1955

Microbiological and functional changes in dried egg albumen stored at elevated temperatures

George J. Banwart
Iowa State College

NOTE TO USERS

MICROBIOLOGICAL AND FUNCTIONAL CHANGES IN DRIED EGG ALBUMEN STORED AT ELEVATED TEMPERATURES

135

by

George J. Banwart

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Food Technology-Bacteriology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
1955

TABLE OF CONTENTS

I. INTRODUCTION

The presence of pathogenic microorganisms belonging to the genus Salmonella in dried egg products has resulted in many investigations concerning the sources of these contaminants and methods to eliminate the organisms from the dried product.

Members of the genus Salmonella have been involved in a number of human ills such as typhoid fever, paratyphoid fever, septicemias, abscesses, bronchopneumonias, meningitis, arthritis, pleurisy, pyelonephritis and gastroenteritis. Some of the symptoms of gastroenteritis include nausea, vomiting, headache, fever, chills, generalized aches and pains (particularly of the lumbar region), rapid pulse and a flushed appearance. Therefore, the presence of these organisms in food is very undesirable and methods for detection and isolation, as well as procedures for eliminating them from food products, are desirable and necessary.

Although media and procedures have been devised for detecting and isolating Salmonella from various materials such as feces, urine, infected tissues and water, none were devised specifically for use with egg products; thus investigators interested in the incidence of Salmonella in eggs have employed broths intended to be used for other

materials. It is evident that egg, with its content of proteins, lipids, vitamins and antibacterial agents (e.g. lysozyme, avidin, conalbumin, ovomucoid and carbon dioxide) changes the nutritional constituents of the broths when added to them.

Plant sanitation and pasteurization of the liquid egg have been employed in an effort to obtain dried eggs devoid of Salmonella; however, even with these procedures, organisms belonging to this genus have been isolated from the product after drying.

This investigation was undertaken to study media used to detect and isolate Salmonella and to investigate methods to reduce and eliminate these pathogens from dried albumen.

The commonly used enrichment broths and selective agars were tested in order to determine their ability to support the growth of Salmonella.

In order to reduce multiplication of organisms during holding of the liquid albumen, the liquid egg white was altered by the addition of antibiotics (chlortetracycline, oxytetracycline and streptomycin) and by varying the hydrogen ion concentration. The effect of elevated temperatures of storage (50°, 60° or 70° C.) and the moisture content of the dried albumen (1.5, 3, 6 or 12 per cent) upon the microbiological population and functional properties of the albumen were observed in order to determine

if this treatment could be used to obtain a product devoid of *Salmonella*.

II. REVIEW OF LITERATURE

In treating dried egg white to reduce the number of microbial contaminants, two aspects must be considered: (1) reductions in the microbiological populations, and (2) changes in the physical and functional properties of egg white due to the treatment imposed upon the product.

The types of organisms which contaminate dried egg white, the sources of contamination and the means by which contamination may be reduced, as well as the effect of various treatments upon the physical and functional properties of dried egg white, are discussed in the following review of literature.

A. Types of Microorganisms in Eggs and Egg Products

1. The genus Salmonella

Edwards et al. (1948a) presented a review regarding the occurrence and distribution of Salmonella in animals and in various foods. They concluded that eggs and food products containing eggs are important sources for the transmission of Salmonella from animals to man.

It is not the intent of the writer to review all of the literature pertaining to this genus, but rather to consider aspects such as the occurrence and distribution of members of this genus, particularly those involved in food infection, methods and media used to isolate and enumerate members of this genus and the pathogenicity of Salmonella.

a. Occurrence and distribution. Several workers have examined eggs produced by hens which were known S. pullorum "reactors", a reactor hen being a bird whose blood contains agglutinins for the antigens of S. pullorum. The data obtained by these investigators are summarized in Table 1. Evidently this organism was not transmitted to every egg laid by an infected hen. Runnels and Van Roekel (1927) tested the eggs from 14 "reactor" hens and found that only 10 of the 14 birds produced eggs containing S. pullorum; however, over 50 per cent of the eggs laid by one hen were found to contain this organism.

Bornstein et al. (1941), Seligmann et al. (1943), Gibbons and Moore (1944a), Schneider (1951), Solowey et al. (1946, 1947, 1948), Gibbons (1947a), Sutton and McFarlane (1947), McFarlane and Calesnick (1948) and Edwards et al. (1948a), reported the presence of organisms belonging to the genus Salmonella in dried whole egg powder. Goresline (1948) presented a review concerning the occurrence of Salmonella in eggs.

Table 1. Incidence of *Salmonella pullorum* in eggs laid by hens whose blood contained antibodies for *S. pullorum*

Investigator(s)	Number of eggs examined	Per cent infected
Rettger and Stoneburn (1909)	44	20.5
Gage *et al.* (1914)	619	5.2
Hadley *et al.* (1917)	55	10.5
Doyle (1925)	341	2.6
Kaupp and Dearstyne (1927)	3,818	5.94
Rochaix *et al.* (1933)	207	1.93
Tittsler *et al.* (1928)	1,560	5.2
Runnells and Van Roekel (1927)	169	33.7
Weaver and Weldin (1931)	775	6.8
Chase (1947)	1,000	6.1

Edwards *et al.* (1948a) reported the isolation of 23 different types of *Salmonella* from eggs and egg powder and stated that these cultures generally followed the distribution of types of *Salmonella* found in fowl. The hen has long been known to harbor *Salmonella*, and, in particular, *S. pullorum*. The isolation of several species of *Salmonella* from fowl was reported by Edwards and Bruner (1938, 1941, 1943), Edwards (1939), Lerche (1939), Jungherr and Clancy (1939), Mallman *et al.* (1942), Darby and Stafseth (1942), Hinshaw *et al.* (1944), Moore (1946), Buxton (1948), Karlshoj and Marthedal (1948), Hinshaw and McNeil (1948) and Edwards *et al.* (1948a, 1948b).

McFarlane and Calesnick (1948) examined 507 samples of low moisture spray dried whole egg powder and isolated Salmonella from 5 per cent of the samples; 11 serological types were identified. Edwards et al. (1948a) and McFarlane and Calesnick (1948) observed that of those bacteria identified, S. oranienburg was the predominant species.

Schneider (1946a) analyzed 901 samples of egg powder and obtained 32 (3.18 per cent) isolations of several species of Salmonella. Solowey et al. (1948) identified serologically from 2 to 30 Salmonella in each of 171 samples of spray dried whole egg powder. Gibbons (1947), during 1945, isolated Salmonella (other than S. pullorum) from 112 of 400 samples of dried whole egg.

A report by Ayres and Slosberg (1949) showed that sizeable numbers of Salmonella were present in dried egg white.

b. Methods and media for detection and isolation. Since saprophytic organisms are usually more predominant in foods than are pathogenic organisms, it has been necessary to use methods and media which favor the growth of Salmonella and, at the same time, inhibit other organisms. The purpose in using an enrichment broth is to increase the numbers of Salmonella present and, at the same time, to prevent the reproduction of other organisms in order that the probability

of isolating Salmonella will be increased. The results of Watt and Carlton (1945) and Galton and Hardy (1948) have demonstrated the importance of using enrichment methods. The broths selected should exhibit minimum bacteriostatic or bactericidal effects on Salmonella. This is particularly important if only a few organisms of this genus are present in the sample.

The enrichment broths generally used are Selenite-F and tetrathionate. Modifications of tetrathionate are also used.

In 1936 Leifson described the formula now used for the preparation of Selenite-F. By means of growth curves, he showed that, although the medium was not sufficiently toxic to completely inhibit the growth of enterococci or "fecal colon bacilli", there was a decrease in their numbers during the initial incubation period; "typhoid bacilli" were able to multiply rapidly from the start and soon outnumbered other organisms.

Gohar (1943) used sodium selenite as an inhibitor of extraneous organisms. He found that Bacterium enteriditis (S. enteriditis), Bacterium paratyphosum A (S. paratyphi), and Bacillus paratyphosus B (S. schottmuelleri) were more resistant to the action of sodium selenite than was Bacterium coli (Escherichia coli). However, he also

observed that some extraneous organisms were more resistant to the action of sodium selenite than were some members of the genus Salmonella.

Knox et al. (1943) reported that Bacterium paratyphosum B (S. schottmuelleri), most of the common Salmonella, and members of the genus Proteus were powerful tetrathionate reducing organisms. Bacterium typhosum (S. typhosa) was less effective; Bacterium paratyphosum A (S. paratyphi), Bacterium coli (E. coli), Bacterium aerogenes (Aerobacter aerogenes), dysentery bacilli and most fecal organisms were unable to reduce tetrathionate.

As a means for enriching Salmonella in dried egg products, Gibbons and Moore (1944a) utilized tetrathionate while Solowey et al. (1946) and Ayres and Slosberg (1949) used Selenite-F. Cantor and McFarlane (1948) employed both Selenite-F and tetrathionate for enrichment of Salmonella in fresh eggs.

Ruys (1934) reported the use of brilliant green-Esbach broth for isolation of S. schottmuelleri and later (1940) stated that this broth yielded a higher per cent of positives than did other enrichment broths for all species of Salmonella.

Although there have been indications that one enrichment fluid may be better than another, in some instances the results obtained from different samples or from varying

quantities of the same sample were used as a basis for comparison. The conclusions derived from these comparisons do not necessarily mean that one broth is better for all types of samples or for all species of Salmonella.

Many solid media have been suggested and utilized for the detection and isolation of organisms presumptively identified as belonging to the genus Salmonella. Included in this list are Salmonella-Shigella (SS), brilliant green (BG), desoxycholate citrate lactose sucrose (DCLS) and bismuth sulfite (WB) agars. Hajna and Perry (1938) and Gunther and Tuft (1939) reported that WB was superior to all agars that they tested; however, several selective agars have been developed since their studies were reported. Although at one time SS agar was considered to be a satisfactory medium for differentiation of Salmonella, Broh-Kahn (1946) found this medium unsatisfactory for the isolation of these organisms. He considered Kauffmann's BG agar to be a very good medium and recommended its use as an analytical tool.

c. Pathogenicity. Organisms of the genus Salmonella have been involved in a number of cases of sickness in human beings. Outbreaks of food poisoning due to contaminated rice pudding were reported by D'Albora et al. (1945) and Mitchell et al. (1946). D'Albora et al. isolated S. montevideo and Mitchell et al. isolated S. pullorum.

It was believed that eggs used in the formulation of the pudding provided the source of the organisms. Mayonnaise made with contaminated eggs was shown by Watt (1945) to be the cause of an outbreak of salmonellosis involving 28 people.

Schiff (1938) and Hormaeche and Peluffo (1941) found that Salmonella were of more importance in infant pathology than in adult infections. Hormaeche and Peluffo examined 2,141 children and isolated Salmonella from 266. Seligmann et al. (1946) reported that of 2,000 cases of salmonellosis, 40 per cent involved children under 10 years of age. They also found that fatalities due to Salmonella infection were high in people over 50 years of age.

In a series of papers McCullough and Eisele (1951a, 1951b, 1951c) reported the pathogenicity of several species of Salmonella which were isolated from spray dried whole egg. The organisms tested were S. meleagridis, S. anatis, S. pullorum, S. newport, S. derby and S. bareilly. At the lowest level studied (125,000 organisms) S. bareilly caused illness in one of six men, while in order to cause illness in three (or 50 per cent) of the men, an intake of 1,700,000 organisms was required. S. newport, fed at a level of 152,000 organisms, caused symptoms in one of six men. While most of the organisms caused symptoms or illness at

concentrations of hundreds of thousands, S. pullorum was fed at a level of over 1 billion before causing disturbances. It was believed that this resistance to S. pullorum was due to the fact that all of the subjects ingesting these organisms were immunized against S. typhosa, which is antigenically similar to S. pullorum.

2. Other organisms

Since dried egg white is a product of shell eggs, the types of microorganisms (other than Salmonella) as well as the extent of infection of the intact egg deserve some comment. The extent of bacterial infection of eggs has been investigated by many workers and their results are listed in Table 2. Although the per cent of eggs infected varied considerably, factors such as the age of the eggs, method of sampling, amount of sample, type of culture media, and temperature of incubation could account for the differences obtained by these workers. Results showed that there was a higher incidence of infection in the yolk than in the white. The observation by Bushnell and Maurer (1914) that the per cent of infection at an incubation temperature of 20° C. was greater than at 38° C. indicated that the predominant flora was saprophytic soil organisms rather than pathogenic types.

Table 2. Bacterial infection of fresh eggs

Investigator(s)	Number of eggs examined	Part examined	Per cent infected
Maurer (1911)	575	whole	24.0
Rettger (1913)	3,510	yolk	14.24
	582	white	1.20
Bushnell and Maurer (1914)	2,759	whole	23.7
		yolk	23.6
		white	3.40
Hadley and Caldwell (1916)	2,520	whole	8.70
	111	yolk	4.50
	111	white	0.00
Haines (1938)	112	whole	7.0
Wolk et al. (1950)	651	whole	12.13

Forsythe et al. (1953) examined more than 1,000 fresh eggs and found that in every examination there were less than 2 organisms per egg.

The organisms which were isolated from eggs by the workers listed in Table 2 included the following: Bacillus mesentericus, B. subtilis, Micrococcus pyogenes, var. albus, Proteus vulgaris, Pseudomonas aeruginosa, Ps. fluorescens, Streptococcus lactis, E. coli, Achromobacter sp., Serratia sp., Streptothrix sp., Penicillium, Sporotrichum, Mucor and Botrytis.

The presence of streptococci in spray dried whole egg powder was reported by Brown and Gibbons (1950) and Solowey and Watson (1951). Paracolons representing 14 different antigenic groups were isolated from dried whole egg by Solowey (1947). Watson and McFarlane (1948) found Aspergillus flavus, Penicillium, Mucor, Alternaria, and Oidium in whole egg powder. McFarlane and Calesnick (1948) and Sutton and McFarlane (1947) investigated the presence of coliforms in whole egg powder and reported that these organisms were isolated from 51 per cent of the samples that they analyzed. Organisms belonging to the genus Bacillus were isolated from dried egg by Hartsell (1944) and McFarlane and Calesnick (1948).

B. Sources of Microorganisms in Dried Egg White

1. General

A description of the method used in the preparation of egg white prior to drying will enable the reader to understand the manner in which contamination by microorganisms can take place.

Shell eggs procured from buyers are candled to eliminate eggs which are rotten or contain blood spots or blood rings. Cracked or checked eggs, termed "cracks" and "leakers",

are removed and broken separately; the acceptable eggs are transported to the breaking room where they are broken open. In the breaking operation the shell is cracked against a knife edge and is opened by pulling the half-shells apart. The liquid contents (albumen and yolk) are either separated, or the melange is allowed to drop into a small cup. If a rotten egg is broken into the cup, the contents of the cup are discarded and the breaking equipment washed before it is used again. The acceptable eggs are collected in a large bucket, and then poured into a blendor. Following the blending procedure, the liquid egg is screened to remove membranes and pieces of broken shell. The final products are called liquid whole egg, liquid yolks and liquid whites.

The whites are placed in a vat where the glucose is removed by an enzyme complex, glucose oxidase, or by fermentation processes which involve the use of bacteria or yeasts. Then the fermented whites are dried either by a spray drying or by a pan drying process.

During the processing of eggs prior to drying, microbial contaminants may gain entrance from four main sources: the shell egg, the breaker, the utensils, and the fermentation process.

2. Shell egg

a. Mode of infection. Whether or not eggs are contaminated before or after laying is a controversial question. One manner in which eggs are infected is the direct contamination of the yolk from infected ovaries of the hen. According to Lamson (1909), diseased ovaries may cause infection of the egg prior to ovulation. Frank and Edgington (1937) examined 21 "reactor" hens and found that 20 of these birds showed ovarian infection due to S. pullorum.

Chase (1947) mixed S. bareilly into the feed of a small group of hens and collected their eggs during a 40-day period. Of 124 eggs laid by these birds, two were found to contain this organism. Hens fed S. oranienburg laid 25 eggs, none of which were found to contain the enteric pathogen.

Although bacterial invasion of the developing ovum is known to occur, Haines and Moran (1940) consider that microbial contamination of eggs is due mainly to the penetration of microorganisms into the egg after laying. The egg possesses physical barriers which impede the migration of bacteria from the shell surface into the interior of the egg. These barriers include the outer coating of mucin on the shell and the shell and its associated membranes. Fresh unwashed eggs were observed by Haines (1938) to be resistant to bacterial penetration. Eggs

immersed in a culture of Pseudomonas for 12 hours contained no organisms even after they had been stored for 14 days at 20° C. When washed eggs received the same treatment, upon immediate examination, 10 per cent were found to be infected while, after storage for 14 days, 40 per cent were contaminated.

Although the mucin coating on the shell was shown by Haines (1938) to inhibit bacterial penetration, Gillespie et al. (1950) reported that the removal of this coating did not enhance bacterial invasion of the egg. Further, Forsythe et al. (1953) found that eggs properly washed soon after laying had fewer organisms on the inside after storage than did unwashed eggs.

Haines and Moran (1940) found the porosity of the shell to vary at different points on the same shell and with successive eggs from the same hen. They also concluded that, if the temperature of an egg is higher than the fluid in which the egg is immersed, the bacteria in the fluid are readily drawn through the shell by suction as the egg cools.

To show the penetration of the egg shell by Pseudomonas, Rievel (1939) coated the interior of an aseptically obtained shell with nutrient agar and then dipped this prepared shell into a broth culture of Pseudomonas. The appearance of fluorescent spots on the inside of the shell, when observed

with ultraviolet light, showed that Pseudomonas had penetrated the shell. This work was confirmed by Lorenz et al. (1952).

If eggs are removed from cold surroundings to a warm room with a high relative humidity, moisture collects on the shell. In commercial establishments, this condition is commonly called "sweating". Moran and Pique (1926), Rosser et al. (1942), Goresline and McFarlane (1944), and Forsythe et al. (1953) showed that "sweating" of eggs resulted in an increased bacterial content inside the eggs.

Wolk et al. (1950) stored shell eggs at 5°, 15°, 25° or 35° C. and observed the number of eggs that became infected during storage. They noted that at 35° C. the percentage of infected eggs increased rapidly; however, at 25° C. the highest percentage of infected eggs was observed. Storage at 5° or 15° C. resulted in contamination of 20 or 30 per cent of the eggs respectively. Thus the temperature is a factor to be considered when storing eggs.

Eggs examined by Stuart and McNally (1943) were found to possess sterile shells when laid. Blount (1949) suggested that the main source of infection of eggs is dirty, contaminated nests which come into contact with freshly laid eggs while the eggs are warm and moist.

During the breaking operation, dirt on the shell of an egg is a source of bacteria for the contamination of liquid

eggs. Zagaevsky and Lutikova (1944a) reported that liquid egg obtained from eggs with soiled shells contained 100 times as many bacteria as did liquid egg obtained from clean eggs. They reported also that disinfection of shells with chlorine before breaking resulted in liquid egg relatively free from bacteria. Solowey et al. (1946) reported a higher incidence of Salmonella in the melange obtained from dirty eggs than in that secured from clean eggs. Cantor and McFarlane (1948) confirmed these results. It was concluded by Solowey et al. (1946) that the external shell surface was an important source of Salmonella and that dirty eggs were the primary offenders.

When the contents of several eggs are mixed together, one contaminated egg can infect an entire batch of liquid egg. Although the number of organisms in shell eggs may not be excessive, this contamination is important in liquid eggs, since the broken-out liquid egg provides a more accessible source of nutrients for bacterial reproduction than do shell eggs.

b. Growth of microbial contaminants. According to Romanoff and Romanoff (1949), it was first postulated by Wurtz (1890) that egg white possessed germicidal activity. Later, Laschtschenko (1909) observed that liquid albumen placed in petri dishes and exposed to air did not become putrid during drying. He repeated his experiment, and

removed samples to agar and gelatin surfaces for bacterial examination and was unable to isolate any microorganisms even after 6 days of exposure of the albumen. When he added bouillon broth to the albumen, only 2 or 3 days were required for putrefaction to occur. Laschtschenko further observed that cultures of B. subtilis, B. anthracis, and B. megatherium, were destroyed by the albumen. Albumen was heated to 55° to 60° C. for 30 minutes without destroying its bactericidal action; however, when albumen was heated to 65° to 70° C. for 30 minutes and, after cooling, was inoculated with B. subtilis, this organism grew profusely.

Four major antibacterial substances have been detected in hen's egg white. These active principles are: lysozyme, avidin, conalbumin, and ovomucoid. Bacteriostatic action is ascribed also to pH effects in the egg, the presence of carbon dioxide, and the inability of organisms to use native proteins.

The ability of lysozyme either to lyse or to inhibit bacteria was demonstrated by Fleming (1922). Work concerning the observation and characterization of lysozyme in egg white has been reviewed by Haines (1939), Zagaevsky and Lutikova (1944b) and Romanoff and Romanoff (1949). Of the bacteria tested, the ones susceptible to lysis were Gram positive, while Gram negative organisms were, in general,

not lysed. Kern et al. (1951) considered that the action of lysozyme against M. pyogenes var. aureus and M. lysodeikticus, in general, affected the cell membranes.

Avidin, the biotin binding protein, was isolated from fresh egg white by Eakin et al. (1941). Two molecules of biotin are bound by each avidin molecule, according to Fraenkel-Conrat et al. (1952a). The composition and mode of action of avidin was described by Fraenkel-Conrat et al. (1952b).

The existence of an iron binding agent in egg white was reported by Schade and Caroline (1944). Schaible and Bandemer (1946) and Alderton et al. (1946) identified conalbumin as the agent responsible for iron binding and, as a result of the formation of this complex, albumen displayed antibacterial activity. Fraenkel-Conrat and Feeney (1950) found that conalbumin did not prevent the growth of M. pyogenes var. albus but prolonged the lag phase and reduced the rate of subsequent growth of this organism. It was postulated by Fraenkel-Conrat and Feeney and later by Feeney and Nagy (1952) that bacteria assimilated free iron which was in equilibrium with the conalbumin-iron complex and thus the complex would dissociate to form free iron and conalbumin. Feeney and Nagy (1952) reported large differences in the sensitivity of different organisms to conalbumin.

The presence of anti-trypsin in egg white was first noted by Vernon (1904) and later by Balls and Swenson (1934). The trypsin inhibitor of egg white was identified as the ovomucoid fraction by Lineweaver and Murray (1947). Bier et al. (1952, 1953) detected electrophoretically five components in ovomucoid. They separated three of the five components and found that every one of these exerted the same inhibition of trypsin as did the original unfractionated ovomucoid. Anti-tryptic activity is not restricted to liquid egg white since, according to Harte (1945), commercial dried egg albumen contains about as much activity as does fresh egg white.

The pH of a medium is an important factor in the growth of microorganisms. Dernby (1921) studied the effect of the hydrogen ion concentration on the growth of 40 species of bacteria. He found that pathogenic organisms, such as S. typhosa, were able to grow within a rather narrow range of pH values. The limits for growth of S. typhosa were pH 6.2 to pH 7.6; of S. paratyphi, pH 4.5 to pH 7.8; and of S. schottmuelleri, pH 4.5 to pH 8.0. It was shown by Healy and Peter (1925) and more precisely by Sharp and Powell (1931) that a pH 7.5 existed in fresh egg white, whereas after storage this value rose to pH 9.5. Experiments on the effect of pH ranging from pH 5.3 to pH 10.6, on the germicidal action of egg white were performed by Sharp and

Whitaker (1927). The organisms used in their studies included B. subtilis, Ps. fluorescens, Serratia marcescens, Proteus vulgaris, E. coli, B. megatherium, B. cereus, and B. mycoides. Of this list, only B. subtilis and B. mycoides were inhibited by egg white which had a pH 8.5 or lower. Egg white with pH 9.5 or above inhibited the organisms to the greatest extent.

