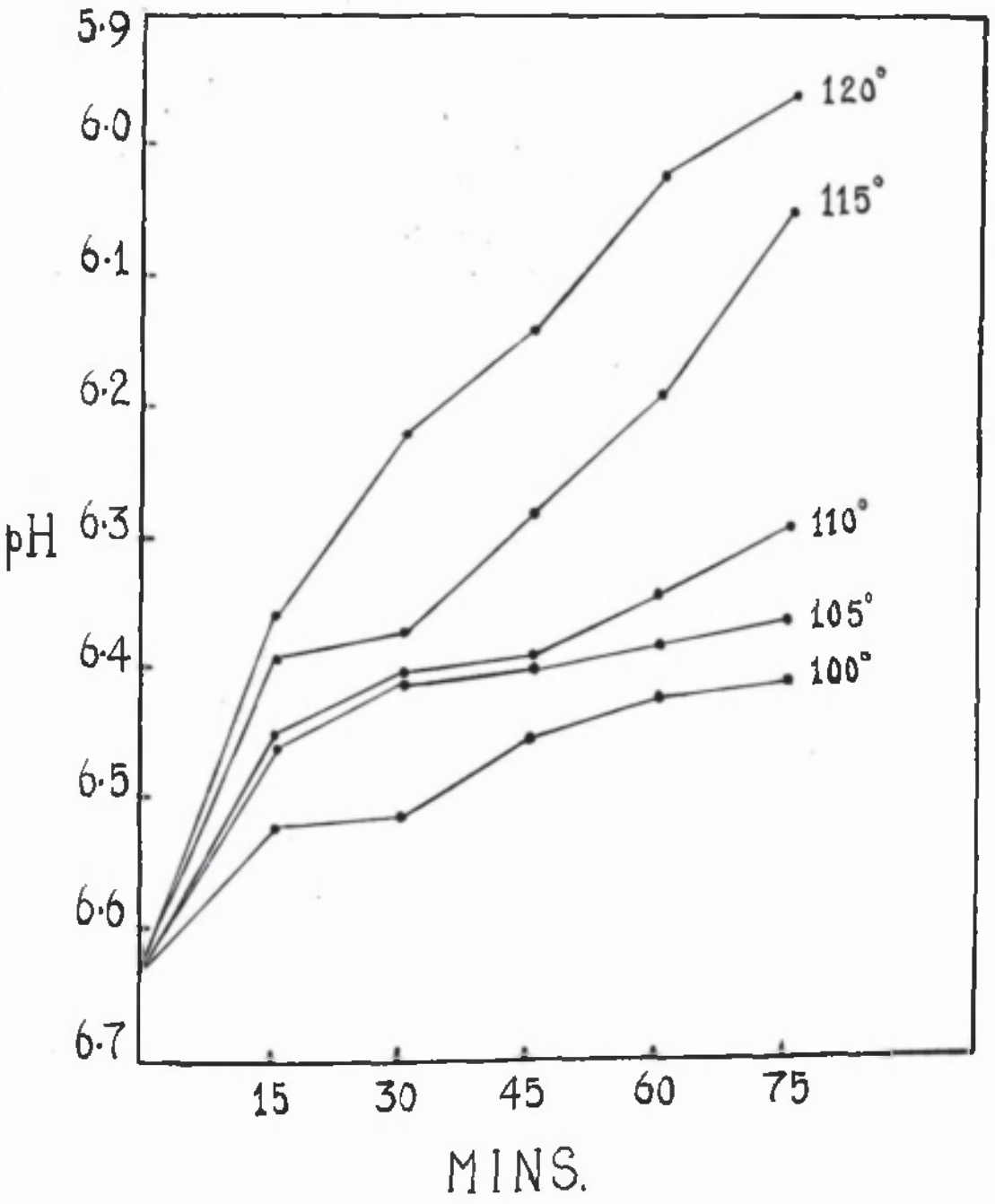
TABLE 17.

pH of a milk heated at 100°-120°C.

Time of Heating mins.	100°	105°	110°	115°	120°
Unheated	6.64	6.64	6.64	6.64	6.64
15	6.52	6.46	6.45	6.39	6.36
30	6.51	6.41	6.40	6.37	6.22
45	6.45	6.40	6.39	6.28	6.14
60	6.42	6.38	6.34	6.19	6.02
75	6.41	6.36	6.29	6.05	5.96

Fig. 3.

The pH of a milk heated at temperatures 100°-120°C.



It may be seen from the graph that at temperatures 100°-115° there was considerable buffer action between the fifteen and thirty minute intervals, but that at 120° this action had been overcome. The samples heated at 105° and 110° kept remarkably close to each other for the first forty-five minutes, there being a difference of only 0.01 of a pH unit. It is significant to note that the increase in pH is more marked at 115° and 120°, since it is in the range of these temperatures that milk products are generally sterilized.

It would be expected that cream with its increased fat phase and consequently smaller amounts of lactose would not show such a rapid increase in pH when heated as was observed in milk.

In Table 18 is recorded the effect of heat on the pH of a cream heated for long periods at 120°C.

TABLE 18.

The pH of a cream heated at 120°C.

Time of Heating hours	pH
Unheated	6.70
½	6.45
1	6.24
1½	6.09
2	5.89
2½	5.84
3	6.67
3½	5.58
4	5.49
4½	5.42

The rise in pH in the above table is slower than that associated with milk similarly treated. A more interesting comparison may be drawn from the heat treatment of cream at temperatures 100°-120°C. (Table 19).

TABLE 19.

The pH of a cream heated at 100°-120°C.

