PREVENTION OF MOLD SPOILAGE OF EGGS UNDER CONDITIONS IN WARMER AREAS

by

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I. INTRODUCTION

This work was undertaken mainly to find out the effect of room temperature storage (76 F to 80 F) on the keeping quality of eggs. This problem obviously arises in places, like India, where due to lack of sufficient cold storage facilities it is a common observation that eggs would not keep more than a week untreated at such a high temperature of storage.

The factors responsible for the penetration of the shell by the microbial flora have not been thoroughly investigated, but is fairly well known that entry takes place under certain conditions by some species of most of the common egg molds.

It has been estimated that in England a loss of 5 per cent occurs in stored imported eggs which means a loss of 150 million eggs annually.

Warmer storage would naturally accelerate the changes that are responsible for spoilage. The object therefore consists in controlling as far as possible the conditions which would facilitate spoilage in warm storage.

About one-fourth of all eggs produced in the United States go into cold storage. For prolonged periods of storage the preservation of eggs by cold is not the ideal one to which the industry would look with favor. Particularly for prolonged periods there is much that could be improved in the present methods of egg preservation. Better storage conditions are necessary to eliminate spoilage by microorganisms and a quality loss due to the evaporation of water from the eggs in storage.

Cold storage certainly does not prevent completely the growth of microorganisms and enzymatic spoilage. Molds particularly grow at low temperature and cause spoilage. The purpose of this investigation was to study the effect of certain substances which would prevent the development of molds on eggs stored especially at room temperature. The growth of microorganisms particularly molds both within the egg and on the outer shell has been mentioned as the principal cause of spoilage.

It is now a well-accepted fact that egg spoilage is due to the organisms which enter the shell. It was therefore thought that if the mold growth on the outside could be prevented one cause of quality deterioration could be removed.

In all the experiments fresh eggs obtained from the Poultry Department of Oregon State College were used,

except in the first series when they were purchased from a retail store. To keep the factors of temperature and humidity constant all the samples were stored at 76 F to 80 F and approximately 100 per cent humidity. It is fairly well known that molds grow well when the relative humidity is above 80 to 85 per cent.

The experiments were performed under conditions closely approximating those existing in commercial warehouses. The saving in cost effected by storing eggs at ordinary temperatures several weeks before being sold is obvious. The object of investigation was to determine the extent of storage life of eggs at room temperatures. It was thought that if the storage life were extended, even for several weeks, it would benefit the industry considerably in warm areas. Eggs spoil rapidly at high temperatures. If treated eggs are kept free of microbial decomposition at high temperatures, it should be possible to keep them in good condition before consumption.

II. REVIEW OF LITERATURE

The fresh egg has been known to contain an efficient system of defenses against microbiological spoilage. Nature provides these defenses to cope with the heavy contamination which would naturally occur after laying.

Haines (7) points out that there are a number of variables involved in the penetration of the egg shell by bacteria, which require adequate study one at a time, with proper controls. He lists the following.

(i) The penetration, if any, of the intact, dry shell by the indigenous micro-flora.

(ii) The effect of the deposition of moisture on the shell on microbial penetration of the intact egg by the indigenous flora.

(iii) The effect of removing the outer muscilaginous coating, ie, washing the eggs or treating with abrasives, on penetration of egg by the indigenous flora.

(iv) The effect of removing the coating of mucin on microbial penetration if the eggs are exposed to a fresh infection and not the indigenous infection.

(v) Any correlation between bacterial penetration and quality, size, or number of pores in the shell, or age of the shell.

Important factors involved in infection according to Haines (7) are (a) washing and possibly (b) quality of the shell. He emphasizes that washing renders the egg much more easily infectable if exposed at any subsequent period to bacteria and moisture. According to him, good quality, fresh unwashed eggs are extraordinarily resistant to bacterial infection. Washing renders the egg much more susceptible to infection if exposed at any subsequent time to moisture and bacteria. It is almost certain that most of the organisms which cause spoilage under commercial conditions are caused by the invasion of the egg through the shell after laying.

True rotting is mainly microbial but according to Haines (7), the conversion of the thick white into thin white and gradual weakening of the membranes of the yolk are biological changes not related to microbial activity.

Miles and Malnan (11) first isolated an organism, Proteus melanovogenes, which causes the most rapid and complete black rotting. Haines (7) emphasizes that when advanced stages of this type of rot are encountered on an extended scale, it is probable that, in addition to the handling of the eggs being suspected from the hygenic standpoint, they have at some period been stored at too high a temperature. According to him other strains of Proteus have been known to give rots of this type.

Two other rots which are common to industry are "red rots", caused by infection with Pseudomonas and "green rots", caused by members of the Pseudomonas group while growing in the egg white. A type of "red rot" is known to be caused by Sporotrichum spreading along the chalazae to the yolk (7).

The most common species of microorganisms found in eggs are micrococci, molds, yeast and sporing bacilli, B. proteus, B. subtilis, Micrococcus aureus, B. pyocyaneus, B. prodigiosus, B. violoaceus, Micrococcus albus, aureus, and luteus, "M. sulphureus nonliquefaciens," B. faecalis alkaligenes, B. proteus, B. mesentericus, and streptococci, and "B. putidus nonliquefacieus," staphylococci, B. mesentericus, B. ramosus, B. coli, B. proteus, streptococci, micrococci, actinomyces, anaerobes, B. fluorescens, B. mucosus, diphteroid bacilli, spirillium, S. tetragena, S. lutea, B. pyogenes, B. albolactis, B. mycoides, corynebacteria and various molds (7) (14).

Haines (7) sums up the above findings by stating that the flora of the small proportion of whites and yolks which may represent a real infection consists of a great diversity of common saprophytic organisms occurring in manure and soil. He is of the opinion that average, clean egg shell carries a heavy and diverse bacterial flora which are responsible for most of the types of spoilage observed

in commercial practice and successful preservation by storage consists in maintaining such conditions as would make it impossible for them to enter and reproduce within the egg.

The most common molds which cause spoilage in eggs were noted by various workers (7) (9) (24) to be Penicillium, Cladosporium, Sporotricum, Thamnidium, Mucor, Alternaria, and Aspergillus. Entry takes place under certain conditions by some species of most of the above genera.

Winter (26) points out that black rot in fresh shell eggs could be prevented by producing clean eggs. According to him the source of infection in most cases is dirt on the shell or in the water used for cleaning. Alcaligenes, Escherichia and Proteus groups of bacteria were found most frequently in such condition. He observed that most cases of black rot were found during or following periods of warm, humid weather.

A. FACTORS INVOLVED IN PRESERVATION OF EGGS

Two most important factors involved in preservation of eggs are the temperature and humidity. They are very closely interrelated and it becomes impossible to rate the two factors separately (10).

Temperature: Higher temperatures have been known to be favorable for reproduction of bacteria and molds. Holding eggs at lower temperatures would help to prevent bacterial and mold growth. High temperature during transport has been indicated as one of the causes of spoilage. In warmer areas (76 F to 80 F) it becomes impossible to preserve quality in eggs unless the eggs are treated in some manner or are immediately stored in cold rooms. Experiments were run to determine the relative merits of different treatments at room temperature storage which was uniformly kept at 76 F to 80 F. Microbial development is greatly dependent on the conditions of storage in which temperature plays an important role.

Humidity: This is another important factor to be taken into consideration in egg preservation. If relative humidity of the surroundings is high, conditions become ideal for mold development due to the condensation of water on shell surface. It has been noted that even if the film of moisture is microscopic it enables the mold to develop.

Rapid temperature changes allow moisture condensation on the surface. Such condensation accelerates the growth of surface molds which may penetrate through the shell.

Extreme care in handling eggs is always emphasized. Rapid temperature changes during handling facilitates "condensate" formation.

It becomes necessary therefore that ideal storage with low humidity and low temperature should be started as soon as the egg is laid. Egg quality as determined microbiologically depends largely on the method of handling after the egg is laid. Quality is determined by the manner in which the egg is handled at its source. The storage house could not possibly do anything in the way of maintaining quality if the egg is contaminated when it is received even though the infection may not be great. This supposes that the only place where the egg quality could be improved is either at the poultry plant or farm (10).

