THE USE OF HYDROGEN PEROXIDE IN MILK AND DAIRY PRODUCTS

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Introduction

During recent years great efforts have been made by many countries with tropical or subtropical climates to develop their dairy industry. In such countries many difficulties in handling fresh milk arise simply from the fact that milk remains sweet for only very short periods of time at the high temperatures prevailing. Moreover, it is often necessary to transport the milk over long distances, and the initial bacterial counts are already generally quite high, partly because of insanitary conditions of milk production. In addition to attempts to achieve more hygienic milk production, efforts have been made to prolong the keeping quality of the milk by artificial cooling on the farm as well as during transport; double or multiple pasteurization; and preservation. Without doubt the first is the ideal method, but its cost prohibits its use at present in many newly developing tropical countries, while the second method is practicable only if milk depots exist. Thus preservation of milk has been tried in certain countries.

General aspects of the use of milk preservatives

A " preservative for milk " may be defined as any chemical compound and/or process which, when applied to milk, retards alterations caused by the growth of micro-organisms, or enables the physical properties, chemical composition and original nutritional value to remain unaffected by microbial spoilage. It is felt that substances such as stabilizers, etc., added to milk to prevent physical changes, should not be included in this definition.

The question of using preservatives for milk and dairy products has been extensively debated. The general attitude in most countries is that it should not be permitted, for two reasons: firstly, the preservation of milk and milk products should be secured through improved production and processing methods rather than through the addition of preservatives; secondly, milk and dairy products are basic foodstuffs, and as such should be free

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of preservatives. On the other hand, it has been maintained that the use of preservatives should not be entirely condemned under certain conditions, in countries where the dairy industry is not well developed or organized, and more particularly in countries with a warm climate. Any country adopting this method should ensure that it presents no obstacles to the production of clean milk. Real and permanent progress of a dairy industry is only possible through clean, hygienic milk production and the use of hygienic manufacturing processes. Recourse to preservatives should therefore be restricted to emergencies, and must be considered only as a temporary aid to farmers and the dairy industry in warm countries until the necessary improvements in production conditions (for instance cooling on the farm as well as during transport) become an economic possibility. If preservatives are employed, control must be exercised to prevent misuse or unnecessary use of them. Various methods of processing milk, in which different preservatives or preserving methods are used, have been introduced not only in countries where the dairy industry is comparatively undeveloped but also—by certain commercial interests—in some highly developed countries.

A good milk preservative should satisfy the following requirements: (1) it must not react with any of the constituents of milk; (2) it should be easily removable in the milk-plant before the milk is made available for human consumption or for industrial purposes (e.g. cheese-making); (3) after it has been eliminated from the milk, the substances remaining must be non-poisonous, odourless and tasteless; (4) the whole preservation process must be easily carried out and should not be expensive.

None of the known milk preservatives fulfils all these demands. Different chemicals have at one time or another been used or recommended officially or unofficially in different countries—e.g., antibiotics (penicillin, aureomycin, streptomycin), quaternary ammonium compounds, chloropicrine (microlysin), menadione, bromine compounds of acetic acid, mercaptopropionic acid or its compounds, formaldehyde, extracts of plants, peroxides, and oxygen under pressure. Very few of these have given satisfactory results. Most of them entail a risk to the health of the consumer and the public health authorities frown upon their use as additives. The problem with antibiotics is that a single substance—e.g. penicillin, which is destructible by penicillinase—is not sufficiently effective, while if a combination of more than one antibiotic is employed, the ultimate elimination of the residual preservative becomes very difficult. The preservation of large quantities of milk through oxygen under pressure is costly and very difficult to handle on a commercial scale.

The general principles of food preservation, which are also valid for milk and dairy products, have been summarized in reports of a Joint FAO/WHO Expert Committee on Food Additives (World Health Organization, 1957, 1962).

General aspects of the use of hydrogen peroxide

In the dairy industry the objections against the use of hydrogen peroxide (H_2O_2) are those applicable to any preservatives, as described above. However, it is the most acceptable at present available. It can be destroyed easily, quickly and completely through catalase; after enzymatic treatment the breakdown products, water and oxygen, are normally undetectable in milk and no toxic residue remains once H_2O_2 has been destroyed.

H_2O_2 is a strong oxidizing, bleaching and germicidal 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 have very little influence on the constituents of milk, especially the native proteins. Generally the influence of H_2O_2 on milk is intensified by an increase of the temperature and prolongation of the period of treatment. Highly concentrated H_2O_2 should be diluted with water before being added to milk.

The germicidal properties of H_2O_2 have been known since its discovery by the French chemist Thenard in 1818. The first experiments in preserving milk with hydrogen peroxide were made at the end of the last and the beginning of this century (Schrodt, 1883; Heidenhein, 1890; Low, 1900; Jablin-Gonnet, 1901; Renard, 1904; Nicolle & Duclaux, 1904; Budde, 1904; Much & Römer, 1906). The quantity of H_2O_2 required for significant inhibition of bacterial growth is small and does not constitute any appreciable dilution of the milk. Most of the H_2O_2 added is decomposed by the catalase of micro-organisms and leucocytes of the milk, while heat treatment can also destroy H_2O_2 to a certain extent.

A disadvantage in using H_2O_2 in tropical countries is its instability, which is accelerated by contamination. Today, very pure H_2O_2 can be manufactured, but the presence of 1 part per million (p.p.m.) of iron or 0.05 p.p.m. of copper can cause rapid decomposition. The stability decreases as the pH increases, decomposition becoming rapid at pH 10. Stability may be maintained by adding small amounts of organic or inorganic stabilizers. For use in the preservation of milk, the H_2O_2 solution must be of analytical quality and free from metallic or other impurities.

Hydrogen peroxide is of interest to the dairy industry in treating milk for two purposes: first, as a *short-time treatment* in place of pasteurization, 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 contact between the preservative and the milk is much longer (several hours or days), hence any reaction of the H_2O_2 with the constituents of the milk will be intensified. If H_2O_2 is used for both purposes, efforts should continue to be made to replace the peroxide treatment as soon as possible by better methods of milk production, transport and processing.

H_2O_2 has also been used in conjunction with other preservatives, for instance 0.2% H_2O_2 and 0.025% propyl-*p*-hydroxybenzoate or 0.05% of the ethyl ester (Ferrara and co-workers, 1957). Both esters, or mixtures of them, prolong the preservative effect of H_2O_2 on milk, but it is impossible to eliminate them subsequently.