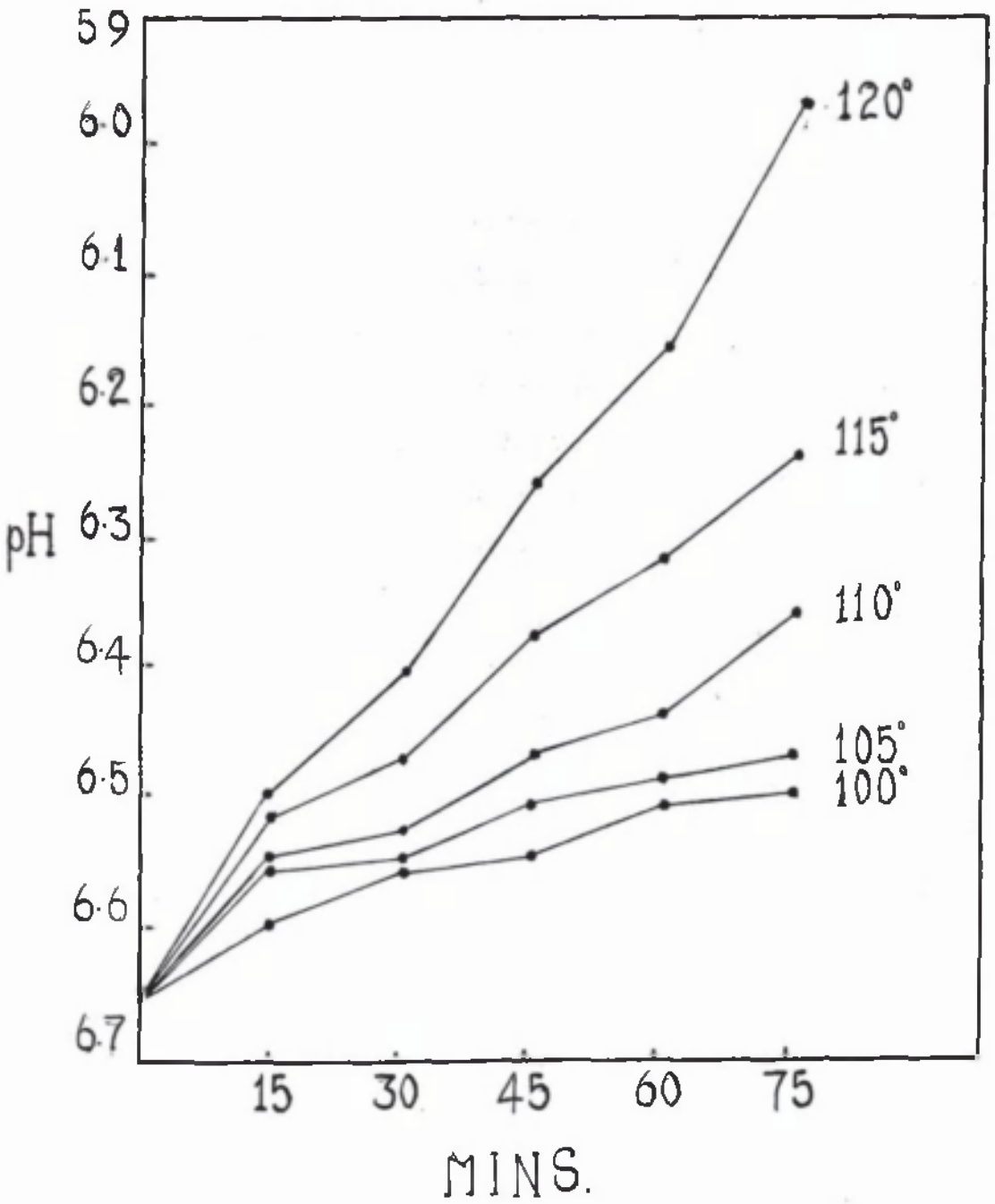
Time of Heating mins.	100°	105°	110°	115°	120°
Unheated	6.65	6.65	6.65	6.65	6.65
15	6.59	6.55	6.54	6.51	6.49
30	6.55	6.54	6.52	6.46	6.40
45	6.54	6.50	6.46	6.37	6.25
60	6.50	6.48	6.43	6.31	6.15
75	6.49	6.46	6.35	6.23	5.97

While at the lower temperatures the fall in pH was slower than that observed in milk, at 120°C. it was very similar. It may be seen from the graph that at 100°C. there was considerable buffer action till the 45 minute period, and at 105° and 110° till the 30 minute period. The marked fall in pH at the higher temperatures 115° and 120°C. is very evident.

The results reported here are an indication of the changes in hydrogen ion concentration that may be expected to take place in milk and milk products as the result of sterilization during manufacture.

Fig. 4.

The pH of a cream heated at temperatures 100°-120°C.



Summary.

The work reported in Part I of this investigation may be summarised as follows:-

(i) The effect of long periods of heating at high temperatures on the nitrogen distribution of milk and cream has been reported.

(ii) Evidence has been presented for the fact that caseinogen as well as the soluble protein was hydrolysed.

(iii) The extent of hydrolysis of the protein was determined by the severity and duration of the heat treatment.

(iv) The production of a free sulphydryl group in heated milk and cream as the result of the denaturation of the albumin and globulin has been reported.

(v) The disappearance of this active sulphur group on continued heating has been observed and a possible theory advanced for the explanation of this behaviour.

(vi) The changes in hydrogen ion concentration of heated milk and cream have been investigated.

PART II.

DISCOLORATION AND CORROSION

IN

CANNED CREAM.

Introduction.

The problem of food preservation, from a chemical viewpoint, has been reviewed by Jordan Lloyd (1926) who says "The difficulties in preserving protein food of both plant and animal origin arise from two causes: firstly, the changes which take place in moribund cells: and secondly, infection from micro-organisms." The difficulties connected with the preservation of milk products come under the second category. The bacterial contamination of evaporated milk and canned cream causes a number of defects by which the product is spoiled. Nichols (1936) has drawn attention to a number of these defects associated with insufficient heat-treatment to ensure sterility, the presence of very heat-resistant spore forming organisms, or to a defect in the can through which contaminating organisms may gain access after sterilization. The temperatures and duration of sterilization commonly used vary quite widely, from between 110°C.-120°C. for 15 to 45 minutes, and are of sufficient intensity to cause considerable protein breakdown, as would be expected from the evidence submitted in the previous section.

It is not surprising that the severe heat treatment which canned dairy products undergo to ensure proper sterilization is capable of causing corrosion of tinplate

containers, since such is the case with other foodstuffs. The effect of foodstuffs on tinplate containers has been extensively investigated, but with particular reference to fish, meat, vegetables and fruit; and from this aspect Morris and Bryan (1931) have compiled a comprehensive survey of existing knowledge. Little work, if any, has been done on the corrosion of cans by dairy foodstuffs and the consequent deterioration of the product. This investigation has been initiated by defects in canned cream which must be considered as a potential source of danger to the cream-canning industry. The defects are discoloration of the can and, what is more serious, of the contents.

Blackening of the surface of the metal in canned foodstuffs, notably marine products, mutton and corn, has been reported by Dill and Clark (1926) and Fitzgerald et al. (1922) as due to the formation of tin sulphide produced by volatile sulphur compounds. It was noticed, however, that while the formation of tin sulphide caused a discoloration which was confined to the surface of the metal, the formation of iron sulphide was more serious since it might become deposited throughout the food. The liberation of sulphide-sulphur by heat is suggestive of proteolysis (Norton (1906)) and denaturation of protein as demonstrated by Harris (1923), and it may be noted that the small incidence of blackening that occurs in canned

fruits is usually ascribed to the introduction of extraneous sulphur.

