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THE USE OF HYDROGEN PEROXIDE AS A DAIRY PRESERVATIVE.

by

H. LÜCK

GERMAN RESEARCH INSTITUTE FOR FOOD CHEMISTRY, MUNICH, GERMANY.

GENERAL

INTRODUCTION

During recent years great efforts have been made by many countries with hot or tropical climates to develop their dairy industry. In these countries there are numerous difficulties in handling fresh milk, simply because milk will only remain sweet for a very short period of time at high temperatures. Furthermore, it is often necessary to transport the milk over long distances. Different methods have, therefore, been tried to prolong the keeping quality, among which the most important are:

(*i*) Artificial cooling on the farm as well as during transport
(*ii*) Double or manifold pasteurization
(*iii*) Preservation

Without doubt the first is the ideal method but it is very costly and, for the present, impossible to be realized in many hot countries. The second method is only practical if milk depôts exist. Preservation of milk has, therefore been tried in some countries, namely Italy, France, India, South America and South West Africa.

A good milk preservative must have the following properties:

(*i*) It must not react with any of the constituent parts of the milk.
(*ii*) It must be easily destructible in the factory before using the milk for human consumption or for industrial purposes.
(*iii*) After destruction the remaining substances must be non-poisonous, odourless and tasteless.

None of the known milk preservatives fulfils all these demands but at the moment hydrogen peroxide is the most preferable.

The application of H_2O_2 in the dairy industry has not been generally accepted. The leaders of public health work frown upon the addition of chemicals to foods. However, H_2O_2 is one of the ideal preservatives, because it can be destroyed easily, quickly and completely through the addition of catalase, the enzyme which splits H_2O_2. The breakdown products, water and oxygen, are not detectable and there is no residual toxic effect after the destruction of the hydrogen peroxide.

Hydrogen peroxide has found interest in the dairy industry in treating milk for two purposes: firstly as a short time treatment in place of pasteurization by heat, in order to reduce the total bacterial count, and secondly as a preservative to maintain the keeping quality of milk for a longer period. In the first method H_2O_2 is present in the milk only for a short period (up to 1 hour); in the second method the contact with the preservative is much longer (several hours or days), hence any reaction of the H_2O_2 with the constituent parts of the milk will be more intensified.

Under certain conditions traces of H_2O_2, produced by anaerobic lactobacilli, can be found naturally in dairy products. Probably the inhibitor lactobacillin, generated by lactobacilli, is identical with H_2O_2 (Wheater *et al.*, 1952).

HISTORY OF H_2O_2 PRESERVATION

The germicidal properties of hydrogen peroxide have been known since its discovery by the French chemist, Thenard, in 1818. The first experiments of preserving milk with H_2O_2 (0·4 per cent of a 3 per cent H_2O_2 solution= 0·012 per cent H_2O_2) were made by Schrodt (1883) followed by Heidenhein (1890), Low (1900), Jablin and Gonnet (1901), Renard (1904) (addition of 0·09 per cent H_2O_2) and Nicolle and Duclaux (1904) (addition of 0·06 per cent H_2O_2). Budde (1904) combined H_2O_2 and low heat treatment (0·05 per cent H_2O_2 for 8–10 hr. at 52°C, or 0·035 per cent H_2O_2 for 30 min. at 48°–50°C and 2–3 hr. at 52°C). Much and Römer (1906) used H_2O_2 decomposing enzymes ("Hamase", "Hepin") to destroy the residual peroxide.

Consequently the methods of using H_2O_2 as a dairy preservative, of treating milk at these temperatures in the presence of H_2O_2 and of adding enzymes to destroy the residual peroxide are very old. In this article the literature, published since the last war, is reviewed.

CHEMICAL ASPECTS

Hydrogen peroxide is a strong oxidizing, bleaching, and germicidally active agent. As mentioned above its decomposition yields water and

oxygen. In dilute H_2O_2 solutions the oxidizing effect is reduced. The concentrations used in the dairy industry appear to influence the constituent parts of milk, especially the native proteins, very little and to a lesser extent than other available preservatives. Generally the influence of H_2O_2 on milk is intensified by an increase of the temperature and by prolonging the period of treatment. Highly concentrated H_2O_2 should be diluted with water before being added to milk.

A disadvantage in using H_2O_2 in tropical countries is its instability which is accelerated by contaminations. To-day, very pure H_2O_2 can be manufactured but the presence of one part per million of iron or 0·05 p.p.m. of copper can cause rapid decomposition. The stability decreases as the *p*H increases, decomposition becoming rapid at *p*H 10. By addition of small amounts of organic or inorganic stabilizers the stability is conserved.

For the preservation of milk the hydrogen peroxide must be of high purity (edible grade), firstly for hygienic reasons and secondly because traces of the heavier metals promote oxidation of the milk. Only stainless steel or other corrosion-resistant material should be used in contact with milk containing H_2O_2. Copper and tin are chemically attacked by H_2O_2. A comparison of the results found in the literature is often difficult because different units are used for the description of the peroxide concentration. Table I allows an easy calculation and comparison*.

Table I.

Equivalent concentrations of hydrogen peroxide.

H_2O_2 concentration	*P.p.m.*	*Mole*	*Per cent (by wt.)*	*Per cent of a 40% soln.*	*Per cent of a 130 vol. soln.*
0·01 mole	340	0·0100	0·034	0·085	0·086
100 p.p.m.	100	0·0029	0·010	0·025	0·025
0·1 % (by wt.)	1000	0·0294	0·100	0·250	0·253
0·1 % (40 %)	400	0·0118	0·040	0·100	0·101
0·1 % (30%)	300	0·0088	0·030	0·075	0·076
0·1 % (130 vol.)	395	0·0116	0·040	0·099	0·100

A 130-volumes H_2O_2 solution contains 39·5 per cent H_2O_2. This means, that the decomposition of one litre of this peroxide solution yields 130 litres of oxygen.

OTHER PEROXIDES USED IN THE DAIRY INDUSTRY

Hydrogen peroxide reacts with bases to form peroxides. Calcium and magnesium peroxide have only been of historical importance as dairy preservatives ("Kalkodat" and "Magnodat" process) (Brichta, 1909; Mayerhofer, 1910). Calcium peroxide (CaO_2) seems to be unsuitable for milk treatment even under exceptional conditions. The addition of 0·5 g. of a preparation of calcium peroxide containing 71·3 per cent CaO_2 per litre of milk, gave an objectionable taste, increased the reduction test and decreased the acidity of milk by 0·8°–1·6° Soxhlet-Henkel (0·018-0·036 per cent lactic acid) (Csiszár, 1944).