The reduction in hydrogen ion concentration is primarily due to diffusion of carbon dioxide from albumen during storage. In order to maintain the white of the egg at its original hydrogen ion concentration of pH 7.9, Moran and Smith (1936) reported that the storage atmosphere should contain 2 to 3 per cent carbon dioxide at 0° C., 3 to 4.5 per cent carbon dioxide at room temperature and 6 per cent carbon dioxide at 38° C. Moran (1934) demonstrated the efficiency of carbon dioxide in inhibiting the growth of molds during storage of eggs. Later, Moran (1937, 1938) stated that when present in the atmosphere, carbon dioxide in any concentration retarded mold growth on eggs. On the other hand, Kaess (1943) reported that a concentration of 50 per cent carbon dioxide was necessary to prevent the growth of microorganisms on the surface of the shells of eggs, and a concentration as high as 70 per cent carbon dioxide was required to maintain all of the eggs

in an edible condition. The diffusion of carbon dioxide in and out of eggs results in two reactions: the presence of carbon dioxide inhibits microorganisms, while, as the carbon dioxide diffuses out of the egg, the pH rises, and hence bacteria are inhibited due to the resulting alkaline condition of the egg white.

The growth of some bacteria in egg white is inhibited due to the inability of those bacteria to utilize for their nutrition the native proteins that are present. Bainbridge (1911) observed that proteolytic organisms were unable to grow in a pure crystalline egg albumen medium. Rettger et al. (1916) stated that coagulated egg albumen was attacked by organisms possessing strongly proteolytic enzymes, but not by bacteria such as E. coli or Micrococcus pyogenes var. aureus. Haines (1939) made an extensive survey of the literature on the resistance of native proteins to bacterial attack. He stated that native proteins are very resistant towards enzymic breakdown. Haines (1937) observed that little decomposition of native egg albumen occurred when inoculated with a culture of Clostridium histolyticum; however, heat denatured albumen was rapidly attacked. It seemed to be the opinion of Haines that a supply of simple compounds was required by bacterial cells before they could elaborate their enzymes, hence the bacteria

were unable to attack native protein but could attack the denatured protein.

3. Egg breaker and utensils

The individuals involved in the breaking of shell eggs provide a means for producing bacterial contamination of the liquid egg. Pennington (1912) and Zagaevsky and Lutikova (1944a) stated that, when the workers' fingers became wet with egg material, a source of microorganisms was acquired. Then too, human carriers of pathogenic organisms could contaminate the egg with these bacteria during breaking. Juenker (1945) reported the isolation of four Salmonella types from one carrier. Although examination of personnel will reveal any permanent carriers, the detection of temporary carriers may prove difficult.

Pennington (1912) observed that utensils used in the breaking plant were heavily seeded with microorganisms, especially after an undetected contaminated egg had been broken out. Data presented by Zagaevsky and Lutikova (1944a) showed that continuous use of equipment for several hours promoted considerable bacterial contamination of the product. They concluded that the main sources of contamination of the liquid were the implements and vessels used in the breaking operation. They suggested that chlorine

might be used to reduce the amount of bacterial contamination of liquid egg.

It was reported by McFarlane et al. (1945) and Ayres and Slosberg (1949) that pumps, sanitary lines, storage vessels and drying pans contained large numbers of microorganisms and hence albumen was contaminated during the drying process. Proper sanitary precautions were necessary to eliminate these sources of organisms.

4. Removal of glucose

The removal of glucose from egg white prior to drying has been found to be necessary for the production of stable, dried albumen. Some of the procedures which have been used for the removal of glucose from liquid egg white include spontaneous fermentation, fermentation by the addition of specific bacterial or yeast species, and the addition of enzymes.

The spontaneous fermentation of the glucose present in liquid albumen was described by Blomberg (1932), Mulvaney (1941), Stewart and Kline (1941) and Watts and Elliot (1941). In this type of fermentation, the glucose in liquid egg white is fermented at room temperature by naturally occurring microbial contaminants. Stuart and Goresline (1942a) reported that during fermentation, bacteria increased to counts of over a billion per ml. regardless of the number

initially present. For example, egg white that contained 230 organisms per cc. before fermentation was found to possess 3.3×10^8 organisms per cc. at the end of the fermentation period; an initial count of 1.5×10^5 per cc. increased during fermentation to 3.7×10^9 organisms per cc. They isolated bacteria from eight commercial lots of fermenting egg white and found that bacteria of the genera Aerobacter and Escherichia were present in such predominating numbers that other types of organisms were practically excluded. They also isolated Proteus, Serratia and Pseudomonas from the fermenting egg white.

Using a selected strain of A. aerogenes, Stuart and Goresline (1942b) found that the rate of fermentation was directly proportional to the amount of the inoculum. They studied fermentations made with selected strains of Serratia marcescens, Proteus sp., and Ps. aeruginosa. These organisms did not decrease the glucose content of albumen as rapidly as did either A. aerogenes or E. freundii; also, the fermentations were characterized by strong proteolytic action. Bollenback (1949) inoculated egg white with A. aerogenes and incubated this mixture at 40°C. for from 4 to 6 hours. She reported that more than a billion organisms per ml. of egg white were present during the fermentation period.

Kaplan et al. (1950) used resting cells of Streptococcus lactis to ferment egg white. They reported that this type of fermentation was rapid and that it eliminated the possibility of microbiological contamination that was associated with the preparation of mother cultures used in other bacterial fermentations.

In another type of controlled fermentation used for the removal of glucose, yeast is inoculated into the raw liquid albumen. It was reported by Hawthorne and Brooks (1944) that a reduction in the glucose content of egg white from 0.5 to 0.05 per cent and 0.09 per cent respectively, occurred in two trials where they used an inoculum of 1 per cent Saccharomyces apiculatum culture and incubated the material for 3 hours at 37° C. The rate of glucose removal by Saccharomyces cerevisiae was influenced by several factors such as the pH of the liquid egg white, the amount of inocula, the surface/volume ratio, added growth factors, and serial fermentations, according to Ayres and Stewart (1947). These workers reported that glucose could be removed from egg white in 2 hours when an actively growing yeast and added yeast extract was used.

Hawthorne (1950) carried out the yeast fermentation procedure at 10° C. and believed that at this temperature bacterial multiplication would be negligible. After

fermentation, he removed the yeast cells by means of centrifugation, thus eliminating the undesirable yeasty flavor sometimes associated with yeast fermented albumen.

A review of the discovery, purification and action of the enzyme glucose oxidase was presented by Baldwin et al. (1953). Glucose oxidase catalyzes the reaction between glucose and oxygen to form gluconic acid and hydrogen peroxide. Addition of catalase to glucose oxidase by the Vita-Zyme laboratories formed the enzyme complex "Deoxygenase"[1]. The reason for adding catalase was to break down hydrogen peroxide to form water and oxygen. Another term used to designate this enzyme complex is Glucatase[2]. Procedures for the use of the enzyme complex for the removal of glucose from egg albumen have been outlined by Baldwin et al. (1953), Carlin and Ayres (1953), Pfizer (1953), Scott (1953) and Snyder (1953).

According to Baldwin et al. (1953) and Snyder (1953) enzyme treatment is conducted under conditions unfavorable to microbial development, inhibition of microorganisms being due to the presence of hydrogen peroxide.

---

[1]Produced by Takamine Laboratories, Clifton, N. J.

[2]Trademark of Chas. Pfizer and Co., Brooklyn, N. Y.

C. Reduction of the Number of Organisms in Dried Albumen

1. General

Since it is evident that microbiological contamination of albumen can occur either before, during or after processing, means for reducing the load of organisms have been developed. These methods utilize the effects of time, heat and desiccation on bacterial cells, to secure a lower level of contamination. The processor makes use of such operations as pasteurization and drying of the liquid, as well as storage of the dried product to reduce the bacterial population. The consumer can reduce the bacterial population further when the product is heated during cooking or baking.

2. Pasteurization

The pasteurization of liquid egg has been used primarily as an attempt to eliminate pathogenic organisms, as well as to reduce the total load of microorganisms.

It was observed by Wilkin and Winter (1947) that pasteurization killed bacteria more quickly in albumen than in yolk, although the albumen had to be pasteurized at a lower temperature to prevent coagulation. Pasteurization

of egg white at 56° C. for 1.6 minutes killed more than 99 per cent of the total bacterial load and all of the coliform bacteria. Ayres and Slosberg (1949) found that flash pasteurization of liquid albumen at 59.4° C. destroyed any viable Salmonella.

Much attention has been given to the pasteurization of liquid yolk and of liquid whole egg. Van Oijen (1940) suggested heating liquid egg to 65° C. for 20 minutes to destroy organisms of the paratyphoid and enteriditis groups which are commonly found in duck eggs. To rid liquid whole egg of coliforms, Gibbons et al. (1946) found that 30 minutes was the shortest time possible for vat pasteurization at 60° C. They observed a 99 per cent reduction in bacterial count by using flash pasteurization at 57° C. Winter et al. (1946) reported that pasteurization destroyed pathogenic bacteria, especially those belonging to the genus Salmonella. In a study reported by Goresline et al. (1951) it was shown that flash pasteurization of liquid whole egg at temperatures as high as 63° C. was not sufficient to destroy all organisms of the genus Salmonella; however, pasteurization at 60° C. with a holding period of 3 minutes was sufficient to produce a product from which no Salmonella were recovered.

Solowey et al. (1948) tested 91 cultures of Salmonella and observed that in liquid whole egg at 58° C., the time

required to reduce the number of bacteria by one log cycle ranged from 0.48 to 0.91 minutes.

Although primarily intended to lower the moisture content of liquid egg, the preheater has also been found to be effective in eliminating Salmonella from powdered whole egg due to the heat imposed upon the liquid egg. In some instances, however, when liquid egg was passed through a preheater before spray drying, there were significant increases in the bacterial population. Johns (1944) found that, in such cases, material adhered to the walls of the preheater tubes and established a nucleus of infection. Multiplication of organisms also occurred in preheaters at temperatures below 57° C. This work was confirmed by McFarlane et al. (1945) who suggested that consideration should be given to whether the preheater was to be used merely to warm the liquid egg or primarily for pasteurization purposes.

In his work with high quality egg powder, Schneider (1946a) isolated Salmonella from 13 of 797 lots of whole egg spray dried with the aid of a preheater, and from 19 of 104 lots processed without the use of the preheater. Schneider (1946b) reported that during commercial processing only one isolation, a culture of S. pullorum, was made from 45 specimens of preheated liquid egg. More

recently, Schneider (1951) stated that it was only when the liquid egg was preheated to a minimum of 60° C. prior to spray drying that organisms of the genus Salmonella were reduced in numbers to the extent that their isolation from egg powder became a rarity.

Anellis et al. (1954) and Osborne et al. (1954) studied the heat resistance of several species of Salmonella. They found that of the organisms studied, S. pullorum WRRL 3083 was the least heat resistant and S. senftenberg 775W was the most resistant to heat. Ten species of organisms belonging to the genus Salmonella were isolated by Osborne et al. (1954) from dried whole egg which had been pasteurized. They found that these organisms were no more heat resistant than were ordinary strains and suggested that their presence in the powder could have resulted from improper operation of the pasteurizer, heavy original contamination, or recontamination after drying.

3. Dehydration

The review by Rahn (1945) discussed the effect of desiccation on bacterial cells. According to his article, bacteria survived fairly well when dried in culture media such as broth or milk, while bacteria dried in a suspension of water have only a very small percentage of survivors. According to Rahn the severity of the drying process on

bacterial cells is reduced by the presence of proteins which act as protective colloids. He also observed that the effect of desiccation on the death of bacterial cells depends upon the species of bacteria being dried. Stark and Herrington (1931) found that Streptococci sp. tolerated desiccation much better than did E. coli or Lactobacillus acidophilus; yeast and micrococci showed intermediate tolerances.

The temperature at which eggs are dried is an important factor in the determination of the quality of the powder; the lower the drying temperature, the better are the physical qualities, but the greater is the survival of bacteria. It was shown by Gibbons and Fulton (1943) that a decrease of 4° or 15° C. in the temperature of the exhaust air from a spray drier was accompanied by a 3- to 5-fold increase in the number of bacteria in the powder. Bartram (1943) reported reductions of 63.7 per cent and 88.1 per cent in the bacterial count due to the effect of drying whole egg. Using an air outlet temperature of 66° C., Gibbons and Moore (1944b) observed a reduction in total viable count of 81.5 per cent and a reduction in number of S. bareilly of 99.99 per cent. At an outlet air temperature of 60° to 66° C. the total viable count was reduced 64.6 per cent while the count of S. manhattan was reduced by 99.99 per cent. McFarlane et al. (1945) found that an average of 35 per cent of the bacterial cells present in liquid whole

egg were killed during the drying process. Using an experimental spray drier with the average inlet air temperature of 116° C. and the average outlet air temperature of 65° C., Brown and Gibbons (1950) reported the following per cent reductions in the count of organisms: total, 97.17; coliforms, 99.93; E. coli, 100.0; S. bareilly, 99.98; and Streptococcus faecalis, 59.09. Hirschmann and Lightbody (1947) found that 99.9 per cent of the cells of Ps. fluorescens inoculated into liquid whole egg did not survive lyophilization of the egg.

A review of the literature revealed no reports concerning the reduction in numbers of organisms due to pan drying of liquid albumen.

4. Storage

a. Air. According to Rahn (1945) dry bacterial cells display no life functions, their enzymes are not active, and even endogenous catabolism ceases. Rahn stated that death of dried cells is due to oxidation rather than heat since dry proteins do not coagulate and dry enzymes retain their activity. Bacteria in the dried state die more rapidly as the temperature is elevated due to an increase in the rate of oxidation.

Orr and Moore (1953) inoculated S. gallinarum on cloth and then stored this cloth in the dark at room temperature.

The organism remained viable for as long as 228 days. In addition, S. gallinarum remained viable for 88 days in water at room temperature.

Commercially dried whole egg powder was stored at 1° C. for 18 months by Stiles and Bates (1912). One-third to one-half of the number of microorganisms originally present in this egg powder survived the storage period. Goresline et al. (1943) reported that, at temperatures below 7° C., there was little or no change in the viable count during the storage of dried whole egg. When egg powder was stored at temperatures above 7° C., the per cent of survivors was inversely related to length of storage. It was found by Johns (1944) that at 4° C. the bacterial counts showed no appreciable change; at 21° C. a moderate decline was noted in 2 days; and at 30° to 32° C. a sharp reduction was noted after 1 day of storage. A decrease of 56 per cent in the total counts was observed by McFarlane et al. (1945) when dried whole egg was stored for 6 days at 16° C. Hartsell (1944) reported that Escherichia, Aerobacter, Proteus, and Pseudomonas did not survive in spray dried whole egg powder for long storage periods.

Dried whole egg powder was stored at 10° and 21° C. by Brown and Gibbons (1950). They reported that at 21° C., the coliforms and Salmonella died rapidly and could not be

detected after 2 weeks of storage. However, the number of enterococci had decreased only slightly by 12 weeks of storage. At 10° C., the counts of Salmonella and coliforms dropped to less than 1 per gram after 4 and 6 weeks of storage, respectively. The enterococci were still present in almost undiminished numbers after storage for 18 weeks. Hirschmann and Lightbody (1947) observed that when whole egg powder inoculated with Ps. fluorescens was stored at 35° C. for 2 weeks, 99 per cent of the inoculated cells failed to grow.

According to Wilson (1948) Salmonella remained viable for several months in dried whole egg powder stored at 27° C. On the other hand, the results of Ayres and Slosberg (1949) showed that dried albumen could be stored at elevated temperatures for a period sufficient to destroy Salmonella without seriously affecting the functional properties of the powder. Storage at 49°, 54°, or 57° C. for 20, 8 or 4 days, respectively, freed the products of these organisms.

b. Carbon dioxide. Work regarding the use of carbon dioxide to retard the growth of molds in shell eggs during storage was mentioned previously.

Several workers have investigated the use of carbon dioxide in the atmosphere of storage of foods. Thistle et al. (1944) found that solubility was retained better

when dried whole egg powder was stored in carbon dioxide (97 to 98 per cent) than when it was stored in air. With shell eggs, Sharp and Stewart (1931) reported that deterioration was delayed if a little carbon dioxide was added to the storage atmosphere.

Brooks et al. (1936), Allen and Smock (1937) and Gerhardt (1939) observed that fruits and vegetables stored in an atmosphere containing carbon dioxide showed less fungal growth and decay than fruits stored without added carbon dioxide. The work of Ogilvy and Ayres (1951a, 1951b, 1953) showed that multiplication of bacterial cells was inhibited when meats were stored in carbon dioxide.

The rate of death of S. typhosa was increased three-fold when 3 volumes of carbon dioxide were added to water (Shillinglaw, 1941). Thus, carbon dioxide was an effective inhibitor of bacterial growth.

c. Ethylene oxide. The gaseous compound, ethylene oxide, is primarily used as a chemical intermediate according to McClellan (1950). The use of this compound in the food industry for controlling microorganisms was reported by Kirby et al. (1936). Yesair and Cameron (1938) and Hall (1938, 1951) discussed the effectiveness of ethylene oxide for the sterilization of spices and Lorenz et al. (1950) used the gaseous substance in the fumigation of shell eggs. Thermophilic organisms could, in most cases, be eliminated

from flour by using ethylene oxide according to Pappas and Hall (1952). Whelton et al. (1946) summarized the use of ethylene oxide as a preservative for foods. They mentioned that since ethylene oxide is explosive at concentrations of from 3 to 80 per cent in air, in order to reduce the explosive potential, mixtures of 7 to 9 parts of carbon dioxide to 1 part of ethylene oxide should be used.

5. Cooking or baking

Rettger et al. (1916) observed that frying on one side only, coddling, or soft-cooking eggs did not necessarily result in the yolks becoming free of viable bacteria. In further work, Rettger et al. (1926) found that S. pullorum was not killed in the yolk of eggs soft-cooked for 3 minutes; a cooking time of at least 4 minutes was required to destroy this organism.

Cathcart et al. (1942) inoculated both stirred custards and baked custard pies with S. enteriditis. After inoculation, custards were cooked for 2 to 5 minutes; the pies were baked for 25 to 35 minutes; at 246° C. S. enteriditis did not survive in either of the prepared products.

Dried whole egg contaminated with S. oranienburg, S. bareilly, or S. typhimurium was used by Gibbons and Moore (1944b) to prepare scrambles, omelets, sponge cakes,

custards and muffins. Their results indicated that as many as 12 to 13 million Salmonella per gram of powder were destroyed by ordinary, adequate cooking practices. On the other hand, Solowey and Calesnick (1948) recovered Salmonella from test scrambles prepared both from artificially contaminated and from naturally contaminated reconstituted egg powders. (They recovered Salmonella from a scramble cooked 17 minutes, 11 seconds at 56° C.) They found that Salmonella were recovered more frequently from scrambles prepared from artificially contaminated powder than from products prepared from naturally contaminated powder, probably because of the higher initial contamination of the artificially infected powder.

Kintner and Mangel (1953b, 1953c) found that Salmonella did not remain viable in either stirred or baked custards heated to 91° to 93° C. They further stated that the danger of food poisoning from the use of contaminated eggs in products such as puddings and custards appeared to be remote if these products were adequately heated and then refrigerated until they were used.

In their work using dried whole eggs contaminated with Salmonella in cooked salad dressing, Kintner and Mangel (1953a, 1953c) observed that cooking the salad dressing to 84° to 86° C. was sufficient to destroy the food poisoning bacteria.

No reports were observed concerning the destruction of Salmonella during the baking of products containing dried albumen.

6. Antibiotics

Antibiotics have been used primarily as remedial agents for infectious diseases of humans; however, these antibacterial substances have been found to be useful in many other ways, including the control of animal diseases, growth stimulation of animals, control of plant diseases (blight, crown gall, scab), treatment of seeds prior to planting, growth of plants, isolation of bacterial mutants and preservation of foods.

Perhaps the earliest work in which the preservative action of antibiotics was tested was that reported by Curran and Evans (1946). These workers evaluated penicillin as a preservative in milk; however, the results did not indicate that much benefit was derived from the use of this antibacterial substance. Burroughs and Wheaton (1951) reported that in 1946 they attempted to use penicillin to aid in the preservation of canned corn. All of the resultant cans swelled and this work was therefore discontinued.

Andersen and Michener (1950) reported that subtilin, in combination with mild heat treatment, exerted a preservative effect in canned vegetables. Since this work was reported,

many publications have appeared in the literature concerning the use of this antibiotic. Adams et al. (1951, 1952) reported that a high level of subtilin and a relatively drastic thermal process were required to inhibit spores in beef.

Burroughs and Wheaton (1951) reported the results of tests of using antibiotics in preserving canned food. The antibiotics used included subtilin, germicidin, bacitracin, and streptomycin. None of these antibiotics, except subtilin, controlled the natural bacterial flora of vegetables. Subtilin, in a concentration of as high as 80 ppm., permitted 100 per cent spoilage of inoculated packs. Subtilin, when used in tomato juice, appeared to inhibit flat-sour spoilage. Bohrer (1951) and Olcott (1951) reviewed the work concerning the use of subtilin in canned foods and came to the conclusion that this antibiotic did not prevent the growth of the usual spoilage types of organisms found in low-acid canned foods.

Godkin and Cathcart (1952) reported that aureomycin (0.6 to 1 ppm.) or terramycin (0.6 to 1 ppm.) effectively retarded the growth of M. pyogenes var. aureus in custard. On the other hand, bacitracin (40 to 60 ppm.), chloromycetin (4 to 5 ppm.) or streptomycin (4 to 5 ppm.) did not inhibit this pathogenic organism in custard.

Work by Cahill et al. (1952), Goldberg et al. (1953) and Weiser et al. (1953) using antibiotics to preserve fresh meat, Kohler et al. (1955), testing the preservative action of these substances for poultry and Tarr and Deas (1948) and Tarr et al. (1952), attempting to preserve fresh fish, has indicated that antibiotics, especially aureomycin were effective in inhibiting growth of spoilage organisms. Some beneficial aspects were derived from terramycin and chloromycetin (Goldberg et al., 1953).

In general it appears that antibiotics such as chlortetracycline, oxytetracycline, streptomycin and chloramphenicol show promise of being able to inhibit the reproduction of microorganisms in foods. The ultimate use of these substances as food preservatives will depend upon future decisions of the Food and Drug Administration. The present opinion of this federal agency was stated by Hobby (1953, p. 1077), and is as follows:

> Direct or indirect addition of antibiotic drugs to foods for human consumption. (a) The Food and Drug Administration has received inquiries concerning the use of antibiotic drugs as food preservatives. Careful consideration has been given to this question and the conclusion has been reached that such use constitutes a public-health hazard. Consumption of food so treated may cause sensitization of the consumer to such antibiotics and may result in the emergence of strains of pathogenic microorganisms resistant to these drugs.

(b) The presence of antibiotic drugs in foods intended for human consumption, or the direct or indirect addition of such drugs to such foods, may be deemed an adulteration within the meaning of section 402 of the Federal Food, Drug, and Cosmetic Act (Sec. 402, 52 Stat. 1046; 21 U.S.C. 342).

D. Processes Affecting Functional and Physical Properties of Dried Albumen

1. General

Dried albumen is used in products such as angel cakes, nougat creams, icings, meringues, and marshmallow whips, because of its ability to form a stable foam. Some of the measurements that have been used experimentally to indicate the ability of albumen to be whipped include the beating rate, the specific gravity of the meringue, the stability of the foam and the volume of angel cakes. Owing to the use of dried albumen, its solubility is an important property to be considered. Other measurements that are used to determine changes include pH, fluorescence and moisture content of the dried albumen.