Extensive research has been carried out on all these aspects in several countries (for a review of the literature see Lück (1956a)). The question of adding H_2O_2 to milk has also been discussed at a meeting of experts from different countries which was convened at Interlaken by the Food and Agriculture Organization of the United Nations in 1957. In their report [1] the following points are made on the practical aspects of the use of this preservative.

The use of H_2O_2 should be permitted only when: (1) adequate control can be maintained by official agencies; (2) the availability of tested and approved grades of H_2O_2 can be ensured, and its distribution organized through authorized agencies; (3) the absence from milk destined for liquid consumption of any residual preservatives can be checked before distribution. Provided that these essential controls can be established, the local conditions under which this use of H_2O_2 might possibly be permitted are as follows: (1) where a preservative is urgently necessary in an emergency (e.g., as a result of the mechanical breakdown of a refrigeration plant); (2) where, in a technically less developed country, the production and collection of milk is as yet insufficiently well organized; (3) where, in a warm country, milk is produced on scattered farms, usually in small quantities, and has to be bulked and transported over considerable distances before reaching a cooling or pasteurizing centre; (4) where, in a warm country, roads and transportation conditions are such as will not allow milk to reach the consuming area within a sufficiently short time—in some warm countries, less than four hours; (5) where, in a warm country, atmospheric temperatures are so high at certain seasons of the year as to cause very rapid bacterial multiplication and spoilage of milk, and no refrigeration is available.

Trial distribution of H_2O_2-treated milk has been made in some countries—namely, Italy, France, Spain, Nigeria, and areas in south-west Africa and South America.

The use of other peroxides

Besides H_2O_2 other peroxides—in particular, solid peroxides—have been tried as preservatives for milk. Solid substances are easier to handle than liquid preservatives, especially highly concentrated H_2O_2 solutions. Calcium and magnesium peroxides (CaO_2, MgO_2) which are of only historical

[1] Food and Agriculture Organization of the United Nations (1957) *Report on the meeting of experts on the use of hydrogen peroxide and other preservatives in milk, Interlaken, Switzerland, 23-27 September 1957*, Rome (Unpublished mimeographed document FAO/57/11/8655).

interest (" Kalkodat " and " Magnodat " processes, Mayerhofer & Přibram (1910)) seem to be unsuitable for treatment of milk, even under exceptional conditions. They impart an objectionable taste, increase the reduction test, and decrease the acidity (Csiszar, 1944).

Several inorganic salts which form peroxyhydrates have proved of interest, but for similar reasons to those advanced against CaO_2 sodium carbonate-peroxyhydrate cannot be recommended for practical use (Csiszar, Tomka & Bittera, 1949). Rudy (1944) has used alkali phosphate-peroxyhydrates to prevent microbial infections of processed cheese.

Hydrogen peroxide also forms solid molecule compounds with organic amines and their derivatives which are very considerably dissociated in aqueous solution. As a milk preservative, the urea compound $CO(NH_2)_2.H_2O_2$ containing 64 % urea and 36 % H_2O_2 has been tested with good results (Dahlen & Crossley, 1945; Banerjee, 1947). The addition of urea to milk may, however, be objectionable from the hygienic point of view. If, for instance, skim milk powder is made from urea- and H_2O_2-preserved milk, the urea concentration per 100 g of powder will be approximately 0.2 g-1.4 g. Urea is not very toxic, but its use as a food additive in such an unusually high concentration is very undesirable.

No peroxides other than H_2O_2 (and this only within the limitations previously stated) should therefore be considered as preservatives for milk used for human consumption.

Effects of H_2O_2 on the Constituents of Milk

Taste

Milk treated with H_2O_2 in the concentrations normally used retains no undesirable taste once complete decomposition of the H_2O_2 has taken place. As long as H_2O_2 is demonstrable by chemical reaction the milk has an objectionable flavour of the preservative. When 0.05 % by weight of H_2O_2 and above is used to preserve milk having excessive bacterial counts, off-flavour may develop. Milk containing an appreciable amount of undecomposed peroxide may, depending on the material of the container, develop a slight " oxidized " flavour after prolonged storage (Nambudripad, Laxminarayana & Iya, 1952).

On the other hand, the treatment of milk with H_2O_2 can prevent the development of a typical " solar-activated " flavour in homogenized milk samples (Weinstein & Trout, 1951), and can delay the formation of an " oxidized " flavour (Bell & Mucha, 1949; Morris, 1950), and the development of a " tallowy " flavour (Krukovsky & Guthrie, 1946) during cold storage, probably by chemical reaction. When H_2O_2-treated milk is subsequently heat-processed, no undesirable flavour is detectable even after long storage.

Vitamins

The vitamins of milk are very little damaged by normal treatment with H_2O_2. Only ascorbic acid is seriously affected, but this is not important because milk is not an important source of this vitamin. After addition of 0.04 % by weight of H_2O_2, the loss of ascorbic acid in milk held for 20 hours at 15°, 22°, 26° and 32°C was 54 %, 78 %, 85 % and 92.5 % against 84 % in the controls (Satta et al., 1943). Higher H_2O_2 concentrations and higher temperatures intensify the destruction. The instability of vitamin C in milk containing peroxide has also been confirmed by other authors (Bisogni & Calendoli, 1943; Banerjee, 1947; Krukovsky, 1949).

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 (0.03 % by weight H_2O_2 added, and the milk pasteurized at 63°C for 1-2 hours) as in the control milk, which was only pasteurized. Lück & Schillinger (1958a) have found that vitamin B_1 is partly destroyed but vitamin B_2 is quite stable even under relatively extreme conditions (see Table 1).

TABLE 1

EFFECTS OF H_2O_2 TREATMENT ON THE VITAMIN B_1 AND B_2 CONTENT OF MILK *

Treatment	Vitamin B_1 (µg/100 ml)	Vitamin B_2 (µg/100 ml)
Milk, untreated	49	158
Milk + 0.075 % by weight H_2O_2, 21 hours, 20°C	47	158
Milk + 0.30 % by weight H_2O_2, 23 hours, 20°C	22	160

* After Lück & Schillinger (1958a)

Janiček & Pokorny (1958) have shown that a peroxide concentration of 0.25 % destroys 20 %-25 % of the riboflavin. A similar destruction of the thiamine was observed after 5 minutes' heating of the milk at 60°-65°C.