Several salts form peroxyhydrates. Sodium carbonate-peroxyhydrate cannot be recommended for practical use. Although 0·1 per cent of this substance (containing 23·7 per cent H_2O_2) gave satisfactory preserving qualities for 48 hours at room temperature (Csiszár *et al.*, 1949), the treatment decreased the palatability, had an adverse effect upon the colour and diminished the acidity by 1·2°–2·0° S.H. (0·027–0·045 per cent lactic acid).

Hydrogen peroxide forms solid molecule compounds with organic amines and their derivatives (Fialkov & Shokol, 1949). These addition compounds are very considerably dissociated in aqueous solution. As a milk preservative the urea compound, $CO(NH_2)_2 \cdot H_2O_2$ ("Hyperol", "Ortizon"), containing 64 per cent urea and 36 per cent H_2O_2, has been tested with good results (Dahlem *et al.*, 19[illegible]; Banerjee, 1947). Solid substances are easier to handle than liquid preservatives, but the addition of urea to the milk may be objectionable from the hygienic point of view.

Another peroxide, benzoyl peroxide, has been recommended for the bleaching of milk and cream for cheese manufacture (Kuramoto & Jezeski, 19[illegible]).

INFLUENCE OF H_2O_2 ON THE CONSTITUENT PARTS OF MILK

TASTE

The influence of hydrogen peroxide on the flavour of milk is transitory unless too high a concentration of the preservative is used. As long as H_2O_2 is demonstrable by chemical reactions, the milk has an objectionable taste (it tastes of H_2O_2), but as soon as it is destroyed by catalase, there is no such taste. The breakdown products, water and oxygen, are not detectable. In literature the flavour of treated milk, containing undecomposed H_2O_2, is called "metallic" (Voitkevitch *et al.*, 1944; *et al.*, 1943).

* Unfortunately the H_2O_2 concentrations used in different papers are often not stated clearly. In order to avoid mistakes, the same concentration units are retained in this article as in the original papers.

When H_2O_2-treated milk was subsequently heat-processed, there was no undesirable flavour detectable, even after long storage.

In cases where high concentrations of the preservative (500 p.p.m. and above) are used to preserve milk having excessive bacterial counts, off-flavour may develop. Milk containing an appreciable amount of undecomposed peroxide developed a slight "oxidized" flavour after prolonged storage (Nambudripad *et al.*, 1952) These authors have further found that the material of the containers influences the development of off-flavours. The milk samples treated in aluminium cans gave rise to a slight oxidised flavour. Glass containers were the best, tinned brass, porcelain and aluminium containers coming next in order of preference. Transport of milk preserved on the farm with 0·2 per cent (130 vol. H_2O_2 (Romani, 1944) caused a smoky flavour.

All the above-mentioned disadvantages are not considered big enough to be of great importance. Furthermore, the treatment of milk with H_2O_2 prior to pasteurization and homogenization can prevent the development of a typical solar-activated flavour in homogenized milk samples (Weinstein & Trout, 1951) whilst high-temperature heat-treatment (176°F, 80°C for 5 min.) neither retarded nor prevented its development. The H_2O_2 treatment delays the development of an oxidized flavour in milk during storage at −17°C (1·4°F) (Bell & Mucha, 1949). Morris (1950) found that oxidized flavour developed in 70 per cent of the pasteurized milk samples, but in none of the peroxide-treated samples (0·2 per cent H_2O_2, followed by addition of catalase), the odour and flavour of which was satisfactory apart from a slight malty flavour. In the same way the development of a "tallowy" flavour during cold storage of milk can be retarded by addition of H_2O_2(Krukovsky &Guthrie, 1946).

VITAMINS

The vitamins of milk are damaged very little by treatment with H_2O_2. Only ascorbic acid is seriously influenced, but this is not important because milk is not an important source of this vitamin. After addition of 0·1 per cent H_2O_2 (130 vol.) the loss of ascorbic acid in milk held for 20 hours at 15°, 22°, 26° and 32°C (59°, 72°, 79°, 90°F) was 54, 78, 85 and 92·5 per cent against 84 per cent in the controls (Satta *et al.*, 1943). Higher peroxide concentrations and higher temperatures intensify the destruction. The instability of ascorbic acid in milk containing peroxide has been confirmed by other authors (Bisogni & Calendoli, 1943; Banerjee, 1947; Krukovsky, 1949). The ascorbic acid oxidation varies inversely with the quantity of H_2O_2 added to the milk (Krukovsky & Guthrie, 1946). The reaction was retarded appreciably when the amount of the preservative added was in excess of that required to oxidize ascorbic acid completely. Peroxidase may be responsible for the loss of ascorbic acid (Krukovsky, 1949) and destruction of this enzyme with higher H_2O_2 concentrations may explain the above-mentioned inverse relationship between the retardation of ascorbic acid destruction and peroxide concentration.

After treating milk with 0·1 per cent H_2O_2 (130 vol.) the vitamin *A* content decreased by 22–42 per cent (Giolitti, 1949). Satta *et al.*, (1943) have shown that the addition of 0·3 per cent H_2O_2 (130 vol.) at 20° or 32°C (68° or 90°F) for 36 hours reduced the vitamin *A* content from 158 I.U. to 125 I.U. per 100 g. The corresponding figures for the stored controls without H_2O_2 were 130–135 I.U. The thiamine content dropped in the same treated sample from 25–30 I.U. to 12–15 I.U. and in the controls to 15—18 I.U. per 100 g. In biological assays of vitamin *D* with rats, no appreciable change in the milk treated with up to 0·4 per cent H_2O_2 (130 vol.) and held at 22°C (72°F) for 36 hours could be noted.

None of the *B*-complex vitamins examined by Nambudripad *et al.* (1952) was found to be affected by peroxide treatment of milk. The thiamine, riboflavin, nicotinamide and cobalamin contents were nearly the same in H_2O_2-treated pasteurized milk (300 p.p.m. of H_2O_2 added and the milk pasteurized at 63°C (145°F) for 1–2 hours) as in the control milk which was only pasteurized.

MILK SUGAR AND BUTTERFAT

The lactose content of peroxide treated milk is somewhat lower than of untreated samples (Banerjee, 1947). The same results were confirmed by Arnaudi and Treccani (1953). The sugar content of untreated milk was 5·01 per cent but, after treatment with 0·025, 0·05, 0·1 or 0·2 per cent H_2O_2 (130 vol.) at 30°C (86°F) for 16 hours, the figures were 5·01, 4·95, 4·95 and 4·60 per cent respectively. Giolitti (1949) found no changes for lactose, fat, total N and *p*H.