According to Mallmann and Davidson (10) the bacterial invasion is exceedingly rapid when the egg is first laid because the shell and egg membranes have little if any protective value against infection. It has been noted that the natural defenses of shell eggs are not sufficient to prevent spoilage when they are subjected to unfavorable conditions of temperature and humidity. Clean eggs need great care in handling. The object of any method of preservation should be to prevent handling practices which would facilitate bacterial and mold growth. It has been noted that under practical conditions bacterial invasion occurs largely before storage. This, however, presupposes that storage conditions are not conducive to bacterial development.

Moran (13) is of the opinion that storage of eggs in air at ordinary or chilling temperatures is marked by several changes among which are: (a) Evaporation of water giving rise to large air chamber, (b) Thinning of the thick white and shrinkage of the bag of thick white (enclosing the inner thin), (c) Weakening of the yolk membrane, (d) A decrease in the viscosity of the yolk, and (e) The appearance of "storage taste" in the yolk.

Evaporation at constant humidity is independent of the speed of the air passing over the eggs. There is a gradual decrease in the rate as evaporation proceeds as noted by Moran (13). He also notes that the quality of the "white" as a whole is best preserved if the pH is maintained at about 7.8, ie, in air containing approximately 2-1/2 per cent CO_2. He, however, emphasizes that the pH is not the only factor in preservation of quality and some shrinkage of the bag of thick white occurs, whatever the conditions of storage. In this respect the white at laying seems to be in an unstable system.

There seems to be doubts as to the origin of "storage taste." Eggs have been known to absorb stray odors. The presence of CO_2 has been known to delay the appearance of "storage taste" in the yolk (13). Treatment of eggs with mineral oil reduces the storage odor of eggs as shown by Gross, Hall, and Smock (6).

B. SPOILAGE OF EGGS AND ITS PREVENTION

It is an accepted fact that the egg as it comes out of the hen is generally free from microorganisms except in case of pullorum (S. Pullorum) egg (10). The consensus of opinion is that microbial spoilage is due to contamination from the outside. The extent of such contamination is an indication of the manner in which an egg is handled from the time the egg leaves the oviduct of the hen to the time it is consumed.

It has been known that the egg contains an efficient system of defenses aginst microbial spoilage. If these defenses are augmented, we could materially increase the storage life of eggs.

The use of inhibitors of microbial growth in egg preservation has been widely attempted. The preservation of eggs by dipping them in water glass has been known for a long time. It is recorded in history that eggs could be kept in good condition for several years by storage in water glass. However, with the discovery of newer compounds attention seems to be focused on elimination of the difficulties encountered in older compounds. Thus water glass, though in itself a good preservative, presents quite a problem in commercial practice because of the bulk of the packing material.

A number of compounds have been suggested for preservation of eggs. They are all dependent on three simple principles, namely, (a) decreasing evaporation, (b) setting up more or less conditions which prevent air from getting in, and (c) prevention of microbial growth and eventual spoilage (7).

Quaternary Ammonium compounds have recently been shown to have high germicidal efficiency. Emulsept and E 607 have already been shown to be quite effective in reducing microbial flora on shell eggs.

The most commonly used quaternary ammonium compounds listed by Rahn (19) are as follows:

(1) CTAB (Cetavlon) = cetyl trimethyl ammonium bromide

(2) Zephiran=Roccol=BTC= alkyl dimethyl benzyl ammonium chloride

(3) Phemerol = Hyamine 1622 = Polymine D = p-tert octylphenoxy ethoxy ethyl dimethyl benzyl ammonium chloride

(4) Ceepryn = cetyl pyridinium chloride

As far back as 1911, Riddle (22) showed that if hexamethylene tetramine were fed to the laying hen, the eggs

obtained would preserve for five months at 53.6 F to 64.4 F from bacterial spoilage. The controls in the same time become unpalatable. It was thought by him that this substance passed unchanged through the follicular and vitelline membranes and became incorporated in the developing ovum. Hexamethylene tetramine decomposed into formaldehyde after laying and its presence was revealed in both white and yolk. According to him this was sufficient to preserve the egg from bacterial decomposition for five months at 53.6 F to 64.4 F.

Washing even when antiseptic washes are used seems to be harmful because wetting the surface gives the microorganisms an opportunity to invade the egg through the shell pore as observed by Hallmann (10).

In experiments using a germicide in wash water to reduce bacterial contamination on the egg shell, Penniston and Hedrick (16) noted that E 607 special is not quite as effective on molds as it is on bacteria though it kills a large percentage of molds. E 607 special is a quaternary derivative of approximately equal mixture of lauric and myristic fatty acid esters of colamino formyl methyl pyridinium chloride. They observed that when dirty eggs were washed with various concentrations of E 607 special, the bacterial count on the egg shell was reduced from over 20,000,000 to a little over 1,000 or

none. In contrast, dirty eggs which were washed in water still retained $3x10^6$ to $6x10^4$ microorganisms on the shell surface.

They also observed that when washed dirty eggs of the above series are rinsed in 500 ppm or less of E 607 special for three to five minutes, the count was reduced to a little over 1,000 or none.

Penniston and Hedrick (17) compared the comparative values of chlorine and Emulsept as germicides in washing dirty eggs. Emulsept is the lauric acid ester of calamino formyl methyl pyridinium chloride. They used successive groups of twelve dozen eggs each in their experiments and determined the percentage of microorganisms not killed by germicides in relation to the number of microorganisms present when no disinfectant was used in rinse water and wash water. They used Emulsept in concentration of 400 ppm and chlorine in concentration of 100 ppm and found that the percentage of microorganisms killed with Emulsept in these experiments for the same series of eggs were many times greater than the percentage of those killed when 100 ppm chlorine was used.

In more recent work, Penniston and Hedrick (18) report that when shells were pulverized or rinsed in sterile water, the number of microorganisms washed off were approximately the same. When 400 ppm of Emulsept or

100 ppm of active chlorine was used to wash the eggs, six to twelve times as many microorganisms were washed off. Four hundred ppm of Emulsept solution was six to ten times as effective as chlorine solution in causing the same reduction in the number of bacteria on the shell surface. They also noted that the bacterial count in the egg meat per egg when no fungicide was used in the wash water and when either 0.04 per cent Emulsept or 100 ppm chlorine was used, was respectively 1.5 million and 3,000.

According to Mallmann (9) the molds found on eggs and egg case materials are extremely common to fresh eggs and even to new egg packing materials. He emphasizes that the sanitary handling of the eggs, although desirable, does not in any sense prevent the development of molds in the egg package during cold storage.

The elimination of moisture from the egg surfaces and from the fillers and flats would prevent the development of mold mycelia, but to maintain such conditions would mean a loss of moisture of eggs. At 85 to 88 per cent relative humidity (which is common practice in cold storage rooms), the molds sometimes appear particularly if the eggs are held longer than the usual storage period which is about six months. If, however, humidities are kept at 90 per cent or above, molds appear after four or five months' storage in cold rooms (9).

It is not feasible to destroy the molds but the incorporation of chemical agents which would suppress the growth of molds is possible.

Mallmann and Michael (9) put down four necessary criteria for such a substance.

(a) It should have maximum toxicity towards the fungi common to eggs.

(b) Should have a low vapor pressure to envelop the egg in a mycostatic vapor.

(c) Should be available at low cost.

(d) Should be free from odor.

In their experiments, they observed that sodium pentachlorphenate which is marketed by Dow Chemical Company under the trade name of Dowicide G to be satisfactory as far as the above criteria are concerned.

Based on this observation, Mallmann and Michael (9) developed a process for the prevention of molds on egg surfaces, fillers, flats, and cases. This process consisted in impregnating fillers and flats with approximately 0.4 per cent sodium pentachlorophenate (Dowicide G). This has some importance because the mycostatic activity was sufficient even to suppress the molds at relative humidity much higher than could be maintained practically in cold storage room and the compound does not import any taste or odor to the eggs during storage.