After milk has been treated with 0.04 % by weight of H_2O_2, the vitamin A content decreased by 22 %-42 % (Giolitti, 1949). Satta et al. (1943) have shown that the addition of 0.12 % by weight of H_2O_2 at 20°or 32°C for 36 hours reduces the vitamin A content from 158 International Units (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 changes could be noted for milk treated with H_2O_2 up to 0.16 % by weight and held at 22°C for 36 hours.

Other experiments have shown (see Tables 2, 3) that the fat-soluble vitamins are quite stable, probably being protected by the fat of the fat

globules. Even H_2O_2 in a concentration of 0.3 % by weight could not destroy β-carotene, vitamin A and vitamin E (Lück & Schillinger, 1958a). However, fat-soluble vitamins added to milk are more sensitive to H_2O_2 treatment.

TABLE 2

EFFECTS OF H_2O_2 TREATMENT ON THE CONTENT IN MILK OF CERTAIN FAT-SOLUBLE VITAMINS *

Treatment	β-Carotene (μg/100 ml)	Vitamin A μg/100 ml)	Vitamin E (μg/100 ml)
Milk, untreated	22.8	37	110 **
Milk + 0.075 % by weight H_2O_2, 30 minutes, 51°C	22.1	38	—
Milk + 0.30 % by weight H_2O_2, 24 hours, 30°C	22.5	37	112 **
Vitamin-enriched milk, untreated	42.0	302	404 †
Vitamin-enriched milk + 0.30 % by weight H_2O_2, 24 hours, 30°C	40.2	285	200 †

* After Lück & Schillinger (1958a)
** Total tocopherols
† Total tocopherols + DL-α-tocopherol

TABLE 3

EFFECTS OF H_2O_2 SHORT-TIME TREATMENT * ON THE CONTENT IN MILK OF CERTAIN WATER-SOLUBLE VITAMINS **

Vitamin	Mg/100 ml		Loss %
	before treatment	after treatment	
B_1	0.055	0.054	0
B_2: chemically determined	0.220	0.224	0
microbiologically determined	0.197	0.181	8
B_6	0.023	0.023	0
C: ascorbic acid	1.57	0.10	94
ascorbic acid + dehydroascorbic acid	1.73	0.14	92

* 0.07 % by weight H_2O_2, 30 minutes, 51°C
** After Lück & Schillinger (1958b)

Milk sugar and butterfat

Giolitti (1949) has found no changes for lactose, fat, total nitrogen and pH after the addition of 0.04 % by weight of H_2O_2 to milk. According to other experiments, the lactose content of peroxide-treated milk is somewhat lower than that of untreated samples (Banerjee, 1947). The same results were confirmed by Arnaudi & Treccani (1953). The sugar content of untreated milk was 5.01 % but, after treatment with 0.01 %, 0.02 %, 0.04 % or 0.08 % by weight of H_2O_2 at 30°C for 16 hours, the figures were 5.01 %, 4.95 %, 4.95 % and 4.60 % respectively.

The higher unsaturated fatty acids do not, in practice, react with hydrogen peroxide. Even under relatively severe conditions of treatment (0.3 % by weight of H_2O_2 for 24 hours at 51 °C) the ultraviolet absorption spectrum of the butterfat did not change significantly (Lück & Schillinger, 1958a). Butter made from preserved milk (0.03 % by weight of H_2O_2) did not differ appreciably in quality from that made from fresh milk (Nambudripad et al., 1952; Negretti, 1952).

Casein

Hydrogen peroxide in higher concentration oxidizes proteins and aldehydes, ketones and acids being formed. Dilute peroxide solutions do not show this effect. According to the experimental results of cheese-making from H_2O_2-treated milk, changes of the casein molecule are to be expected. Generally, peroxide has a softening action on curd. Arnaudi, Cartasegna & Passani (1949) have investigated the influence of H_2O_2 treatment on the coagulation of milk by rennet and found that the addition of H_2O_2 up to 0.04 % by weight improves the coagulum; the addition of 0.08 % or more, however, decreases the firmness of the curd. The coagulation time using rennet is increased (Lück & Joubert, 1955b).

Compared with untreated samples, the size of the casein particles in milk shows no significant change when examined under the electron microscope. The alcohol titre is a little lower; this means that the stability is reduced and the alcohol concentration for the coagulation of treated milk is a little lower than that for the same milk when untreated (Lück & Joubert, 1955b). H_2O_2 treatment of pure casein solutions (0.1 %-0.4 % by weight) increases the proportion of nitrogen not precipitated by trichloracetic acid and reduces the viscosity of the solutions (Lück, 1956b). This may be the reason why H_2O_2 treatment (0.04 % by weight) of milk increases the albumin content and decreases the casein content (Giolitti, 1949). Experiments with ultra-centrifugation have shown that part of the casein has dissociated after 5-6 days' preservation with 0.1 % or 0.4 % by weight of H_2O_2 at 4°C. The sedimentation constant of the more rapidly sedimenting component was reduced; however, electrophoresis did not indicate any difference between the casein of treated and that of untreated milk (Lück & Joubert, 1955a,b).

The reaction described above may explain the longer period of time required for coagulation by rennet observed in milk (as well as in pure sodium caseinate solution after addition of $CaCl_2$). The effects of H_2O_2 on casein are not as serious as may appear because untreated milk, if kept for long at a low temperature, undergoes similar alterations.

Whey proteins

The β-lactoglobulin in milk shows similar changes to those noted with casein. The storage of skim milk to which has been added 0.4 % by weight of H_2O_2 for 7 days at 4°C resulted in a complete breakdown of the β-lacto-

globulin into a component of lower molecular weight. At lower peroxide concentration the effect is reduced. Solutions of pure crystalline β-lactoglobulin appeared to be more resistant to dilute H_2O_2 solution than the natural β-lactoglobulin in milk (Lück & Joubert, 1955c). Compared with β-lactoglobulin, the immune globulins are far less susceptible.