No exact figures are available yet on the influence of H_2O_2 (at concentrations used for milk preservation) on the butterfat, especially on the unsaturated fatty acids. Butterfat was not changed in treated samples of milk containing 0·1 per cent H_2O_2 (130 vol.) (Giolitti, 1949). Butter made from preserved milk (300 p.p.m. H_2O_2) did not differ appreciably in quality from that made from fresh milk. (Nambudripad *et al.*, 1952; Negretti, 1952).

PROTEINS

Hydrogen peroxide in higher concentrations oxidizes proteins, aldehydes, ketones and acids being formed. Dilute peroxide solutions do not show this effect. Rondoni and Bassi (1951) have studied the influence of dilute H_2O_2 solutions (approximately 0·3 per cent of H_2O_2, 40 per cent) on partly autolysed protein solutions (liver extracts, serum). The addition of peroxide brings about an

aggregation of the protein molecules, judging from the increase of the trichloracetic acid precipitable N. Among the many proteins investigated by Sizer (1947), only pepsin, trypsin, chymotrypsin, bovine fibrinogen, casein, mucin, and soya bean proteins were oxidized by H_2O_2 in the presence of peroxidase. The tyrosine part of the suspectible proteins seems to be changed.

Amino acids.

Certain amino acids are very sensitive towards H_2O_2 particularly cysteine, cystine (Schormüller, 1949) and methionine (Mohan *et al.*, 1949). Tyrosine and tryptophan can also be easily oxidized. Fortunately, the –SH groups of the milk proteins are relatively resistant to oxidizing agents. Addition of 0·03 per cent H_2O_2 (by wt.) or 0·03 per cent H_2O_2 with 8×10^{-5} per cent Cu had no effect on the –SH groups in skim-milk after 20 hours at room temperature (Zweig & Block, 1953). Even the addition of 0·25 per cent H_2O_2 (40 per cent) for one day at 30°C (86°F) or 20 minutes at 55°C (131°F) did not noticeably reduce the –SH content (Lück & Joubert, 1955 *c*). Treatment with higher peroxide concentrations or at higher temperatures and for longer periods of time diminishes the sulphydryl groups content. Addition of 0·3 per cent H_2O_2 (39 per cent) to milk does not affect the amino acid content (Previtera, 1945) by giving rise to the formation of keto acids and ammonia.

In spite of this fact changes in the nitrogen content are noticeable. Giolitti (1949) found that H_2O_2 treatment (0·1 per cent, 130 vol.) had increased the albumin content from 0·042 to 0·061 (in N percentage and decreased the casein content from 0·367 to 0·317 per cent as N. The soluble N expressed as a percentage of the total N amounted to about 30 per cent in peroxide sterilized milk (1 per cent H_2O_2) after one month at room temperature (Peltola & Mattson, 1950). The same figure for milk sterilized by autoclaving for 20 minutes at 120°C was 10 per cent.

In the presence of metal ions (Cu, Fe) or of peroxidase the oxidizing effect is catalytically accelerated. With a peroxidase derived from haemoglobin H_2O_2 was able to deaminate asparagine but did not deaminate the amino acids glycine, alanine, leucine, glutamic acid and aspartic acid (Solsone & Mora, 1949). Of all amino acids studied by Sizer (1947, 1953) only tyrosine, tryptophan, lysine and possibly cystine were oxidized in a peroxidase–H_2O_2 system (37°C (99°F) and *p*H 7·3), the most readily oxidized being tyrosine.

Casein.

According to the experimental results on cheese-making from H_2O_2–treated milk, changes of the casein molecule are to be expected. Generally, peroxide has a softening action on casein. Arnaudi *et al.* (1949*b*) have investigated the influence of H_2O_2 treatment on the coagulation of milk by rennet and found that the addition of up to 0·1 per cent H_2O_2 (130 vol.) improves the coagulum. The addition of 0·2 per cent and more decreases the firmness of the curd. Schormüller and Ballschmieter (1952) have observed that at the beginning, after the addition of sodium hypochlorite solution, the CO_2 development of peroxide-treated casein (0·09 per cent H_2O_2) is more intensified than in untreated samples.

Electrophoresis experiments did not indicate any difference between the casein in treated and untreated milk (Lück & Joubert, 1955*c*). Experiments with the ultracentrifuge have shown that after 5–6 days' preservation with 0·25 or 1 per cent H_2O_2 (39 per cent) at 4°C (39°F) a part of the casein has dissociated. The sedimentation constant of the fast sedimenting component was reduced from $6{\cdot}2 \times 10^{-13}$ in untreated milk to $3{\cdot}8 \times 10^{-13}$ (...) in treated samples (Lück & Joubert, 1955*d*.) The slowly sedimenting component ($s_{20} = 1{\cdot}3 \times 10^{-13}$) has not been changed. The effect mentioned may explain the longer period of time required for the coagulation by rennet, observed in milk as well as in pure sodium caseinate solution (after addition of $CaCl_2$). Compared with untreated samples the size of the casein particles in milk was not changed signficantly when examined under the electron microscope. The alcohol titre is a little lower. This means that the alcohol concentration for the coagulation of treated milk is a little lower than for the same milk when untreated. The decribed H_2O_2 effect on casein is not as serious as it may seem because untreated milk, if kept for a longer period of time at a low temperature, undergoes the same alteration (11 days at 4°C). Recent investigations (Lück & Joubert, unpublished) have shown that preservation for 24 hours at 25°C (77°F) causes similar changes. When the sedimentation diagrams of the casein and casein from treated milk (0·25 per cent H_2O_2 (40 per cent) for 24 hours at 25°C) are compared, a considerable increase in the concentration of the $s_{1.3}$ component is noticed. The viscosity of treated alkaline casein solutions is somewhat lower than that of untreated samples.

Whey proteins.

As in the case of casein, the β–lactoglobulin in milk also shows similar changes. The storage of skim-milk with 1 per cent H_2O_2 (40 per cent) for 7 days at 4°C resulted in a complete breakdown of the β–lactoglobulin ($s_{20} = 3{\cdot}0 \times 10^{-13}$) into a component of much lower sedimentation constant ($s_{20} = 1{\cdot}3 \times 10^{-13}$) (Lück & Joubert, 1955*e*). After the addition of 0·25 per cent H_2O_2 (39 per cent) (6 days at 4°C), separation of the broken-down β–lactoglobulin component indicates heterogeneity. No significant change in the fast sedimenting component (immune globulin, $s_{20} = 6{\cdot}9 \times 10^{-13}$) can be found after peroxide preservation (1 per cent H_2O_2 (40 per cent) for 7 days at 4°C). Solu...