Description of Defects.

Detailed examination of a number of cans of a proprietary brand of cream which had undergone some months of storage revealed several distinct kinds of trouble.

(1) The can showed marked "bronzing" or "purpling" due to the formation of an adherent film on the tin surface (page 40, Figs. 1b, 2b). The metal underneath was very slightly attacked, giving the "spangling" or "feathering" effect similar to that often found in fruit cans.

(2) The can exhibited a "corroded" appearance, due to the formation in the tin of large areas of minute pits very close together (page 40, Fig. 1c). Large areas of discoloured cream were found at the sites of attack when this was severe (Fig. 3a), and loose black specks were found throughout the body of the cream (Fig. 3b).

Generally both types of defect occurred together, but certain of the cans showed only the one or the other. There was a tendency for purpling to be worse on one end of the can, and pitting on the other.

The bronze or purple film was found to be not soluble in dilute hydrochloric acid, though the concentrated acid at once dissolved the film. It was soluble in caustic soda, and the solution on acidifying with acetic acid gave

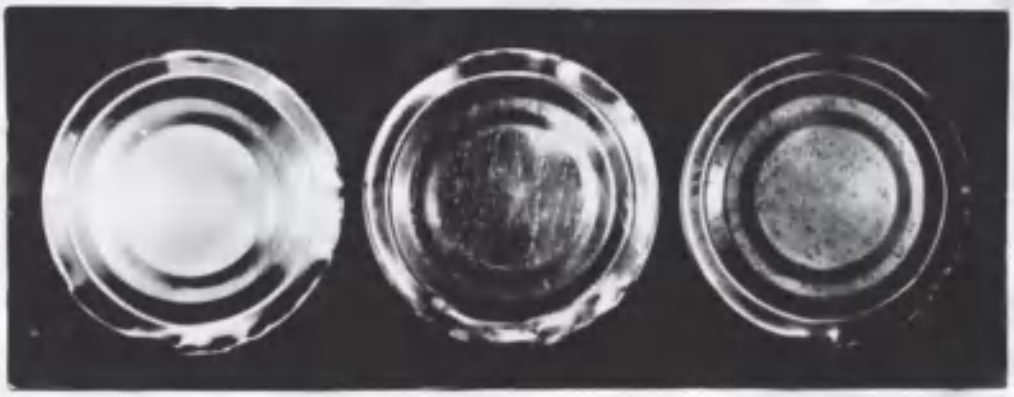


Fig.1a. Unattacked. Fig.1b. Purpling. Fig.1c. Pitting

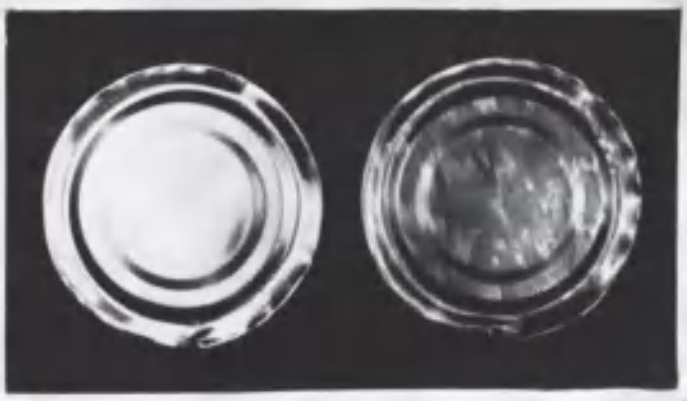


Fig.2a. Unattacked.

Fig.2b. Purpling (produced experimentally).

Fig.3a. Discoloured patches on defective tin.

Fig.3b. Typical black specks in affected cream.

a positive sulphide test with lead acetate. The nitroprusside test was also positive. There is thus no doubt that the film consists of stannous or stannic sulphide.

The contents of twenty-five of the above cans were examined for pH, presence of sulphide, tin and iron. Sulphide was detected by the nitroprusside test. Tin was detected, in a sample previously boiled with 1:1-hydrochloric acid, by means of 4-methyl-1:2-dimercaptobenzene, introduced by Clark (1936). Iron was detected in a sample similarly treated, with thioglycollic acid. The results of the tests are given in Table 20.

It may be seen from the results that when black specks were found, either iron or tin, and usually both, were found in a sample of the stirred cream. Tests on the black specks themselves showed that they consisted of ferrous sulphide or stannous oxide, being usually a mixture containing iron, tin and sulphide.

There appears to be some tendency for greater amounts of tin to be taken up by the more acid creams, but there is no evident correlation between the acidity and the amount of iron taken up, or purpling produced. It is notable that all but one of the samples gave positive tests for sulphur with nitroprusside.

TABLE 20.

Tests of commercial cream.

Can	pH	Cream				Can	
		Nitro-prusside	Iron	Tin	Black Specks	Purpling	Pitting
1	6.71	-	-	+++	+	-	+++
2	6.93	++	+++	-	+	+	+
3	6.93	+	+	-	-	++	++
4	6.85	+	++	++	+	-	++
5	6.78	+	+	-	-	++	++
6	6.73	+	-	-	-	+	-
7	6.63	++	(+)	-	+	+	+
8	6.59	++	++	++	-	+	++
9	6.80	++	(+)	-	+	++	+
10	6.63	++	+++	+++	+	(+)	+++
11	6.81	++	+	+	-	+	+
12	6.87	++	++	-	+	+	+
13	6.73	+++	+	++	+	+	+
14	6.78	++	+	-	-	+	+
15	6.65	++	+	+	-	++	+
16	6.85	+	+++	-	+	+	+
17	6.92	+++	+	(+)	-	++	++
18	6.78	++	+	(+)	-	++	+
19	7.87	++	+	-	+	+	+
20	6.78	++	+	-	-	++	++
21	6.81	+	++	-	-	-	+
22	6.75	+++	+	-	+	+	+
23	6.87	++	(+)	-	-	++	+
24	6.61	++	++	++	+	-	++
25	6.58	++	+	+++	+	++	++

Source of Sulphur in Cream.

Cysteine and methionine are the most probable forms of volatile sulphur in cream, being produced by the effect of heat with consequent denaturation of the protein. The liberation of such compounds containing free-SH groups and their effect on strips of tinplate has been demonstrated experimentally as follows.