The above workers (9) discovered the effectiveness of sodium 2, 4, 5 trichlorophenate (Dowicide B), sodium 2, 4, 5, 6 tetrachlorophenate (Dowicide F) and sodium pentrachlorophenate (Dowicide G) in controlling the development of mold on eggs in cold storage. They observed that although sodium 2, 4, 5 trichlorophenate (Dowicide B) was the best mycostatic agent under the drastic experimental conditions used, sodium pentachlorophenate (Dowicide G) was observed to be the most satisfactory mycostatic agent under ordinary commercial conditions. The use of Dowicide G was advantageous because it is better from the standpoint of low cost, slight odor, and a relatively low vapor pressure. They observed that the eggs stored in flats and fillers treated with these phenol preparations were no different in odor and taste than the eggs stored under identical conditions in ordinary flats and fillers. He recommends that the most effective method of applying the mycostat is by impregnating the fillers, flats, and cases during the time of manufacture.

Rosser, White, Woodcock, and Fletcher (21) have demonstrated the fungicidal value of urea and dimethylol urea and the effectiveness of the vapors of certain volatile solids as mold growth preventives.

In their experiments for preserving eggs at high temperatures, they noted that the best results would be obtained by conditioning with carbon dioxide to lower the pH, treating shell surfaces with a disinfectant to reduce contamination followed by effective sealing, preferably with a substance having properties that would prevent both growth and entrance of contaminants during subsequent storage. Dimethylol urea in their experiments proved to be the most effective growth inhibitor for microorganisms and vaseline proved to be the best sealing agent. They obtained satisfactory results by dipping eggs in polyvinyl alcohol treated with dimethylol urea and by packing oil dipped eggs in moisture resistant bags.

C. INCORPORATION OF MYCOSTATIC AGENTS IN OIL

Oil treatment has been used quite extensively in commercial practice. It prevents evaporation from the egg and prevents spoilage.

Mallmann and Davidson (10) maintain that the effectiveness of the oil in preventing moisture loss depends on the thickness of the residual film left on the egg after treatment. They observed good results with 30-60 S_{ay}. viscosity oil. In his experiments he noted that a viscosity of 50 to 60 permits dipping eggs at temperature of 60 F to 70 F without slowing drainage too much. He

incorporated two of oil-soluble Dowicides namely Dowicide 1 and Dowicide 7 in oil and treated eggs with different concentrations of each of them. The concentrations he used (both the compounds) were 0.1 per cent, 0.25 per cent, 0.5 per cent, 1.0 per cent, 2.0 per cent. The treated eggs were placed in large mouth mason-type jars and the air was kept saturated with moisture throughout the experiment to produce extremely adverse conditions for maintaining keeping quality. The eggs were stored at 68 F with 100 per cent relative humidity. The eggs were examined after forty-two days' storage for spoilage and the interior quality was determined by candling.

They observed that 0.1 per cent pentachlorophenol (Dowicide 7) failed to stop mold formation entirely and that orthophenylphenol (Dowicide 1), in all concentrations from 0.1 to 2.5 per cent, failed to stop mold formation on the shell surfaces. The eggs were held for 71 days when they were re-examined. The eggs treated with 0.25 per cent or more of pentachlorophenol (Dowicide 7) were observed at this time to be still free of microbial decomposition. Hallmann and Davidson (10) however emphasized that oiling with or without the aid of antibiotic agent is not a substitute in any sense for refrigeration.

This method of preventing mold growth on eggs seems to have some importance because this process, if applied

to receiving stations' eggs in place of the present oiling practices, will enable the eggs to withstand poor storage conditions, such as; marked temperature changes, excessive humidities, sweating, and poor ventilation. The antibiotic agent on the shell prevents penetration of the shell by molds and bacteria encountered during storage.

Reedmand and Hopkins (20) in their studies on the effect of oil treatment and egg case liner bags in the preservation of shell eggs under adverse conditions observed no resulting effect of initial condensate on the eggs and packaging material. They conditioned different sets of eggs at 32 F, 40 F, 60 F and 70F before placing them in humidity chamber.

They observed that oil treatment by the best commercial applicable method, followed by packaging in sealed egg-case liner bags, was found to retard greatly the development of internal defects and severe external mold. They noted that oil treatment alone was definitely beneficial but the use of egg-case liner bags on untreated eggs was detrimental. They, however, emphasize that somewhat different storage conditions may be encountered in the export of shell eggs in nonrefrigerated holds. According to them the length of time seems to influence directly the degree of spoilage. The workers put some importance to humidity

gradients within the case because differential spoilage of eggs located in the central and top and bottom trays within the case and at the periphery and in the interior of individual trays was also noted under certain conditions.

In further studies, Gibbons, Fulton, and Hopkins (4) stored untreated eggs and eggs oiled and sealed in egg-case liner bags for six weeks in standard export cases (i) at 70 F and 90 per cent relative humidity continuously, (ii) at 65 F and 95 per cent relative humidity alternating with 75 F and 70 per cent every two days, the dew point consequently always remaining below 65 F, and (iii) at 60 F alternating with 80 F every three days, both at 90 per cent relative humidity, but the dew point varying from 57 F to 77 F respectively. In their experiments they observed that within rather wide limits, microbiological development is dependent primarily upon storage conditions rather than upon the quantity of inoculum on the surface of eggs at the beginning of storage. They noticed that the bags used had little effect on intracase temperatures.

Under (i) and (iii) the workers noted that oiling and bagging reduced internal mold and rot but did not significantly affect the development of external mold. In (ii) internal spoilage was uniformly low in both treated and control eggs. They observed that oiled and bagged

eggs were in better physical condition after storage than were the untreated, having smaller air cells, freer yolks and less distinct yolk shadows. The variations which the workers observed in the incidence of spoilage within cases are attributed by them to intracase temperature and humidity differentials.

Gibbons, Michael, and Irish (5) noted that mineral oils with Saybolt viscosities of 70 to 100 at 100 F maintained egg quality and prevented weight loss better than oils of lower viscosity. They observed that oiled eggs lost from one-tenth to one-fourth as much weight. The addition of vaseline and magnesium stearate improved the action of the light oils. They found also that heavy oil diluted with mineral spirits did not give as good results as lightoils of comparable viscosity. The quality difference between the oils (maintained at 76 F, 100 F, and 130 F) used for dipping was not marked.

Treatment of eggs with mineral oil seems to have some importance in reduction of storage odor of eggs as is pointed out by Gross, Hall, and Smock (6).

Kaess (8) in his evaluation of some treatment processes for storing eggs at environmental temperatures under continental and tropical conditions compared Osagit, a commercial oil emulsion, Osagit with bactericide, Biosoter (bactericidal fat used to rub on eggs), Hilsol (oil plus

bactericide), ethylene glycol plus 3 per cent Nipagin and lime water treatment of eggs in storage tests at 0 to 28 degrees and at various humidities. He observed that quality decrease was related to taste and unrelated to increase in size of air cell. He observed that the content of free NH_3 in yolks depended on storage temperature with no significant differences between controls and impregnated eggs, except that the yolk of eggs preserved in lime water had more free NH_3 than controls. He noted that decline of quality of taste was small at nine months' storage in $Ca(OH)_2$.

Among the impregnating methods he found that dipping in 5 per cent Osagit solution at 60 degrees best preserved the eggs. The pH was observed to be closely related to the taste value. For storage at relative humidity of more than 80 per cent, Kaess recommends a bactericidal treatment.

Rievel (23) in his experiments on egg preservation by oil treatment with lubricating oils, bacon rind, petrolatum, paraffin, mineral oils, and wool fat, either pure, dissolved in pentane, or with various additions observed that washing the eggs with disinfecting solutions previous to oil treatment decreased keeping quality. He noted that Ovanol of unknown composition gave satisfactory results.

Heavy metals like silver, copper, and mercury are known to have a mold inhibiting value. Accordingly Mallmann and Davidson (9) tried out cupric nitrate, cupric sulphate, cupric ammonium sulphate, cupric chloride on Pencillium puberulum, Aspergillus niger and Alternaria species. They, however, observed that the concentration necessary to inhibit growth is far too strong. They are of the opinion that the marked variability in resistance of three molds would not allow the use of such compounds for inhibiting mold growth.