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of pure crystalline β–lactoglobulin appear to be more resistant to dilute H_2O_2 solutions than the same protein in milk. Identical sedimentation diagrams were obtained for treated (1 per cent H_2O_2 1 per cent) 7 days at 4°C) and untreated samples. However, the sedimentation constant is somewhat less for the treated pure protein (treated: $s_{20} =$ [illegible] $\times 10^{-13}$; untreated: $s_{20} = 2·57 \times 10^{-13}$). Electrophoretic studies carried out by the same authors (1955*e*, 1955*c*) also show an influence of H_2O_2 on the whey proteins of treated milk. Spreading of the boundary is noticed and this is accompanied by a decrease in the electrophoretic mobility. Addition of 1 per cent H_2O_2 (40 per cent) for 6 days at 4°C splits the β–lactoglobulin into two components with considerably lower mobilities. Moreover a large number of minor components are observed. Again, the pure β–lactoglobulin is affected less than the same protein in milk.

Enzymes.

Hydrogen peroxide preservation influences the enzymes of milk to a certain extent. The phosphatase test for distinguishing raw and heated milk is applicable to peroxide-treated milk. The addition of 0·2–0·3 per cent H_2O_2 (39 per cent) both at freezing temperature and at 20°–30°C did not affect amylase, lipase, tryptase and phosphatase, but nearly destroyed peroxidase, catalase and reductase (Cimino, 1945).

If H_2O_2 (0·06–0·15 per cent) is used as a milk preservative, and the preserved milk is kept for a long period (10 days to 3 weeks) under summer conditions at room temperature, the activity of the milk phosphatase is seriously inhibited (Sanders & Sager, 1949). The delay of milk coagulation by rennet is caused by an H_2O_2 influence on the casein and not on the rennet enzyme (Lück & Joubert, 1955*d*). Two per cent of a 10 volumes H_2O_2 solution is able to destroy all peroxidase in milk, whereas smaller doses of peroxide disappear and allow some peroxidase to persist (Banerjee, 1947). Fortunately, catalase and peroxidase are destroyed by H_2O_2. If peroxidase would persist the danger of oxidizing amino acids and proteins would be much greater. If catalase would persist, all the preservative would be decomposed after a very short period of treatment and the preserving effect would be much reduced. Yamasaki (1921) and Maximowitsch and Awtonomowa (1928) were the first authors to investigate the kinetics of the destruction of catalase accompanied by the decomposition of H_2O_2. Later, Molland (1947) made intensive studies on the kinetics of the decomposition of H_2O_2 by bacteria and the inactivation of the catalase. Both reactions take place simultaneously. The quantity of catalase inactivated is proportional to the quantity of H_2O_2 decomposed. The inactivation of catalase by H_2O_2 is intensified by increasing temperature (Morgulis *et al.*, 1926).

Other enzymes are influenced by H_2O_2 with varying results. Papain (Bersin & Logemann, 1933) is inactivated with $M/500$ H_2O_2 in 5 minutes at 40°C. The effect of H_2O_2 on urease was not greater than uncatalysed aeration (Hellermann *et al.*, 1933). The following amounts of enzyme inactivation by H_2O_2 (Barron *et al.*, 1952) are given: urease 0 per cent, yeast hexokinase 0 per cent, papain 40 per cent, yeast alcoholic dehydrogenase 23 per cent.

DESTRUCTION OF BACTERIA BY H_2O_2 TREATMENT

INFLUENCE ON PURE CULTURES

Hydrogen peroxide is known as an effective bactericidal and bacteriostatic agent. The mechanism of its action is not clear yet. In the older literature the effect has been attributed to the developed oxygen *in statu nascendi* but, as recognized today, the undecomposed H_2O_2 is effective (Müller, 1921). The bactericidal efficiency varies with different organisms, with the bacterial count, the concentration of H_2O_2, the period of time, and the temperature of the treatment.

Molland (1947) has investigated the catalase production of bacteria and their H_2O_2 tolerance. The strains which produced catalase are as a rule most resistant towards H_2O_2. The aerobic strains (about 10^{10} cells per ml.) were able to grow in a broth containing 0·0125 per cent H_2O_2 (estimated by means of the turbidity of the medium after incubation at 37°C (99°F)). Even bacteria which do not produce catalase or produce very little such as the pneumococcus, the streptococci, *Salmonella typhosa*, *Bacillus anthracis*, and *Erysopelothrix rhusiopathiae* could grow in a medium containing 0·0125 per cent H_2O_2. The H_2O_2 concentrations tolerated by different strains of bacteria were as follows: *Proteus* spp. 0·2, *Bacterium coli*, 0·05–0·1; *Staphylococcus aureus* strain 1, 0·0125 –0·025; strain 2, 0·1– 0·2; *Brucella abortus*, 0·4; *Streptococcus agalactiae*, 0·4; *Str. zymogenes*, 0·0125; *Neisseria catarrhalis*, 0·0125–0·025 per cent.

Nambudripad *et al.* (1949, 1951) have studied the bactericidal efficiency of H_2O_2 with respect to micro-organisms isolated from milk. In general, the Gram-negative group of bacteria (coliforms) are more susceptible to destruction by H_2O_2 than are Gram-positive species (spore formers). The susceptibility of the lactic acid bacteria was intermediate. The time required for 100 per cent destruction after addition of 50, 300 or 500 p.p.m. H_2O_2 (by wt.) to nutrient broth at 37°C was as follows: *Str. lactis* 6 hr., 4 hr., and 2hr. 30 min.; *Str. liquefaciens* 16 hr., 7hr., and 4 hr.; *Sarcina* sp. 8 hr. 10 min., 5 hr., and 2 hr. 30 min.; *Lactobacillus bulgaricus* 6 hr., 4 hr. 30 min., and 2 hr. 30 min.; spores of *B. subtilis* 36 hr., 24 hr. and 18 hr.; spores of *B. cereus* 24 hr., 14 hr., and 7 hr.; spores of *B. megatherium*, not determinable, 24 hr., and 16 hr.;

Bact. coli 5 hr., 45 min., and 45 min.; *Bact aerogenes* 6 hr., 1 hr., and 30 min.; *Alkaligenes viscosus* 2 hr., 1 hr., and 40 min.; *Torula* sp. 24 hr., 7 hr., and 3 hr.; *Oidium* sp. 26 hr., 8 hr., and 3 hr. 30 min. The surviving cells of *Str. lactis* exposed to 500 p.p.m. of H_2O_2, plated and inoculated (an isolated colony) into sterile skim-milk showed a definite retardation in acid production after 3 days (untreated: 0·71 per cent lactic acid; treated: 0·48 per cent). The ability of the organisms to reduce methylene blue was not significantly changed.

Other results with anomalous coliforms from treated milk (Manzari, 1951) suggest that peroxide treatment may change the metabolism of the surviving bacteria (damage or mutation).

The germicidal effect of H_2O_2 in protein-free solutions is more pronounced than in protein-containing liquids (milk).