Amino acids

Certain amino acids are very susceptible to H_2O_2, particularly cysteine, cystine, and methionine. Tyrosine and tryptophan can also be easily oxidized. Fortunately, the -SH groups of the non-denatured milk proteins are relatively resistant to oxidizing agents. Addition of 0.03 % H_2O_2 (by weight) or 0.03 % H_2O_2 with 8.10^{-5}% Cu had no effect on the -SH groups in skim milk after 20 hours at room temperature (Zweig & Block, 1953). Even the addition of 0.1 % H_2O_2 (by weight) for 1 day at 30°C or for 30 minutes at 55°C did not noticeably reduce the -SH content (Lück & Joubert, 1955a; Lück & Schillinger, 1958b). Treatment with higher peroxide concentrations or at higher temperatures and for longer periods of time diminishes the sulfhydryl groups content. In the presence of metal ions (Cu) or of peroxidases, the oxidizing effect is catalytically accelerated. Fortunately the milk peroxidase is quickly destroyed by H_2O_2.

Enzymes

H_2O_2 preservation affects milk enzymes to a certain extent. The phosphatase test for distinguishing raw and heat-treated milk is applicable to peroxide-treated milk. It was found that 0.08 %-0.12 % by weight H_2O_2, 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). The phosphatase is seriously inhibited when the H_2O_2-treated milk is kept for a longer period (10-20 days) (Sanders & Sager, 1949).

H_2O_2, 0.06% by weight, is able to destroy all peroxidase in milk, where smaller doses of peroxide disappear and allow some peroxidase to persist (Banerjee, 1947). The destruction of catalase, which is accelerated at increasing temperature, is accompanied by the decomposition of H_2O_2 (Lück & Schillinger, 1958b). Fortunately, catalase and peroxidase are destroyed. If peroxidase were to persist, the danger of oxidizing amino acids and proteins would be much greater; while if catalase were to persist, all the preservative would be decomposed after a very short period of treatment, and the preserving effect would be much reduced.

Nutritive value

Biological protein evaluation, approximate composition and vitamin analysis indicate little change in the composition and nutritive value of milk treated with 0.1, 0.2 or 0.5 % of H_2O_2, or of whey or cheese obtained

from such milk (Teply, Derse & Price, 1958). It must be recognized that H_2O_2 destroys a large proportion of vitamin C, but this is of little consequence because milk is not an important source of ascorbic acid supply to man. The tryptic digestion of casein pre-treated with hydrogen peroxide is augmented (Muset, Calvet & Valls, 1954; Lück, 1956b).

Effects of H_2O_2 Treatment on Bacteria

Pure cultures

H_2O_2 is known to be an effective bactericidal and bacteriostatic agent, although its mechanism of action is as yet unclear. In the older literature its effectiveness was attributed to the oxygen developed *in statu nascendi* but, as recognized today, it is the undecomposed H_2O_2 which is effective. 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 treatment. Therefore the rate of bacterial reduction by H_2O_2 depends on the initial quality of the milk, i.e., the initial bacterial count (Nambudripad et al., 1952). The germicidal effect of H_2O_2 in protein-free solutions is more pronounced than in protein-containing liquids (milk).

Molland (1947) found that some aerobic strains (about 10^{10} cells per ml) were able to grow in a broth containing 0.0125 % H_2O_2 after incubation at 37°C. Even bacteria which produce very little or no catalase, such as the pneumococcus, the streptococcus, *Salmonella typhosa, Bacillus anthracis* and *Erysopelothrix rhusiopathiae*, grew in a medium containing 0.0125 % 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 %.

Nambudripad & Iya (1951), Nambudripad, Laxminarayana & Iya (1949) 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 inactivation by H_2O_2 than are Gram-positive species (spore-formers). The susceptibility of the lactic acid bacteria fell between these two. The time required for 100 % destruction of some micro-organisms of dairy importance can be seen in Table 4. The surviving cells of *Str. lactis* exposed to 0.05 % by weight of H_2O_2, plated and inoculated (an isolated colony) into sterile skim milk, showed a definite retardation in acid production after three days (untreated : 0.71 % lactic acid ; treated: 0.48 %). 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.

TABLE 4

TIME REQUIRED FOR COMPLETE INACTIVATION OF CERTAIN MICRO-ORGANISMS BY H_2O_2 TREATMENT *

Micro-organism	0.005 %		0.03 %		0.05 %	
	hr.	min.	hr.	min.	hr.	min.
Streptococcus lactis	6	0	4	0	2	30
Streptococcus liquefaciens	16	0	7	0	4	0
Sarcina sp.	8	10	5	0	2	30
Lactobacillus bulgaricus	6	0	4	30	2	30
Bacillus subtilis	36	0	24		18	0
Bacillus cereus	24	0	14	0	7	0
Bacillus kaustophilus	120	0	32	0	18	0
Bacillus megatherium	**	**	24	0	16	0
Escherichia coli	5	45	0	45	0	45
Aerobacter aerogenes	6	0	1	0	0	30
Alcaligenes viscosus	2	0	1	0	0	40
Serratia marcescens	6	0	3	30	2	0
Torula sp.	24	0	7	0	3	0
Oidium sp.	26	0	8	0	3	30

* After Nambudripad & Iya (1951); Nambudripad, Laxminarayana & Iya (1949)
** Not determinable

Non-pathogenic bacteria in milk

Under certain conditions, H_2O_2 treatment causes a higher bacterial reduction than does pasteurization. Anaerobic spore-formers were entirely eliminated (Morris, 1950). Satta et al. (1943) have determined the percentage reduction of the bacterial count in milk treated with different concentrations of H_2O_2 for 20 hours; the results are given in Table 5. Addition of 0.05 % of the peroxide solution arrested the multiplication of the bacteria for more than 15 hours at 15° and 20°C. A reduction of the initial count for 24 hours was effected by the addition of 0.1 %. Onset of multiplication was delayed for 36 hours beyond that of the control.