Winkler (25) reports on a new preserving fluid (developed by Plast-O-Trete, Inc, New York) for shell eggs. It is a triple compound of water dispersed, stripped of monomers, polyvinyl acetate, specific fruit preservatives against mycoids, yeast and bacteria and nonionic wetting agents. It is a milky-white odorless, tasteless, and entirely non-offensive preservative developed by them. Its specific gravity at 20 C is given as 1.1 and viscosity as cps/25 C 300-600. He recommends that eggs can be treated with this compound in the standard machines now used in oil processing. The compound is diluted with twice its volume of water for treatment of eggs. It is claimed that shell eggs when treated with this compound will retain their original grade in cold storage for nine months and longer at temperatures

between 33 F to 45 F. It is recommended that if the eggs are to be kept only for a month or two they could be safely kept above 45 F but not higher than 65 F. He is of the opinion that this treatment allows greater facilities in handling storage eggs. He points out that unprocessed or oil processed eggs when removed from cold storage for one reason or another impairs their quality. He is of the opinion that the eggs so treated could be put back into cold storage because the protective plastic film prevents spoilage from condensation of moisture on the surface of the egg caused by the change in temperature. Dirty eggs which are washed are recommended to undergo treatment otherwise they would spoil easily because washing of dirty eggs removes their natural "bloom", their natural waxy material which fills the pores of the shell and to a certain degree protects the egg from spoilage. The film is absolutely invisible and surface of the treated egg is no different from an untreated egg.

However, if the eggs are stored at temperatures above 35 F, a thicker plastic film must be built up in the shell of the eggs. However, this treatment is effective at temperatures above 69 F for only a few days.

D. USE OF CARBON DIOXIDE AND OZONE IN PREVENTION OF MOLD GROWTH

Ewell (2) (3) has shown that Ozone has some protective action against molds in concentrations of 1.5 ppm at relative humidity of approximately 90 per cent in the aisles of cold storage rooms.

Gross, Hall, and Smock (6) have recently shown that Ozone does not remove volatile odors evolved by case materials but does control mold growth.

However, some workers (1) do not think Ozone to have importance in prevention of mold growth because of the cost of installing and maintenance of ducts and also the fact that Ozone has a corrosive action on ducts.

The use of carbon dioxide in the prevention of mold growth was tried out by Moran (13). He observed that carbon dioxide in concentrations of 2.5 per cent did prevent mold growth on eggs stored at 32 F and 85 per cent relative humidity. This would permit higher relative humidity. However, at saturation and 32 F storage, 60 per cent carbon dioxide was required to get the same effect. This concentration was observed to be detrimental to the consistency of egg white.

Moran (13) is of the opinion that gas storage is a more elaborate form of cold storage involving control of the composition of the gaseous atmosphere of the storage

room as well as the control of temperature and relative humidity. He maintains that the use of this factor of control makes storage more flexible. According to him, with many foodstuffs it will also prevent changes during storage which control of temperature and humidity alone cannot do.

Penniston (15) believes that it is not feasible to maintain definite concentration of gas in rooms not adapted to gas storage because the average cold storage rooms as a rule are not designed for gas storage.

E. UNDESIRABLE FLAVOR DEVELOPMENT IN STORAGE EGGS

Development of undesirable flavor in eggs without producing much decomposition obvious to the eye is quite marked in commercial practice. "Mushiness," "Fishiness," and "Cabbage water" in the white and a "strong" flavor in the yolk are the best recognized flavors or odors in stored eggs. The first three are known to be microbiological in origin. The cause of the last is not clear but "strong" or "cold storage" storage taste appearing after about seven months' storage in commercial practice is said by some to be due to absorption of odors from surrounding environment, especially strawboard fillers (7).

III. EXPERIMENTAL

A. OIL TREATMENT OF EGGS - SERIES I

The first experiment was carried out to determine the effect of room temperature storage and 40 F storage of treated eggs. Identical series were set up, one stored at ordinary room temperature and the other stored at 40 F.

Oil of 70 to 80 Saybolt viscosity was obtained from Union Oil Company of California. This oil was used for making up the solutions.

(i) With Phygon (2-3 dichbro - 1 - 4 - naptha-quinone) using the following concentrations.

(a) 2500 ppm Phygon in Blandol (oil of 70-80 Saybolt viscosity)

(b) 750 ppm Phygon in Blandol

(c) 250 ppm Phygon in Blandol

(ii) With Dowicide 7 (pentachloro phenol) using the following concentrations.

(a) 2500 ppm Dowicide 7 in Blandol

(b) 750 ppm Dowicide 7 in Blandol

(iii) Plain Blandol oil with nothing added.

(iv) 100 ppm Phygon in 40 per cent propylene glycol, 60 per cent water solution, and 0.1 per cent Aresket (a wetting agent).

Oil used as a solvent in the preceding solutions was heated to incorporate the Phygon or Dowicide. It was cooled to room temperature before dipping the eggs.

Procedure: Eggs were purchased from a retail store and were dipped for about two minutes in the particular solution and allowed to drain a few minutes to eliminate excess oil. Two series (one stored at 40 F and the other at room temperature) with sufficient controls were set up. The eggs were placed on wire racks over warm water in a large-mouth pan. The pans were covered with pliofilm and fastened with tape. No spraying of molds whatsoever was used on the eggs.

The room temperature series was examined after a week to note results. The results are tabulated in Table I.

The cold storage series was examined after five weeks and the results are noted in Table II.

In order to determine the internal quality of the above series, one egg of each group of this series was opened after about five weeks. The control eggs were found to be good along with the others.

Eggs from this same cold storage group were opened after three months' storage. It showed very good internal quality though mold was observed on the shell.

To determine the effectiveness of the above treatments in preventing internal spoilage, one egg of each

TABLE I

EGGS STORED AT ROOM TEMPERATURE FOR ONE WEEK

Solution	I	II	III	IV
Control	+	+	++	++
2500 ppm Phygon in Blandol	±	±	+	+
750 ppm Phygon in Blandol	+	±	+	+
250 ppm Phygon in Blandol	+	++	++	+++
75 ppm Phygon in Blandol	±	±	±	±
2500 ppm Dowicide 7 in Blandol	±	++	±	+
750 ppm Dowicide 7 in Blandol	++	+++	+++	+
100 ppm Phygon in 40 per cent propylene glycol, 60 per cent water, and 1 per cent Aresket (a wetting agent)	o[¶]	o[¶]	o[¶]	o[¶]
Plain Blandol oil with nothing added	+	+	±	++

\+ Mold growth evident
++ Some mold growth
+++ Profuse mold growth
± Doubtful

[¶] It was noticed that it is more a case of disinfection rather than protection.

TABLE II

EGGS IN COLD STORAGE FOR FIVE WEEKS

Solution	I	II	III
Control	Trace	++	++
2500 ppm Phygon in Blandol	+	+	+
750 ppm Phygon in Blandol	±	±	+
250 ppm Phygon in Blandol	++	++	++
75 ppm Phygon in Blandol	++	+	++
2500 ppm Dowicide 7 in Blandol	++	o	o
750 ppm Dowicide 7 in Blandol	+	o	+
100 ppm Phygon in 40 per cent Propylene glycol, 60 per cent water, and 0.1 per cent Aresket (a wetting agent)	o	o	o
Plain Blandol oil with nothing added	+	++	++

o No mold growth
+ Mold growth evident
++ Some mold growth
± Doubtful

group stored at room temperature was opened after a storage of five weeks. The results were noted as in Table III.

Some eggs from the above series were opened after 96 days and the results were observed to be as in Table IV.