INFLUENCE ON PATHOGENIC ORGANISMS IN MILK

Treatment with H_2O_2 reduces the bacterial counts and destroys most of the pathogenic organisms but *Mycobacterium tuberculosis* is more resistant than other pathogenic organisms. It resisted H_2O_2 concentrations as high as 2·0 per cent (130 vol.) (Giolitti, 1947*b*). Treatment with H_2O_2 is therefore recommended only for tubercle-free milk. Bovine tubercle bacilli added to milk survived up to 25 hours (0·2 per cent H_2O_2, 130 vol.) (Peregallo, 1945). Similar results were obtained by Monaci (1949). Two of nine milk samples, inoculated with suspensions of *Myco. tuberculosis bovis* and treated with H_2O_2 were positive (guinea-pig inoculation test).

The experimental results of the destruction of *Brucella* organisms in milk by H_2O_2 are not uniform. Complete destruction of *Brucella abortus* and *Br. melitensis* has been obtained by treating milk with 0·2 per cent H_2O_2 (130 vol.) for three hours (Biffi & Romagnoli, 1949), for 14–24 hours at 20°–22°C and 28°–30°C (Monaci, 1949) and for 30 minutes at 20° and 32°C (Satta *et al.*, 1943). *Br. melitensis* is less resistant than *Br. abortus* (Rosati & Mitrovic, 1951). The first mentioned organism survived addition of 0·2 per cent (130 vol. H_2O_2) at 20°–28°C (68°–82°F) for 12 hours, and the latter for 24 hours. The authors conclude that although H_2O_2 treatment of milk does not necessarily result in the elimination of brucellae, it is the safest chemical method yet devised and deserves to be used more widely.

Other authors, however, have found that an H_2O_2 concentration of 0·2 per cent (130 vol.) is not sufficient to kill brucellae completely. Treatment with 0·6 per cent H_2O_2 (130 vol.) for 12 hours gives complete destruction (Maestone, 1952; Giolitti, 1952).

Salmonella typhosa was killed in 8–9 hours by 0·2 per cent H_2O_2 (130 vol.) at 17°–32°C (63°–90°F) and in 4–5 hours by 0·25–0·3 per cent at the same temperature (Satta *et al.*, 1943). Typh... organisms could be isolated (Monaci, 1949) fr... only one of 20 milk samples, inoculated with *S... typhosa* suspension and treated with 0·2 per c... H_2O_2 (130 vol.) for 14–24 hours at 20°–22°C ... 28°–30°C.

REDUCTION OF THE TOTAL BACTERIAL COUNT IN MI...

In the same way as pathogenic organisms ... killed, the total bacterial count of milk is also ... duced. The H_2O_2 treatment causes a higher ... terial reduction than pasteurization by heat. An... obic spore-formers were entirely eliminated (Mon... 1950).

Satta *et al.*, (1943) have determined the p... centage reduction of the bacterial count in m... treated with 0·2, 0·25, and 0·3 per cent of 130 ... H_2O_2 and held for 20 hours. The results we... 99·82, 99·88, and 99·88 at 15°–17°C; 98·64, 99·... and 99·79 at 20°–22°C and 93·57, 99·53 and 99·... at 32°C. Addition of 0·05 per cent of the perox... solution arrested the multiplication of bacteria ... more than 15 hours at 15° and 22°C. A reduct... of the initial count for 24 hours was effected by ... per cent. Onset of multiplication was delayed ... hours over the control. Monaci (1949) has found ... reduction in total bacterial count varying fro... 74·3 to 96·3 per cent with 0·2 per cent H_2O_2 (1... vol.) for 14–24 hours at 20°–22° and 28°–30°... Similar results were obtained by Morandi (19... Nai and Giolitti (1947) recommend contact wi... 0·7–1·0 per cent of the disinfectant (130 vol.) ... 8–10 hours, because addition of 0·25–0·3 per c... is said not to allow a sufficient safety margin ... winter the dose could be lowered to 0·4–0·6 per c... (Giolitti & Nardi, 1949) The rate of bacterial ... duction by H_2O_2 depends on the initial quality ... the milk *i.e.* the initial bacterial count (Nambud... pad *et al.*, 1952).

A short H_2O_2 treatment at higher temperatur... —(0·2 per cent H_2O_2, probably 35 per cent sol... for 30 minutes at 120°F (49°C) (Morris *et al.*, 19... —reduced the average bacterial count from 304,... per ml. in raw milk to 1,090 per ml. in peroxid... treated samples and to 11,485 in pasteurized mi... The coliform counts were 4,339 per ml. (raw), ... (treated), and 10 (pasteurized). The aerobic spor... formers were reduced from 1,730 per ml. (raw) ... 68 in treated milk and to 88 in pasteurized sampl... *Clostridium butyricum* was greatly reduced in ... hours and disappeared in 24 hours. Thermobacter... (Orla-Jensen) and *Streptococcus lactis* are very s... sitive to H_2O_2 (Arnaudi & Treccani, 1953). ... results are available as to how tubercle bacilli re... to such a treatment.

HYDROGEN PEROXIDE IN DAIRY PRACTICE

TESTS ON H_2O_2

In the manufacture of dairy produce (chee...

making) the main problem is to ensure that no trace of H_2O_2 is left in the milk. Several tests are recommended. For the quantitative determination of H_2O_2 the milk (20 ml.) is mixed with trichloracetic acid (4 ml. of a 40 per cent solution) to coagulate the proteins. The solution is filtered and a few drops of sulphuric acid and potassium iodide (KI) are added to 10 ml. of the filtrate. The free iodine is titrated with sodium thiosulphate ($Na_2S_2O_3$) or is determined spectrophotometrically (Ovenston & Rees, 1950; Patrick & Wagner, 1949). As a rule, for a qualitative test, the separation of the proteins is not necessary. The colour of the milk, after addition of some drops of sulphuric acid and some crystals of potassiun iodide, is compared with an untreated sample. If the treated sample becomes yellow, the H_2O_2 is not completely destroyed. With the quantitative method 3 mg. H_2O_2 per litre of milk can be detected. Hypochlorites, giving the same colour, are bound by the coagulated casein and are separated by filtration together with the casein (Pien *et al.*, 1954). The presence of formaldehyde and dichromate interfere (Pien *et al.*, 1953). In milk containing dichromate, H_2O_2 can be determined by the blue colour after shaking with ether and sulphuric acid (2 ml. milk + 2 ml. ether + 5 drops of dilute sulphuric acid) (Rouquette, 1955). This test can only be made up to 2 hours after the dichromate has been added, because H_2O_2 reacts with dichromate at the *p*H of milk. In biological material H_2O_2 is traceable through the reaction of skopoletin (6-methyl-7-oxy-1,2-benzopyrone) with H_2O_2 in the presence of peroxidase giving a decrease of the skopoletin-fluorescence (Andreae, 1955). This method is very much more sensitive than any of the known tests since approximately 10^{-10} per cent H_2O_2 can be detected. Ascorbic acid, glutathione and Mn^{++} ions interfere.