Blackening of tinplate strips was produced in tubed samples of cream by severe heat treatment, but the time and temperature of heating necessary to produce such blackening was decreased by the addition of small amounts of alkali to the cream; the presence of blackening was generally accompanied by a positive nitroprusside test. Furthermore, dilute solutions of cysteine hydrochloride processed in cans gave typical "purpling", which was accentuated by the addition of small amounts of sodium bicarbonate. This evidence suggested that similar factors might control this particular defect in commercially canned cream, since in processing the cream is exposed to various degrees of heat for varying times, varying amounts of alkali are added as stabilizer and the cream must of necessity come in contact with the metal surface of the can. Accordingly a number of batches of cream have been processed under different conditions, in an attempt to reproduce the defects recorded.

Processing Procedures.

A sufficient quantity of milk to produce 4 gallons of cream of approximately 23 per cent. butterfat was separated at 95°F. Stabilizer, if desired, was then added in aqueous solution made up to 200 ml. After vigorous stirring the cream was homogenized at 110°F. and emerging from this process at 125°F. was immediately poured into 6 oz. cans, seamed and sterilized in a rotating sterilizer. At the conclusion of the sterilizing period the sterilizer was filled with cold water and the cans allowed to rotate until they were just warm to the touch. The cans were then stored at room temperature, which varied from 15° to 20°C. Twenty hours after processing a sample from each can was taken, the condition of the can was observed and the pH of the cream (by quinhydrone) determined. These determinations were repeated with other cans after storage for 2 weeks, 6 weeks and 12 months. Tests for the presence of iron and tin in the cream were made at the 12 month period.

Results and Discussion - First Series.

Table 21 records the analyses of the original creams used in the experimental batches.

These twelve batches were processed in such a way as to accentuate individually the effect of different factors in the processing procedure. The four factors controlled

TABLE 21.

Analysis of experimental creams.

Batch	Butter-fat	Titratable acidity (ml. N/10 NaOH)	pH (quin-hydrone)	Nitrogen (mg./100 g.) Total	Non-casein	Non-protein
1	25.0	1.1	6.75	472	103	30
2	24.0	1.3	6.67	456	135	69
3	25.0	1.1	6.73	371	134	34
4	26.0	1.3	6.69	449	123	28
5	23.0	1.2	6.57	370	86	22
6	26.0	1.2	6.55	464	110	37
7	25.0	1.5	6.71	449	132	35
8	26.0	1.3	6.60	457	118	34
9	23.0	1.3	6.58	322	92	26
10	25.0	1.0	6.74	465	97	27
11	23.0	1.2	6.79	428	105	21
12	23.0	0.9	6.66	308	109	92

were (i) The effect of sterilization time, (ii) The effect of sterilization temperature, (iii) The effect of homogenization pressure, (iv) The effect of stabilizer. Tables 22, 23, 24, 25, under these headings, record observations on cans from each batch at various intervals after packing.

It is evident from Table 22 that increases in the time of sterilization lead to considerable increase in the amount of "purpling" produced. There was also a tendency for the "purpling" to prevent or mask pitting. The two lots sterilized for the longest periods exhibited pronounced "purpling" but pitting was not in evidence till the twelve month period. The duplicate batches in Table 23 revealed the marked increase in "purpling" produced by a rise of even $3^{o}C.$ in sterilization temperature and confirmed the effect of sterilization time. The addition of sodium bicarbonate as a stabilizer appeared to be on the whole beneficial, the greatest attack was associated with the addition of little or no bicarbonate. The homogenization pressure appeared to have no definite influence on the subsequent attack of the can. The results of the batches in Table 25, however, have further confirmed the marked influence of sterilization time. It will be noticed that pitting was slight in the short period tests, and that the black film and

TABLE 22.

Effect of sterilization time.

Batch	Stabilizer (g. $NaHCO_3$ per gallon)	Homogenization pressure ($lb./in.^2$)	pH before sterilization	Sterilization Temp. °C.	Sterilization Time mins.	20 hours pH	20 hours Purpling	20 hours Pitting	2 weeks pH	2 weeks Purpling	2 weeks Pitting	6 weeks pH	6 weeks Purpling	6 weeks Pitting	12 months pH	12 months Purpling	12 months Pitting	12 months Fe	12 months Sn	Condition of Cream
1	5.0	3300	6.89	117-118	25	6.47	-	-	6.45	-	+	6.43	-	+	6.39	-	+	-	-	-
	5.0	3300	6.89	117-118	30	6.53	-	-	6.48	-	-	6.43	-	+	6.35	-	+	-	-	-
	5.0	3300	6.89	117-118	40	6.43	+	-	6.34	+	-	6.38	+	-	6.35	+	+	-	-	-
	5.0	3300	6.89	117-118	50	6.63	++	-	6.48	++	-	6.45	++	-	6.40	+++	+	-	-	-

TABLE 23.

Effect of sterilization temperature.

Batch	Stabilizer (g. $NaHCO_3$ per gallon)	Homogenization pressure ($lb./in.^2$)	pH before sterilization	Sterilization Temp. °C.	Sterilization Time mins.	20 hours pH	20 hours Purpling	20 hours Pitting	2 weeks pH	2 weeks Purpling	2 weeks Pitting	6 weeks pH	6 weeks Purpling	6 weeks Pitting	12 months pH	12 months Purpling	12 months Pitting	12 months Fe	12 months Sn	Condition of Cream
2	5.0	3300	6.83	114-115	25	6.65	-	-	6.69	-	-	6.67	-	-						
	5.0	3300	6.83	114-115	40	6.65	-	-	6.45	-	+	6.43	-	+	6.36	-	+	-	-	-
	5.0	3300	6.83	117-118	25	6.43	-	-	6.40	-	-	6.43	-	+	6.36	-	+	+	-	-
	5.0	3300	6.83	117-118	40	6.48	++	-	6.38	+	-	6.36	-	+	6.29	++	+	-	-	-
3	5.0	3300	6.91	114-115	25	6.48	-	-	6.47	-	-	6.49	-	+	6.43	-	-	-	-	-
	5.0	3300	6.91	114-115	40	6.40	-	-	6.40	-	-	6.43	-	+	6.35	-	+	-	-	-
	5.0	3300	6.91	117-118	25	6.53	-	-	6.50	-	-	6.48	-	+	6.45	-	-	-	-	-
	5.0	3300	6.91	117-118	40	6.68	++	-	6.56	++	-	6.53	++	-	6.45	++	-	-	-	-

TABLE 24.

Effect of stabilizer.