As mentioned previously, processes such as fermentation, pasteurization, drying and storage alter the microbiological population of albumen. The effects of

these processes upon the functional and physical properties of dried albumen are discussed in the following section.

2. Removal of glucose

It was believed by Balls and Swenson (1936) that the chief function of fermentation of egg white was to thin the thick albumen and subsequently to increase its whipping property. Therefore, they used trypsin to reduce the viscosity of natural albumen to that of fermented albumen. However, they found that the trypsin-treated albumen darkened and became insoluble after storage.

The role of glucose in the deterioration of dried albumen was demonstrated by Stewart and Kline (1941). These workers compared the properties of dried albumen prepared from a fermented liquid egg white with those prepared from unfermented liquid white. When stored at 40° C., the latter showed a progressive change in color from pale yellow to dark brown and became relatively insoluble within 2 weeks. The fermented sample, however, displayed no change in color or in solubility during 12 weeks of storage at 40° C. The effect of glucose was demonstrated again by adding glucose to the fermented product. As with albumen not subjected to fermentation, this product turned dark and became insoluble after storage.

It was shown by Stewart *et al.* (1943) that dried albumen stored at 50° C. and containing 0.4 per cent glucose decreased in solubility during a 2 week period, while a fermented sample held under the same conditions retained its solubility. Stuart and Goresline (1942b) stored glucose-free albumen for 4 months at room temperature without causing a change in its original color; however, an unfermented sample darkened and became insoluble.

The effect of glucose on the color and solubility of dried albumen was also demonstrated by Hawthorne and Brooks (1944). They fermented a sample of albumen with yeast to a glucose content of 0.05 per cent, and, after drying, stored it at 46° C. This sample retained its color and solubility for 2 weeks, while a dried sample of untreated albumen containing 0.55 per cent of glucose lost 62 per cent of its solubility during this period.

Carlin and Ayres (1951) compared the color stability of dried albumens and observed that, after 2 weeks of storage at 40° C., a definite orange color was evident in an unfermented sample, whereas the color of yeast-fermented samples had not changed. The color of the unfermented albumen was a deep brown after 6 weeks of storage, while the fermented product showed no visible color change even after 5 months of storage at 40° C. They observed also that the beating rate was maintained much better in the

fermented sample than in the unfermented product. Angel cakes made from unfermented albumen stored at 40° C. for 2 weeks showed a 25 per cent decrease in volume but angel cakes made with fermented albumen showed only a 5 per cent decrease in volume.

3. Pasteurization

Gibbons et al. (1946) reported that, except for a tendency to pack and to become slightly less soluble, there was little or no difference between the original and the keeping qualities of whole egg powders prepared from unpasteurized and from vat pasteurized liquid egg.

According to Hanson et al. (1947), pasteurization exerted no harmful effects on the custard-making properties of whole eggs. However, they reported that sponge cakes made from whole egg samples pasteurized in the range of 60° to 68° C. had volumes approximately 4 per cent lower than those of sponge cakes made from control samples. Also, cakes made from eggs pasteurized at 71° C. had volumes approximately 8 per cent lower than those of sponge cakes produced from control samples. Cakes in which pasteurized egg was incorporated were somewhat less desirable in texture and shape than were cakes made from control samples of egg.

Using consistency, stability, and palatability of the stored mayonnaise as criteria of quality, Miller and

Winter (1951) observed that pasteurization of whole egg and egg yolk for 4 minutes at temperatures ranging from 60° to 63° C. did not impair their value for making mayonnaise.

Wilkin and Winter (1947) reported that pasteurization of albumen at 57° C. adversely affected the beating time and decreased the stability of the resulting foam. However, no data were presented to support this statement.

Changes occurred in the appearance and viscosity of egg white when the material was heated above 58° C., according to Payawal et al. (1946). It was shown by Barmore (1936) that heating egg white to 60° C. decreased the stability of foams produced from this product.

Slosberg et al. (1948) reported that there was a gradual decrease in the beating rate of egg white while it was being heated to 59° C. and heating above this temperature caused a rapid decrease in beating rate. They found that heating of the egg white had a greater effect upon the beating rate than on angel cake volume.

Bernard et al. (1948) showed that at temperatures above 59° C. egg white lost its beating properties and cake-making properties very rapidly. They reported that lowering the pH increased the stability of albumen toward heat. Bernard et al. (1948) and Slosberg et al. (1948) suggested that protein denaturation was responsible for the loss in functional properties of the heated egg white.

4. Dehydration

Some of the properties of egg albumen are, by necessity, changed by the removal of water; one of the most important of these properties is the ability of albumen to be whipped into a stable foam. It was believed by Parsons and Mink (1945) that egg white could be concentrated to 60 per cent solids, but not to dryness, and still retain its angel cake-making properties. However, Hanson (1945) was able to concentrate egg white to 92 per cent solids by lyophilization without causing noticeable changes in the angel cake-making properties of the reconstituted product. Also, Slosberg (1946) lyophilized egg white to 95 per cent solids and observed little change in its beating power, although when liquid albumen was pan-dried at 40.5° to 46° C., an inferior product was obtained. Bollenback (1949) pan-dried albumen at temperatures below 40° C. She found that drying of liquid egg albumen at these temperatures caused no significant effect on the volume of angel cakes made from the reconstituted material.

With egg white dried over phosphorus pentoxide, Smith (1935) found that the reconstituted product was similar in appearance to fresh white, since the thick and thin portions reappeared in almost their initial proportions.

It was concluded by Bergquist (1951) that spray drying of egg white resulted in a definite loss of whipping ability. He believed that this loss was due to atomization of the egg white, and not to the drying of the droplets. He found that the loss in beating power due to spray drying was partially reversible by storage of the dried product.

5. Storage

Some of the reasons that egg white is dried include: the suitability of the dried product for prepared mixes, the resistance of the dried product to the reproduction of organisms, and the ease of handling and shipping the dried product. Dried albumen can be stored more conveniently than can either liquid or frozen albumen. Some of the factors to consider when dried egg is to be stored are: the moisture content of the powder, the temperature and duration of storage and the atmosphere in which the product is held.

a. Moisture content. It was reported by Barker (1933) that the denaturation rate of partially dried egg white was greatly reduced by decreasing the water contant. Bumzahov (1944) showed that denaturation was less likely to occur when the moisture content of egg white was less than 30 per cent. When dried

albumen, containing "very little moisture", was heated to 80° C. for 4 hours, the solubility was lowered by only 8 per cent.

Samples of flake albumen were powdered and adjusted to levels of from 8.4 per cent to 1.5 per cent moisture by Stewart and Kline (1941). After 23 weeks of storage at 40° C. the difference in solubility between the samples was negligible, but after 61 weeks, the high-moisture sample was only two-thirds as soluble as was the low-moisture sample. Best (1944) stored albumen samples containing as much as 10.6 per cent or as little as 2 per cent moisture at 60° C. The solubility of albumen with 10.6 per cent moisture decreased rapidly reaching a minimum after 65 hours. The 2 per cent moisture sample, however, retained 90 per cent of its original solubility at this time. Kline (1945) found that the effect of the moisture content of dried albumen on the solubility during storage was quite evident, especially in the range from 10 per cent down to 2 per cent moisture. It was reported by Ayres and Slosberg (1949) that the amount of moisture in albumen samples affected the solubility when the albumen was subjected to heat.

b. Temperature. Stewart and Kline (1941) stored dried albumen produced from unfermented liquid egg white, and reported that, as the temperature of storage increased

from room temperature to 40° C. the time required to produce adverse effects upon the solubility of the dried product decreased from 10 weeks to 2 weeks. Yeast-fermented dried albumen was stored at room temperature and at 40° C. by Carlin and Ayres (1951). After 16 weeks of storage at room temperature, there was no detectable change in angel cake-making properties, while storage at 40° C. for only 2 weeks caused a 5 per cent decrease in the volume of angel cakes made from this product. After this initial loss, however, no further change was noted at 3, 4, 5, 6 or 8 weeks of storage. However, angel cakes made from the dried albumen after 12 weeks of storage at 40° C. showed an additional loss of 5 per cent in volume.

Ayres and Slosberg (1949) reported that the functional properties of dried albumen deteriorated slowly at room temperature. Changes in solubility and beating rate increased directly with increased storage temperature; beating rate was retained for 3 weeks at 49° C., for 8 days at 54° C., and began to decrease after 4 days at 57° C. Ayres and Slosberg obtained similar results using solubility as the criterion for measuring the changes in stored dried egg white.

c. Length of storage period. The length of time that dried albumen can be stored with no adverse effect is dependent upon such factors as the glucose content, the

moisture content, and the temperature of storage. Any one of these factors can affect the shelf-life of the product. It has been suggested by Kline (1945) that albumen could be stored for as long as 2 years with no loss of functional properties if the moisture content were low (1.3 per cent) and the temperature of storage were 30° C. or lower.

III. METHODS AND MATERIALS

A. Preparation of Dried Albumens

1. Pan-dried albumen

a. Source of liquid egg white. Fresh eggs were obtained from the Iowa State College Poultry Farm and broken and separated by the use of a commercial breaking tray and separator. After separation, the whites were blended in a Waring Blendor at 50 volts for 60 seconds as described by Carlin and Ayres (1951).

b. Adjustment of pH. In order to lower the pH of the liquid albumen, 5 per cent hydrochloric acid was carefully added; to raise the pH, ammonium hydroxide was used. pH was measured by means of a Beckman pH meter (model H).

c. Removal of glucose. The blended whites were placed in a beaker, adjusted to pH 7.3 and then treated with an enzyme complex, "Deoxygenase",[1] to remove the glucose. The method used was that described by Carlin and Ayres (1953) except that in some trials 0.12 gram of dried enzyme complex was substituted for 1 cc. of the liquid form. Treatment was

---

[1]Produced by Takamine Laboratories, Clifton, N.J.

continued until the test for residual glucose by the rapid plate method (Bollenback, 1949) showed that insufficient glucose was present to give the browning reaction.

d. Pan drying. The methods and materials used for pan drying were similar to those described by Carlin and Ayres (1951).

e. Milling and screening. The dried albumen was removed from the trays and milled in a ball mill until particles were small enough to sift through a 100-mesh screen. The dried product was stored in a refrigerator at 4° C. until used.

2. Spray dried albumen

a. Source of liquid egg white. Spray dried egg white was acquired from two commercial installations. In Plant A the source of the liquid albumen was not known, while in Plant B frozen albumen was used.

b. Removal of glucose. In Plant A, the egg white was adjusted to pH 6.2 with lactic acid and fermented with bakers' yeast for 16 hours. The method used to check residual glucose was that described by Somogyi (1945).

In Plant B, the liquid egg white was adjusted to pH 7.0 with lactic acid and the glucose was removed by treatment with the enzyme complex, "Deoxygenase", until a negative Somogyi test was obtained.

c. Spray drying. In both commercial plants the liquid white was dried in a pilot plant spray drier. In Plant A, the inlet air temperature was about 135° C. and the outlet air temperature was approximately 68° C. In Plant B, the inlet temperature was 77° to 92° C. and the outlet temperature was 51° to 60° C.

In Plant A, the dried powder was caught in a cloth screen and hence subjected to the heat from the warm air during the entire drying run. The spray drier in Plant B was equipped with a so-called "cyclone collector", so that the powder was not subjected to prolonged heating.

B. Microbiological

1. Salmonella

a. Organisms used. The following organisms were employed in the experiments reported herein: S. paratyphi (ISC 8A24; group A), S. bredeney (WRRL[1] 4393; group B), S. typhimurium (WRRL 5982; group B), S. oranienburg (ISC 8A9 and WRRL 6266; group C), S. pullorum (ISC 8A26 and WRRL 3083; group D), S. anatis (WRRL 5343; group E), S. senftenberg (WRRL 775W; group E), S. give (WRRL 5392; group E) and S. worthington (WRRL 4661; group F).

[1]Western Regional Research Laboratory, Albany, California.

b. Enrichment broths. Tetrathionate (Difco), Selenite-F (BBL) and Ruys' medium[1] were the enrichment broths used in the experiments wherein the broths were compared.

The following procedure was used to determine the ability of enrichment fluids to support the growth of Salmonella. A 24-hour broth culture of a single species of Salmonella was diluted 1:10,000 in saline. Two ml. of this dilution were inoculated into tubes containing 18 ml. of test broth so that there were approximately 10,000 organisms per ml. The inoculated tubes were incubated in a water bath at 37° C. and sampled at 0, 1, 2, 4, 8, 14, 24 and 36 hours. These samplings were diluted in saline and three subsamples were plated using nutrient agar as the substrate. The inoculated plates were incubated for 24 hours at 37° C. and colonies counted with the aid of a Quebec, Spencer Darkfield Model colony counter.

For those studies wherein egg was incorporated into the enrichment broths, 1 ml. of a 1:100 dilution of the organism in saline was incorporated in 99 ml. of homogeneous fresh whole egg suspension and mixed in a Waring micro-blendor jar. A 2 ml. aliquot of this material was introduced into the tubes of test broth and incubation, sampling and enumeration were made as previously described.

---

[1]The dehydrated medium was supplied by Difco and listed as Experimental Control Broth No. 410840.

c. Selective agars. The agars employed in these studies included Salmonella-Shigella (SS; Difco), bismuth sulfite (WB; Difco), brilliant green (BG; Difco), desoxycholate citrate lactose sucrose (DCLS; BBL) and nutrient (NA; Difco).

The method for testing agars consisted of the following steps: (1) A 24-hour broth culture of a single species of Salmonella was diluted in 1:1,000,000 in saline. (2) Three subsamples of this dilution were plated with each test agar. (3) The inoculated plates were incubated and counted as previously described.

The procedures were repeated for each of the organisms included in this study.

d. Growth of Salmonella in liquid egg white. A 24-hour broth culture of S. senftenberg, S. oranienburg or S. pullorum was inoculated onto a nutrient agar surface and incubated at 37° C. for 24 hours. Growth was washed free from the agar, diluted 1:10,000 and inoculated into liquid egg white adjusted to pH 6.0, pH 7.0, pH 8.0, pH 9.0 or pH 10.0, as well as into nutrient broth. The tubes were incubated at 30° C. (the temperature suggested for removal of glucose) and sampled at 0, 2, 4, 8 and 24 hours. The samples were diluted in sterile distilled water and the number of Salmonella were estimated by spreading 0.5 ml.

of the appropriate dilution onto a brilliant green agar surface in a Petri dish. After incubation for 24 hours at 37° C., the colonies of Salmonella were counted with the aid of a Spencer colony counter.

e. Antibiotic studies. The antibiotic substances, chlortetracycline (aureomycin), oxytetracycline (terramycin) and streptomycin were utilized in these studies to ascertain if these agents would retard the growth of Salmonella.

The antibiotics were dissolved in distilled water and sterilized by means of a Seitz filter. After sterilization, appropriate dilutions were made in sterile distilled water.

S. senftenberg, S. oranienburg or S. pullorum were grown on agar slants, the cells harvested and dilutions of 10^0, 10^2, 10^4 or 10^6 were inoculated into test tubes containing 8 mls. of sterile nutrient broth and 1 ml. of antibiotic diluted so that the tubes contained the following amounts of antibiotic: 50, 25, 10, 5, 2.5, 1.0, 0.5, 0.25 or 0.1 ppm. Each of the three antibiotics were utilized in this manner. Tests were run in triplicate.

After inoculation the tubes were incubated at 37° C. and observed for turbidity at 8, 16, 24, 36, 60, 84, 168, 336 and 504 hours. A turbid tube was considered positive for growth of Salmonella. Control tubes, without antibiotic, were utilized to determine the numbers of Salmonella

inoculated and tubes without Salmonella were prepared to determine the sterility of the media and antibiotic solutions.

For those studies wherein albumen was utilized, the three species of Salmonella were grown and harvested as described previously and 1 ml. of the appropriate dilution of cells and 1 ml. of prepared antibiotic solution were inoculated into tubes containing 8 mls. of liquid egg white adjusted to pH 7.0. These prepared tubes were incubated at 37° C. and the numbers of Salmonella were determined at 0, 3, 6, 12 and 24 hours by spreading 0.5 ml. of the appropriate dilution of the sample onto a brilliant green agar surface. After incubation of these plates for 24 hours at 37° C., the colonies of Salmonella were counted.

f. Enumeration in dried albumen. Enumeration of Salmonella in dried albumen was accomplished by the method described by Ayres (1949), except that, in using the most probable numbers (MPN) technique, 5 tubes of Selenite-F were employed for each series instead of the 3 tubes suggested. By means of this procedure it was possible to detect as few as 0.18 Salmonella per gram.

2. Coliforms

In order to determine the number of coliforms in dried albumen, the MPN technique was employed. Lactose broth

tubes (five per dilution) were inoculated with appropriate dilutions of egg white and then incubated at 37° C. for 24 hours. Growth from those tubes showing gas formation was streaked on plates containing Levine's eosin methylene blue agar (EMB) and these plates were incubated for 24 hours at 37° C. Typical colonies were considered positive and Gram stains were made at intervals to check any colonies considered questionable. An estimate of the number of coliforms was determined by comparing the number of positive tubes with an MPN table (Hoskins, 1934).

3. Total count

The total count of organisms in the powder was obtained by the MPN technique. Serial dilutions of egg white were inoculated into tubes of lactose broth, using five tubes per dilution and the tubes were incubated at 37° C. for 24 hours. Turbid tubes were considered positive. Those tubes showing no turbidity were incubated an additional 24 hours. After a total of 48 hours incubation, a loop of material from each of the remaining tubes was streaked on nutrient agar to determine the presence of organisms. The number of positive tubes was compared with an MPN table to estimate the number of organisms present in the albumen.

C. Storage of Dried Albumen

1. Moisture content

a. Determination. The moisture content of dried albumen was determined by the vacuum oven method (A.O.A.C., 1950).

b. Changing the moisture content. The dried albumen obtained from the drier contained from 6 to 8 per cent moisture. In order to reduce the moisture content of the albumen below this level the material was dried in a vacuum oven at 30° to 35° C. To increase the moisture content, the dried albumen was placed in a sealed box in which a fan circulated air over a pan of water until the desired moisture level was obtained.

c. Levels of moisture. In preliminary experiments moisture levels of albumen used were 4, 8 or 12 per cent. However, storage studies of albumens containing from 1.8 to 18 per cent moisture indicated that 3 per cent and 6 per cent were critical moisture levels. In order to complete the geometric series and remain within practical limits, levels of 1.5, 3, 6 or 12 per cent were used in subsequent experiments.

2. Containers

Albumen of the desired moisture concentration was transferred into sterile, 2-ounce, screw-capped jars which were then sealed with tackiwax. These jars of albumen were stored in incubators at the selected temperatures.

3. Temperature of storage

Preliminary experiments indicated that storage of albumen at 50° C. required extended time periods to eliminate Salmonella from the product. Since shorter time periods were desirable, higher temperatures of storage were necessary. However, Maurer (1911) observed that when dried egg was heated to 75° C. the solubility was reduced very rapidly; he reported that heating to 70° C. did not result in drastic changes. Therefore the temperatures of storage selected for these experiments were 50°, 60° and 70° C.

4. Times for sampling

The storage times used in this study were selected on the basis of preliminary experiments using pan dried egg white. The observed reduction in numbers of S. oranienburg during storage of dried albumen at the selected temperatures and moisture levels, was plotted against time of storage. The lines obtained were extrapolated until,

theoretically, no S. oranienburg would exist in the product. In cases of long storage times, these intervals were subdivided to obtain a better estimate of the reduction of bacteria during storage. All samples were analyzed after storage for 0, 1 and 3 days so that comparisons could be made between treatments.

During the experiments using spray dried egg white it became evident that the number of Salmonella were reduced at a faster rate than they were in the case of pan dried albumen. Therefore, samples of spray dried albumen were analyzed for the number of viable Salmonella after 0, 1, 2, 3, 4, 5, 6, 8 and 9 days of storage.

5. Storage in ethylene oxide and/or carbon dioxide

Spray dried albumens inoculated with known numbers of S. senftenberg, S. oranienburg or S. pullorum and containing 6 per cent moisture were placed in 6-inch vacuum desiccators. By means of an Orsat-type gas analyzer the air in these desiccators was replaced with ethylene oxide and/or carbon dioxide. Levels of 0, 25, 50, 75 and 100 per cent carbon dioxide were used. To avoid flammability of ethylene oxide, carbon dioxide was combined with this compound so that the atmosphere in the desiccators was 90 per cent carbon dioxide and 10 per cent ethylene oxide. By means of a reaction unit, described by Branham and

Shepherd (1939), that was attached to the Orsat gas analyzer, the per cent ethylene oxide was determined. The prepared desiccators were stored in an incubator at 50° C. for 3 days, at which time the enclosed gas was sampled and analyzed for carbon dioxide and ethylene oxide and the albumen was sampled and analyzed for the numbers of viable Salmonella.

D. Functional Tests

1. pH

Albumen in the proportion of 27 grams to 73 ml. of distilled water was reconstituted for 30 minutes. The pH of this liquid was measured by means of a Beckman pH meter (model H).

2. Solubility

One and one-half grams of albumen were reconstituted in 50 mls. of distilled water for 30 minutes in an Erlenmeyer flask. The flask and its contents were shaken 50 times and the resulting suspension was filtered by means of Whatman number 12 filter paper as outlined by Stuart et al. (1942). Approximately 5 ml. of this filtrate were accurately weighed into a tared 50 ml. beaker and most of the liquid was evaporated in a 70° C. incubator.

The per cent solids was then determined by using the vacuum oven method (A.O.A.C., 1950, p. 342).

3. Meringue test

The two meringue tests used were those described by Slosberg et al. (1948) and Carlin and Ayres (1951).

4. Angel cakes

The procedure used to prepare angel cakes and measure their volume was that described by Carlin and Ayres (1951), except that the amount of cream of tartar in the recipe was reduced from 0.90 to 0.23 grams.

E. Calculations

1. Generation times

Using the formula $g = \frac{t_2 - t_1}{3.22 (\log f - \log i)}$ (g = generation time, f = final number of organisms at time t_2, and i = initial number of organisms at time t_1), the generation times were calculated.

2. Death rate (velocity) constants

The velocity equation $K = \frac{1}{t} \log \left(\frac{i}{f} \right)$, (where K = death rate, t = time in days, i = initial number of cells, and f = final number of cells) was used to calculate the rate of death of microorganisms in dried albumen during storage.

3. Q_{10} values

The Q_{10} values (temperature coefficients) were determined by using the formula $Q_{10} = \frac{K_2}{K_1}$ where K_2 = death rate at temperature T_2, K_1 = death rate at temperature T_1.

4. Statistical analysis

Use was made of analysis of variance and correlation coefficient determinations, as outlined in Snedecor (1946).

IV. RESULTS AND DISCUSSION

A. Proliferation of *Salmonella*

1. Enrichment broths

Although some information is available in the literature comparing the ability of enrichment broths to support the growth of *Salmonella*, generally the comparisons are based on qualitative results rather than on quantitative estimations. In Figure 1, the curves represent the growth of eight species of *Salmonella* in each of the broths tested. Using nutrient broth as the control medium, these curves show that no single enrichment broth supported the growth of all organisms as well as did nutrient broth during the first 8 hours of incubation. It can be seen that at 37° C. the lag phase persisted for from 1 to 2 hours, and that the logarithmic phase terminated at about 14 hours.