TABLE 5

PERCENTAGE REDUCTION OF BACTERIAL COUNT IN H_2O_2-TREATED MILK AFTER 20 HOURS *

Temperature (°C)	Percentage reduction by weight H_2O_2		
	0.08	0.10	0.12
15-17	99.82	99.88	99.88
20-22	98.64	99.76	99.79
32	93.57	99.53	99.96

* After Satta et al. (1943)

TABLE 6

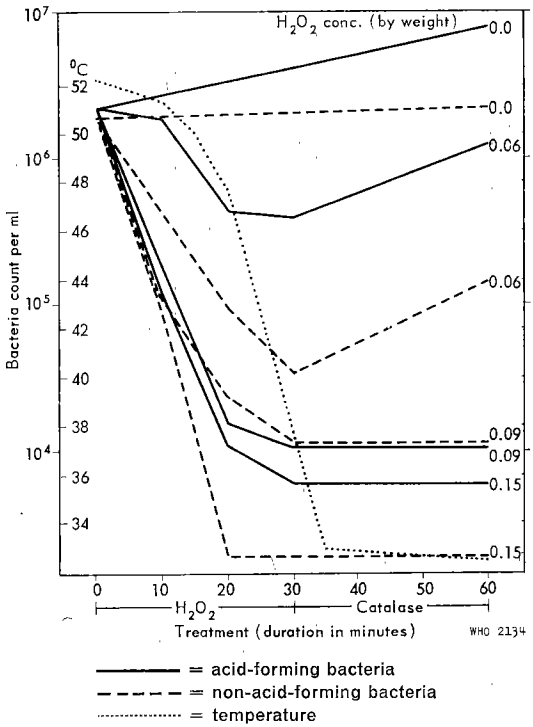
REDUCTION OF AVERAGE BACTERIAL COUNT AFTER SHORT-TIME * H_2O_2 TREATMENT **

Micro-organisms	Bacterial count per ml in		
	raw milk	pasteurized † milk	H_2O_2-treated milk
Total bacteria	304 280	11 485	1 090
Coliforms	4 339	10	4
Aerobic spore-formers	1 730	88	68

* 0.07 % H_2O_2, 30 minutes, 49°C
** After Morris, Larson & Johnson (1951)
† Temperature not stated

Monaci (1949) has found a reduction in total bacterial count varying from 74.3 % to 96.3 % with 0.08 % by weight of H_2O_2 for 14-24 hours at 20°-22°C and 28°-30°C. Similar results were obtained by Morandi (1943).

EFFECTS OF H_2O_2 TREATMENT (0.06% BY WEIGHT H_2O_2) ON BACTERIAL REDUCTION IN RAW MILK *



* After Demeter et al. (1959)

Nai & Giolitti (1947) recommend contact with 0.28 %-0.4 % by weight of the preservative for 8-10 hours, because addition of 0.1 %-0.12 % by weight is said not to allow a sufficient safety margin. In winter the dose could be lower : 0.16-0.24 % by weight (Giolitti & Nardi, 1949).

Clostridium butyricum was greatly reduced in 11 hours and disappeared in 24 hours. *Thermobacteria* and *Streptococcus lactis* were very sensitive to H_2O_2 (Arnaudi & Treccani, 1953). Babad, Boros & Baier (1959) report that the bactericidal effect of H_2O_2 is reduced in milk from penicillin-treated cows, probably as a result of the selection of bacteria which are also heat-resistant.

As mentioned earlier H_2O_2 treatment has also been recommended as a short-time treatment (30 minutes, 49°-51°C, 0.08 % by weight H_2O_2) in place of pasteurization by heat. Data on the effectiveness of this method are given in Table 6. Generally the non-acid-formers are better inactivated by this process (98.5 % reduction) than are the acid-formers (84 % reduction). The coliforms and anaerobic spore-formers are totally destroyed (Demeter et al., 1959). For this reason the method has been particularly recommended for cheese-making. The results on bacterial reduction are shown in the accompanying figure.

Pathogenic organisms

Treatment with H_2O_2 destroys most of the pathogenic organisms, but *Mycobacterium tuberculosis* is more resistant than other pathogenic organisms, withstanding an H_2O_2 concentration as high as 0.8 % by weight (Giolitti, 1947b). Treatment with H_2O_2 is therefore recommended only for tubercle-free milk. Bovine tubercle bacilli added to milk survived up to 25 hours at H_2O_2 concentrations of 0.08 % by weight (Bertarelli et al., 1945). Similar results were obtained by Monaci (1949). Two of nine milk samples inoculated with suspensions of *Myco. tuberculosis var. bovis* and treated with H_2O_2 were positive in a guinea-pig inoculation test.

With the short-time treatment *Myco. tuberculosis bovis* is inactivated by addition of 0.15 % by weight of H_2O_2 only if the infection of the milk is not excessive (Demeter et al., 1959).

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 0.08 % by weight of H_2O_2 for 3 hours (Biffi & Romagnoli, 1949), for 14-24 hours at 20°-22°C and 28°-30°C (Monaci, 1949) and for 30 minutes at 20°C and 32°C (Satta et al., 1943). *Br. melitensis* is less resistant than *Br. abortus* (Rosati & Mitrovic, 1951), the first surviving the addition of 0.08 % by weight of H_2O_2 at 20°-28°C for 12 hours, and the second 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 for this purpose and deserves to be used more widely.

Other authors, however, have found that an H_2O_2 concentration of 0.08 % by weight is not sufficient to kill brucellae completely. Treatment with 0.24 % by weight H_2O_2 for 12 hours gave complete destruction (Maestone, 1952; Giolitti, 1952). The short-time treatment inactivated *Br. abortus* at 0.06 % by weight H_2O_2 (Demeter et al., 1959).

Salmonella typhosa was killed in 8-9 hours by 0.08 % by weight H_2O_2 at 17°-32°C and in 4-5 hours by 0.1-0.12 % by weight H_2O_2 at the same temperature (Satta et al., 1943). Typhoid organisms could be isolated (Monaci, 1949) from only one of 20 milk samples, inoculated with *S. typhosa* suspension and treated with 0.08 % by weight H_2O_2 for 14-24 hours at 20°-22°C and 28°-30°C.

Given the results with mycobacteria, it seems clear that the use of H_2O_2 should be recognized only as a means of increasing the keeping quality and of reducing the total bacterial count; it does not completely destroy certain pathogenic micro-organisms in milk under normal conditions. Consequently H_2O_2 treatment can be recommended for the manufacture only of dairy products which are also usually made from raw milk. *If milk is to be distributed for liquid consumption, it is essential that H_2O_2 treatment should be followed by approved methods of processing to ensure the destruction of pathogens.*

Conditions giving a maximum preserving effect

Theoretically, H_2O_2 should not be able to preserve milk for long; like other biological substances, milk contains catalase both originally present, and to a greater extent formed additionally from cell and bacterial catalase. This enzyme decomposes H_2O_2. After a certain period of time all H_2O_2 should therefore have been destroyed and no further preserving effect should exist. Fortunately, as mentioned above, catalase is inactivated by H_2O_2 in the same way as the peroxide is decomposed by catalase (Lück, 1957). By this means some H_2O_2, split by catalase, is exhausted in the destruction of the catalase, while the remainder exerts a preservative effect for a certain period. It is known that the speed of destruction of the H_2O_2 by catalase is increased by higher temperatures (in the physiological range), but the destruction of catalase itself is also intensified at higher temperatures. The quantities of H_2O_2 decomposed by milk, which was highly contaminated with coliform bacteria (Lück, 1955a; Lück & Schillinger, 1958b) after 24 hours at 4°,13°, 20° and 30°C were 0.17 %, 0.12 %, 0.078 % and 0.043 % by weight of H_2O_2 respectively. According to these results, the addition of 0.1 % would satisfactorily preserve this milk at temperatures 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 the addition of catalase, the milk should be relatively 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 the milk. For a maximum preservative and germicidal effect, therefore, the H_2O_2 must be added soon after milking, when the bacterial content is low, and while the milk is at a temperature of 37°C (blood temperature).