Batch	Stabilizer (g. $NaHCO_3$ per gallon)	Homogenization pressure (lb./in.^{2})	pH before sterilization	Sterilization Temp. °C.	Sterilization Time mins.	20 hours pH	20 hours Purpling	20 hours Pitting	2 weeks pH	2 weeks Purpling	2 weeks Pitting	6 weeks pH	6 weeks Purpling	6 weeks Pitting	12 months pH	12 months Purpling	12 months Pitting	12 months Fe	12 months Sn	Condition of Cream
4	None	3300	6.56	117-118	40	6.38	+	-	6.30	+	-	6.28	+-	-	6.28	+	-	-	-	-
	1.0	3300	6.63	117-118	40	6.38	-	-	6.28	-	-	6.24	-	+	6.15	-	++	+	-	-
	3.0	3300	6.71	117-118	40	6.47	-	-	6.26	-	-	6.46	-	+	6.18	-	+	-	-	-
	5.0	3300	6.88	117-118	40	6.57	-	-	6.30	-	+	6.34	+-	-	6.25	-	+	-	-	-
5	3.0	3300	6.81	117-118	25	6.54	-	-	6.51	-	-	6.46	-	-	6.45	-	-	+	-	-
	4.0	3300	6.84	117-118	25	6.58	-	-	6.46	-	-	6.41	-	-	6.42	-	+	-	-	-
6	None	3300	6.36	117-118	25	6.34	-	-	6.43	+	-	6.29	+	-	6.26	-	+	+	-	-
	None	3300	6.36	117-118	40	6.19	+	-	6.07	-	-	6.05	++	-	6.01	+	++	-	-	-
	5.0	3300	6.78	117-118	25	6.66	-	-	6.50	-	-	6.48	-	+	6.46	-	+	-	-	-
	5.0	3300	6.78	117-118	40	6.29	-	-	6.34	-	-	6.31	-	+	6.28	++	-	-	-	-
7	None	3300	6.54	114-115	25	6.38	-	-	6.39	-	-	6.37	-	-	6.29	-	+	+	-	-
	None	3300	6.54	114-115	40	6.31	-	-	6.19	-	-	6.22	-	+	6.27	-	-	-	-	-
	5.0	3300	6.91	114-115	25	6.65	-	-	6.52	-	-	6.56	-	+	6.47	-	-	+	-	-
	5.0	3300	6.91	114-115	40	6.46	-	-	6.35	+?	-	6.36	-	+	6.29	-	+	-	-	-
8	None	3300	6.50	114-115	25	6.38	-	-	6.35	-	-	6.37	--	-	6.27	-	+	-	-	Black film and spots, Fe.
	None	3300	6.50	114-115	40	6.29	-	-	6.18	-	-	6.22	+-	-	6.05	-	+	+	-	Slight black film.
	5.0	3300	6.95	114-115	25	6.54	-	-	6.55	-	-	6.52	--	-	6.45	-	+	-	-	Black film and spots Fe and Sn.
	5.0	3300	6.95	114-115	40	6.49	-	-	6.40	-	-	6.37	-	+	6.35	-	+	-	-	-

TABLE 25.

Effect of homogenization pressure.

Batch	Stabilizer (g. $NaHCO_3$ per gallon)	Homogenization pressure (lb./in^2)	pH before sterilization	Sterilization Temp. °C.	Sterilization Time mins.	20 hours pH	20 hours Purpling	20 hours Pitting	2 weeks pH	2 weeks Purpling	2 weeks Pitting	6 weeks pH	6 weeks Purpling	6 weeks Pitting	12 months pH	12 months Purpling	12 months Pitting	12 months Fe	12 months Sn	Condition of Cream
9	None	711	6.58	117-118	25	6.38	-	-	6.37	-	-	-	-	-	6.26	-	+	++	-	-
	None	2136	6.58	117-118	25	6.36	-	-	6.44	-	-	6.37	-	-	6.29	-	+	++	-	-
	None	2854	6.58	117-118	25	6.33	-	-	6.39	-	-	6.28	-	-	6.21	-	+	+++	-	-
	None	4266	6.58	117-118	25	6.37	-	-	6.42	-	-	6.39	-	-	6.33	-	-	+	-	-
	1.5	711	6.75	117-118	25	6.48	-	-	6.51	-	-	6.49	-	-	6.43	-	+	++	-	Black film at pits.
	1.5	2136	6.75	117-118	25	6.48	-	-	6.48	+	-	6.48	+	-	6.40	+	+++	-	-	-
	1.5	2854	6.75	117-118	25	6.47	-	-	6.49	+	-	6.48	+	-	6.45	++	+	-	-	-
	1.5	4266	6.75	117-118	25	6.47	+?	-	6.49	+	-	6.49	++	-	6.45	+	+	+++	-	Black spots Fe and Sn.
10	4.5	711	7.06	117-118	25	6.59	-	-	6.43	-	-	6.45	-	+	6.40	-	+	+	-	-
	4.5	711	7.06	117-118	40	6.53	-	-	6.25	-	-	6.31	-	+	6.22	-	+	-	-	-
	4.5	2854	7.05	117-118	25	6.56	-	-	6.53	-	-	6.60	-	-	6.57	-	+	+	-	-
	4.5	2854	7.05	117-118	40	6.65	+	-	6.29	+	-	6.40	+	-	6.38	-	-	-	-	-
11	4.5	711	6.98	117-118	25	Not determined	Not determined	Not determined	6.34	-	-	6.30	-	-	6.31	-	-	-	-	-
	4.5	711	6.98	117-118	40				6.20	+?	-	6.17	++	-	6.17	+++	+	-	+++	-
	4.5	2854	7.01	117-118	25				6.45	-	-	6.50	-	-	6.49	-	+	-	-	Black film Fe and Sn.
	4.5	2854	7.01	117-118	40				6.32	-	-	6.40	-	-	6.28	-	+	+	-	Black film.
12	4.5	711	6.88	117-118	25	6.56	-	-	6.40	-	-	6.47	-	-	6.38	-	-	-	-	-
	4.5	711	6.88	117-118	40	6.47	+	-	6.37	-	-	6.29	-	+	6.22	+	-	-	-	-
	4.5	3300	6.91	117-118	25	6.68	-	-	6.54	-	-	6.56	-	-	6.56	-	+	-	-	Black spots at pits.
	4.5	3300	6.91	117-118	40	6.50	-	-	6.40	-	-	6.35	-	+	6.30	-	+	-	-	-

spots found after the longer period of storage were always associated with the presence of pitting. A few cans in which the steel was deliberately exposed by filing gave on processing a non-adherent black film at the exposed steel; in the course of a few weeks black specks were found throughout the cream. The effect of exposed steel illustrated the potential dangers of inferior cans, and through the courtesy of the Metal Box Co. a special supply of selected cans was obtained. Every can used in these batches was examined for signs of porosity, and only sound cans used.