TABLE III

EGGS STORED FOR FIVE WEEKS AT ROOM TEMPERATURE

Treatment	Observations
Controls	Rotten
2500 ppm Phygon in Blandol	Good odor and appearance of yolk and white. Red spots on inside shell.
750 ppm Phygon in Blandol	Good odor and appearance of yolk and white. Slight red spots on inside shell.
250 ppm Phygon in Blandol	Good odor and appearance of yolk and white. Slight spots on inside shell.
2500 ppm Dowicide 7 in Blandol	Mold on inside shell.
750 ppm Dowicide 7 in Blandol	Mold on inside shell. Poor aroma.
100 ppm Phygon in 40% Propylene glycol. 60% water solution and 0.1% Aresket (a wetting agent)	Outside shell good. Inside very moldy and spoiled egg.
Plain Blandol oil with nothing added	Bad

TABLE IV

CONDITION OF EGGS STORED FOR 96 DAYS

Treatment	Storage Temp	Results
(1) 100 ppm Phygon in 40% Propylene glycol, 60% water solution and 0.1% Aresket (a wetting agent)	Room Temp	Outside good. Very bad from inside.
(2) 250 ppm Phygon in Blandol	40 F	Good appearance inside. No mold inside. No off smell but mold growth on the outside.
(3) Plain oil dipped	40 F	Mold on the outside. No mold on the inside. White is thinner and yolk good and firm (stands up). No off smell.
(4) 2500 ppm Dowicide in Blandol	40 F	No smell. Good equal to Phygon. Mold on the outside. No mold on the inside.

B. OIL TREATMENT OF EGGS - SERIES NO. II

The next series was set up using the following compounds incorporated in 70 to 80 Saybolt viscosity oil.

(1) Plain 70-80 Saybolt viscosity oil

(2) 0.36% Benzoic acid in Blandol

(3) 0.10% Benzoic acid in Blandol

(4) 0.10% Hexyl[1] in Blandol

(5) 0.36% Hexyl in Blandol

(6) 0.10% Hyamine 10x[2] in Blandol

(7) 0.36% Hyamine 10 in Blandol

(8) 0.10% Phemerol[3] in Blandol

(9) 0.36% Phemerol in Blandol

(10) 2500 ppm Dowicide 7 in Blandol

Procedure: The eggs were dipped for one-half minute to one minute in oil solutions and were allowed to drain overnight. They were placed on standard egg flat in large metal pan over water and were sprayed with a mold suspension taken from a moldy egg. The whole set was then

---

[1] Hexylresorcinol

[2] Hyamine 10x is Di-isobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride monohydrate

[3] Phemerol is p-tert octylphenoxyethoxy ethyl dimethyl benzyl ammonium chloride

sealed with pliofilm and was held at room temperature for observation.

The pliofilm was opened up each week and the set was sprayed each time with mold culture taken from moldy eggs. Since the investigation was more of a technological rather than of a scientific nature, no attempt was made to find out the type of mold used for spraying. The mold used for spraying everytime was taken from control moldy eggs. It was later found out that such a mold would be the best for artificial inoculation of eggs.

The eggs were examined periodically for more than three weeks but repeated inoculations did not show any mold growth. Since the controls did not show any mold growth on the outside, no conclusions were made as to the relative effectiveness of the compounds used in this series.

In order to determine the inside condition, the eggs were opened up after storage for 42 days. The observations are recorded in Table V.

TABLE V

EGGS STORED AT ROOM TEMPERATURE FOR 42 DAYS

	Treatment	Observations
1.	Control	(1) Leached out (2) Rotten inside (3) Very rotten
2.	Plain Oil (Blandol) with nothing added	(1) Very liquid-thin white, yolk broke easily, no mold or spoilage apparent. (2) Very liquid-thin white, yolk broke easily, no mold or spoilage apparent. (3) Very nice appearance, some thick white, yolk holds shape, very slight off smell. No mold.
3.	Benzoic acid 0.36% in Blandol	(1) Very liquid-thin white, yolk broke easily, no mold or spoilage apparent, slightly rotten smell. (2) Very slight amount of thick white, yolk slightly cloudy, broke fairly easily, some off smell. (3) Very nice. Some thick white, yolk holds shape. No mold but slightly more off smell.
4.	Benzoic acid 0.10% in Blandol	(1) Very nice, some thick white, yolk holds shape. No mold but slightly more off smell. Trace of darkening on the embryo was observed which was thought to be physiological.

TABLE V (CONTINUED)

Treatment	Observations
	(2) Good odor, stands up good. (3) Very nice, some thick white, yolk holds shape, no mold but slightly more off smell.
5. Hexyl in 0.10% in Blandol	(1) Very nice, some thick white, yolk holds shape, very slight off smell, no mold, slight dark spot in the yolk was observed. (2) Very nice, some thick white, yolk holds shape, slightly more off smell. (3) Slight green discoloration of white was observed. There was slightly more off smell.
6. Hexyl 0.36% in Blandol	(1) Shell very spotty (as when dipped), very nice, slightly less thick white yolk holds shape, very slight off smell. No mold. (2) Shell very spotty (as when dipped), very nice. slightly less thick white, yolk holds shape, very slight off smell. No mold.
7. Hyamine 10x, 0.10% in Blandol	(1) Very nice, some thick white, yolk holds shape. No mold but there was more off smell. (2) Yolk didnot stand up. thick white thin-rotten smell. (3) Thin white was observed to be very liquid, thick

TABLE V (CONTINUED)

	Treatment	Observations
		white fairly good, yolk was observed to be cloudy; there was a brown discoloration on air-sac. The odor was not good but was better than (2).
8.	Hyamine 10x, 0.36% in Blandol	(1) Very liquid, smell slightly worse than the (3) egg of Hyamine 10x (0.10%). It was nearly as bad as (2) egg of Hyamine 10x (0.10%). (2) Very nice, some thick white. Yolk holds shape and has very slight off smell. No mold.
9.	Phemerol 0.10% in Blandol	(1) Very similar to above except that it had slightly better smell. (2) Very rotten. (3) Stinks. Shell cracked.
10.	Phemerol 0.36% in Blandol	(1) Like egg (2) of Hyamine 10x (0.36%). It was not as good as (1) egg of phemerol (0.10%) in smell. (2) Rotten.
11.	Dowicide 7, 0.25% in Blandol	(1) Like (2) egg of Hyamine 10x (0.36%) in smell. (2) Slightly more off smell. (3) Soft yolk, slightly cloudy.

N. B. Rotten means discolored and possessing very bad smell.

C. TREATMENT OF EGGS WITH QUATERNARY AMMONIUM COMPOUNDS

In later experiments a wild culture obtained from moldy control eggs growing in a commercial storage house was used for artificial inoculation of eggs to find out the relative merits of different compounds used for preventing mold growth on storage eggs. No attempt was made to determine the type used. Probably it was a mixture of several common molds common to commercial egg storage houses like Aspergillus, Mucor, Penicillium, etc.

It was decided to eliminate as many variables as possible and at the same time maintain conditions which would be ideal for storage. The cold storage series were entirely eliminated since it was observed that it would take a longer time for showing definite results and consequently it would be impossible to determine the mold inhibiting values of the compounds used. Therefore, it was decided to confine the attention to room temperature (76-80 F) storage which would have some importance in warmer areas where cold storage facilities are not available.

In preliminary experiments four molds were used for experiments.

(1) This mold was originally obtained from the mold growing on control, untreated storage

eggs held in cold room approximately three months.

(2) This mold was originally obtained from the mold growing on control, untreated and treated eggs held in cold room approximately three months.

(3) A variety of Mucor (M_1).

(4) A variety of Mucor (M_2).

Both (3) and (4) were obtained from Dr. Bollen, Department of Bacteriology, Oregon State College. The species name was not known.

The same procedure was used for plating of all the four molds. They were grown on acidified dextrose peptone agar of pH = 4 for a week before being used for spraying. The composition of the agar used was as follows:

Bacto - beef extract	3 gm
Bacto - peptone	5 gm
Dextrose	20 gm
Bacto Agar	15 gm

The above ingredients were suspended in 1000 cc of distilled water and heated to boiling to dissolve the medium completely. It was then sterilized in the autoclave at 15 lb pressure (121 C) for 15 minutes.