Of 11 mentioned methods (Funk, 1949) for detecting H_2O_2 in whey, only three are recommended: (*i*) vanadic acid dissolved in dilute sulphuric acid forming a red colour with H_2O_2; (*ii*) benzidine in alcohol solution (10 drops per 10 ml. milk or whey) forming a blue colour in the presence of peroxidase; (*iii*) very sensitive method (10^{-9} per cent) with aminophthalic acid (500 ml. whey + 0·3 g. 3-aminophthalic acid + 30 ml. 1 per cent NaOH solution) giving a yellow-green luminescence.

Other tests which may be of interest are the following: (*iv*) the decoloration test of black PbS by H_2O_2, modified by Freytag (1950) (fluorescence picture of the spotted PbS test paper in ultra-violet light); (*v*) the oxidation of Fe^{++} to Fe^{+++} by H_2O_2, Fe^{+++} forming a dark green complex with feron (7-iodo-8-quinlinol-5-sulfonic acid) (Musha *et al.*, 1951); (*vi*) an improved method with $Ti(SO_4)_2$ (Aquino, 1950) in which a 10 per cent solution of $Ti(SO_4)_2$ in 6*N* H_2SO_4 is shaken in a test tube with pure amyl alcohol. After the two layers have separated, 1 ml. of the alcoholic layer is transferred to another test tube and one drop of the H_2O_2—containing solution is added giving a yellow colour.

CONDITIONS GIVING A MAXIMUM PRESERVING EFFECT

Theoretically H_2O_2 should be unable to preserve milk for any long period. Milk—like other biological substances—contains catalase which is originally present in the milk and additionally so from cell and bacterial catalase. This enzyme decomposes H_2O_2. After a certain period of time all H_2O_2 should therefore be split and no further preserving effect should exist. Fortunately, as mentioned above, catalase is inactivated by H_2O_2 in the same way as H_2O_2 is decomposed by catalase. By this means some H_2O_2, split by catalase, is used up for the destruction of the catalase. The rest, the undestroyed H_2O_2, exerts the real preservative effect for a certain period. It is known that the speed of the H_2O_2 destruction by catalase is increased by higher temperatures (in the physiological range), but the destruction of catalase itself is also increased at higher temperatures. The quantities of H_2O_2 decomposed by milk which was highly contaminated with coliform bacteria (Lück, 1955*a*) after 24 hours at 4°, 13°, 20°, and 30°C (39°, 55°, 68° and 86°F) were 0·44, 0·31 0·20 and 0·11 per cent H_2O_2 (39 per cent) respectively. According to these results the addition of 0·25 per cent H_2O_2 (39 per cent) would satisfactorily preserve this milk at a temperature of 20° and 30°C, but not at 13°C.

On the other hand if surplus H_2O_2 in milk must be destroyed by addition of catalase, the milk should be cold. At lower temperatures it takes a longer time to decompose the preservative, but much less catalase is necessary for splitting the same quantity of peroxide than at higher temperatures. The quantity of H_2O_2 decomposed by milk at a constant temperature is proportional to the quantity of catalase (cells, aerobic bacteria) in milk. For a maximum preservative effect therefore, the H_2O_2 must be added after milking when the bacterial content is low and the milk has a temperature of 37°C (blood temperature). Moreover the germicidal action of the H_2O_2 is more pronounced at higher temperatures.

As pasteurization by heat destroys most of the catalase, H_2O_2 has a better preserving effect on pasteurized milk than on raw milk.

Experiments have been done to improve the preserving effect of H_2O_2 by the addition of other preservatives. Combination of 0·1 per cent methyl *p*-oxybenzoate ("Abiol") and 0·2 per cent H_2O_2 (130 vol.) (Ferrara & Salerno, 1954) increased the time, during which milk kept satisfactorily, to 6 days at 15°C (59°F) and 9 days at 25°C (77°F). Without peroxide "Abiol" had a very poor preservative action on milk.

PEROXIDE TREATMENT INSTEAD OF PASTEURIZATION BY HEAT

Review articles on the H_2O_2 treatment of milk have been published by Minut (1946, 1947), Demeter (1953) and Rosell (1954).

In this paragraph the methods are described which use H_2O_2 instead of pasteurization by heat to reduce the bacterial count. H_2O_2 treatment has been recommended as a method of retaining the raw milk properties when making high quality Swiss cheese (Morris *et al.*, 1951, 1952, 1953), in processing and canning whole sweet milk (Winger, 1952*a*) and in treating milk for human consumption (Lewis, 1953).

For the manufacture of cheese, H_2O_2 (edible grade) is added to the milk and, after the effective germicidal period needed to kill the micro-organisms present, the rest of the H_2O_2 is removed through the addition of catalase. Normally, a treatment with 0·2 per cent H_2O_2 (35 per cent) for 20–40 minutes at 120°–130°F (49°–54·4°C) is recommended (Morris *et al.*, 1951; Armour & Co., 1953). At this temperature the peroxide should not remain in the milk for more than 1 hour. Prolonged treatment causes the casein to become gummy. After the milk is cooled to 100°F (38°C) catalase is added at a rate of 0·5 g. (dissolved in water) per 2,000 lb. of milk (Morris *et al.*, 1951), or at a rate of 0·5 g. "Armour Catalase 10" for each pound (450 g.) of 35 per cent H_2O_2 previously used (Armour & Co., 1953). Generally, the cheese made from this milk (Swiss, Cheddar and Jack cheese) was superior in flavour, body and texture when compared with products made from raw and pasteurized milk (Morris *et al.*, 1951). The cheesemaking procedure is normal although the use of larger quantities of starter and cooking at slightly higher temperatures than is customary are advisable.

The advantages of the process mentioned are summarized as follows: (Armour & Co., 1953):

(*i*) Superior quality and fine flavour of the cheese.
(*ii*) Improved body and texture.
(*iii*) Control of fermentation by variation in temperature and in amount of peroxide.
(*iv*) Elimination of "late gas formers".
(*v*) Pre-treatment with H_2O_2 does not interfere with later cheesemaking operations.
(*vi*) Avoid the harmful action of high temperatures (pasteurization by heat).