The widespread use of sodium bicarbonate as a stabilizer dictated its use in these batches. Where no stabilizer was added coagulation invariably occurred, although the milk from which the cream was derived was of Grade A (T.T.) standard.

While in general an unduly large air space in a can might be expected under some conditions to enhance attack, there was no evidence for this effect in some special experiments with varying air space. Nor did variation of pH of the cream within the limits studied appear to have any particular effect. The results of these preliminary batches, while reproducing the defects observed in commercial canned cream, suggested that further investigations were desirable, in particular to ascertain the effect of

stabilizers other than sodium bicarbonate and the incidence of pitting in artificially blackened cans. Hunziker (1926) has described the relative merits of sodium bicarbonate, sodium citrate and di-sodium phosphate as a means of raising the heat coagulation point in evaporated milk. The disadvantages of bicarbonate are that in addition to balancing excess calcium, it will when used in large amounts change the reaction and magnify the excess calcium by combining with the casein and replacing the calcium in the calcium-casein combination. In addition large quantities of bicarbonate cause a distinct darkening in the colour of the product. Accordingly the effect of these three stabilizers in canned cream has been investigated.

Since it was shown in the first series that variations in homogenization pressure had no effect on the cream from the point of view of can attack, the homogenization pressure in the second series was kept constant at 3300 lb./in^2. The temperature of sterilization was kept constant at 117^o-118^{o}C. By minimising the number of variable factors in the process of manufacture it was hoped to get more comparable results.

Results and Discussion - Second Series.

Table 26 records the analysis of the original creams used in the second series.

TABLE 26.

Analysis of experimental creams - second series.

Batch	Butter-fat %	Titratable acidity (ml. N/10 NaOH)	pH	N. mgs. % Total	T.C.A.	A.C.
1	23	1.4	6.83	428	131	32
2	23	1.3	6.93	407	128	23
3	23	1.4	6.88	415	131	34
4			Not determined.			
5	23	1.2	6.83	436	124	31
6	22	1.3	6.77	410	125	35
7	25	1.0	6.90	386	116	23
8	25	1.0	6.95	387	144	30
9	25	1.3	6.76	396	110	25
10	23	1.0	6.83	349	98	21
11	25	1.3	6.89	352	116	29

These eleven batches were processed so that comparison might be made between the effect of sodium bicarbonate, sodium di-phosphate and sodium citrate. Thus Table 27 illustrates the comparative effect of sodium bicarbonate and sodium di-phosphate, Table 28 the comparative effect of sodium bicarbonate and sodium citrate, and finally Table 29 the comparative effect of the three salts together. Stabilizer was always added in equimolecular amounts.

The Effect of Bicarbonate and Phosphate.

It will be seen from Table 27 that an increase in the amount of either stabilizer added caused an immediate increase of "purpling". At the six month period all the phosphate samples showed pitting, whereas only two of the bicarbonate samples showed this form of attack. In no instance was there any attack of the can, either "purpling" or pitting, where bicarbonate had been added and the period of sterilization limited to 25 minutes. This again demonstrates the effect of sterilization time. The pH of the phosphate samples were uniformly lower than those of bicarbonate. In addition the samples to which the maximum amount of phosphate had been added tended to become very thin. From these three batches it is evident that sodium di-phosphate is inferior to sodium bicarbonate as a stabilizer for canned cream.

TABLE 27.

The effect of bicarbonate and Phosphate.

Batch	Stabilizer	g. Stabilizer per gallon	Homogenization pressure lb./in^2.	pH before sterilization	Sterilization Temperature °C.	Sterilization Time mins.	20 hours pH	20 hours Purpling	20 hours Pitting	4 weeks pH	4 weeks Purpling	4 weeks Pitting	4 months pH	4 months Purpling	4 months Pitting	6 months pH	6 months Purpling	6 months Pitting	Condition of Cream
1	$NaHCO_3$	1.0	3300	6.90	117-118	25	6.64	-	-	6.60	-	-	6.49	-	-	6.42	-	-	-
	$NaHCO_3$	1.0	3300	6.90	117-118	40	6.61	-	-	6.66	+	-	6.51	++	-	6.43	++	++	-
	Na_2HPO_4	1.7	3300	6.83	117-118	25	6.57	-	-	6.53	+	-	6.39	+	-	6.23	+	+	-
	Na_2HPO_4	1.7	3300	6.83	117-118	40	6.57	-	-	6.48	+	-	6.33	-	+	6.29	-	+++	Black film.
2	$NaHCO_3$	3.0	3300	7.04	117-118	25	6.78	-	-	6.59	-	-	6.62	-	-	6.42	-	-	-
	$NaHCO_3$	3.0	3300	7.04	117-118	40	6.79	+	-	6.55	++	-	6.60	++	-	6.47	++	-	-
	Na_2HPO_4	4.9	3300	6.92	117-118	25	6.74	+	-	6.63	++	-	6.63	+	+	6.36	-	+	-
	Na_2HPO_4	4.9	3300	6.92	117-118	40	6.65	+	-	6.55	++	-	6.54	++	-	6.38	++	+	-
3	$NaHCO_3$	5.0	3300	7.05	117-118	25	6.88	-	-	6.73	-	-	6.66	-	-	6.57	-	-	-
	$NaHCO_3$	5.0	3300	7.05	117-118	40	6.74	++	-	6.66	++	-	6.52	++	+	6.34	++	+	-
	Na_2HPO_4	6.15	3300	6.96	117-118	25	6.63	+++	-	6.61	+++	-	6.56	+	+	6.49	+	+	-
	Na_2HPO_4	6.15	3300	6.96	117-118	40	6.52	++++	-	6.48	++++	-	6.48	+++	++	6.35	+	+++	-

TABLE 28.

The effect of bicarbonate and Citrate.