The mold suspension was made up as follows: 18 cc of distilled water was poured over the mold growth (7 days' growth). The plates were rotated for a few seconds and

the heavy suspension so obtained was poured off into a spray bottle which had a capacity of about 18 cc. This procedure was followed for all molds. The mold suspension so prepared was uniformly sprayed over the treated eggs each week by means of a spray bottle to obtain a fairly uniform inoculation so that a fairly reasonable estimate could be made about the relative effectiveness of the particular compound used for preventing mold growth on storage eggs. Fresh eggs (controls) were sprayed in the same manner and added each week.

Experiments were initially run on a small scale using desiccators for storage of the treated eggs. The desiccators had a capacity for six eggs each. Humidity in the container was kept at approximately 100% by pouring warm water on the bottom of the dessicator. The desiccators containing the treated eggs were stored at 76 F to accelerate mold growth.

Separate series were set up using the four molds. The eggs were initially treated with Bional, Isothan, Dowicide G, and Onyxide in concentration of 2500 ppm each. They were stored along with controls.

It was found that using this method extreme difficulty was encountered in getting mold growth on storage eggs even on the controls. Repeated inoculations failed to show mold growth on treated and untreated eggs for more

than 20 days. The molds did grow on some of the control eggs after 20 days but this was not taken into account because the problem was to find out a mold which would show reasonable growth on control eggs, say within 7 to 10 days. Of all the four molds none of them showed promising results. It was, therefore, decided to vary the conditions of experiments and to discover a mold which would grow on control eggs in a short time so that it could serve as a standard for evaluating the mycostatic power of the chemicals being used for experiments.

The next experiments therefore consisted in finding out the most effective mold of the four mold cultures which were on hand. Accordingly one group of one dozen untreated eggs each were sprayed with the correct mold suspension and stored in large pans over water in a wooden cabinet. The temperature of the wooden cabinet was kept uniformly at 80 F and the humidity in the pan was kept high by pouring warm water on the bottom of the pan and sealing the top with pliofilm.

It was found that if this procedure was followed the growth of mold on storage eggs was facilitated in that a fairly good growth was obtained after about 10 days of storage and the first growth on the eggs was observed on the seventh day of storage. Out of the four molds, the one originally taken from control, untreated eggs did show the best growth as is seen in Table VI. This mold was used in

TABLE VI

GROWTH OF DIFFERENT MOLDS ON UNTREATED EGGS IN TEN DAYS

	Type of Mold	Control Eggs (Unsprayed)	Eggs Sprayed with Mold
(1)	Mold originally obtained from mold growing on control untreated storage eggs.	+	++
		+	+++
		+	+
(2)	The mold originally obtained from the mold growing on both untreated and treated storage eggs.	o	++
		o	+
		+	o
		o	o
(3)	Mucor M_1	+	o
		+	o
		+	o
		+	o
		+	o
(4)	Mucor M_2	++	o
		o	o
		o	o
		o	o

o No mold growth
+ Slight evidence of mold growth
++ Evidence of mold growth
+++ Mold growth very marked

further experiments. The other three molds were not used in later experiments.

It was thought that incorporation of 1 per cent dextrose in mold suspension would enhance the mold growth but no significant improvement was noticed if this technique was followed as is seen in Table VII.

In other experiments that followed this mold was used without incorporation of 1 per cent dextrose. The mold was used for spraying after 7 days' growth on dextrose peptone agar at pH = 4 after the fruiting bodies had developed on plates. The method of preparing the mold suspension was kept the same in all experiments. This mold suspension was sprayed on the treated eggs every week and also on the new controls added each week.

After an active mold was obtained, it was decided to test the relative merits of some quaternary ammonium compounds along with Dowicides in preventing mold growth on eggs stored at room temperature which was uniformly kept at 80 F.

Two series were set up -- one containing water soluble compounds (Table VIII) and the other containing oil soluble compounds (Table IX) along with other compounds.

Identical method of treatment was followed. After treatment they were stored at 80 F over water in large-mouth pans covered with pliofilm. All the series were

TABLE VII

THE EFFECT OF 1% DEXTROSE ON THE GROWTH OF MOLD

Sprayed with 1% dextrose in mold suspension. The mold was taken from control, untreated egg.	Sprayed without 1% dextrose in mold suspension, which was originally taken from control untreated egg.
+++	+++
++	+++
+++	++
++	+++
+++	++
+++	+++

++ Evidence of mold growth

+++ Mold growth very marked

TABLE VIII

WATER SOLUBLE QUATERNARY AMMONIUM COMPOUNDS
USED IN EXPERIMENTS

	Chemical Name	Tradename	Manufactured by
(1)	Liquid containing alkyl dimethyl benzyl ammonium chloride (50%)	B.T.C.	Onyx Oil & Chemical Company
(2)	Powder containing cetyl dimethyl ethyl ammonium bromide (100%)	Bional EC	General Dyestuff Corporation
(3)	Powder of following composition: cetyl dimethylethyl ammonium bromide (10%) octadecyl dimethylethyl ammonium bromide (85%) octadecenyl dimethylethyl ammonium bromide (5%)	Bional ST	General Dyestuff Corporation
(4)	Acqueous mixture of high molecular alkyl-dimethyl-benzyl-ammonium chlorides (25%)	Bional A	General Dyestuff Corporation
(5)	Powder contains approximately: stearyl dimethyl ethyl ammonium bromide (10%) cetyl dimethyl ethyl ammonium bromide (10%) oleyl dimethyl ethyl ammonium bromide (35%) linoleyl dimethyl ethyl ammonium bromide (45%)	Ethyl Decab	Rhodes Chemical Corporation
(6)	Powder containing cetyl dimethyl ethyl ammonium bromide (100%)	Ethyl Cetab	Rhodes Chemical Corporation

TABLE VIII (CONTINUED)

	Chemical Name	Tradename	Manufactured by
(7)	See Table VIII-a	Arquad 16	Armour Chemical Co
(8)	See Table VIII-a	Arquad 12	Armour Chemical Co
(9)	See Table VIII-a	Arquad S	Armour Chemical Co
(10)	Liquid containing lauryl isoquinolinium bromide (about 20%)	Isothan Q-15	Onyx Oil & Chemical Company
(11)	An acqueous concentrate of N acyl colamino formyl-methyl pyridinium chloride (10%)	Emulsept	Emulsol Corporation
(12)	Sodium pentra chlorophenate (100%)	Dowicide G	Dow Chemical Company
(13)	An acqueous solution of alkenyl dimethyl ethyl ammonium bromide (10%)	Onyxide	Onyx Oil & Chemical Company
(14)	Powder containing di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride monohydrate (10%)	Timsol	Theo Ross Associates
(15)	Liquid containing cetyl dimethyl benzyl ammonium chloride (25%)	Ammonyx T	Onyx Oil & Chemical Company
(16)	Liquid containing alkyl dimethyl 3,4 dichloro benzyl ammonium chlorides (60%)	Tetrosan	Onyx Oil & Chemical Company

TABLE VIII (CONTINUED)

	Chemical Name	Tradename	Manufactured by
(17)	Paste containing alkyl dimethyl benzyl ammonium chlorides (90%)	Rodalon	Rhodes Chemical Co
(18)	Powder containing 85% octadecyl dimethyl benzyl ammonium chloride and 15% oleyl dimethyl benzyl ammonium chloride	Octab	Rhodes Chemical Co

The name "ARQUAD" is the tradename given to a series of quaternary ammonium compounds manufactured by the Armour Chemical Division. This series varies as to the alkyl chain length attached to the nitrogen atom as is seen below in Table VIII-a.