The calculated generation times are listed in Table 3. In nutrient broth the range of generation times was 29 minutes for *S. worthington* to 48 minutes for *S. pullorum*, with the average for the eight organisms being 34 minutes.

Knox (1945) indicated that growth of *Bacterium paratyphosum B* (*S. schottmuelleri*) in broth was increased

Figure 1. Growth curves of 8 species of *Salmonella* in various broths

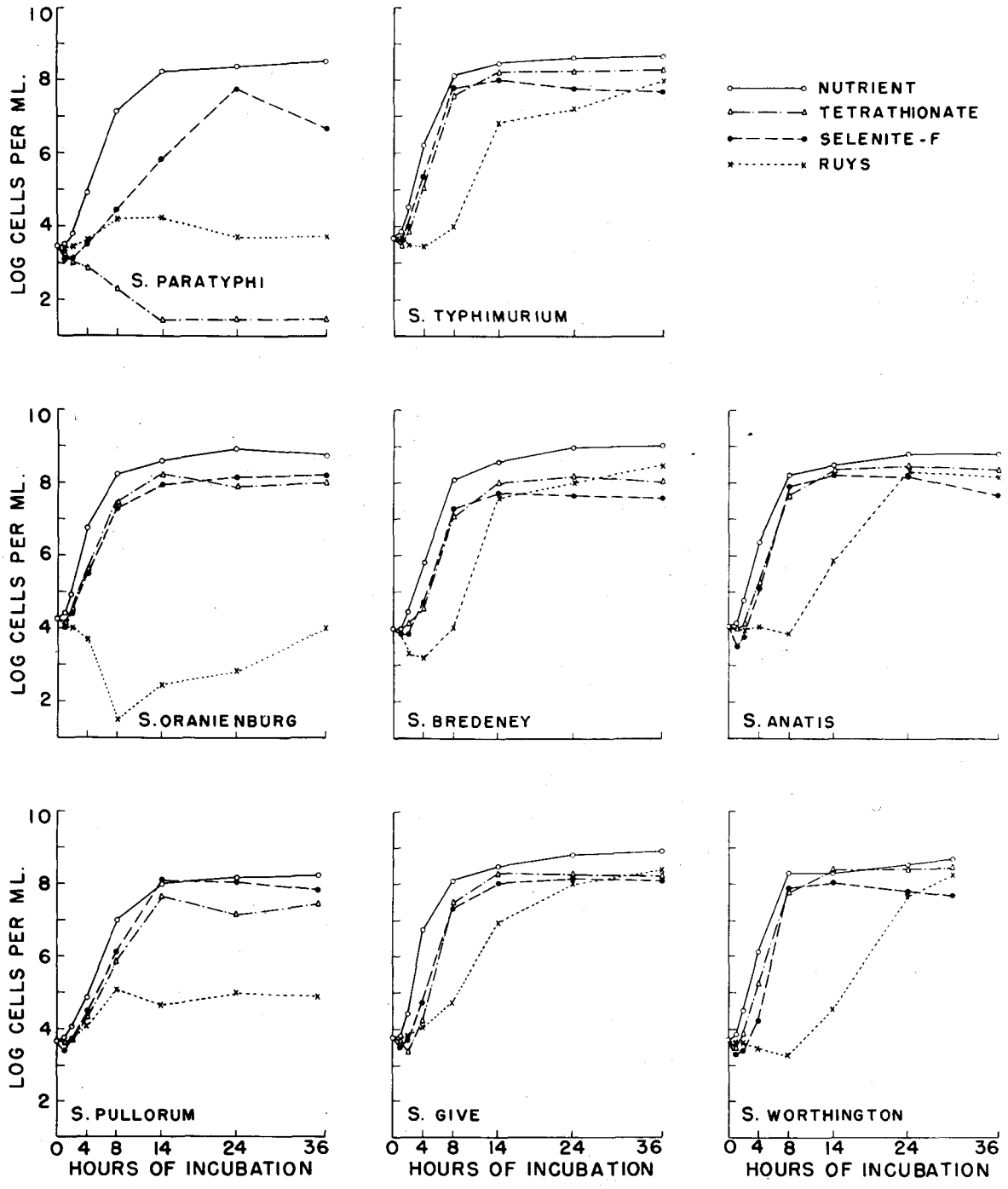

LOG CELLS PER ML.
10
8
6
4
2
S. PARATYPHI
S. TYPHIMURIUM
NUTRIENT
TETRATHIONATE
SELENITE-F
RUYS
S. ORANIENBURG
S. BREDENEY
S. ANATIS
S. PULLORUM
S. GIVE
S. WORTHINGTON
0 4 8 14 24 36
HOURS OF INCUBATION

Table 3. Generation times of eight species of *Salmonella* in various broths

Organism	Generation time in minutes			
	Broths			
	Nutrient	Tetrathionate	Selenite-F	Ruys
S. anatis	32	35	28	62
S. bredeney	32	38	33	31
S. give	31	27	30	93
S. oranienburg	31	33	39	184
S. paratyphi	31	--	71	396
S. pullorum	48	66	52	88
S. typhimurium	31	33	33	75
S. worthington	29	28	21	67
Total	269	260	307	996
Average	34	37	38	125

two- or three-fold if tetrathionate was added. With the organisms used in this study, stimulation of growth was not observed in tetrathionate broth. Growth of *S. paratyphi* in this enrichment broth was definitely inhibited; there were fewer viable cells after incubation for 18 or for 36 hours than there were immediately after inoculation. This is particularly undesirable since the object in using an enrichment broth is to increase the number of *Salmonella*

present. Since S. paratyphi did not reproduce in tetrathionate, it was not possible to obtain its generation time. Excluding S. paratyphi, the generation times of the organisms in tetrathionate ranged from 27 minutes for S. give to 66 minutes for S. pullorum; the average time for the seven organisms was 37 minutes.

The principal objection to the use of Selenite-F was that the numbers of viable cells decreased during the initial incubation period. Analysis of variance was used to study the decline in numbers of cells and, in the case of S. anatis, was found to be statistically significant ($P = .01$). Since destruction during the lag phase could subsequently result in failure to isolate organisms, reduction in viable count is particularly undesirable in the case of samples with low counts of Salmonella. The other organisms tested also showed a decrease in numbers during the initial incubation period. In most cases, logarithmic growth ceased by 14 hours and, by 24 or 36 hours, the number of viable cells began to decrease.

When the eight species of Salmonella were grown in Selenite-F, the generation times ranged from 21 minutes, in the case of S. worthington, to 71 minutes for S. paratyphi; the average generation time of 38 minutes compared favorably with the 37 minutes which were needed in tetrathionate.

In general, Ruys' medium was the least satisfactory of those tested, since it induced an extended lag phase and exerted inhibitive action on all of the organisms tested. In the case of S. oranienburg, the lag phase was so prolonged that the original number of cells was not attained during the entire 36-hour incubation period. In Ruys' medium the growth of S. pullorum was similar to that of S. paratyphi; no decrease in their numbers was noted during the lag phase, but only a slight increase was noted after incubation for 36 hours. Even though S. typhimurium, S. anatis, S. worthington, and S. bredeney were inhibited during the initial incubation period in Ruys' medium, the number of viable cells compared favorably with the number found in the other broths at the end of 24 hours of incubation. Only in the case of S. bredeney did the generation time in Ruys' medium compare favorably with the generation times of these organisms in the other test broths. S. paratyphi had a generation time of 396 minutes; this was the longest period of time required for any of the organisms to reproduce in any of the broths. For the eight organisms in Ruys' medium the average generation time of 125 minutes was considerably longer than was that observed in the other broths.

Under the conditions of this study, Selenite-F, tetrathionate or Ruys' medium did not support the growth of

eight species of Salmonella as well as did nutrient broth. Although tetrathionate supported the growth of most of these species of Salmonella, it was definitely inhibitory to S. paratyphi. Selenite-F appeared to be satisfactory; however, during the initial incubation period, the inhibition of S. anatis was statistically significant (P = .01). Ruys' medium inhibited all of the organisms during the initial growth phase and, further, inhibited S. paratyphi, S. oranienburg and S. pullorum during the entire period of incubation.

2. Effect of adding whole egg to enrichment broths

The enumeration of Salmonella in substances such as whole egg is often necessary; hence, information is desirable regarding the effect of the addition of whole egg to the broths in which these organisms are grown.

When whole egg was incorporated into the media, it appeared that the properties of the enrichment broths that were inhibitory to Salmonella were masked. Figure 2 illustrates the growth of eight species of Salmonella in the test broths containing whole egg. The growth curves of S. typhimurium, S. anatis, S. worthington and S. oranienburg showed that the reproduction of these organisms was similar in the four broths in which whole egg was incorporated.

S. paratyphi was inhibited to a considerable extent in tetrathionate broth to which whole egg was added. However, the other organisms grew as well in tetrathionate to which egg was added as they did in that in which no egg was introduced. For the Salmonella species studied, the addition of egg to tetrathionate altered the average reproduction time by only 3 minutes.

The reduction in numbers of viable cells during the initial incubation period was not as pronounced in Selenite-F when whole egg was present in the medium as that noted in Selenite-F without whole egg. For these eight species of Salmonella, the average generation time was reduced by 7 minutes when whole egg was incorporated into the Selenite-F broth.

With whole egg added, Ruys' medium apparently supported the proliferation of all of the organisms studied as well as did nutrient broth; this can be observed from the curves in Figure 2 and by the comparison of the average generation time for the organisms (Table 4). It can be seen (Tables 3 and 4) that the average generation time was reduced from 125 minutes to 33 minutes by the incorporation of whole egg into the media.

The overall growth of Salmonella in nutrient, tetrathionate or Selenite-F broths was not alterated

Figure 2. Growth curves of 8 species of *Salmonella* in various broths with whole egg added

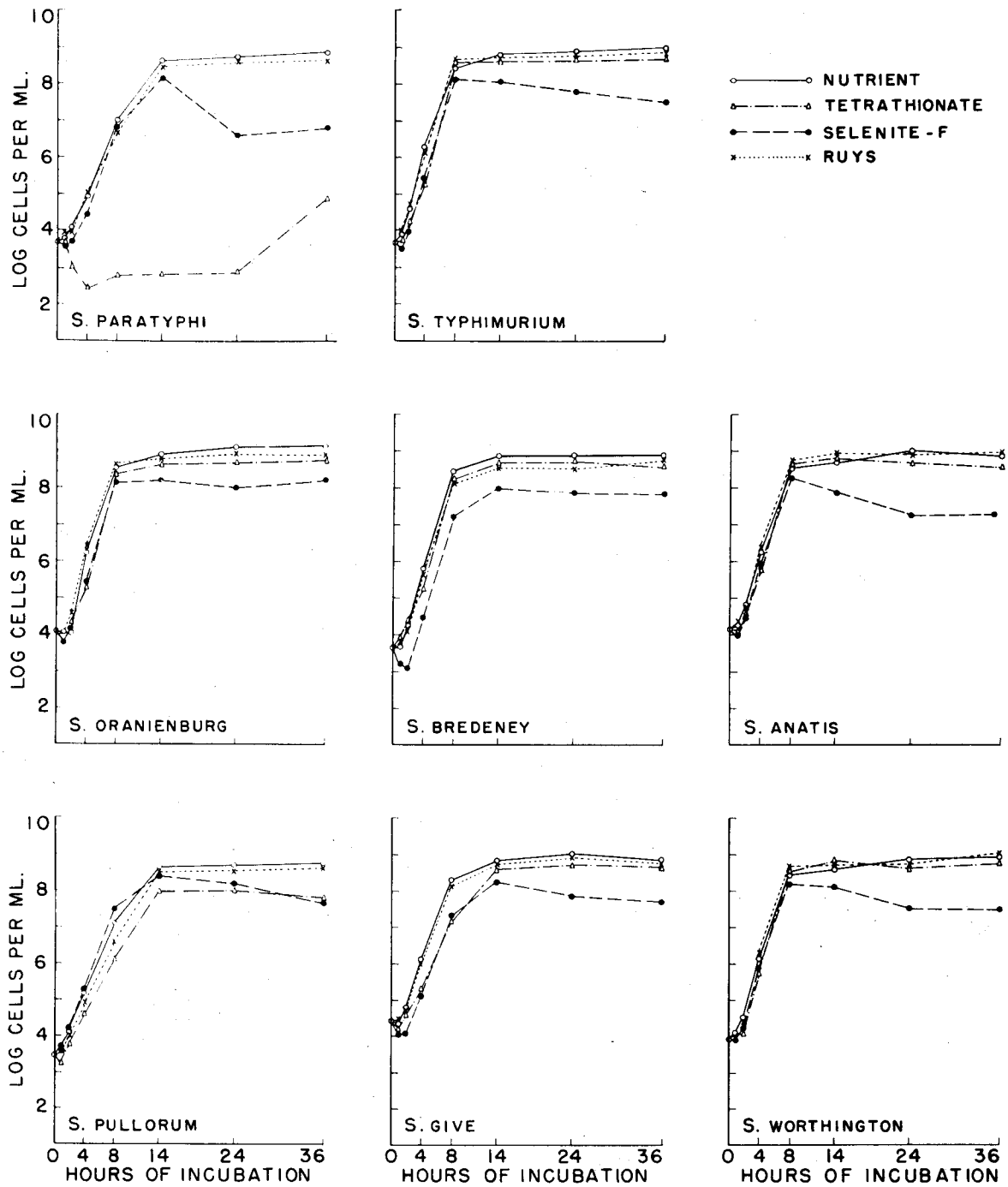

LOG CELLS PER ML.
10
8
6
4
2
S. PARATYPHI
S. TYPHIMURIUM
NUTRIENT
TETRATHIONATE
SELENITE - F
RUYS
S. ORANIENBURG
S. BREDENEY
S. ANATIS
S. PULLORUM
S. GIVE
S. WORTHINGTON
0 4 8 14 24 36
HOURS OF INCUBATION

Table 4. Generation times of eight species of Salmonella in various broths with whole egg added

Organism	Generation time in minutes			
	Broths			
	Nutrient	Tetrathionate	Selenite-F	Ruys
S. anatis	30	27	29	28
S. bredeney	27	28	27	28
S. give	32	43	34	33
S. oranienburg	25	27	28	28
S. paratyphi	39	44	36	41
S. pullorum	37	48	34	45
S. typhimurium	29	27	27	29
S. worthington	29	27	30	29
Total	248	271	245	261
Average	31	34	31	33

appreciably when whole egg was added to these broths. S. paratyphi grew in tetrathionate if whole egg was introduced; however its growth was definitely inhibited. The improvement in growth of Salmonella in Ruys' medium due to the presence of whole egg is evident; the average generation time in this medium was reduced to one-fourth that in the basal medium. With seven of the eight organisms used in

this study, no apparent difference in the generation times could be observed with either Selenite-F or tetrathionate. The use of tetrathionate broth requires that iodine solution for oxidizing sodium thiosulfate to sodium tetrathionate be transferred aseptically; this extra operation not only affords a source of contamination, but is time consuming as well. Therefore, Selenite-F was chosen throughout the remainder of the study as the broth to be used for enumerating Salmonella.

3. Selective agars

Selective agars employed for the isolation of Salmonella should possess a minimum degree of inhibition of this genus and should have maximum bacteriostatic action on extraneous organisms. Several agars could be used to facilitate the isolation of Salmonella; however, the use of more than one agar increases the amount of labor and expense; therefore, it would be advantageous if a single agar could be used to detect and isolate these organisms.

The mean colony counts on the selective agars (BG, SS, DCLS and WB) are shown in Table 5. Using nutrient agar as the reference, it was demonstrated that the several agars varied greatly in providing satisfactory conditions for the reproduction of the various microorganisms studied. DCLS agar was somewhat inhibitory to all of these organisms,

Table 5. Comparison of colony counts[a] on various agars

Organism	Plating agars				
	Nutrient	Brilliant green	Salmonella-Shigella	Desoxycholate citrate lactose sucrose	Bismuth sulfite
Salmonella paratyphi	232	208	145[b]	102[b]	209
S. typhimurium	700	590[b]	85[b]	6[b]	490[b]
S. oranienburg	620	550	312[b]	77[b]	71[b]
S. pullorum	186	173	66[b]	37[b]	112
S. anatis	360	340	272[b]	66[b]	73[b]
S. worthington	410	370	249[b]	129[b]	330[b]

[a]Counts reported are means of nine plates.

[b]Significant difference as compared to nutrient (P = .01).

producing less than 1 per cent of the number of colonies of S. typhimurium that were produced on nutrient agar. The other selective agars showed a tendency to inhibit some organisms more than they did others.

When the colony counts for the various species of Salmonella were analyzed statistically, SS and DCLS agars showed a significantly ($P = .01$) smaller number of colonies with every species tested. On bismuth sulfite agar, the number of colonies of all the organisms except S. paratyphi and S. pullorum was significantly ($P = .01$) smaller than the number observed on nutrient agar. However, no difference was observed between the results obtained with brilliant green and nutrient agar, except in the case of S. typhimurium.

4. Liquid egg white

During the removal of glucose the liquid egg white is subjected to conditions favorable for the multiplication of contaminating bacteria. If Salmonella gain access to the liquid egg white, the numbers of these organisms might increase to a level whereby a health hazard would result; however, bacteriostatic agents present in egg white could prevent excessive reproduction of bacteria during the fermentation period.

Data regarding the reproduction of S. pullorum in egg white adjusted to pH 7.0 and in nutrient broth are shown in Figure 3. Similar results were obtained in the case of S. oranienburg and S. senftenberg. In every case the organisms grew more profusely in nutrient broth than in egg white during the 24-hour incubation period. Examination of the generation times for the three organisms (Table 6) revealed that S. oranienburg and S. senftenberg reproduced at a faster rate in nutrient broth than in egg white; however, S. pullorum reproduced at approximately the same rate in egg white at pH 6.0 as it did in nutrient broth. Since during the initial incubation period the organisms grew as well in egg white as they did in nutrient broth, there evidently was not any greater adjustment period required for growth in egg white than for growth in nutrient broth.

Limitation in the reproduction of these organisms in egg white could be due to the presence of inhibitory factors (avidin, lysozyme, conalbumin and ovomucoid) or to the lack of essential nutrients in the albumen.

Although the species of Salmonella studied were inhibited in egg white, the extent of their reproduction indicated that egg white should not be allowed to remain at a temperature favorable for growth of these organisms. Rather, albumen should be handled in a sanitary manner and removal of glucose should be conducted as quickly as

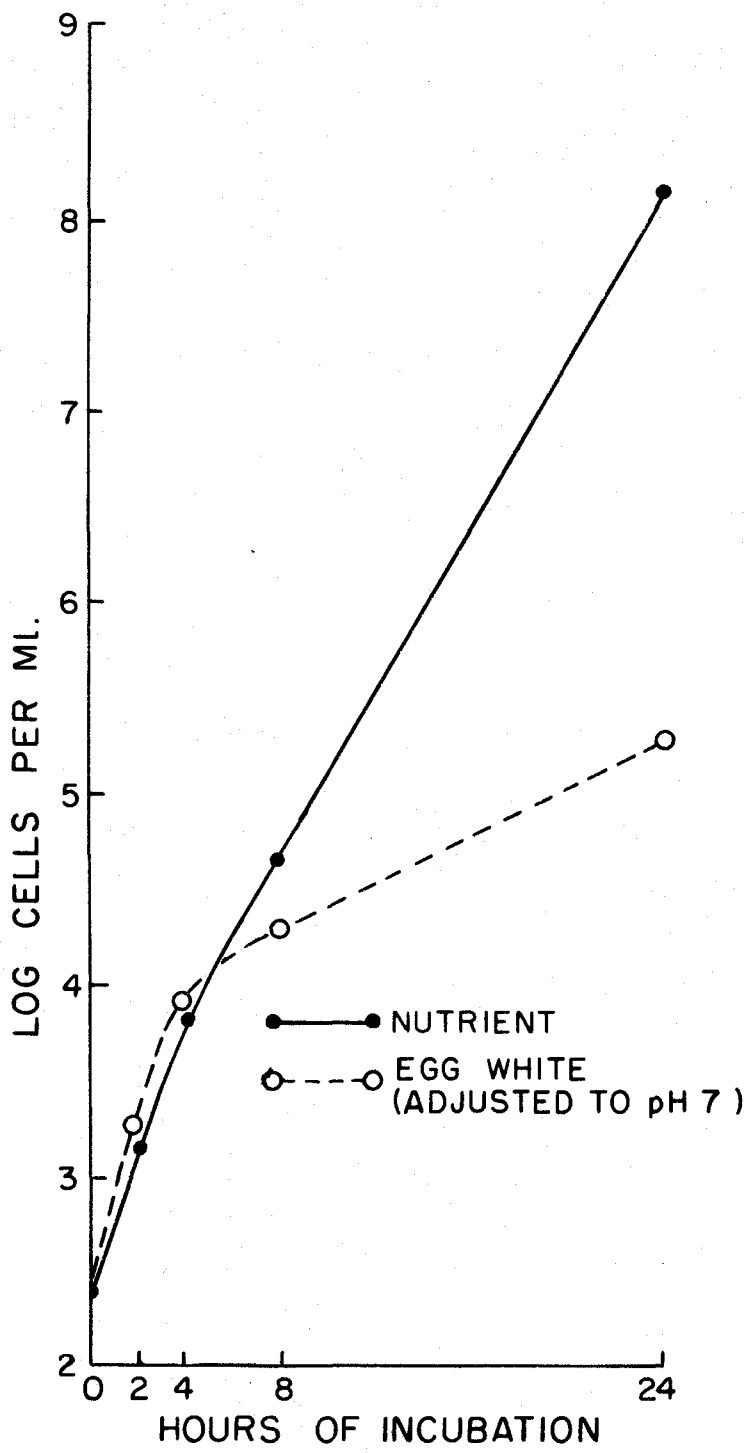


Figure 3. Growth curves of S. pullorum in liquid egg white and in nutrient broth

Table 6. Generation time of Salmonella in egg white and in nutrient broth

Organism	Time in minutes			
	Nutrient broth	Egg white		
		pH 6.0	7.0	8.0
S. pullorum	75	72	173	144
S. oranienburg	33	47	65	72
S. senftenberg	34	46	51	53

possible under conditions that are unfavorable for reproduction of Salmonella.

5. Effect of hydrogen-ion concentration of egg white

It was shown by Sharp and Whitaker (1927) that the bactericidal activity of egg white was influenced by changes in the hydrogen-ion concentration; inhibition of bacteria was more pronounced at pH 9.0 than at pH 7.0. Since Salmonella multiplied in liquid egg white adjusted to pH 7.0 (commonly used in commercial products) it was decided to determine whether adjusting the pH to other levels would affect the growth of these organisms. S. senftenberg, S. oranienburg and S. pullorum were therefore grown in egg white adjusted to pH 6.0, pH 7.0, pH 8.0, pH 9.0 and pH 10.0. The growth curves obtained with S. pullorum are

given in Figure 4. The growth of S. senftenberg and S. oranienburg was similar to that of S. pullorum. It is evident that this organism grew profusely in egg white at pH 6.0 to pH 8.0; however, at pH 9.0 or pH 10.0 the growth of the organisms was inhibited. The effect of pH of albumen on the growth of Salmonella is further exemplified by the generation times listed in Table 6. In the case of S. pullorum, alteration of the hydrogen-ion concentration of liquid albumen from pH 6.0 to pH 7.0 resulted in a doubling of the generation time. Increases in the generation times for S. oranienburg and S. senftenberg were evident when the hydrogen-ion concentration of the egg white was altered from pH 6.0 to pH 8.0.