Batch	Stabilizer	g. Stabilizer per gallon	Homogenization pressure lb./in^2.	pH before sterilization	Sterilization Temperature °C.	Sterilization Time mins.	20 hours pH	20 hours Purpling	20 hours Pitting	4 weeks pH	4 weeks Purpling	4 weeks Pitting	4 months pH	4 months Purpling	4 months Pitting	6 months pH	6 months Purpling	6 months Pitting	Condition of Cream
4	$NaHCO_3$	1.0	3300	6.87	117-118	25	6.63	-	-	6.54	+	-	6.42	+	-	6.36	+	-	-
	$NaHCO_3$	1.0	3300	6.87	117-118	40	6.58	-	-	6.44	-	-	6.30	+	-	6.29	-	+	-
	$Na_3C_6H_5O_7$	3.07	3300	6.83	117-118	25	6.70	-	-	6.61	+	-	6.52	-	+	6.47	-	++	-
	$Na_3C_6H_5O_7$	3.07	3300	6.83	117-118	40	6.58	-	-	6.54	-	-	6.49	-	+	6.35	-	++	-
5	$NaHCO_3$	3.0	3300	7.23	117-118	25	6.84	-	-	6.89	-	-	6.76	-	-	6.74	+	+	-
	$NaHCO_3$	3.0	3300	7.23	117-118	40	6.89	+	-	6.78	+	-	6.74	+	-	6.63	++	-	-
	$Na_3C_6H_5O_7$	9.2	3300	7.17	117-118	25	6.83	+	-	6.80	+	-	6.80	-	+	6.68	++	+	-
	$Na_3C_6H_5O_7$	9.2	3300	7.17	117-118	40	6.73	++	-	6.88	++	+	6.68	++	++	6.57	++	+	-
6	$NaHCO_3$	5.0	3300	7.12	117-118	25	6.89	-	-	6.75	-	-	6.67	-	-	6.61	-	-	-
	$NaHCO_3$	5.0	3300	7.12	117-118	40	6.81	++	-	6.64	+++	-	6.58	-	++	6.42	-	++	-
	$Na_3C_6H_5O_7$	15.4	3300	7.0	117-118	25	6.76	+	-	6.54	+++	-	6.52	++	+	6.34	+	++	-
	$Na_3C_6H_5O_7$	15.4	3300	7.0	117-118	40	6.63	++++	-	6.58	++++	-	6.50	++	+++	6.33	++	+++	-

TABLE 29.

The effect of bicarbonate, Phosphate and Citrate.

Batch	Stabilizer	g. Stabilizer per gallon	Homogenization pressure lb./in^2.	pH before sterilization	Sterilization Temperature °C.	Sterilization Time mins.	20 hours pH	20 hours Purpling	20 hours Pitting	4 weeks pH	4 weeks Purpling	4 weeks Pitting	4 months pH	4 months Purpling	4 months Pitting	6 months pH	6 months Purpling	6 months Pitting	Condition of Cream
7	$NaHCO_3$	3.0	3300	7.11	117-118	25	7.00	-	-	6.78	-	-	6.71	+	-	6.56	-	++++	Black spots.
	Na_2HPO_4	4.9	3300	6.95	117-118	25	6.72	-	-	6.56	-	-	6.51	+	-	6.42	-	+	-
	$Na_3C_6H_5O_7$	9.2	3300	6.95	117-118	25	6.76	+	-	6.61	-	-	6.58	-	+	6.50	-	+	-
8	$NaHCO_3$	5.0	3300	7.13	117-118	25	6.77	-	-	6.70	-	-	6.66	+	+	6.57	+	+	-
	Na_2HPO_4	6.15	3300	7.03	117-118	25	6.70	++	-	6.60	++	-	6.60	++	-	6.49	+	++	-
	$Na_3C_6H_5O_7$	15.4	3300	7.00	117-118	25	6.75	++	-	6.59	++	-	6.49	++	-	6.53	-	+++	-
9	$NaHCO_3$	5.0	3300	7.14	117-118	40	6.82	+++	-	6.73	+++	-	6.82	+++	-	6.61	++	+	-
	Na_2HPO_4	6.15	3300	6.97	117-118	40	6.48	+++	-	6.51	+++	-	6.42	+	++	6.38	+	++	-
	$Na_3C_6H_5O_7$	15.4	3300	6.97	117-118	40	6.63	+	-	6.63	+	-	6.56	-	++	6.46	+	+	-
10	$NaHCO_3$	3.0	3300	7.07	117-118	25	6.89	-	-	6.82	-	-	6.79	-	-	6.60	-	+	-
	$NaHCO_3$	3.0	3300	7.07	117-118	40	6.86	+	-	6.66	+++	-	6.60	-	++	6.45	+++	-	-
	Na_2HPO_4	4.9	3300	6.90	117-118	25	6.71	-	-	6.66	-	-	6.60	-	+	6.46	-	++	-
	Na_2HPO_4	4.9	3300	6.90	117-118	40	6.64	+	-	6.54	-	-	6.46	+	-	6.30	-	++	-
	$Na_3C_6H_5O_7$	9.2	3300	7.00	117-118	25	6.94	-	-	6.81	-	-	6.74	-	++	6.62	-	++	Black film.
	$Na_3C_6H_5O_7$	9.2	3300	7.00	117-118	40	6.75	+	-	6.62	++	+	6.50	-	+++*	6.38	-	+++	Black film.
11	$NaHCO_3$	5.0	3300	7.20	117-118	25	6.83	-	-	6.96	+	-	6.78	-	+	6.62	-	+	-
	$NaHCO_3$	5.0	3300	7.20	117-118	40	6.80	+	-	6.93	++	-	6.57	-	+	6.49	-	++	-
	Na_2HPO_4	6.15	3300	6.92	117-118	25	6.60	-	-	6.75	+	-	6.51	+	+*	6.41	-	++	-
	Na_2HPO_4	6.15	3300	6.92	117-118	40	6.53	+	-	6.66	+++	-	6.45	++	+	6.36	++	++	Black film.
	$Na_3C_6H_5O_7$	15.4	3300	7.00	117-118	25	6.87	+	-	6.93	++	-	6.74	+	-	6.64	-	++	-
	$Na_3C_6H_5O_7$	15.4	3300	7.00	117-118	40	6.67	++	-	6.79	++++	-	6.62	++	++	6.47	+	++++	Black film and spots.

The Effect of Bicarbonate and Citrate.

As in the previous batches increased amounts of either stabilizer caused an immediate increase of "purpling". In all three batches the addition of citrate was more injurious than the addition of bicarbonate. There was slight can attack in only one of the samples, where bicarbonate had been added and the period of sterilization limited to 25 minutes. Addition of the citrate in Batches 5 and 6 caused a severe thinning in the final product.

The Effect of Bicarbonate, Phosphate and Citrate.