TABLE VIII-a

AVERAGE COMPOSITION OF ARQUADS

"R" group (Alkyl)	Carbon chain length	Arquad 16	Arquad 12	Arquad S
Octyl	8	--	--	--
Decyl	10	--	--	--
Dodecyl	12	--	90	--
Tetradecyl	14	--	9	--
Hexadecyl	16	90	--	10
Octadecyl	18	6	--	10
Octadecenyl	18	4	1	35
Octadecadienyl	18	--	--	45
Active Ingredients				
Nacl (Approx)		33	33	33
Water (Approx)		17	17	17
Form at room temp		Fluid	Fluid	Heavy Fluid

TABLE IX

OIL SOLUBLE QUATERNARY AMMONIUM CPD
USED IN EXPERIMENTS

	Chemical Name	Tradename	Manufactured by
(1)	Liquid containing 75% concentration of dilauryl dimethyl ammonium bromide	Iconal	Onyx Oil & Chemical Company
(2)	Pure crystalline quaternary. It is di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride	Phemerol Hyamine 1622	Parke Davis & Co Rohm & Haas Co
(3)	Pure crystalline quaternary. It is di-isobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride	Hyamine 10x	Rohm & Haas Co
(4)	Light tan paste containing 38% active fungicide. The active ingredient is a quaternary ammonium pentachlorophenate	Hyamine 3258	Rohm & Haas Co
(5)	Crystalline quaternary containing 85% Octadecyl dimethyl benzyl ammonium chloride and 15% oleyl dimethyl benzyl ammonium chloride	Octab	Rhodes Chemical Co

sprayed every week with freshly grown mold. Fresh controls were put in every week. Following this method it was noticed that control sprayed eggs regularly showed mold growth in ten to twelve days. Results are recorded in Tables X and XI. Results of duplicate runs are recorded on Tables XII and XIII.

It is seen from Tables X and XII that out of all the water soluble compounds used, alkyl dimethyl benzyl ammonium chloride, Bional EC, Bional ST, Bional A, Ethyl Decab, Isothan, Dowicide G, Arquad 12, onyxide, and Timsen do show some promise in preventing mold growth on surface of eggs for about a month under extreme conditions used in these experiments.

On the other hand,the protection noticed by the use of Octab, Emulsept, Rodalon, Tetrosan, Ammonyx T, and Arquad S does not seem to be adequate. The protection noticed by the use of Arquad S, Ammonyx T, Tetrosan, and Rodalon seemed to disappear after about 26 days.

The oil soluble compounds however, showed very poor results. None of them showed any promise except Dowicide 7. It is even doubtful whether it has any protective value unless used in higher concentrations. (See Tables XI and XIII). The quaternary ammonium compounds have been known to be adversely affected in their efficiency by some incompatible substances. In this case it was thought

that oil proved incompatible.

In order to check the internal condition of the treated eggs which showed no mold growth on the outside after 19 days' storage, one egg from each series was cooked in boiling water for a period of four minutes. After cooling, the egg was cut open and tasted. Eggs which showed mold growth on the outside after 19 days were not tasted. The results of this experiment are recorded in Table XIV.

TABLE X

EGGS TREATED WITH QUATERNARY AMMONIUM COMPOUNDS IN ACQUEOUS SOLUTIONS

1st Experiment

Compound and Concentration used	Observations made after 14 days	26 days	33 days
(1) 2500 ppm alkyl dimethyl benzyl ammonium chloride	o	o	o
(2) 2500 ppm Bional EC	o	o	o
(3) 2500 ppm Arquad S	o	o	++
(4) 2500 ppm Bional ST	o	o	o
(5) 2500 ppm Ammonyx T	o	o	++
(6) 2500 ppm Bional A	o	o	o
(7) 2500 ppm Ethyl Decab	o	o	o
(8) 2500 ppm Ethyl Cetab	o	o	?
(9) 2500 ppm Octab	o	++	+++
(10) 2500 ppm Tetrosan	o	o	++
(11) 2500 ppm Isothan 215	o	o	o
(12) 2500 ppm Emulsept	o	++	++
(13) 2500 ppm Dowicide G	o	o	o
(14) 2500 ppm Arquad 12	o	o	o
(15) 2500 ppm Onyxide	o	o	o
(16) 2500 ppm Rodalon	o	o	++
(17) 2500 ppm Timsen	o	o	o
(18) 2500 ppm Arquad 16	o	o	?
Controls	All badly molded after 10 to 12 days.		

o No mold growth
++ Mold growth evident
+++ Mold growth very marked
? Doubtful

TABLE XI

EGGS TREATED WITH VARIOUS COMPOUNDS IN OIL SOLUTION STORED 40 DAYS

1st Experiment

Compound and Concentration used	I	II	III	IV
(1) Oil dipped controls	+++	++	++	
(2) 700 ppm Dowicide 7	o	+	o	++
(3) 2500 ppm Dowicide 7	o	o	o	o
(4) 1000 ppm Benzoic acid	+++	++++	+++	+++
(5) 3600 ppm Benzoic acid	++++	+++	++++	+++
(6) 1000 ppm Phemerol	+++	++++	++++	+++
(7) 3600 ppm Phemerol	++++	+++	+++	++++
(8) 1000 ppm Hyamine 10x	++++	++++	+++	+++
(9) 3600 ppm Hyamine 10x	+++	++++	++++	+++
(10) 500 ppm Hyamine 3258	+++	+++	+++	++++
(11) 2500 ppm Isonal	+++	+++	++++	+++
(12) 2500 ppm Octab	+++	+++	++	+++
Controls	All badly molded after 10 to 12 days.			

N. B. All the eggs showed mold growth about the same time as controls.

o No mold growth
++ Mold growth evident
+++ Mold growth very marked
++++ Profuse mold growth

TABLE XII

EGGS TREATED WITH QUATERNARY AMMONIUM COMPOUNDS IN ACQUEOUS SOLUTIONS

2nd Experiment

Compound and Concentration used	Observations made after 10 days	17 days
(1) 250 ppm alkyl dimethyl benzyl ammonium chloride	o	o
(2) 2500 ppm Bional EC	o	o
(3) 2500 ppm Arquad S	o	o
(4) 2500 ppm Bional ST	o	o
(5) 2500 ppm Ammonyx T	o	o
(6) 2500 ppm Bional A	o	o
(7) 2500 ppm Ethyl decab	o	o
(8) 2500 ppm Ethyl cetab	o	o
(9) 2500 ppm Octab	o	++
(10) 2500 ppm Tetrosan	o	o
(11) 2500 ppm Isothan 215	o	o
(12) 2500 ppm Emulsept	o	++
(13) 2500 ppm Dowicide G	o	o
(14) 2500 ppm Arquad 12	o	o
(15) 2500 ppm Onyxide	o	o
(16) 2500 ppm Rodalon	o	o
(17) 2500 ppm Timsen	o	o
(18) 2500 ppm Arquad 16	o	o
Controls	All badly molded after 10 to 12 days.	

o No mold growth
++ Mold growth evident

TABLE XIII

EGGS TREATED WITH VARIOUS COMPOUNDS IN OIL SOLUTION STORED 40 DAYS

2nd Experiments

Compound and Concentration used	Observations made after 20 days				Observations made after 29 days			
	I	II	III	IV	I	II	III	IV
(1) Oil dipped controls	+++	++	+++		+++	+++	+++	
(2) 700 ppm Dowicide 7	o	o	o	o	o	o	o	o
(3) 2500 ppm Dowicide 7	o	o	o	o	o	o	o	o
(4) 1000 ppm Benzoic acid	+++	++++	+++	++	+++	++++	+++	++++
(5) 3600 ppm Benzoic acid	++++	+++	+++	++++	++++	+++	++++	++++
(6) 1000 ppm Phemerol	++++	+++	++++	++++	++++	++++	++++	++++
(7) 3600 ppm Phemerol	++++	++	+++	+++	++++	++++	++++	+++
(8) 1000 ppm Hyamine 10x	+++	++++	+++	++++	++++	++++	+++	++++
(9) 3600 ppm Hyamine 10x	++++	+++	+++	++++	++++	+++	++++	++++
(10) 500 ppm Hyamine 3258	+++	++++	+++	+++	+++	++++	+++	+++
(11) 2500 ppm Isonal	+++	++++	+++	+++	+++	++++	+++	++++
(12) 2500 ppm Octab	++++	++++	+++	+++	++++	++++	+++	+++
Controls	All badly molded after 10 to 12 days.							