It was possible to restrict the reproduction of Salmonella in liquid albumen merely by adjusting the egg white to pH 9.0 or pH 10.0; however, with present methods, it would be difficult to remove the glucose at these pH levels. If a method to remove glucose could be devised which would be efficient at these pH values it would aid the problem of ridding dried albumen of pathogenic organisms. With the present methods of removing glucose, therefore, care should be taken to maintain as high pH as possible so that the process is efficient and, at the same time, retards the reproduction of Salmonella.

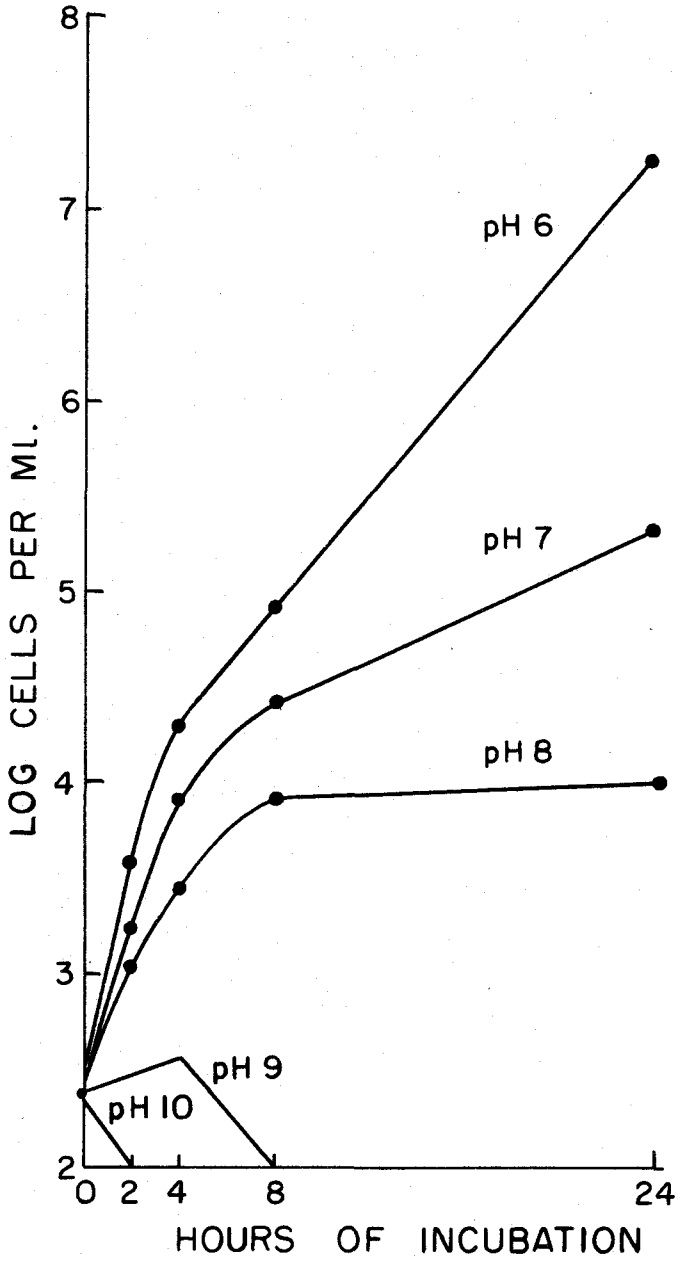


Figure 4. Growth curves of S. pullorum in liquid egg white at 5 pH levels

6. Effect of antibiotics

Although antibiotics are not allowed in foods, these substances have been shown to inhibit bacterial growth, and thus prolong the length of time that foods can be stored. The addition of antibiotics to egg white could act to inhibit the growth of any Salmonella contaminating the liquid albumen.

Three antibiotic substances, chlortetracycline, oxytetracycline and streptomycin, were tested for their ability to inhibit the growth of Salmonella in nutrient broth and in egg white.

a. Nutrient broth. It is evident (Table 7) that the number of cells originally present determined to a great extent the concentration of antibiotic necessary to inhibit bacterial growth. In some cases a ten-fold increase in antibiotic was required to retard the growth of the larger number of cells as compared to the smaller number of cells.

The antibiotics, chlortetracycline and oxytetracycline lost their inhibitive properties considerably during the 336 hour incubation period. While a loss was observed in the case of streptomycin, it was not as pronounced as that noted with the other antibiotics. In any case, a concentration of antibiotic of 5 ppm. or less was sufficient to

Table 7. Concentration of antibiotics needed to inhibit the growth of *Salmonella* in nutrient broth

Time (hours)	Number of cells introduced per ml. of media					
	S. senftenberg		*S.* oranienburg		*S.* pullorum	
	14,000	1.4	170,000	17	150,000	15
	Ppm. chlortetracycline					
8	0.25	0.10	1.0	0.10	0.25	0.10
16	0.50	0.25	1.0	0.50	0.50	0.10
24	1.0	1.0	2.5	1.0	0.50	0.10
336	50.0	5.0	50.0	50.0	50.0	2.5
	Ppm. oxytetracycline					
8	0.50	0.10	2.5	0.10	0.50	0.10
16	1.0	0.50	2.5	0.10	1.0	0.10
24	1.0	1.0	2.5	1.0	5.0	0.25
336	50.0	5.0	50.0	50.0	50.0	25.0
	Ppm. streptomycin					
8	0.50	0.10	2.5	0.25	0.50	0.10
16	1.0	0.50	5.0	0.50	1.0	0.10
24	1.0	0.50	5.0	0.50	2.5	0.25
336	2.5	0.50	10.0	1.0	25.0	2.5

retard the growth of these three species of Salmonella for 24 hours in nutrient broth.

b. Egg white. The results wherein S. senftenberg was grown in liquid egg white that contained 0.1, 1 or 10 ppm. of chlortetracycline, oxytetracycline or streptomycin are depicted in Figure 5. The curves obtained with S. pullorum and S. oranienburg were similar to those of S. senftenberg.

In most cases there was no apparent difference in the reproduction of these Salmonella in the presence of antibiotics at a concentration of either 0.1 or 1 ppm.; generally the rate of reproduction was considerably less when the concentration of antibiotic was 10 ppm. than at either of the other two levels.

At any of the three concentrations the antibiotics definitely retarded the reproduction of S. senftenberg and S. oranienburg at 12 hours of incubation; this was not as pronounced for S. pullorum.

Examination at 24 hours showed that the number of S. oranienburg was less in the presence of oxytetracycline at 10 ppm. than in the absence of this antibiotic; however, in all other cases, at 24 hours, no difference in numbers was observed due to the presence of added antibiotics. Thus it was evident that the antibiotics were not as effective in retarding the reproduction of these species of Salmonella in egg white as they were in nutrient broth,

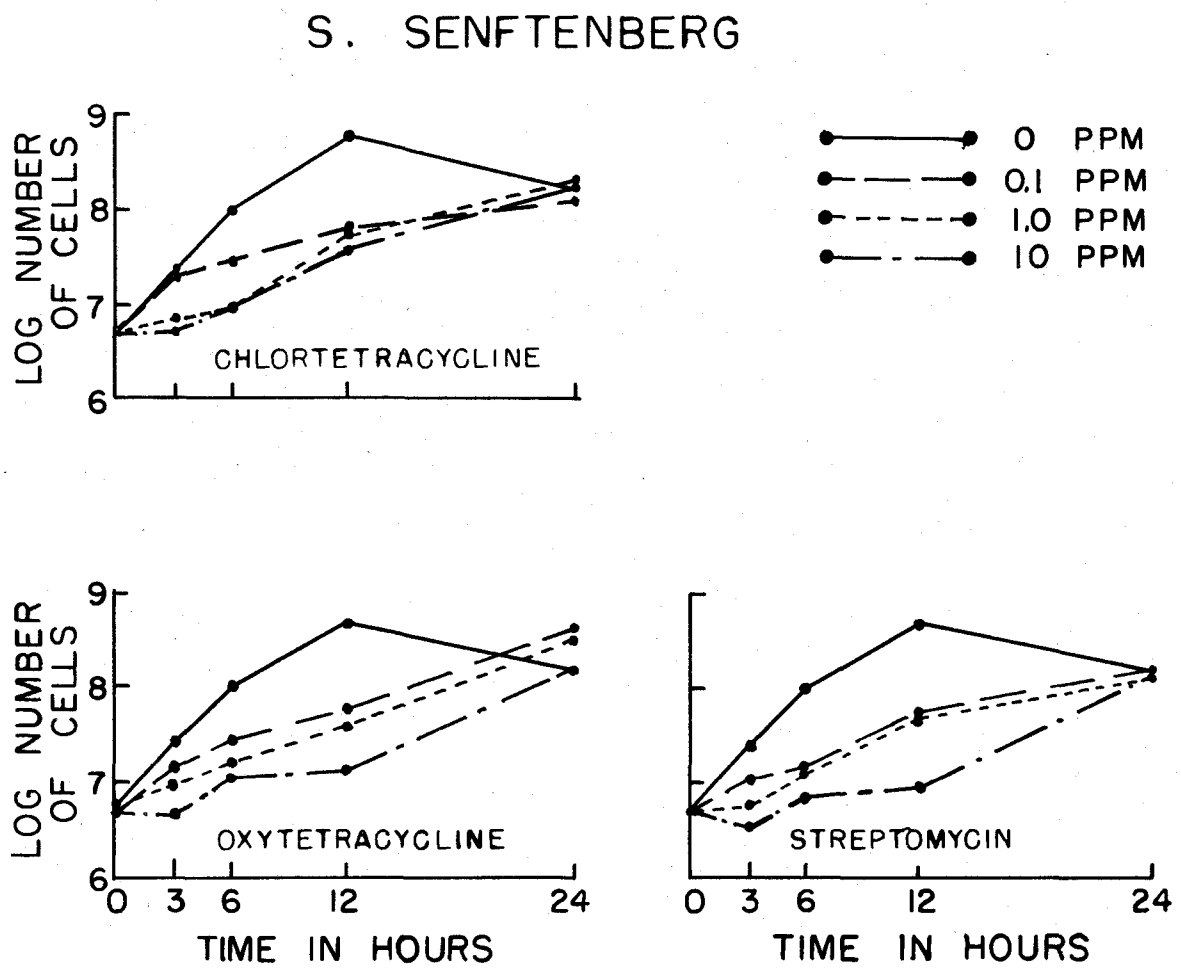


Figure 5. Effect of 0, 0.1, 1 or 10 ppm. of chlortetracycline, oxytetracycline or streptomycin on the reproduction of S. senftenberg in liquid egg white

since it was shown (Table 7) that concentrations of from 0.1 ppm. to 5 ppm. successfully retarded growth in nutrient broth for 24 hours. Beigelman (1949) reported similar results when human serum or horse blood was added to a medium containing chlortetracycline. He believed that the serum or blood enriched the medium to the extent that bacterial growth was more prolific after prolonged incubation, rather than that the added substances inactivated the chlortetracycline.

Although the organisms utilized in these studies were able to reproduce in egg white containing as much as 10 ppm. antibiotic, there was definitely less rapid proliferation for 12 hours. This time period is sufficient to remove the glucose from egg white and hence the addition of antibiotics to the fermenting whites would be of value in retarding the multiplication of these enteric pathogens.

B. Effect of Drying Albumen on Viability of Inoculated Salmonella

It was pointed out by Rahn (1945) that the percentage of organisms surviving desiccation varied from 0 to nearly 100. The percentage depended not only upon the species and age of the culture, but also upon the medium in which

the cells were suspended, the surface on which they were dried and the rate and temperature of drying.

1. Effect of pan drying

The conditions of pan drying are such that the temperature is kept below 40° C., and usually considerable time is required in order to obtain a dried product. The per cent survivors of three organisms, S. senftenberg, S. oranienburg and S. pullorum during pan drying of albumens are listed in Table 8. During the pan drying process 18 per cent of the numbers of S. oranienburg survived, while only 4 per cent of the population of S. pullorum remained viable. Since the pan drying operation was conducted in an open room, the conditions varied somewhat for the experiments. The product was considered sufficiently dry when it could be removed from the pans without any particles adhering to the surface. In most cases an exposure to drying conditions for about 8 hours was sufficient although, when atmospheric conditions were less favorable, as much as 12 hours were necessary to obtain a dried product. Thus, part of the differences in the percentage of survivors could be accounted for by the fact that times for drying varied.

Table 8. Per cent reduction of numbers of *Salmonella* due to drying

Type of drying	Organism	Temperature °C.		Per cent survivors
Pan	*S. senftenberg*[a]	Room		8
	S. oranienburg[b]	Room		18
	S. pullorum[a]	Room		4
		Inlet	Outlet	
Spray[c]	*S. senftenberg*	94	60	.01
	S. senftenberg	77	51	.05
	S. oranienburg	77	51	0.2
	S. oranienburg	135	68	0.1
	S. pullorum	140	68	.01

[a]Average of 3 trials.

[b]Average of 8 trials.

[c]Data for each from 1 trial.

2. Effect of spray drying

The spray drying operations were conducted at two commercial installations. Air inlet temperatures of 94° and 77° C. were used for drying at Plant A and temperatures of 135° and 140° C. were used at Plant B. Since the albumens were dried at two plants and in two different driers,

the results due to the effect of drying upon all three organisms cannot be compared. However, one can examine the results obtained at Plant A and those obtained at Plant B separately.

When an inlet temperature of 94° C. and an outlet temperature of 60° C. was used (Plant A), less than 0.01 per cent of the numbers of S. senftenberg survived drying, while when the temperatures were lowered to 77° and 51° C., respectively, 0.05 per cent of the numbers of this organism remained viable. At the latter temperatures (inlet, 77° C., outlet, 51° C.) 0.2 per cent of the numbers of S. oranienburg survived spray drying. Thus S. oranienburg appeared to be more tolerant to this treatment than did S. senftenberg.

At Plant B, the inlet and outlet temperatures were considerably higher than at Plant A. Less than 0.01 per cent of the cells of S. pullorum survived the conditions imposed upon the egg white while 0.1 per cent of the cells of S. oranienburg remained viable, indicating that S. oranienburg was more resistant to this treatment than was S. pullorum.

3. Discussion

In the spray drying of albumen the conditions are such that fine particles of liquid albumen are subjected to a high temperature for a short time. In pan drying, however,

the albumen is in contact with air at room temperature for a relatively long time. Also, a film forms on the surface of the albumen during pan drying, and the albumen on the bottom of the pan remains in a liquid state under conditions wherein bacterial multiplication could occur. Thus, these two methods for drying differ with regard to the temperature and rate of drying as well as the surface exposed. During spray drying, atomization, with the resulting shearing action of the albumen, could affect the viability of the cells. The results in Table 8 indicated that fewer organisms survived spray drying than survived pan drying of albumen. Differences in survival during pan drying and spray drying could explain the results obtained by Byrne (1954) when she examined 10 samples of spray dried albumen and found no Salmonella, whereas she reported that of seven samples of pan dried albumen, all were contaminated with Salmonella.

C. Microbiological Changes During Storage of Dried Albumen

Storage of dried albumen at elevated temperatures was suggested by Ayres and Slosberg (1949) as a means of eliminating Salmonella from this commodity. In the present study, work was undertaken to determine the significance of

the moisture content of the powder, the temperature of storage, the type of dried product (spray dried or pan dried), the species or type of organism present, and the alteration of the atmosphere upon the survival of organisms during storage of dried albumen.

1. Salmonella

Pan dried albumen containing a known number of S. oranienburg was adjusted to moisture levels ranging from 1.8 per cent to 18 per cent; these albumens were stored in a 50° C. incubator and analyzed for the presence of S. oranienburg after 0, 12 and 38 days of storage. The percentages of survivors were calculated and are shown in Figure 6.

After storage for 12 days there was a noticeable difference in the per cent of cells surviving in albumen containing 5 per cent moisture when contrasted with the per cent surviving in albumen having 6 per cent moisture. No isolations of Salmonella were made from albumens with moisture levels above 10 per cent on the 12th day of storage. The per cent of survivors was considerably greater in albumen having 3 per cent moisture than it was in albumen with 6 per cent moisture on the 38th day of storage. Since the results indicated that levels of 3 per cent and 6 per cent moisture were critical, these two moisture concentrations were selected for further study. Moisture levels of

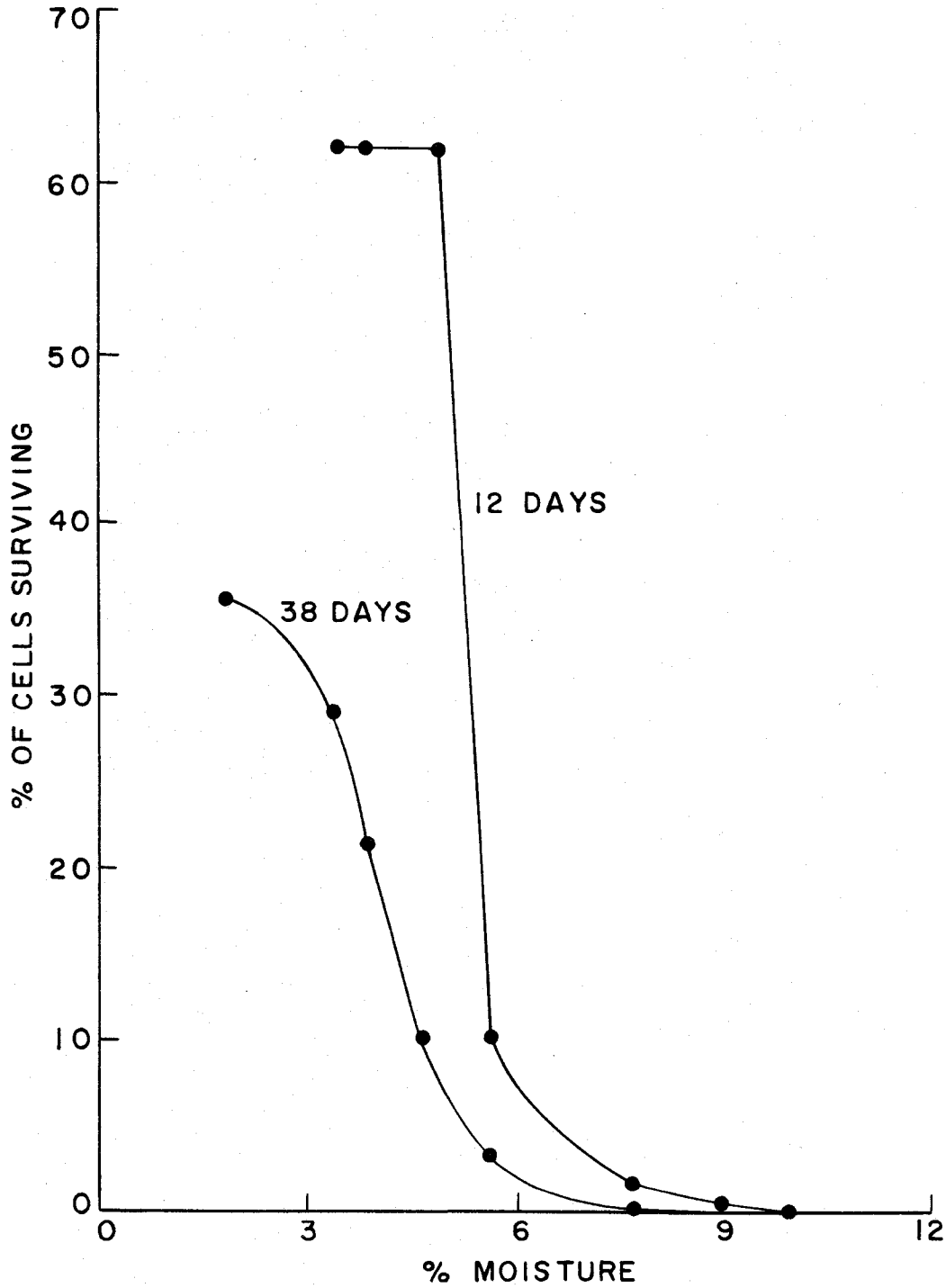
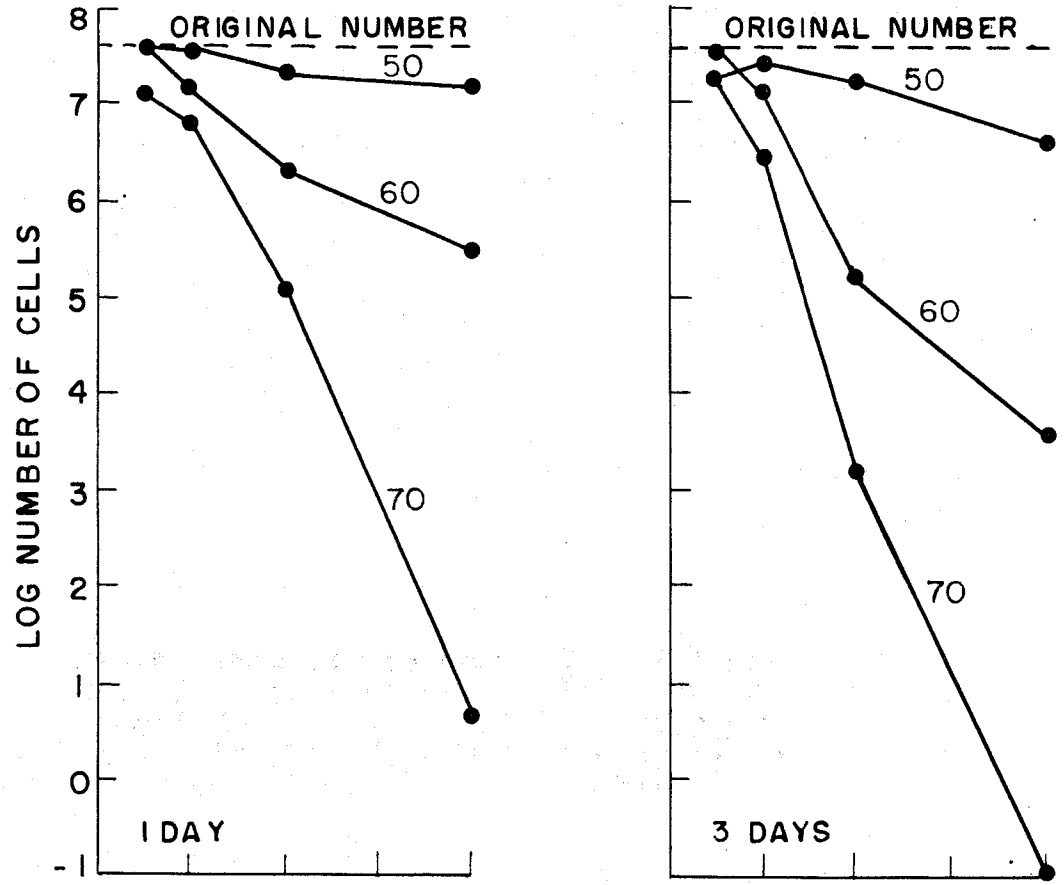


Figure 6. Effect of moisture on the per cent survivors of S. oranienburg in dried albumen stored at 50° C. for 12 or 38 days

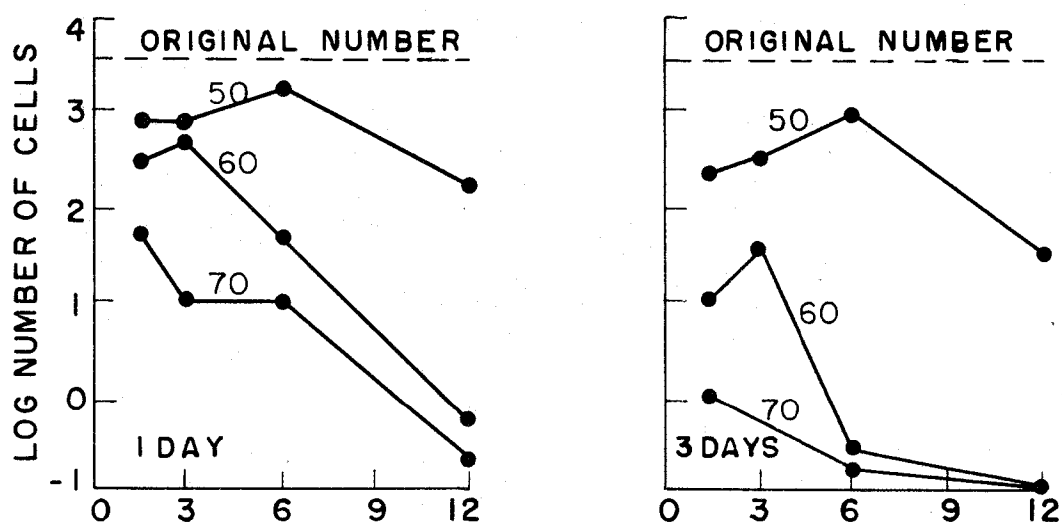
1.5 per cent and 12 per cent were also selected in order to provide a geometric series representing reasonable minimum and maximum moisture concentrations that might be encountered in commercial albumen.