A similar peroxide treatment is used for the manufacture of sterilized milk. The milk is treated with 0·1–0·2 per cent of 35 per cent H_2O_2 at 120°F (49°C) for 30 minutes (Winger, 1952*a*). After removal of the H_2O_2 with the catalase (0·1–0·5 g. per 1,000 lb. (450 kg.) milk), the milk is homogenized, canned and sterilized at a temperature above 212°F (100°C). The product produced by this process does not contain the objectionable cooked and scorched flavour common to normally heat-sterilized milk.

In another patent, Winger (1952*b*) recommends pasteurization before peroxide treatment by the addition of only 0·015 per cent of 35 per cent H_2O_2 (2 oz. H_2O_2 for each 860 lb. of milk) at 142°–144°F (61°–62°C) for 30–35 minutes and no addition of catalase before sterilization.

The low-heat-H_2O_2-catalase process has been recommended for the treatment of fresh milk in tropical countries (Lewis, 1953). The effect of this method on tubercle bacilli has not been investigated yet. At room temperature the period of treatment must be prolonged and higher H_2O_2 concentration must be used (Nai & Giolitti, 1947) in order to obtain the same destruction as with pasteurization by heat.

IMPROVEMENT OF THE KEEPING QUALITY OF MILK BY ADDITION OF H_2O_2

The improvement in the keeping quality of milk obtained by treating with H_2O_2 has been pointed out by many authors. Two methods are recommended: (*i*) addition of a relatively small amount of H_2O_2 to improve the keeping quality between milking and arrival at the pasteurization plant, and (*ii*) addition of a relatively high amount of H_2O_2 to improve the keeping quality and to render pasteurization by heat unnecessary. Both methods reduce losses from souring during transport which renders milk unfit for pasteurization and consumption.

Romani (1944) notes, however, that H_2O_2 treatment at the farm causes the sediment to rise, and promotes churning during transport.

The peroxide concentrations to be added depend on the climate, the quality of the milk and the period of preservation desired. The following quantities are recommended: 0·3 per cent of a 0·3 per cent solution (0·023 per cent H_2O_2, 40 per cent) (Vintika, 1948), 300 p.p.m. (0·075 per cent H_2O_2, 40 per cent) (Nambudripad *et al.*, 1952), 0·1–0·2 per cent H_2O_2, 40 per cent (Pien, 1948), 0·2–0·3 per cent H_2O_2, 40 per cent (Satta *et al.*, 1943, Satta, 1948). Because of the complete destruction of the H_2O_2, addition of catalase, is not necessary. Pasteurized milk, in which most of the catalase is destroyed by heat, can be preserved with less H_2O_2 than is normally used for raw milk. With this method it is possible to supply big towns in tropical countries with fresh pasteurized milk from great distances.

Treatment of milk for food purposes by adding 0·2–0·3 per cent H_2O_2 (130 vol.) is useful in extending the period of freshness but cannot replace pasteurization (Rosati, 1945, 1947). Milk preserved with H_2O_2 must be pasteurized before using it for human consumption unless the peroxide concentration or the temperature of treatment is increased. The second method mentioned above corresponds

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to these demands (Giolitti, 1947*a*). It has found practical application in Italy (Nai *et al.*, 1951). From 0·25 to 0·6 per cent H_2O_2 (130 vol.)—according to the catalase number—is added to raw milk at a small collecting centre. After 8 hours contact the excess peroxide is eliminated by the addition of catalase, and the milk is bottled and distributed. The cost of the method is slightly less than 6·25 lire per litre ($\frac{1}{2}d.$ per pint). The H_2O_2-treated samples have a better keeping quality than pasteurized milk especially at 13°C (55·4°F) (Nemec, 1950). Preservation with peroxide concentrations higher than 1 per cent has also been tried. Milk can be kept at 5°C (41°F) by the addition of 1, 2, or 3 per cent H_2O_2 (130 vol.) for 24–35 days, 32–40 days and up to 100–110 days (Romani, 1947). This method cannot satisfactorily, however, replace methods at present accepted for preserving milk (Anselmi, 1946). Romani (1944) considered that use might be made of the liberated oxygen, as in the Höfus process. Comparison of the keeping quality of H_2O_2-treated milk in tightly stoppered bottles with that of samples in loosely stoppered ones has shown that pressures up to 2·5, 4 and 6 atmospheres were developed in the presence of 0·1, 0·2 and 0·3 per cent H_2O_2 (130 vol.) at 28°C (82·4°F) respectively. The keeping quality under pressure of oxygen was better. An acidity of 0·21 per cent was normally reached in the presence of 0·3 per cent H_2O_2 (130 vol.) in 1 day at 30°C (86°F) and in 4 or 5 days at 10°C (50°F). In the tightly stoppered bottles these times were 2 days and 30–40 days respectively. Skim-milk treated with H_2O_2 (0·2 per cent H_2O_2, 130 vol.) can replace fresh skim-milk for calf feeding (Mainradi, 1952).

PRESERVATION OF WHEY AND CREAM BY H_2O_2

The use of H_2O_2 for the preservation of deproteinized sweet whey has not been very successful (Plöttner, 1947). Addition of 0·005 per cent H_2O_2 (by wt.) produces a disagreeable taste (that of H_2O_2) and even the addition of 0·01 per cent did not prevent the growth of yeast. Better preserving results were obtained by the same author through the addition of 0·015 and 0·03 per cent H_2O_2 (by wt.) to raw sweet whey. After the precipitation of the proteins by heating at 98°C for 10 minutes or after the addition of small quantities of yeast, the taste of the H_2O_2 disappears. The addition of 0·09 per cent H_2O_2 to raw whey only limits the extent of souring.

In contrast to these results, Yunus (1953) reported that the addition of 0·1 per cent H_2O_2 has no preservative value for whey. Formaldehyde is more effective and prevents acid formation for at least 21 days.

The preservation of cream by H_2O_2 has also been tried. In a pre-war patent (Reichert *et al.*, 1936) the addition of H_2O_2 in amounts ranging from 0·01 to 0·09 per cent by weight and heating at a temperature of 61°–63C for 15–30 minutes is recommended. For the control of infections in synthetic cream, 0·005–0·02 per cent H_2O_2 should be added (Hobbs & Smith, 1954). In the presence of butter, milk, and egg yolk, at least three times the concentration of H_2O_2 is necessary as when emulsified fat without added protein is used.