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

Hydrogen Peroxide in Dairy Practice

The practical application of H_2O_2 as a dairy preservative has been described by many authors (such as Minut, 1946, 1947; Demeter, 1953; Rosell, 1954). As mentioned earlier, two methods are normally used: a short-time treatment to reduce the bacterial content of the milk (e.g., " Per-zym " process, H_2O_2-catalase process), and the addition of H_2O_2 to improve the keeping quality.

Short-time treatment as substitute for pasteurization

Rosell (1957) and others have described the method of using H_2O_2 instead of pasteurization by heat to reduce the bacterial count. This treatment has been recommended as a method of retaining the raw milk properties when making cheese (Morris, Larson & Johnson, 1951, 1952, 1953; Roundy, 1958, 1959), in processing and canning whole sweet milk (Winger, 1952a), and in treating milk for human consumption (Lewis, 1953).

For the manufacture of cheese, H_2O_2 is added to the milk and, after the necessary period for effective germicidal action has elapsed, the rest of the H_2O_2 is removed through the addition of catalase. Normally, a treatment with 0.08-0.1 % by weight of H_2O_2 for 20-40 minutes at 49°-54°C is recommended. At this temperature the peroxide should not remain in the milk for more than one hour. Prolonged treatment causes the curd to become gummy. After the milk has been cooled to about 38°C, catalase is added. Cheese made from this milk (Swiss, Cheddar and Jack cheese) has been reported to be superior in flavour, body and texture to products made from raw and pasteurized milk (Morris, Larson & Johnson, 1951), but according to other experiments, no outstanding differences in texture, " eye "-formation or flavour were detected between cheese such as Emmental and Tilsit made from H_2O_2-treated, raw or pasteurized milk (Heidrich, 1956). Only milk rich in butyric-acid-producing bacteria gave more " eyes " when treated with H_2O_2. The cheese-making procedure follows the usual methods, although the use of larger quantities of starter and slightly higher cooking-temperatures than is customary are advisable, because slight variations in reaction may be noted at different stages of manufacture.

The advantages of the process have been summarized (Armour & Company, Inc., 1953) as follows:

(1) Superior quality and fine flavour

(2) Improved body and texture

(3) Control of fermentation by variation in temperature and in amount of H_2O_2

(4) Elimination of " late gas formers "

(5) Pre-treatment with H_2O_2 does not interfere with later cheese-making operations

(6) The deleterious action of high pasteurization temperatures is avoided

The disadvantages are that the treatment with H_2O_2 and catalase of milk for cheese-making takes longer than the normal pasteurization process; and that butyric-acid-forming bacilli are not completely killed by 0.1 % by weight of H_2O_2, if the milk is highly infected (Wassermann, 1959).

A similar peroxide treatment is used for the preparation of sterilized milk. The milk is treated with 0.035 %-0.07 % by weight of H_2O_2 at 49°C for 30 minutes (Winger, 1952a). After removal of H_2O_2 with catalase, the milk is homogenized, canned and sterilized at a temperature above 100°C. The product manufactured by this process does not contain the objectionable cooked and scorched flavour usually noted in normally heat-sterilized milk. In another patent, Winger (1952b) recommends pasteurization before peroxide treatment by the addition of only 0.0053 % by weight of H_2O_2 at 61°-62°C for 30-35 minutes and no addition of catalase before sterilization.

The low-heat-H_2O_2-catalase process has also been recommended for the treatment of fresh milk in tropical countries (Lewis, 1953). The results reported above on the effect of this method on *Myco. tuberculosis* suggest that it is unsuitable for milk which is to be distributed for liquid consumption, unless it is known to have been effectively pasteurized. The peroxide treatment must be prolonged, and a higher H_2O_2 concentration must be used (Nai & Giolitti, 1947) in order to obtain the same destruction-rate as with pasteurization by heat.

Addition of H_2O_2 to improve keeping quality

The improvement in keeping quality of milk obtained by H_2O_2 treatment has been pointed out by many authors. Two methods are known:

(1) addition of a relatively *small* amount of H_2O_2 to improve the keeping quality between milking and arrival at the pasteurization plant, and

(2) addition of a relatively *high* amount of H_2O_2 to improve the keeping quality and to render pasteurization unnecessary.

Both methods reduce the losses from souring during transport which renders milk unfit for pasteurization and consumption. From the nutritive point of view, only the first method can be recommended. As mentioned above, concentrations higher than 0.1 % by weight of H_2O_2 may unfavourably influence the constituents of the milk.

The advantage of using a low peroxide concentration to preserve milk lies in the fact that its transport in hot countries then becomes practicable, and valuable animal protein is thus available for human consumption, while milk production in remote areas is encouraged. The disadvantage of the method lies in its delaying effect on the development of hygienic milk production.

The peroxide concentrations to be added depend on the climate, the quality of the milk and the period of preservation desired. The following quantities (% by weight) are recommended: 0.009 (Vintika, 1948), 0.03 (Nambudripad et al., 1952), 0.03-0.07 (Rosell, 1957), 0.04-0.08 (Pien, 1948), 0.08-0.12 (Satta, 1948; Satta et al., 1943). Because of the complete destruction of H_2O_2, addition of catalase may not be necessary at lower H_2O_2 concentrations. 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. Skim milk treated with H_2O_2 (0.08 % by weight) can replace fresh skim milk for calf-feeding (Mainradi, 1952). To summarize, the concentrations of H_2O_2 for preservation should range from 0.01 % to 0.08 % by weight, but this treatment cannot replace pasteurization.