Albumens which contained 1.5, 3, 6 or 12 per cent moisture were analyzed for the numbers of Salmonella (S. senftenberg, S. oranienburg or S. pullorum) that survived storage at 50°, 60° or 70° C. The effect of moisture content of the albumen and temperature of storage on the survival of S. oranienburg can be observed in Figure 7. Similar results were obtained in the case of S. senftenberg and S. pullorum. Fewer organisms were isolated from albumen containing 12 per cent moisture than from those powders with 1.5, 3 or 6 per cent moisture. In all cases except one, fewer viable organisms were found in albumen containing 6 per cent moisture than in those albumens with either 1.5 or 3 per cent. Comparison of the survivors of the Salmonella in albumens containing 1.5 or 3 per cent moisture showed that, in 11 of the 36 cases, there were more survivors in those samples with 3 per cent. These results agreed with those shown in Figure 6 that, in general, there was no apparent difference between the number of survivors in albumen with 1.5 or 3 per cent moisture; however, as the moisture content of the powder increased, the number of survivors was greatly reduced.

Figure 7. Numbers of S. oranienburg detected in pan dried and in spray dried albumens containing 1.5, 3, 6 or 12 per cent moisture and stored at 50°, 60° or 70° C. for 1 and 3 days



PAN DRIED ALBUMEN



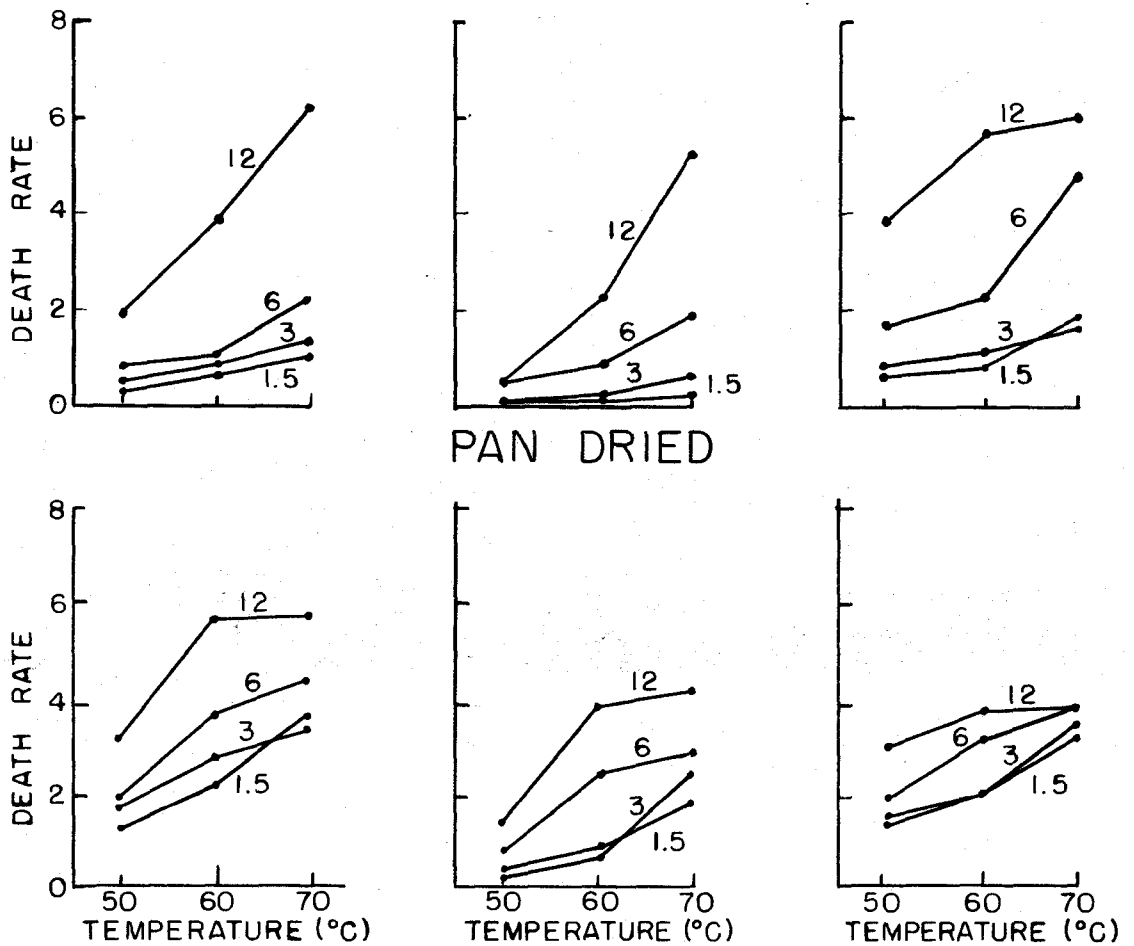
SPRAY DRIED ALBUMEN

The effect of temperature upon the viability of cells of S. oranienburg can be seen in Figure 7. As the temperature of storage was increased, the number of viable cells isolated after storage was decreased. In every case there were fewer survivors of Salmonella in the albumen stored at 70° C. than in that stored at 60° or 50° C. Also, in every case except one, there were fewer survivors in albumen exposed to 60° C. than in that at 50° C.

Since the original numbers of Salmonella in the several albumens varied, it was difficult to compare the effect of the conditions of storage imposed by merely observing the number of survivors. Therefore, death rate (or velocity) constants were calculated and plotted in Figure 8. The rate of death was influenced by the moisture content of the albumen, the temperature of storage, the species of organism and the type of dried product.

a. Effect of moisture. The death rate constants were averaged for each moisture level and are plotted in Figure 9. It can be readily observed that the rate of death decreased with decreasing moisture content of the egg white solids. Statistical analysis of the data revealed that there was a significant difference ($P = .01$) in the death rates due to moisture. Comparing individual degrees of freedom showed that there was no significant difference between the rates

Figure 8. Death rates of S. senftenberg, S. oranienburg and S. pullorum in pan dried or spray dried albumens with 1.5, 3, 6 or 12 per cent moisture and stored at 50°, 60° or 70° C.


DEATH RATE
0
2
4
6
8
12
6
3
1.5
PAN DRIED
50
60
70
TEMPERATURE (°C)
SPRAY DRIED
S. SENFTENBERG
S. ORANIENBURG
S. PULLORUM

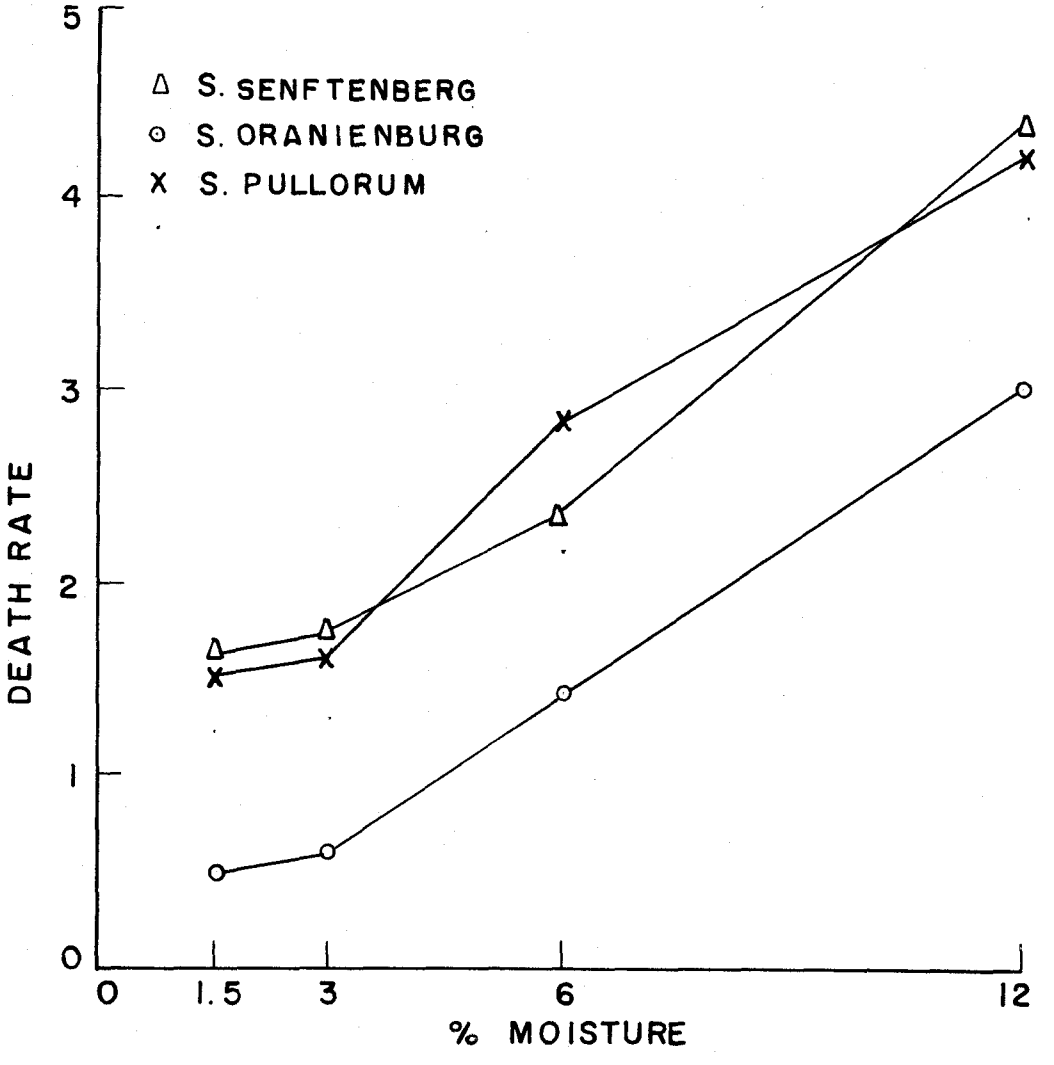


Figure 9. Effect of moisture content on the rate of death of three species of Salmonella in pan dried and spray dried albumen stored at elevated temperatures (50°, 60° and 70° C.)

of death representing 1.5 and 3 per cent moisture; nearly all of the difference existed among the death rates of 3, 6 and 12 per cent moisture. Although the curves representing S. senftenberg and S. pullorum were not straight lines between 3 per cent moisture and 12 per cent moisture, as was the case with S. oranienburg, all of the curves showed the same trend. The rate of death did not appear to be different between 1.5 per cent and 3 per cent moisture; however, the slope of the line increased considerably when the moisture content was greater than 3 per cent.

In order to determine if the increase in the death rate constants was directly proportional to the moisture content, these constants were divided by the per cent moisture of the powder they represented. Thus, the curves in Figure 10 represent the rate of death/per cent moisture content of the powder. Comparing the average values for rate of death/per cent moisture showed that the value for 1.5 per cent moisture was approximately twice as large as the value obtained at the other three moisture levels; a slight decline in rate of death/per cent moisture was noted as the moisture content of the powder increased. It would appear that when albumen contained 1.5 per cent moisture, there was a different effect on the cells than when the moisture content was 3 per cent or higher. It could be postulated that when the moisture content of albumen was less than

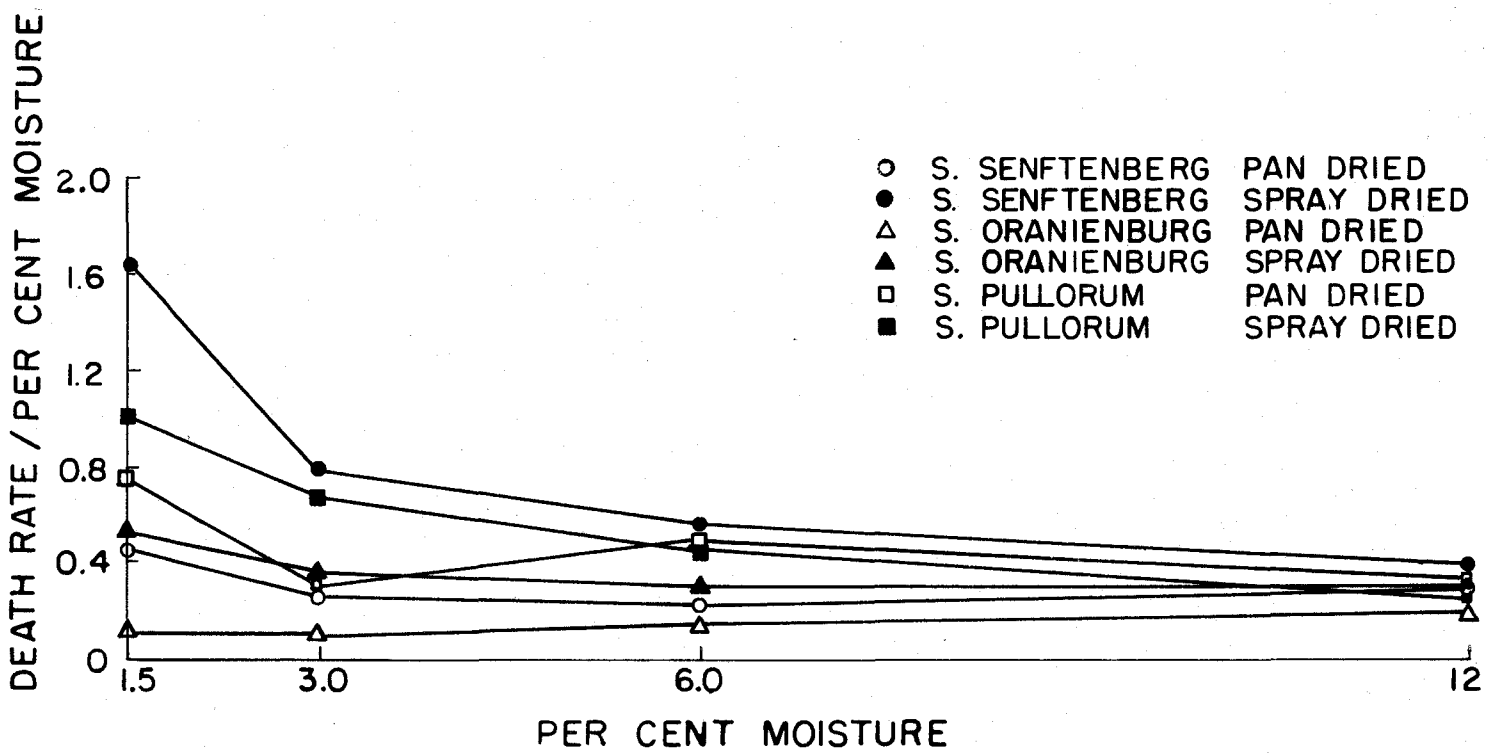


Figure 10. Death rate/per cent moisture of S. senftenberg, S. oranienburg and S. pullorum in spray dried or pan dried albumens stored at elevated temperatures (50°, 60° and 70° C.)

3 per cent, the cells were desiccated to the extent that they lost their viability; however, with 3 per cent moisture or above, the rate of death was apparently proportional to the moisture content of the powder.

b. Temperature of storage. Rahn (1945) stated that the temperature coefficients for the death of dry bacteria were those to be expected for an oxidation process; hence, raising the temperature of storage resulted in a faster rate of destruction of bacterial cells due to the increase in the rate of oxidation.

The death rate constants for S. senftenberg, S. oranienburg and S. pullorum were averaged at each of the storage temperatures (50°, 60° or 70° C.) and plotted in Figure 11. The curves in this figure approximate straight lines, indicating that a direct relationship exists between temperature of storage of albumen and the rate of death of inoculated Salmonella. Differences in the rate of death due to temperature of storage were found to be statistically significant ($P = .01$). The temperature coefficients, Q_{10} values, listed in Table 9 were calculated from the death rate constants for these organisms. All of these Q_{10} values except one, fell within the range of 1.44 to 1.96; the exception was for S. oranienburg, where the temperature coefficient was 4.30.

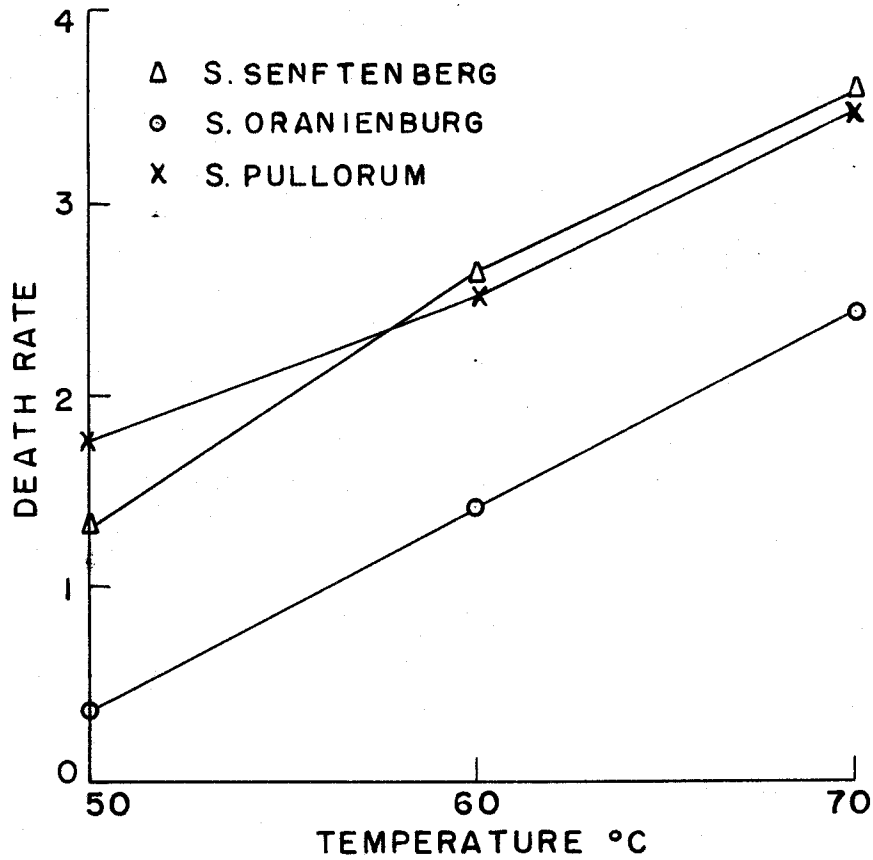


Figure 11. Effect of temperature of storage on the death rate of three species of Salmonella in pan dried and spray dried albumens

Table 9. Effect of temperature on death rates of organisms

Organism	Temperature° C. 50	60	70	Temperature Coefficients (Q_{10})[a] 1	2	3
	Death rates					
S. senftenberg	1.35	2.65	3.62	1.96	1.37	1.66
S. oranienburg	0.33	1.42	2.45	4.30	1.73	3.02
S. pullorum	1.70	2.48	3.58	1.46	1.44	1.45
Average of Salmonella	1.13	2.18	3.19	1.93	1.46	1.70
Coliforms	0.56	1.10	1.58	1.96	1.44	1.70
Total count	0.30	1.44	2.31	1.80	1.60	1.70

[a]1 = Q_{10} for 50° to 60° C.

2 = Q_{10} for 60° to 70° C.

3 = Average Q_{10}.

c. Type of dried product. Several lots of spray dried albumen and pan dried albumen were stored and analyzed for the number of surviving microorganisms in order to determine if, during storage, organisms would survive for longer periods in one type of powder than in the other.

The death rates of the three species of Salmonella both in spray and in pan dried albumens are shown in Figure 12. There was no significant difference in the rate of death of S. pullorum either in pan or in spray dried albumen.

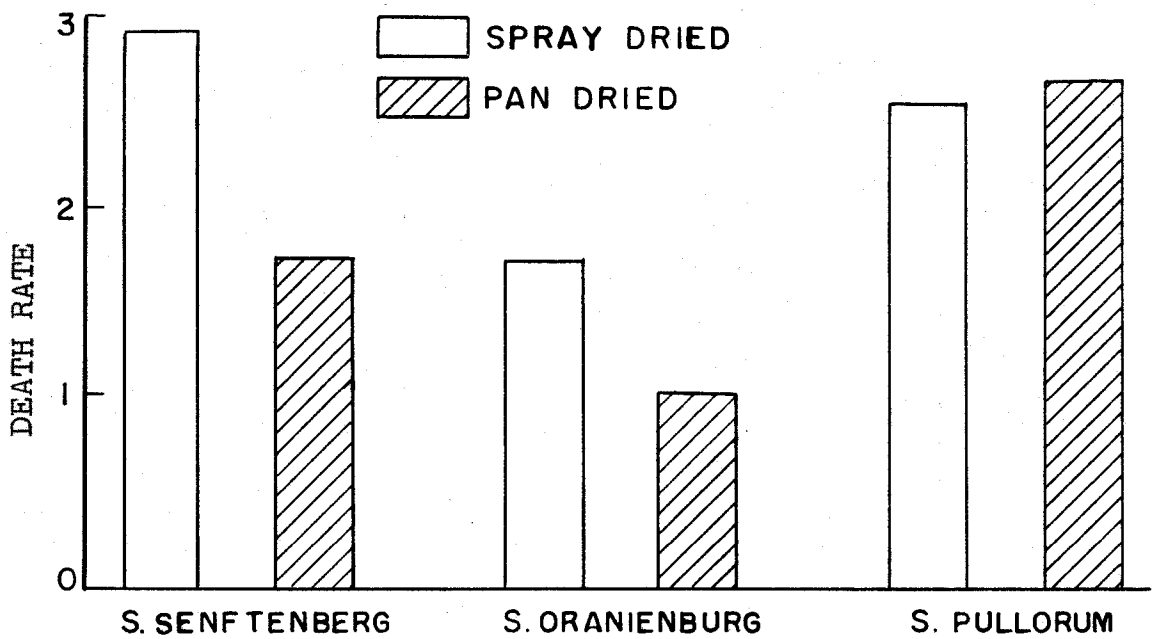


Figure 12. Average death rates of S. senftenberg, S. oranienburg, or S. pullorum in pan dried or spray dried albumens due to storage at elevated temperatures

S. senftenberg and S. oranienburg lost their viability more readily in spray dried than in pan dried albumen; this difference was evident at the 1 per cent level of significance.