N. B. All the eggs showed mold growth about the same time as controls.

o No mold growth
++ Some mold growth
+++ Mold growth very marked
++++ Profuse mold growth

TABLE XIV

EGGS COOKED AND TASTED AFTER 19 DAYS' STORAGE

Compound of Concentration used	Observation
(1) 2500 ppm Alkyl dimethyl benzyl ammonium chloride	Taste good; protection on outside.
(2) 2500 ppm Bional EC	Taste good; protection on outside.
(3) 2500 ppm Bional ST	Taste good; protection on outside.
(4) 2500 ppm Bional A	Taste good; protection on outside.
(5) 2500 ppm Ethyl Decab	Taste good; protection on outside.
(6) 2500 ppm Ethyl Cetab	Taste good; protection on outside.
(7) 2500 ppm Arquad 16	Definitely off smell but protection on outside.
(8) 2500 ppm Isothan Q-15	Taste good; protection on outside.
(9) 2500 ppm Emulsept	No protection on outside.
(10) 2500 ppm Dowicide G	Taste good; protection on outside.
(11) 2500 ppm Arquad 12	Taste good; protection on outside.
(12) 2500 ppm Onyxide	Taste good; protection on outside.
(13) 2500 ppm Timsen	Taste good; protection on outside.

TABLE XIV (CONTINUED)

Compound of Concentration used	Observation
(14) 2500 ppm Arquad S	Taste good; protection on outside.
(15) 2500 ppm Ammonyx T	Taste good; protection on outside.
(16) 2500 ppm Tetrosan	Taste good; protection on outside.
(17) 2500 ppm Rodalon	Taste good; protection on outside.
(18) 2500 ppm Octab	No protection on outside.
Controls	Bad. Had decidedly bad off smell.

IV. DISCUSSION

It is apparent that for reduction of spoilage brought about by activities of microorganisms during storage two most important points to be considered are control of temperature and humidity. In the problem at hand, extreme conditions of temperature and humidity were created to find out the relative merits of different compounds used in egg preservation.

The problem was started out by storing both treated and untreated control eggs at room temperature (76 F to 80 F) and cold storage temperature (40 F). However, it was realized that it would take a long time for definite results to be apparent in cold storage. After the first experiments cold storage series were entirely eliminated and attention was mainly directed to room temperature storage. It was thought that at room temperature it would take a shorter time for definite results to be apparent. At the same time attention was directed towards finding an effective mold which would serve as a standard for comparing the mycostatic power of different compounds used in experiments.

Extreme difficulty was encountered in finding such a mold. After repeated experiments a mold was obtained which served as a standard of comparison of mycostatic

activity of different compounds in later experiments. This mold was originally obtained from the mold growing on control, untreated storage eggs held in cold storage approximately three months. It was found that using this mold control untreated eggs regularly molded after 10 to 12 days.

In all experiments identical storage conditions were created to approximate closely the conditions existing in commercial storehouses. Since the problem at hand was more of a technological nature, no attempt was made to find out the species name of the mold used as a standard. Probably it was a mixture of several common molds regularly found in commercial storehouses.

In initial experiments an attempt was made to find out the merit of propylene glycol because it has been recommended as a mold inhibitor in recent times. However, it was noticed that it is more of a disinfecting compound rather than an inhibiting compound. It was thought that it would hardly stand up to infection in commercial storehouses.

Phygon and Dowicides have recently been shown to have some promise in preservation. Accordingly an attempt was made to determine their efficiency. On a similar basis, hexyl resorcinol and benzoic acid were also tried out.

It was noticed that good protection is afforded internally by phygon in concentrations of 250 and 750 ppm in Blandol at room temperature. The eggs had good odor and appearance after storage for five weeks at room temperature except for slight red spots observed on the inside shell. However, it was not known whether such an improvement could be noticed in the internal condition on repeated infection with egg molds.

Dowicides have been shown to have good value in egg preservation by Mallmann. Accordingly both water-soluble and oil-soluble Dowicides were tried out in egg preservation. Oil-soluble Dowicide in concentrations of 2500 ppm seem to have some mold inhibiting value on the egg surface at room temperature but it is doubtful whether they could be effective for prevention of mold growth on the inside at room temperature. However, water-soluble Dowicide in concentrations of 2500 ppm seem to have mold inhibiting value both on the outside and internally at room temperature storage. It also showed a normal taste except that it has a slight storage flavor when tasted after a storage period of 19 days. The controls were bad in the same period.

The quaternary ammonium compounds have been known for their high germicidal power and for non-toxicity in lower dilutions. Accordingly an attempt was also made to test the efficiency of certain quaternary ammonium compounds

in preventing mold growth on storage eggs. It is now definitely established that if we could prevent the microorganisms from entering the shell, we could materially reduce the incidence of spoilage.

In experiments with water-soluble compounds two series were set up using 2500 ppm of Alkyl dimethyl benzyl ammonium chloride, Bional EC, Bional ST, Bional A, Ethyl Decab, Ethyl Cetab, Arquad 16, Isothan Q-15, Emulsept, Dowicide G, Arquad 12, Onyxide, Timsen, Arquad S, Ammonyx T, Tetrosan, Rodalon, and Octab. It was noticed that all of them showed protection for 12 days. The control sprayed eggs in the same period were very badly molded. However, at the end of 17 day period Emulsept and Octab did not show protection. It was also noticed that after about 33 days of storage Arquad S, Ammonyx T, Tetrosan, and Rodalon lost their protective value.

At the end of 33 day storage period with spraying every week, only Alkyl dimethyl benzyl ammonium chloride, Bional EC, Bional ST, Bional A, Ethyl decab, and Isothan did show definite protection while the results with Ethyl Cetab and Arquad 16 were doubtful.

From the above it is seen that the water-soluble compounds used could be divided into three classes:

(a) Those that show good promise in extending storage life of eggs, eg, Bional, Isothan, Arquad 12, etc.

(b) Those that show relatively little promise, but nevertheless show definite improvement in storage conditions, eg, Rodalon, Arquad S, and Tetrosan.

(c) Those that show very little protection as compared to controls, eg, Emulsept and Octab.

Using the same conditions, Phemerol, Hyamine 10x, Hyamine 3258, Isonal, and Octab were used in concentration of 2500 ppm in oil. In both series all the eggs began to get moldy about the same time as controls. Oil was thought to be one of the substances which proves to be incompatible with the quaternary ammonium compounds.

V. SUMMARY AND CONCLUSIONS

(1) Cold storage treated eggs after three months show good internal quality although mold develops on the shell.

(2) The protection observed with 100 ppm propylene glycol does not seem to be significant because infecting propylene glycol treated eggs with molds taken from moldy egg does not give protection. It is more a case of disinfection rather than protection. Besides it does not give protection in the inside at room temperature.

(3) There seems to be a definite improvement in the inside condition of the eggs by incorporation of phygon in concentration of 250 ppm and 750 ppm in Blandol at room temperature. Blandol as such does not show as much protection in the interior at room temperature. However, it was not known whether the same improvement could be noticed on repeated spraying.

(4) Oil-soluble Dowicide in concentration of 2500 ppm seems to have some mold inhibiting value on the egg surface at room temperature, but it is doubtful whether it could be effective for prevention of mold growth on the inside at room temperature.

However, the water-soluble Dowicide in concentration of 2500 ppm seems to have mold inhibiting value both on the

outside and inside at room temperature storage.

(5) Hexylresorcinol and benzoic acid in concentrations of 1000 ppm and 3600 ppm seem to show some promise as far as the preservation of internal quality at room temperature is concerned.

(6) Some of the water-soluble quaternaries showed very encouraging results, both in prevention of mold on the outside and inside at room temperature storage. Most of the water-soluble compounds used showed good results. Out of all the water-soluble compounds used, Alkyl dimethyl benzyl ammonium chloride, Bional EC, Bional ST, Bional A, Ethyl Decab, Isothan, Dowicide G, Arquad 12, Onyxide, and Timsen did show some promise in concentration of 2500 ppm. They prevented mold growth on surface of eggs for about a month under experimental conditions used while the protection afforded by Arquad S, Ammonyx T, Tetrosan, and Rodalon seemed to disappear after about 26 days of storage.

(7) The oil-soluble quaternaries show very little promise. Out of all the oil-soluble compounds used along with Dowicide 7 (2500 ppm), only the latter showed protection. It is not clear why most of the oil-soluble compounds did not show good results.

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