The second method mentioned above tries to replace the normal pasteurization process through increasing the peroxide concentration, or the temperature, or the period of treatment. In some Italian experiments (Giolitti, 1947a; Nai et al., 1951), from 0.1 % to 0.24 % by weight of H_2O_2—according to the catalase number—was added to raw milk at a small collecting centre. After 8 hours' contact the excess peroxide was eliminated by the addition of catalase, and the milk was bottled and distributed. The cost of the method was slightly less than 6.25 lire per litre.[1] The H_2O_2-treated samples had a better keeping quality than pasteurized milk, especially at 13°C (Nemec, 1950).

Preservation with peroxide concentrations higher than 1 % has also been tried. Milk can be kept at 5°C by the addition of 0.4 %, 0.8 % or 1.2 % by weight of H_2O_2 for 24-35 days, 32-40 days, and up to 100-110 days (Romani, 1947). Similar results have been reported by Negretti (1956), who found that milk could be preserved for up to 39 days at 28°C with 1 % H_2O_2. This method cannot, however, satisfactorily replace methods at present accepted for preserving milk.

Romani (1944) considered that use might be made of the liberated oxygen for preservation, as in the Hofius process ; but, as mentioned above, this is

[1] US $1.00 = 621 lire.

hardly practicable with bulk quantities of milk processed on a commercial scale.

Preservation of whey

The preservation of whey with hydrogen peroxide has also been described in the literature. Good results were obtained through the addition of 0.015 % and 0.03 % by weight of H_2O_2 to raw sweet whey. After 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. Similar results with preservation of whey have been reported by Jasewics & Porges (1959). Cheese whey was preserved for more than 10 days by the addition of 0.02 % by weight of H_2O_2 soon after separation. This concentration killed within 1 hour 97 % of the bacteria in whey which was grossly contaminated with 2.8×10^7 micro-organisms per ml; it was, however, relatively ineffective against greater numbers of bacteria. *Saccharomyces fragilis* was grown successfully in preserved whey in which the excess of H_2O_2 was destroyed by catalase.

Experiments with lower peroxide concentrations have not been very successful. Addition of 0.01 % did not prevent the growth of yeasts (Plöttner, 1947).

In contrast to the above-mentioned results—probably because of considerable contamination with yeasts—Yunus (1953) reported that the addition of H_2O_2 (0.1 %) had no preservative value for whey. Formaldehyde was more effective, and prevented acid formation for at least 21 days.

Preservation of cream

The preservation of cream by H_2O_2 has also been tried. In a pre-war patent (Reichert, McAllister & Hinegardner, 1936), the addition of H_2O_2 in amounts ranging from 0.01 %-0.09 % by weight, followed by heating at temperatures of 61°-63°C for 15-30 minutes, is recommended. For the control of infections in " synthetic " cream, 0.005 %-0.02 % 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.

Manufacture of Dairy Products from Milk Preserved with H_2O_2

Cheese-making

As mentioned above, the manufacture of cheese from milk treated by the short-time process with H_2O_2 (0.07 % by weight of H_2O_2 for 20-40 minutes at 49°-55°C) yields a product which may be superior in quality to that made from raw or pasteurized milk. On the other hand, cheese manufactured from milk preserved with H_2O_2 (0.08 % by weight) for a longer period (8-24 hours) is mostly of inferior quality, having a pasty texture and soft body (Morris, 1950; Lück, 1955b). At higher H_2O_2 concentrations the

effect is still more pronounced; it is difficult to obtain satisfactorily dry cheese from milk treated with 1 % H_2O_2 (Peltola & Mattson, 1950). Two factors are responsible for these anomalies: (1) an inhibiting after-effect on the lactic acid bacteria (Arnaudi, Cartasegna & Passani, 1949; Lück & Schillinger, 1958b); (2) the influence of H_2O_2 on the structure of the casein (Lück & Joubert, 1955b). The delay in acid formation by lactic acid bacteria is partly caused by the high quantity of dissolved oxygen (Lück & Schillinger, 1958b) and probably a small shift of the redox potential. In order to offset these disadvantages, the use of larger quantities of starter and cooking at slightly higher temperatures than is customary are necessary. Arnaudi et al. (1949) have added up to three times the quantity of rennet normally used to obtain a satisfactorily firm curd. As already discussed, H_2O_2 preservation prolongs the coagulation period by rennet, and this period is further extended when a higher peroxide concentration and longer preservation are used (Lück & Joubert, 1955b). The longer the coagulation period necessary, the less firm is the curd.

Cheese-making experiments (Gouda and Cheddar cheese) with preserved milk (0.1 % by weight of H_2O_2 for 24-30 hours at 26°-30°C) were not very successful, in spite of doubling of the quantity of starter culture and addition of calcium chloride. The body of the Gouda cheese was very soft and flabby, it lost shape, and had a foreign and unclean flavour. When such cheese becomes older, it develops a bitter taste. The Cheddar cheese had a body similar to that of the Gouda and after 8 weeks' storage it had developed a strong off-flavour, with a pasty, greasy and pliable texture resembling that of processed cheese (Lück, 1955b). The taste is also typical, best described as a chemical salt flavour with a slight bitterness. According to these experimental results, the manufacture of cheese from H_2O_2-preserved milk (0.1 % by weight, 24 hours) cannot be recommended. However, experiments on cheese-making 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 30 minutes to one hour, to enable the catalase of the fresh milk to decompose the remaining H_2O_2. No further addition of catalase is necessary. The Gouda made from 50 % preserved milk had a slight foreign flavour, but the Cheddar did not. The samples made from 33 % and 25% of H_2O_2-preserved milk were of even better quality than the controls, especially in tropical countries during that season of the year when the milk arrives over-acid at the factory and the body of the cheese has a tendency to become rough, crumbly or even chalky (Lück, 1955b).

The quality of cheese made from H_2O_2-treated milk is better when preservative is added in low concentrations at intervals (e.g., 0.1 % by weight in three equal lots at 8-hour intervals) than when the same quantity is added in a single dose (e.g., 0.1 % in one lot during 24 hours). The

preservation of milk with 0.02 %-0.04 % H_2O_2 (by weight) does not affect the technique of cheese-making (Arnaudi, 1949; Treccani, 1952). Experiments have also been carried out in making Parmesan cheese from H_2O_2-treated milk (Annibaldi, 1958).