The more rapid loss in viability of S. senftenberg or S. oranienburg during storage in spray dried albumen when compared with pan dried albumen could be due to the higher temperature imposed upon the albumen during spray drying. The cells might lose their viability during storage due to distortions caused by shearing during atomization of the droplets of egg white and exposure to relatively high temperatures during the spray drying operation.

d. Species of Salmonella. The death rates for the three species of Salmonella during storage of dried albumen are shown in Figure 12. It can be noted that in spray dried albumen the rate of death was similar for S. pullorum and S. senftenberg, while the death rate for S. oranienburg was significantly less ($P = .01$). In the pan dried albumen the death rate was highest for S. pullorum and was the lowest for S. oranienburg. Thus, one must consider the type of organism that is present in the albumen in order to determine the period necessary for storage so that no Salmonella will be isolated.

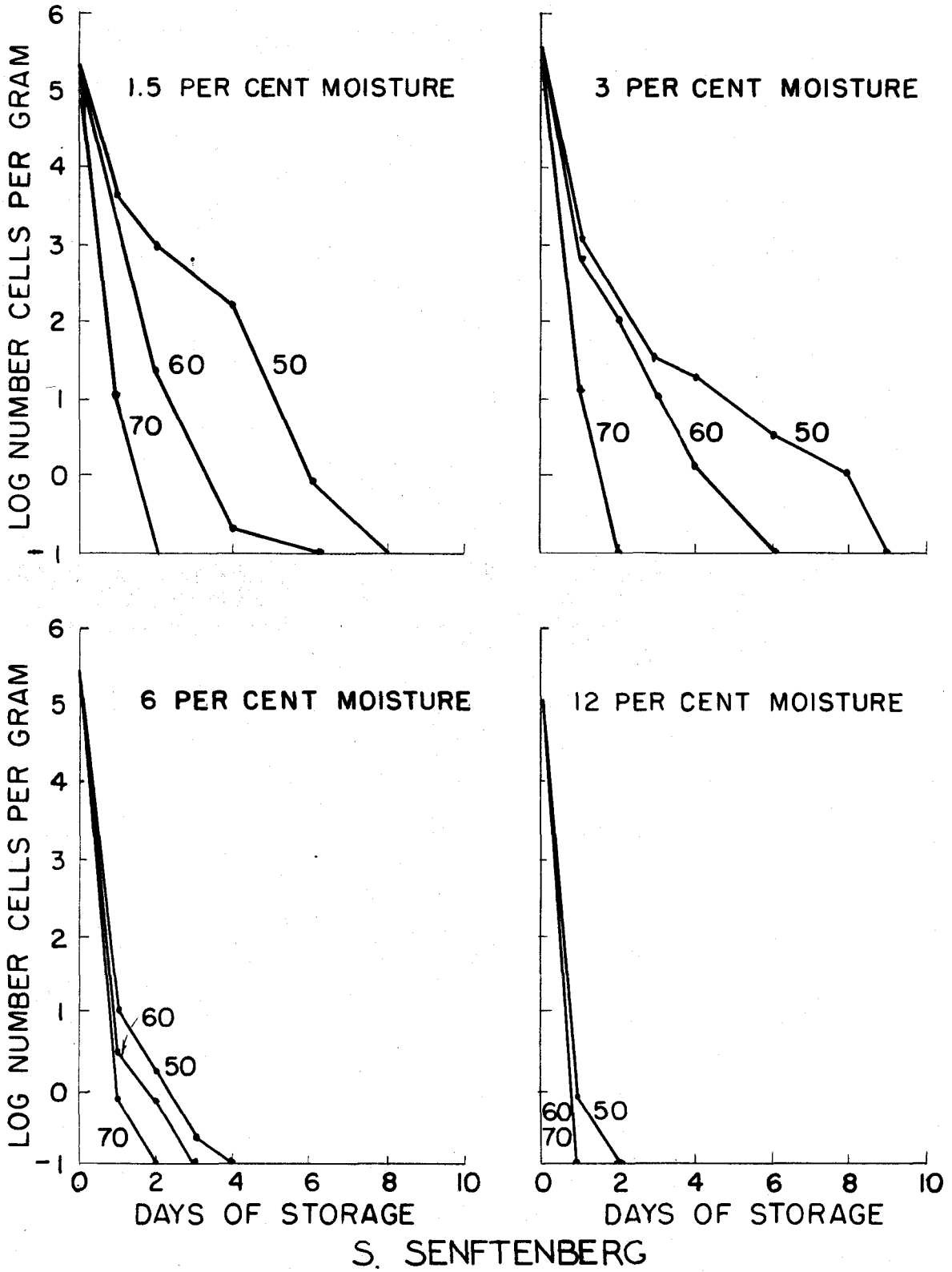
e. Storage time. Spray dried albumens inoculated with S. senftenberg, S. oranienburg or S. pullorum,

containing 1.5, 3, 6 or 12 per cent moisture and stored at 50°, 60° or 70° C. were analyzed and the number of viable cells detected are shown graphically in Figures 13 to 15. The original number of Salmonella in the powder was: S. senftenberg, 304,000; S. oranienburg, 28,500; and S. pullorum, 860. It can be seen that as the moisture content of the powder increased, or as the temperature of storage was increased, the number of days of storage needed to obtain an albumen from which no Salmonella were isolated was reduced.

It was found in previous experiments that the death rate of S. senftenberg was higher than was that of S. oranienburg; therefore one would expect the former organism to be eliminated in fewer days of storage than the latter. However, in these experiments, there were more cells of S. senftenberg originally present than there were of S. oranienburg and, hence, one would expect that the storage time for the former to reach a level of 0.18 organisms per gram would be greater.

With an initial load of as many as 304,000 S. senftenberg per gram of albumen and using the conditions of storage previously mentioned, no Salmonella were detected after only 9 days of storage. In 6 of the 12 conditions of storage, only 2 days were required to produce albumen from which no S. senftenberg was isolated. With albumen

Figure 13. Number of cells of S. senftenberg detected in spray dried albumen containing 1.5, 3, 6 or 12 per cent moisture and stored at 50°, 60° or 70° C.


1.5 PER CENT MOISTURE
3 PER CENT MOISTURE
6 PER CENT MOISTURE
12 PER CENT MOISTURE
LOG NUMBER CELLS PER GRAM
DAYS OF STORAGE
50
60
70
S. SENFTENBERG

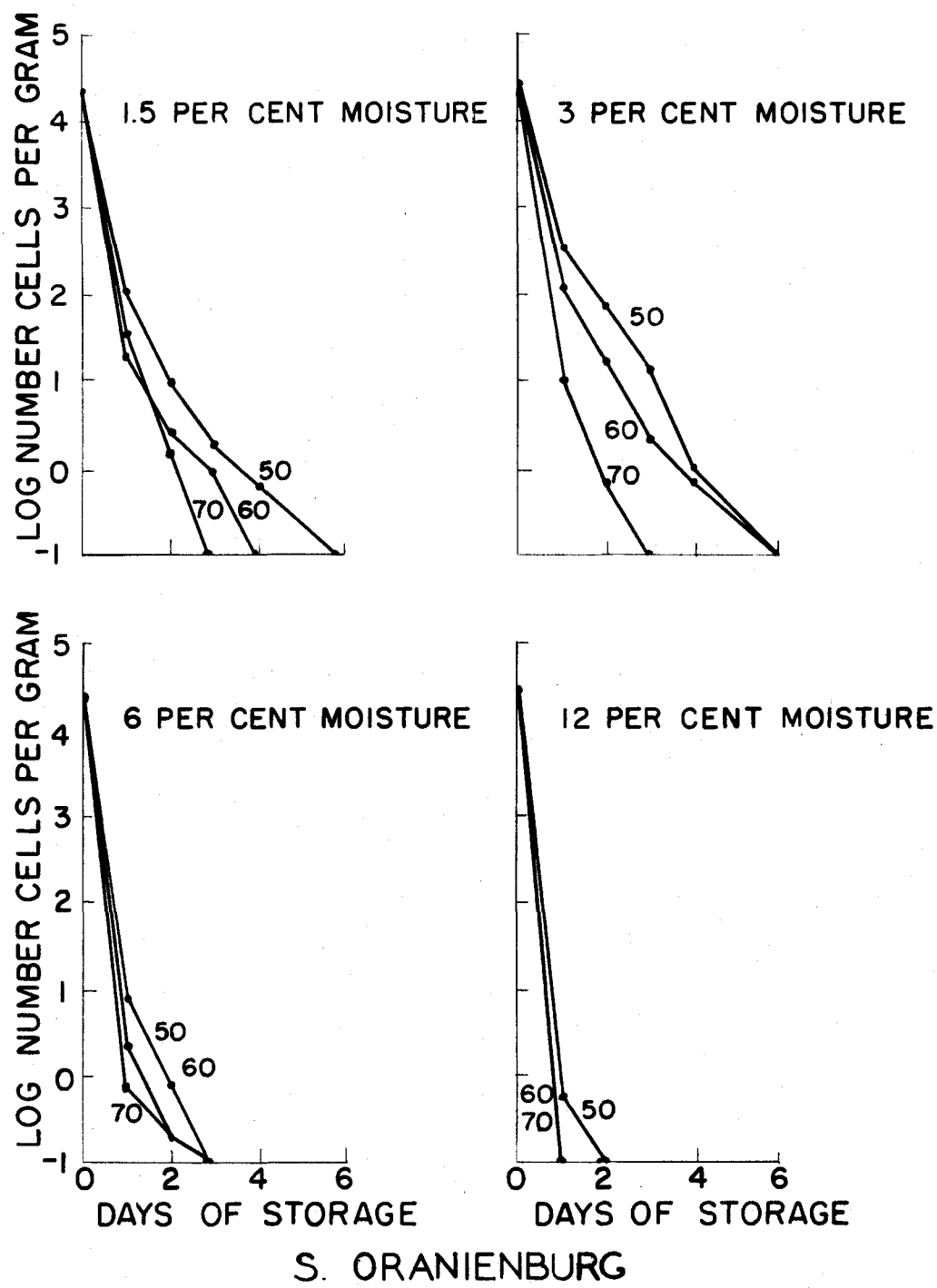


Figure 14. Number of cells of S. oranienburg detected in spray dried albumen containing 1.5, 3, 6 or 12 per cent moisture and stored at 50°, 60° or 70° C.

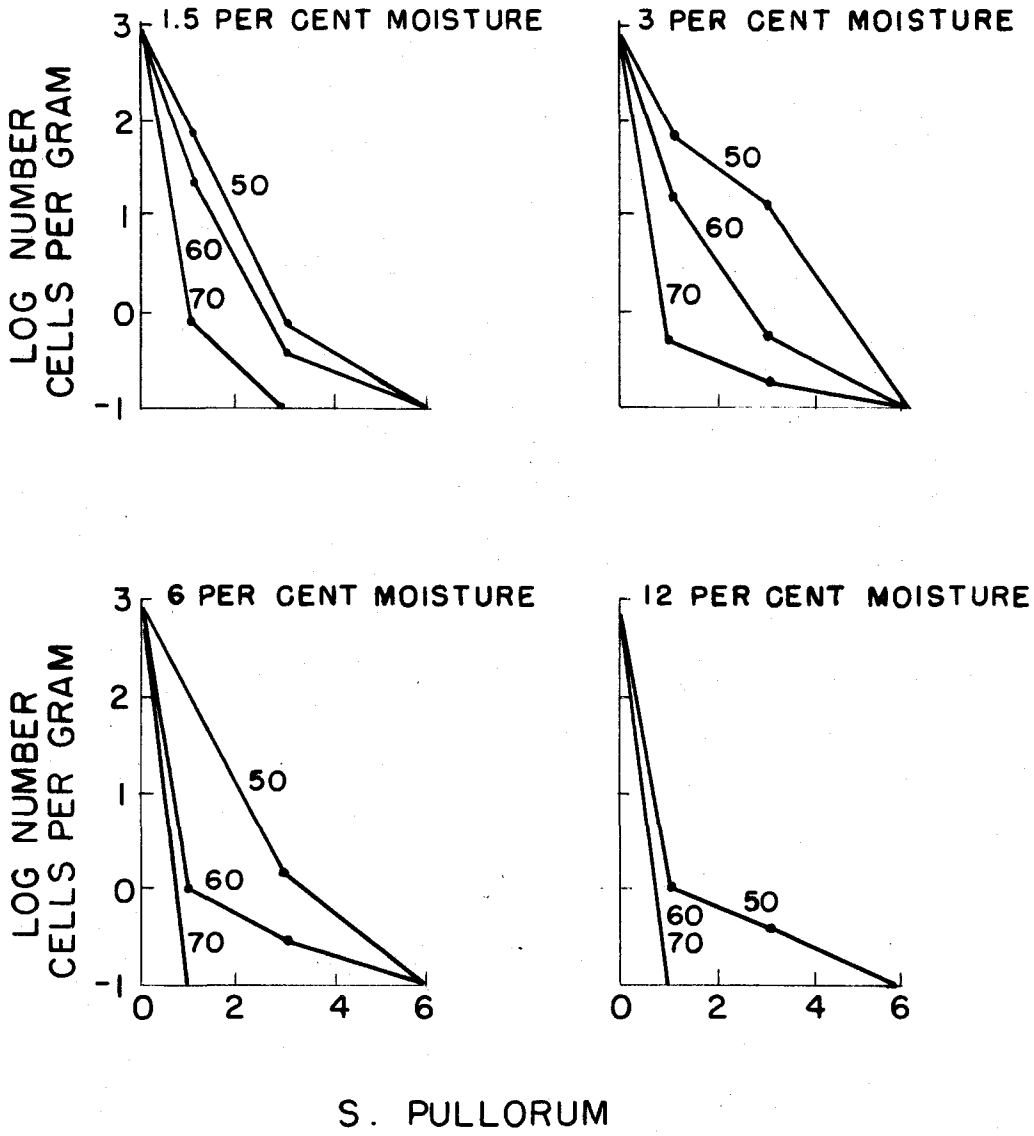


Figure 15. Number of cells of S. pullorum detected in spray dried albumen containing 1.5, 3, 6 or 12 per cent moisture and stored at 50°, 60° or 70° C.

containing 28,500 S. oranienburg per gram, in 7 of the 12 storage conditions, no Salmonella was detected after 3 days of storage, while, using any of the conditions of storage, 6 days was a sufficient time to reduce the number of S. oranienburg to less than 0.18 per gram. In the case of S. pullorum, no survivors were detected in albumens stored for 6 days using any of the storage conditions.

f. Storage in carbon dioxide. Pan dried albumen containing a known load of cells of S. oranienburg was placed in vacuum desiccators and 0, 25, 50, 75 or 100 per cent of the air was replaced with carbon dioxide. These values did not necessarily represent the final concentrations of the carbon dioxide but, as measured by the Orsat apparatus, were within 5 per cent of the concentrations stipulated. The differences in the per cent CO_2 estimated and that obtained by measurement with the Orsat were due to leakage of apparatus and impurities in the commercial CO_2. The desiccators were placed in an incubator at 50° C. for 3 days; the samples were then analyzed for viable Salmonella. A total of four replicates were run and analysis of variance of the data showed that there was no significant difference ($P = .01$) in numbers of survivors due to the effects of the five treatments (0, 25, 50, 75 or 100 per cent CO_2) given this albumen. In the experiments in which albumens containing S. senftenberg, S. oranienburg or S. pullorum

were stored at 50° C. for 3 days in desiccators in which 95 to 97 per cent of the air was replaced with CO_2, the number of survivors was greater than when these powders were stored in air (Figure 16). It was observed (Table 10) that the death rate was reduced when air was replaced by CO_2. If one were to agree with Rahn (1945) who considered death of bacteria in a dried medium to be due to oxidation, one would not expect the presence of CO_2 to be of any significant value in reducing the number of survivors of Salmonella. On the other hand, substitution of an oxidizing agent for air would be expected to give greater reduction in the number of viable cells.

g. Storage in ethylene oxide. The data presented graphically in Figure 16 was obtained from experiments wherein albumen inoculated with S. senftenberg, S. oranienburg or S. pullorum was stored in desiccators in which from 9 to 11 per cent of the air was replaced with ethylene oxide.

There were fewer survivors in those desiccators which contained added ethylene oxide. Death rate constants calculated from these data appear in Table 10. The presence of ethylene oxide increased the death rate significantly ($P = .01$) in the case of S. pullorum and S. oranienburg; however, no difference in rate of death was observed in the case of S. senftenberg.

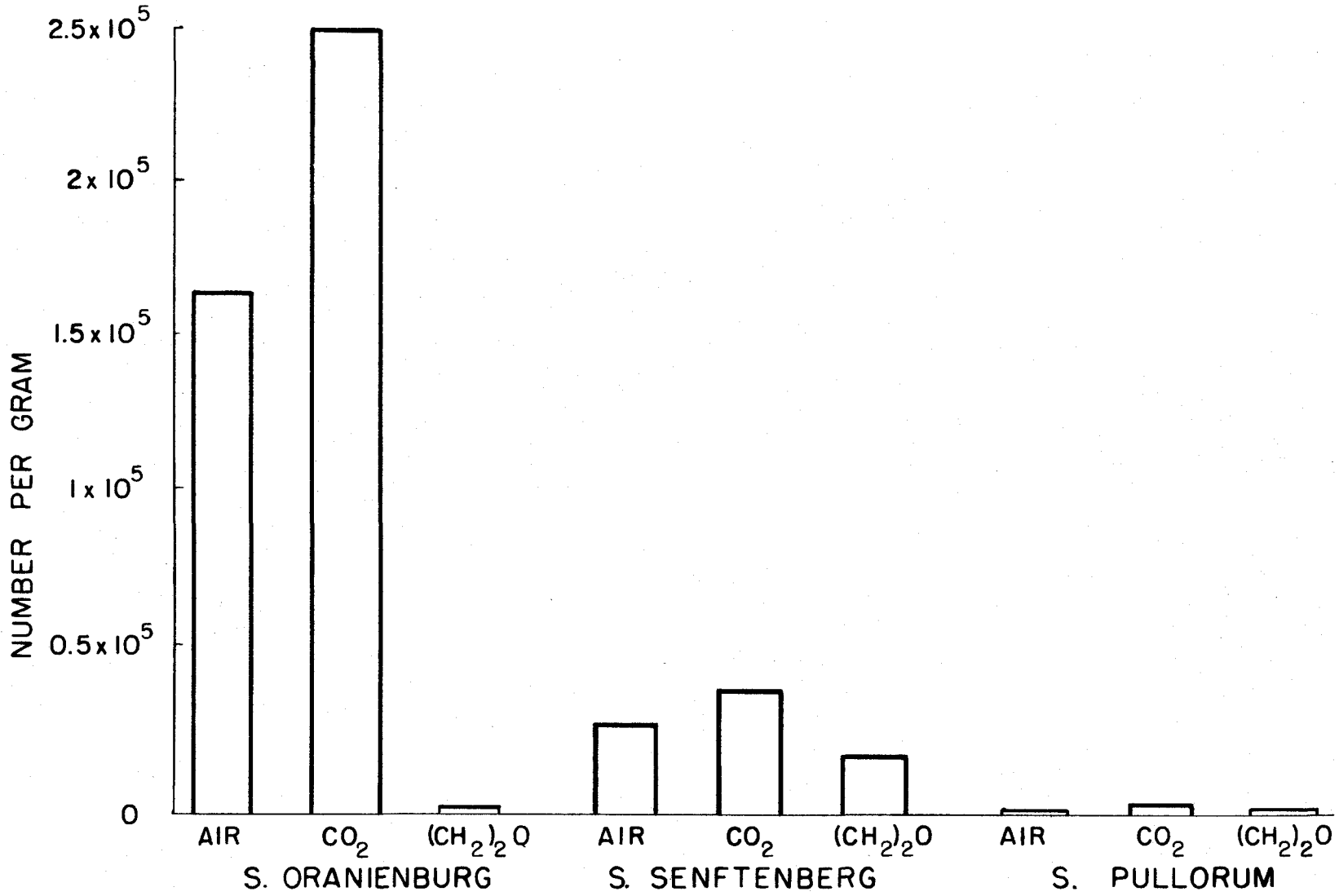


Figure 16. Effect of storage in air, ethylene oxide plus carbon dioxide or carbon dioxide on the survival of Salmonella

Table 10. Effects of storing pan dried albumen in air, carbon dioxide or ethylene oxide on the rate of death of Salmonella

Organism	Death rate constants[a]		
	Air	Carbon dioxide[b]	Ethylene oxide[c]
S. pullorum	1.448	.833	2.093[d]
S. senftenberg	.586	.551	.687
S. oranienburg	.288	.221	1.507[d]

[a] $\frac{1}{3} \log \frac{\text{initial number}}{\text{final number}}$.

[b] 95 to 97 per cent carbon dioxide in 3 to 5 per cent air.

[c] 9 to 11 per cent ethylene oxide, 83 to 88 per cent carbon dioxide plus 3 to 6 per cent air.

[d] Significant increase at 1 per cent level (compared to air).

The reduction in numbers of S. oranienburg due to the addition of ethylene oxide was of particular importance since it was shown previously that the rate of death of this organism at elevated temperatures was lower than was that of either S. senftenberg or S. pullorum.

2. Coliforms

a. Moisture content. The effect of varying the moisture content of albumen on the death rate of organisms

belonging to the coliform group can be observed in Figure 17. The same effects were noted in the case of coliforms as were observed for Salmonella; namely, that a faster rate of death was observed at 12 per cent moisture than at 6 per cent and at 6 per cent than at 3 per cent, while the death rates at 3 and at 1.5 per cent moisture were approximately the same. Statistical analysis revealed that the death rates were significantly affected by the moisture content of the powder but that the difference in death rates at 1.5 and 3 per cent was not statistically significant ($P = .01$).

b. Temperature of storage. The effect of temperature of storage upon the death rate of coliforms was not as noticeable as it was in the case of Salmonella; however there was a difference in the rate of death of coliforms at the three temperatures of storage (Figure 18). Statistical analysis revealed that these death rates were different at the 1 per cent level of significance. The average Q_{10} value listed in Table 9 was 1.70, a value in the range of that associated with chemical or biological reactions.

c. Type of dried product. Statistical analysis revealed no significant difference in the rate of death of coliforms in either spray dried or pan dried albumens ($P = .01$).

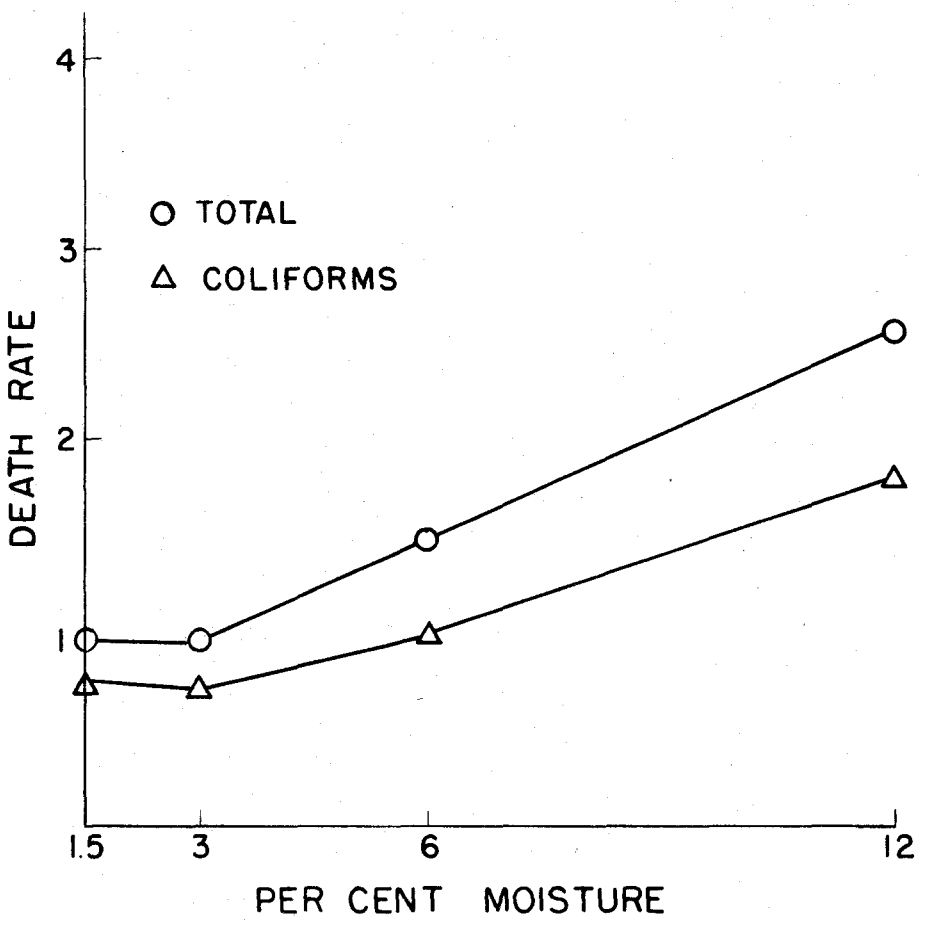


Figure 17. Effect of moisture content on the death rate of coliforms and the total population of organisms in pan dried and spray dried albumens stored at elevated temperatures (50°, 60° and 70° C.)