The five batches in Table 29 demonstrate the comparative effects of the three stabilizers used when added in different amounts and subject to different lengths of sterilization. At the six-month period, with one exception, the phosphate and citrate samples showed more pitting than the bicarbonate samples. This one exception, the bicarbonate sample in Batch 7, showed very intense pitting in one small area which was suggestive of either a defective can or initial exposure of the iron due to mechanical means, the amount of stabilizer added and the heat treatment received did not, from the observations obtained in this investigation, warrant such severe attack. It should be emphasised at this point that owing to variations of cream from batch to batch no exact

comparison can be drawn as between batches; in a similar fashion the cans used in any one batch may be quite variable in quality, although appearing visibly sound, and cause apparent anomalous results. In general, however, excellent comparisons in individual batches has been possible since the cans used reacted in quite a uniform manner.

The marked superiority of the bicarbonate as a stabilizer, and the question of the sulphide film inhibiting or masking pitting in the early stages of storage suggested two further experiments; the addition of very large amounts of bicarbonate, and the effect of processing cream in cans covered with an artificially produced sulphide film.

The Effect of the Addition of Large Amounts of Bicarbonate.

A batch of cream was processed in the normal way with the addition of no, 5, 10 and 20 g. sodium bicarbonate per gallon. Table 30 shows the results of short time storage of this batch.

It is abundantly evident that large amounts of bicarbonate increase can attack, and it would appear as if the increased "purpling" in the fourth sample has protected the can from pitting which is very evident in the third sample. The production of severe can attack and defects in the cream within two months of manufacture illustrates the danger of the indiscriminate use of stabilizer.

The Action of the Sulphide Film.

Since in some of the batches reported it has been

TABLE 30.

The effect of the addition of large amounts of bicarbonate.

Stabilizer	g. Stabilizer per gallon	Homogenization pressure lb./in^2.	pH before sterilization	Sterilization Temperature °C.	Sterilization Time mins.	20 hours pH	20 hours Purpling	20 hours Pitting	2 weeks pH	2 weeks Purpling	2 weeks Pitting	2 months pH	2 months Purpling	2 months Pitting	Condition of Cream
$NaHCO_3$	None	3300	6.75	117-118	25	6.49	-	-	6.38	-	-	6.29	-	-	-
$NaHCO_3$	5	3300	7.03	117-118	25	6.84	-	-	6.76	-	-	6.51	-	-	-
$NaHCO_3$	10	3300	7.22	117-118	25	6.92	++	-	6.84	++	-	6.62	+	++	Black spots and film.
$NaHCO_3$	20	3300	7.45	117-118	25	7.08	+++	-	6.99	+++	-	6.81	+++	-	Purple film.

noticed that "purpling" tends to inhibit or mask pitting, particularly in the early stages of storage, it was decided to process some cream in cans which had been artificially treated. A strong solution of sodium sulphide was poured into the cans for a few minutes and produced typical etching with characteristic "purpling", the lids were treated, on the underside, in similar fashion. In some of these cans a clear area was preserved at the bottom of the can by covering it with paraffin wax while the sodium sulphide was applied. Cream with the addition of 5 g. of bicarbonate per gallon and processed in the usual manner was sterilized for 25 minutes at 117-118° C. in these and a set of untreated cans. At the end of two months there was a slight pitting in the control can, but no visible pitting in either of the two treated cans. It is probable that "purpling" acts in beneficial manner, as far as pitting is concerned, in the early stages of storage, but that severe "purpling" will on long time storage produce serious defects.

Conclusions and Applications.

As a result of this study considerable information has been obtained about the corrosion of the tin-plate containers by canned cream and subsequent defects in the cream itself. Since the initiation of this work, a number of complaints from different sources have emphasised the incidence of trouble of this nature. The marked increase in the production of milk products in the last few years has done much to increase the occurrence of such problems, and has accentuated the necessity of eliminating such causes of wastage.

It is evident from the pH of the commercial cream examined in the first instance that large amounts of stabilizer must have been added, which, in view of the experimental evidence, would be responsible for the severe attack reported. The experimental batches have shown the potential dangers of excessive heat treatment, the use of inferior cans, and of the indiscriminate choice and use of stabilizing salts. The effects of very long periods of storage are at present under investigation, but it is unlikely, however, that the period between production and consumption will exceed twelve months.

It is apparent that the defects, 'purpling' and pitting

of the can and the deposition of black spots throughout the cream are of the same origin. It is also evident that, although these defects appear together, there are also instances where they appear alone, and thus may be considered separately.

Purpling.

Volatile sulphur compounds, liberated from the cream by excess heat treatment and/or excess addition of stabilizing salts, attack the tin surface of the container to form a purple film of tin sulphide. The film may then exhibit three functions.

(i) It may, under certain circumstances, prevent further attack of the iron exposed by pores in the tinplate.

(ii) It may, when particularly severe, stain the cream with which it is in contact with a non-adherent purple film.

(iii) It may remove sufficient tin to expose iron which will be subsequently attacked with consequent 'pitting'.

Pitting.

Pitting is produced by the attack of exposed iron by volatile sulphur compounds. The iron may be exposed in the following manner.

(i) Poorly manufactured cans which exhibit pores through which iron is exposed. Cans manufactured from inferior tin-plate which will tend to crack or develop porosity at

the surface. Iron may also be exposed by the breaking of the tin surface due to mechanical means, such as rough handling or an abrasion. This is particularly noticeable at the seams.

(ii) Tin may be taken up in sufficient quantities by the sulphur to expose the iron.

Black Spots.

Black spots throughout the cream and the black adherent film at the surface of the can are always associated with pitting, the black adherent film which invariably covers severe pitting being produced by iron sulphide which gradually becomes dispersed through the body of the cream as discrete black particles.

This condition will only appear in normal cans after a considerable period of storage, since pitting is generally not in evidence till after a number of weeks.

The application of the experimental evidence to the commercial canning of cream are indeed self-evident, but may be tabulated as follows:-

1. The cream to be processed must be derived from good milk. It is probable that abnormal milks, such as newly calved cows' milk or milk from animals suffering from mastitis, will tend to liberate volatile sulphur very readily on sterilization due to the abnormal condition

of the protein phase.

2. Only sound cans manufactured from good quality tin-plate must be used. The danger of using cans in which iron is exposed through porosity or insufficient tinning of the tin-plate has been demonstrated.

3. The minimum of stabilizer which will prevent coagulation of the cream should be used. The use of excess stabilizer has been shown to cause severe can attack. The use of sodium bicarbonate as a stabilizer is preferable to sodium di-phosphate and sodium citrate.

4. The minimum of heat treatment which will ensure the complete sterilization of the product should be used. While it may be difficult under commercial conditions to control exactly the length of the sterilization period, no difficulty should be experienced in controlling the temperature.

If the above precautions are taken, there seems no reason why the discoloration and corrosion in canned cream should not be eradicated.

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