Butter-making

Several trials in butter-making with H_2O_2-treated milk (0.03 by weight of H_2O_2) (Nambudripad, Laxminarayana & Iya, 1952) have shown no appreciable difference in quality as compared with the product of untreated milk; nor were any differences in yield or flavour observed in butter made on an industrial scale from preserved milk (Negretti, 1952) which had been treated with 0.2 % by weight of H_2O_2, the surplus disinfectant removed by catalase 8 hours later. The butter from the treated milk showed better keeping quality and very low coliform titres and bacterial counts, the latter being almost zero.

Casein-making

No real difference in the casein manufacturing process exists as between H_2O_2-treated (0.1 % by weight, 24 hours at 25°C) or untreated milk, apart from the facts that 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 final wash-water to obtain a pH 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, 1955b).

Milk-powder manufacture

In Northern Nigeria milk is preserved with hydrogen peroxide for transportation from the collecting centres to the dairy. The skim milk is then processed into roller-dried milk powder, by boiling between two rollers, resulting in the complete removal of the H_2O_2. The powder is distributed through welfare centres to children in a ration of two pounds per month, or as a 3: 1 mixture of peanut flour and milk powder (H. Davelaar, personal communication, 1961). The question of the use of peanut flour is at present under review.

Toxic Aspects of H_2O_2 in Dairying

The effect of H_2O_2 treatment or preservation on the constituents of milk is less marked than that of other processes applied and accepted in the

dairy industry. The milk does not lose in nutritional value, apart from a small decrease of some vitamins and a considerable loss of ascorbic acid.

According to present knowledge of the effect of H_2O_2 on the living organism, this preservative should be completely removed before the milk, or its products, are used for human consumption. Hydrogen peroxide causes a decrease in the number of normal mitotic figures in the mouse intestine (Dustin & Gompel, 1949) and induces mutations in micro-organisms (e.g., *Neurospora, Escherichia coli*). Caution should therefore be observed in respect of the risk of undecomposed H_2O_2 in human food, in spite of the facts that H_2O_2 is quickly split in the gastro-intestinal tract, and that living tissues may produce traces of it.

Tests for Presence of H_2O_2

In the manufacture of dairy products (e.g., cheese) and in the processing of liquid milk for human consumption, the main problem is to ensure that no trace of the preservative is left in the milk.

In dairy practice sensitive qualitative tests are important. For this purpose the use of strips of KI-starch paper can be recommended. This method is sensitive to less than 0.001 % by weight H_2O_2. Several ml of milk and the same volume of concentrated hydrochloric acid are mixed, one drop of a weak formalin solution is added, the mixture is warmed to 60°C and a strip of KI-starch paper is dipped into the solution. In the presence of H_2O_2 the paper becomes blue or violet.

Munday (1957) has used vanadium pentoxide solution for testing H_2O_2 in milk: 10-20 drops of a 1 % solution of V_2O_5 in dilute sulfuric acid (6 %) added to 10 ml of milk produce a pink or red colour in the presence of traces of H_2O_2. The test is sensitive to less than 0.008 % by weight H_2O_2.

The reader is referred to the following literature for further information on methods of testing: Funk, 1949; Humpoletz, 1949; Patrick & Wagner, 1949; Aquino, 1950; Freytag, 1950; Ovenston & Rees, 1950; Musha, Higashino & Doi, 1951; Pien, Désirant & Lafontaine, 1953, 1954; Andreae, 1955; Janiček & Pokorny, 1955; Rouquette, 1955; Balley & Boltz, 1959; Meloan, Mauck & Huffman, 1961; Perschke & Broda, 1961.

Conclusions

To conclude, the opinion of the Expert Group which met in 1957 under the auspices of FAO may be quoted: [1]

" (1)... In general the use of any preservative in milk is undesirable—in fact the addition of any preservatives to milk can only be regarded as

[1] Food and Agriculture Organization of the United Nations (1957) *Report on the meeting of experts on the use of hydrogen peroxide and other preservatives in milk, Interlaken, Switzerland, 23-27 September 1957,* Rome (Unpublished mimeographed document FAO/57/11/8655).

being of the nature of a necessary evil. It is a method to be tolerated only in exceptional circumstances, and in warm or technically less developed countries where rapid transport of producers' milk to a processing centre is not possible, or where effective cooling of the milk cannot be carried out and where, if a preservative were not used, serious loss of human foodstuff would result.

" (2)... Of the range of preservatives available at present, the only one that is permissible for milk that is to be used for human consumption or to be manufactured into milk products is a pure grade of hydrogen peroxide (sold commercially in aqueous solution of differing strengths).

" (3)... The addition of hydrogen peroxide should be made at the milk collecting centre, and should not be made by the milk producer unless in exceptional circumstances the sanitary or other competent authority so decides.

" (4)... If, owing to difficult local conditions, permission is given for hydrogen peroxide to be used, either by the producer or by the collector of milk, the quantity to be used should in no circumstances exceed 0.80 g of H_2O_2 (calculated as pure H_2O_2) per litre of milk, and should usually, for milk for liquid consumption, be between 0.10 g and 0.40 g H_2O_2 per litre.

" (5)... Since the function of hydrogen peroxide is merely to delay the souring of the milk, and since this preservative at any permissible strength does not destroy certain types of pathogenic micro-organisms *(including M. tuberculosis)*, any milk treated by hydrogen peroxide must subsequently be subjected to effective heat treatment before being distributed to the consumer or during the course of manufacture.

" (6)... Whether hydrogen peroxide is added to milk destined for liquid consumption or for manufacture, such additions must be very carefully controlled, and official tests made sufficiently frequently to ensure that the preservative has been destroyed before the milk and milk products are distributed to the consumer.

" (7)... If catalase is added to hydrogen-peroxide-treated milk to destroy residual preservative, the enzyme preparation must be entirely satisfactory from the enzymic, chemical and bacteriological standpoints.

" (8)... As the addition of hydrogen peroxide to milk is known to affect to some extent the quality of the milk, further investigations are recommended to evaluate more precisely these changes in relation to human health and nutrition (*vide* the report of the Joint FAO WHO Expert Committee on Food Additives... [World Health Organization (1957)]).

" (9)... Finally, it must clearly be recognized, both by the controlling authorities and the technical personnel concerned, that the use of hydrogen

peroxide is not a hygienic measure, and is no substitute for efficient heat-treatment. In short, it is a method which, in other than exceptional circumstances, is not to be recommended."

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