THE MANUFACTURE OF DAIRY PRODUCTS FROM MILK PRESERVED WITH H_2O_2

Cheesemaking

As mentioned above the manufacture of cheese from milk treated with H_2O_2 (0·2 per cent of a 35 per cent solution for 20–40 minutes) at 120°–140°F) for a short time yields a product which is often superior in quality to that made from raw or pasteurized milk. On the other hand, cheese manufactured from milk preserved with H_2O_2 (0·2 per cent of a 40 per cent solution) for a longer period (8–24 hours) is mostly of inferior quality, having a pasty texture and a soft body (Morris, 1950; Lück, 1955*b*). It is difficult to obtain satisfactorily dry cheese from milk treated with 1 per cent H_2O_2 (Peltola & Mattson, 1950). For these anomalies two factors are responsible: (*i*) an inhibiting after-effect on the lactic acid bacteria (Arnaudi *et al.*, 1949*b*) and (*ii*) the influence of H_2O_2 on the casein (Lück & Joubert, 1955*d*). In order to offset these disadvantages, the use of larger quantities of starter and cooking at a slightly higher temperature than is customary, are necessary. Arnaudi *et al.*, (1949*b*) have added up to three times the quantity of rennet normally used to obtain a satisfactory firmness of the curd.

Hydrogen peroxide-preservation prolongs the coagulation period by rennet and this is increased when a higher peroxide concentration and a longer preservation period are used (Lück & Joubert, 1955*d*). The longer the coagulation period necessary the less firm is the curd.

Cheesemaking experiments (Gouda and Cheddar cheese) with preserved milk (0·25 per cent H_2O_2, 130 vol. for 24–30 hr. at 26°–30°C) were not very successful, in spite of doubling the quantity of starter culture and adding calcium chloride (Lück, 1955*b*). The body of the Gouda was very weak. The cheese sagged, lost shape and had an unclean and foreign flavour. When such cheese becomes older, it develops a bitter flavour. The Cheddar cheese had a Gouda body and after 8 weeks' storage, it had developed a strong off-flavour. The body is pasty, greasy and pliable and similar to the body of processed cheese.

The flavour is also typical. It has been described above as "foreign"; it is like a chemical salt flavour with a slight bitterness. According to these experimental results the manufacture of cheese from H_2O_2-preserved milk (0·25 per cent 130 vol., 24 hr.) cannot be recommended. However, experiments on cheesemaking from mixtures of preserved

and untreated fresh milk, or from milk preserved with a lower H_2O_2 concentration, gave good results. Preserved and fresh milk are mixed before pasteurization. The mixed milk should stand for approximately ½–1 hour, to enable the catalase of the fresh milk to decompose the remaining H_2O_2. No addition of any other catalase is necessary. The Gouda made from 50 per cent preserved milk had a slight foreign flavour, but not the Cheddar. The samples with 33 and 25 per cent of H_2O_2–preserved milk were of even better quality than the controls, especially during that season of the year, when the milk arrives over-acid at the factory and the body of the cheese had a tendency to become rough, crumbly or even chalky (Lück, 1955*b*).

The quality of cheese made from H_2O_2-treated milk is better when the milk is preserved at intervals with low H_2O_2 concentrations than when the same quantity is added in a single dose—for instance, adding 0·24 per cent in three equal lots at 8 hour intervals is better than adding 0·24 per cent in one lot during 24 hours.

The preservation of milk with 0·05–0·1 per cent H_2O_2 (130 vol.) does not affect the technique of cheesemaking (Arnaudi, 1949*a*; Treccani, 1952).

Buttermaking

A few trials on making butter from H_2O_2-treated milk (300 p.p.m. H_2O_2) (Nambudripad *et al.*, 1952), have shown no appreciable difference in the quality as compared with untreated milk. No differences in yield or flavour were observed in butter made on an industrial scale from preserved milk (Negretti, 1952.) The milks were treated with 0·5 per cent H_2O_2 (130 vol.) and the surplus disinfectant was removed with catalase 8 hours later. The butter from treated milk had a better keeping quality and very much lower coliform titres and bacterial counts. The bacterial count was nearly zero.

Casein-making

There is no real difference in the casein manufacturing process from H_2O_2-treated (0·25 per cent H_2O_2, 130 vol., 24 hr at 25°C) or untreated milk. The coagulation period of treated milk is somewhat prolonged and the acidity of the whey before cooking should be somewhat higher. Exact control of the acidity is important, because casein manufactured from treated milk has a stronger tendency to become rubbery and plastic at higher temperatures. This effect can also be observed at higher drying temperatures. It can be prevented by adding some acid to the last wash water to obtain a *p*H of 4·6. Low acid curd from treated milk should not be left overnight in wash water without the addition of acid, otherwise it may become so soft and milky by the next morning that it cannot be pressed and dried (Lück, 1955*b*).

HYGIENIC ASPECTS

The influence of H_2O_2 treatment or preservation on the constituent parts of milk is less than the influence of other processes applied and accepted in the dairy industry. The milk does not lose its nutritional value apart from a small decrease of some vitamins, and a loss of ascorbic acid.

In an experiment by Satta *et al.*, (1943) infants aged 4–24 months were given treated milk (0·2 per cent H_2O_2 130 vol.) in their diet for 12–1 days. The gain in weight was better than for the controls on sterilized milk and a beneficial and dyspeptic action on the gastro-intestinal functi was recorded.

According to our knowledge of the influence of H_2O_2 on the organism, H_2O_2 should be remove completely before using the milk—or other dai products—for human consumption.

Hydrogen peroxide induces mutations *Neurospora* spp. (Jensen *et al.*, 1951; Cleland Lotz, 1949) and in *Bacterium coli* (Demerec *et a* 1951) and causes a decrease in the number of no mal mitotic figures in the mouse intestine (Dus & Gompel, 1949). Organic peroxides are mutage and appear to produce a breakage of the long chro osomes of *Vicia faba* (Ross, 1950). A hypothe on the importance of undecomposed H_2O_2 in tissu for carcinogeneses has also been published (Rond & Cudkowicz, 1953). Care should therefore taken with undecomposed H_2O_2 in human food spite of the fact that H_2O_2 is quickly split in gastro-intestinal tract and in spite of the argume that living tissues actually contain traces of H_2O

DISCUSSIONS AND CONCLUSIONS

The H_2O_2 preservation of milk must be co sidered as an aid to farmers and the dairy indust in countries with hot and tropical climates. Un economic conditions permit the necessary improv ments in production conditions (cooling on farm as well as during transport), the addition H_2O_2 to milk can be recommended. The H_2O_2 added to the milk on the farms which are far fro the receiving dairies, and it has been effective preventing souring during transport. The additi of H_2O_2 may be suggested as a temporary meth if anything unforeseen happens, such as delay milk transport or any mechanical breakdown wh would cause losses through sour milk.

The quantity of H_2O_2 added to milk, depen on the quality of the milk (catalase number) and period of preservation desired, and varies betwe 0·05 and 0·3 per cent H_2O_2 (40 per cent).

This treatment is not absolutely safe for de troying pathogenic organisms. Milk preserved this process should therefore be additionally p teurized by heat before using it for human co sumption. Pasteurization by heat can be rend