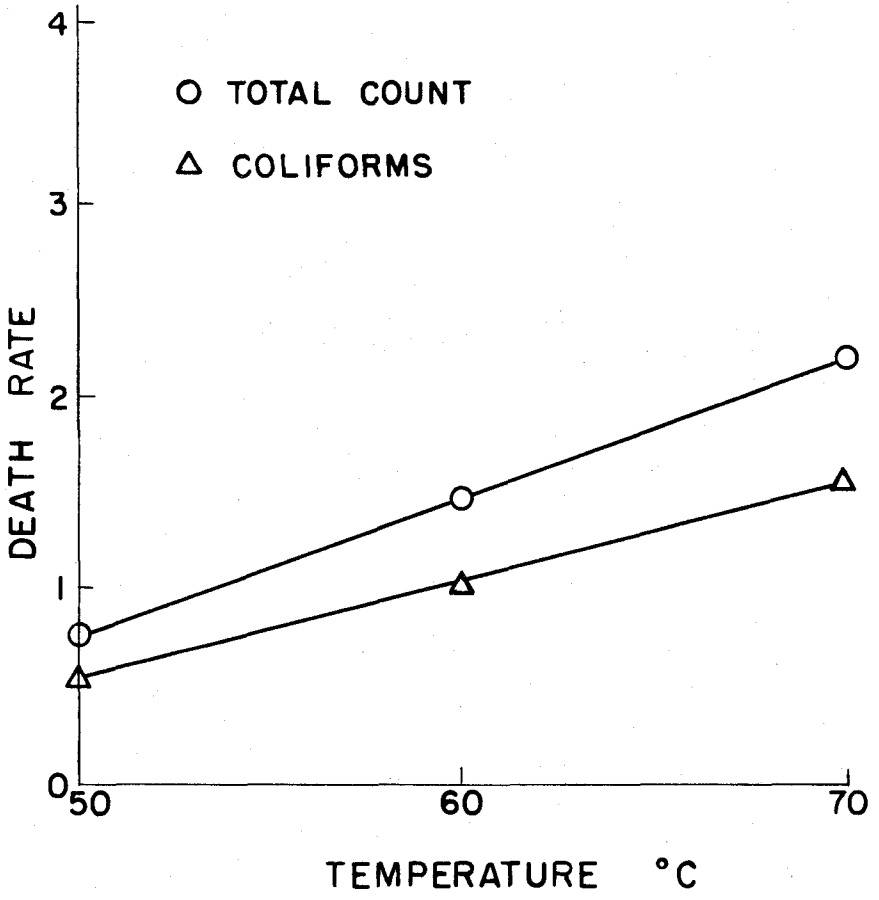


Figure 18. Effect of temperature of storage on the death rate of coliforms and total population of organisms in pan dried and spray dried albumens

3. Total population of organisms

In most cases the total population of organisms in the dried albumens was composed primarily of Salmonella; therefore, the total number of organisms decreased in a manner similar to that observed in the case of Salmonella.

a. Moisture content. The effect of moisture content on the death rate of total population of organisms can be observed in Figure 17. The differences in the rate of death at 3, 6 or 12 per cent moisture were statistically significant ($P = .01$); however, no statistical difference was found between the rate of death in albumen containing 3 per cent moisture and that containing 1.5 per cent moisture.

b. Temperature of storage. The total number of bacterial cells decreased at a faster rate as the temperature of storage increased. This is shown in Figure 18. A straight line function existed when comparing the rate of death with the temperature of storage. The death rates at the three temperatures were analyzed statistically and were found to be significantly different ($P = .01$). The average Q_{10} value (Table 9) was 1.70.

c. Type of dried powder. The rates of reduction in numbers of total population of organisms during storage of spray dried or pan dried albumens were compared. The average rate of death during storage of spray dried albumen was

1.88 and of pan dried albumen was 1.15; when these values were analyzed statistically, they were found to be different at the 1 per cent level of significance.

D. Functional Values of Dried Albumen During Storage

Even though dried albumen can be stored at elevated temperatures so that no Salmonella are subsequently isolated, it is essential that the functional properties of the albumen are not impaired by this treatment; otherwise the use of elevated temperatures to eliminate Salmonella is of little value.

The properties of albumen that are of primary concern are pH, solubility, whipping ability and angel cake-making properties.

1. pH

Measurements of the hydrogen ion concentration listed in Table 11, refer to dried albumen that contained 1.5, 3, 6 or 12 per cent moisture and was stored at 50°, 60° or 70° C. In general, there was a tendency for the pH of the dried albumen to decrease during storage; a change from pH 7.9 to pH 7.4 was noted in albumen containing 1.5 per

Table 11. Changes occurring in the pH[a] of dried albumen during storage

Temperature °C.	Moisture per cent	Days of storage								
		0	1	3	6	30	60	90	120	150
50	1.5	7.9	7.9	7.8				7.7[b]		7.5
	3	7.7	7.7	7.7			7.5[b]		7.6	
	6	7.9	7.8	7.7[b]			7.4	7.3		
	12	7.8	7.8	7.8		7.3[b]	7.3			
60	1.5	7.9	7.8	7.8			7.5[b]		7.4	
	3	7.7	7.7	7.7		7.5[b]	7.4			
	6	7.9	7.8	7.6[b]		7.4				
	12	7.8	7.7	7.6[b]	7.4					
70	1.5	7.9	7.8	7.7[b]		7.5		7.6		
	3	7.7	7.6	7.5[b]		7.3		7.3		
	6	7.9	7.6[b]	7.4	7.4	7.3				
	12	7.8	7.6[b]	7.4	7.4					

[a]Average of 3 determinations.

[b]Significant difference at 1 per cent level.

cent moisture, when it was stored at 60° C. for 120 days. In 7 of the 12 different storage conditions studied, a significant change occurred in the pH of the dried albumen by 3 days of storage; by 90 days, there was a significant change in pH of the albumen with any storage condition used.

2. Solubility

In order to compensate for the range of moisture levels in the stored powder (1.5 per cent to 12 per cent) the solubility values obtained by analysis were determined on a per gram dry solids basis. These values are listed in Table 12.

In general, there did not appear to be any serious loss of solubility of dried albumen under the conditions of storage used in these experiments. Statistical analysis of the data revealed that in only three instances did a significant ($P = .01$) loss of solubility occur due to conditions imposed upon the product. Significant changes were observed in those albumens that contained 6 per cent moisture and that were stored at 70° C. for 30 days, and albumens containing 12 per cent moisture and stored at 60° C. for 6 days or at 70° C. for 1 day.

Table 12. Changes in solubility[a] of dried albumen during storage

Storage temperature °C.	Albumen moisture content per cent	Per cent soluble material								
		Days of storage								
		0	1	3	6	30	60	90	120	150
50	1.5	96.3	92.7	98.0				96.3		92.0
	3	95.3	95.3	94.3			96.3		96.7	
	6	95.0	96.0	95.0			97.7	95.7		
	12	97.3	95.7	93.3		91.3	95.0			
60	1.5	96.3	94.7	97.3			95.3		95.7	
	3	95.3	96.0	96.7		96.3	97.0			
	6	95.0	95.7	94.7		95.3				
	12	97.3	87.7	90.0	79.7[b]					
70	1.5	96.3	94.3	98.7		94.7	95.0			
	3	95.3	94.0	95.7		94.7	92.3			
	6	95.0	95.0	92.3	93.0	83.3[b]				
	12	97.3	71.3[b]	54.0	47.0					

[a]Average of three determinations corrected for moisture content.

[b]Significant at 1 per cent level.

3. Beating rate

a. General. The beating rates during storage of albumens containing 1.5, 3, 6 or 12 per cent moisture and stored at 50°, 60° or 70° C. are depicted in Figure 19 and listed in Tables 13 and 14.

Analysis of variance was used to determine if the changes occurring in the beating rates shown in Figure 19 were of consequence. The beating rate was significantly reduced ($P = .01$) when albumen with 12 per cent moisture was stored at 60° or at 70° C. for 1 day. The other conditions of storage tested in these experiments did not reveal significant change in the beating rate of the albumen.

In some cases the beating rate increased after storage of the albumen, indicating that the albumen improved in this characteristic. The graph of changes in beating rate presented by Carlin and Ayres (1951) also showed this occurrence. Bergquist (1951) observed that beating properties of dried albumen improved during storage. Errors and variations incurred in the analysis might be suggested as possible reasons for an apparent improvement in whipping properties of albumen during storage, but the number of times that this occurred indicated that a more likely answer is that there was a change in the protein structure of the albumen which resulted in an improvement in the

Figure 19. Changes occurring in the beating rate of spray dried albumen with 1.5, 3, 6 or 12 per cent moisture and stored at 50°, 60° and 70° C.

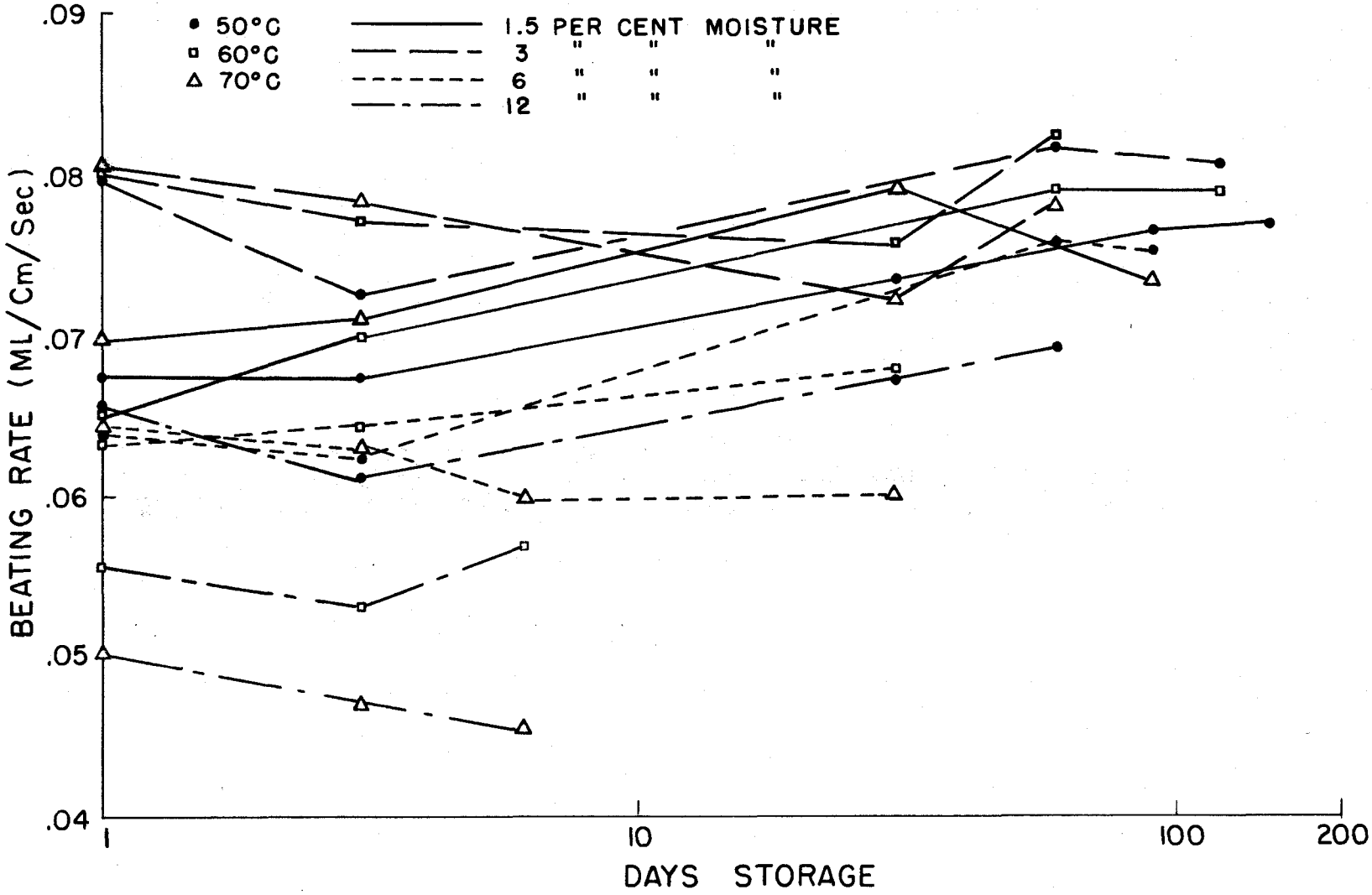

.09
.08
.07
.06
.05
.04
BEATING RATE (ML/Cm/Sec)
• 50°C
▫ 60°C
△ 70°C
1.5 PER CENT MOISTURE
3 " " "
6 " " "
12 " " "
1
10
100
200
DAYS STORAGE

Table 13. Beating rate[1] of spray dried albumen during storage

Storage temperature °C.	Albumen moisture content per cent	Days of storage								
		0	1	3	6	30	60	90	120	150
		ml./ gm./ sec.								
50	1.5	.083		.083				.084		.095
	3	.081		.079			.084		.087	
	6	.082	.077	.079			.085	.088		
	12	.079	.079	.072		.074	.083			
60	1.5	.083		.078			.084		.089	
	3	.081		.083		.079	.083			
	6	.082	.081	.084		.080				
	12	.079	.071	.072	.074					
70	1.5	.083		.086		.082		.083		
	3	.081		.083		.079	.081			
	6	.082	.075	.081	.075	.078				
	12	.079	.075	.073	.067					

[1]Calculated from data obtained from whip test described by Slosberg et al. (1948).

Table 14. Beating rate[1] of spray dried albumen during storage

Storage temperature °C.	Albumen moisture content per cent	Days of storage								
		0	1	3	6	30	60	90	120	150
		ml./ gm./ sec.								
50	1.5	.207		.211				.206		.222
	3	.252		.228			.197		.215	
	6	.202	.195	.182			.184	.191		
	12	.180	.180	.140		.148	.199			
60	1.5	.207		.188			.218		.204	
	3	.252		.229		.221	.219			
	6	.202	.195	.179		.151				
	12	.180	.117[a]	.108	.120					
70	1.5	.207		.186		.203		.172		
	3	.252		.229		.219	.202			
	6	.202	.195	.180	.180	.197				
	12	.180	.105[a]	.113	.146					

[1]Calculated from data obtained from 'soft peak' test described by Carlin and Ayres (1951).

[a]Significant difference (P = .01).

whipping properties. Analysis of variance of the data revealed that in many cases the increase in beating rate was significant.

b. Comparison of two whip tests. The whip tests described by Slosberg et al. (1948) and by Carlin and Ayres (1951) were used to obtain data to calculate the beating rate of albumens that were stored at elevated temperatures. The beating rates are listed in Tables 13 and 14.

It can be seen that the beating rates calculated from data obtained by the method of Slosberg et al. were lower than were those obtained from the whipping method of Carlin and Ayres. Probably this was due to the fact that in the former method a standard whipping time of 75 seconds was used and, in many cases, the albumen was over-whipped. In the latter method, the albumen was whipped to a point whereby tracks from the wire whip were observed in the meringue; hence the whipping times in this procedure were not constant but were dependent upon the quality of the albumen.

Comparison was made of the calculated beating rates obtained from data of two whipping methods by determining the correlation of these values. It was found that the correlation coefficient (r) was equal to .65 which showed that a relationship existed between the results acquired by using the two methods for determining the whipping properties of dried albumen.

4. Volume of angel cakes

The ultimate criterion for the functional properties of albumen is its ability to form a stable foam for use in producing angel cakes of good volume and texture. The volumes of angel cakes made from albumens containing 1.5, 3, 6 or 12 per cent moisture and stored at 50°, 60° or 70° C. for from 0 to 150 days, are listed in Table 15. At a storage temperature of 50° C., albumens containing 1.5, 3 or 6 per cent moisture were stored for 150, 120 or 90 days, respectively, without any significant loss in volume of angel cakes, while the albumen with 12 per cent moisture showed a significant decrease in volume of the resulting angel cakes at 3 days of storage. At a storage temperature of 60° C. albumen having 1.5 per cent moisture was stored for 120 days without a significant loss in volume in the resultant angel cakes. The volume of angel cakes showed no significant loss when made from albumen containing 1.5 per cent moisture and stored at 70° C. for 30 days; however, a significant ($P = .01$) loss in volume was noted after storage for 1 day when the albumens contained 12 per cent moisture.

Thus it is evident that moisture content and temperature of storage are important aspects to be considered in the storage of albumen which is intended for use in angel cakes.

Table 15. Volume[a] of angel cakes baked with albumen stored at elevated temperatures

Storage temperature °C.	Albumen moisture content per cent	Days of storage								
		0	1	3	6	30	60	90	120	150
		Vol. in ml.								
50	1.5	581		577				569		582
	3	579		570			572		583	
	6	572	562	562			550	571		
	12	546	558	514[b]		494	534			
60	1.5	581		555			555		562	
	3	579		576		550	559			
	6	572	566	541[b]		519				
	12	546	479[b]	385	416					
70	1.5	581		554		535[b]		503		
	3	579		564		545[b]	545			
	6	572	565	562	522[b]	517				
	12	546	473[b]	391	367					

[a]Volume in ml. per 100 gm. of batter (average of 3 cakes).

[b]Significant difference at 1 per cent level.

A limited number of studies were conducted to ascertain if albumen stored in atmospheres containing ethylene oxide would lose its angel cake-making properties to a greater extent than would albumen stored in air. Albumens containing 6 per cent moisture were stored at 50° C. for 3 days in desiccators in which air or a mixture of 10 per cent ethylene oxide and 90 per cent carbon dioxide was the atmosphere of storage. The volume of angel cakes made from these albumens (587 cc. for the air and 555 cc. for the ethylene oxide mixture) indicated that there was a 5.5 per cent loss in volume of resulting angel cakes due to the presence of ethylene oxide and carbon dioxide in the atmosphere during storage. Analyzing these values revealed that there was a significant loss ($P = .01$) of volume of angel cakes due to storing albumen in the presence of ethylene oxide.

5. Discussion

Measurements of pH, solubility, beating rate and volume of angel cake were made to determine the effect of moisture content, temperature of storage and length of storage period on the functional properties of albumen. Of these characteristics, the pH of the albumen appeared to change more significantly than did the other properties. The changes exhibited in the pH indicated that acidic type

reactions occurred. This increase in acidity could be due to exposure of the acidic group of amino acids during denaturation of the protein, particularly if dicarboxylic amino acids were prevalent. Stuart et al. (1942), Hirschmann and Lightbody (1947) and Wilson (1948) observed a similar change in pH of dried whole egg during storage. The only explanation of this observation was given by Hirschmann and Lightbody who suggested that changes in pH were related to the activity of non-reproducing bacteria, or to cell components (presumably enzymes) remaining active after the death of the organism.

The results of solubility measurements revealed that albumen can be stored under rather drastic conditions and still retain its solubility. In some cases an apparent increase in solubility was observed. This type of result was also reported by Kline (1945), but no reason was given. Some variation could be expected due to the method of analysis; however, it is more likely that apparent increases in solubility are due to the formation of smaller components of the protein during denaturation. These smaller compounds could then polymerize and become insoluble and, in turn, result in a decrease in the solubility of the powder. Kline (1945) believed that the loss in solubility of albumen was due to reactions of the proteins with the aldehyde group of glucose. The albumen used in this study could have

contained some residual glucose. However, the quantity was too small to be detected by the Somogyi method; thus it is doubtful if losses of solubility were due to the amino-carbonyl reaction. In most cases the solubility of the albumen did not decrease significantly due to the conditions imposed upon the product. Only in the case of albumen containing 12 per cent moisture and which was stored at 60° or 70° C. did a serious loss of solubility occur.

The whip test measures the foaming action of egg white. According to Barmore (1934) the colloidal constituents of the albumen form an interfacial film which becomes sufficiently viscous to result in a stable foam. Most of the loss in beating rate in these experiments was probably due to insolubility of the albumen, since, in the two cases in which beating rate of the meringue was significantly decreased, there was also a significant decrease in the solubility of the albumen. If the albumen failed to reconstitute, less protein would be available for formation of interfacial surfaces, hence less volume would be obtained and the beating rate of the meringue would be decreased.

Calculations were made to determine the correlation between the 75-second whip test and volumes of angel cakes and the "soft peak" whip test and volumes of angel cakes. It was found that a direct relationship existed in both cases; a value of 0.74 was found for the 75-second whip

test and 0.77 was determined for the "soft peak" test. These correlations were tested for significance with the 't' test and were found to be significant at the 1 per cent level. It is interesting to note that the difference between the correlation values was only 0.03, which indicated that both tests were of almost the same value in measuring the angel cake-making ability of the albumen.

The use of albumen in angel cakes requires not only that albumen can be whipped, but also that the resulting foam is stable so that, during baking, this foam will coagulate and retain the entrapped air, thus producing an angel cake of large volume.

The results of these experiments showed that dried albumen could be stored for 150 days at 50° C. if the moisture content were 1.5 per cent, without a significant loss in angel cake-making properties. At 60° C. this albumen (1.5 per cent moisture) was stored for 120 days and still retained its angel cake-making ability. However, as the moisture content and temperature of storage of the albumen was increased, the ability of the albumen to form angel cakes of desirable volume was decreased rapidly, so that at a moisture content of 12 per cent and storage temperatures of 60° or 70° C., a significant loss was observed at 1 day of storage; at 50° C., a storage period of

3 days resulted in a significant loss of angel cake-making properties.

E. Comparison of the Effects of Storage on Microorganisms and on the Functional Properties of Dried Albumen

It was shown that high initial numbers of S. senftenberg, S. oranienburg or S. pullorum were reduced to less than 0.18 organisms per gram during storage of albumen at 50°, 60° or 70° C. However, it was likewise found that the functional properties of dried albumen were changed when the powder was stored at the three temperatures.

Previously it was pointed out that in order for storage of dried albumen to be practical in the elimination of Salmonella, the functional properties must not be impaired. The time required to eliminate S. senftenberg, S. oranienburg and S. pullorum from spray dried albumens was compared with the days the albumen could be stored without significant changes occurring in the functional properties. These data are presented in Figures 20 to 22. The curves depicting storage at 50° C. (Figure 20) showed that the albumens with 6 per cent moisture or less retained their functional properties for a period sufficiently long to obtain a powder

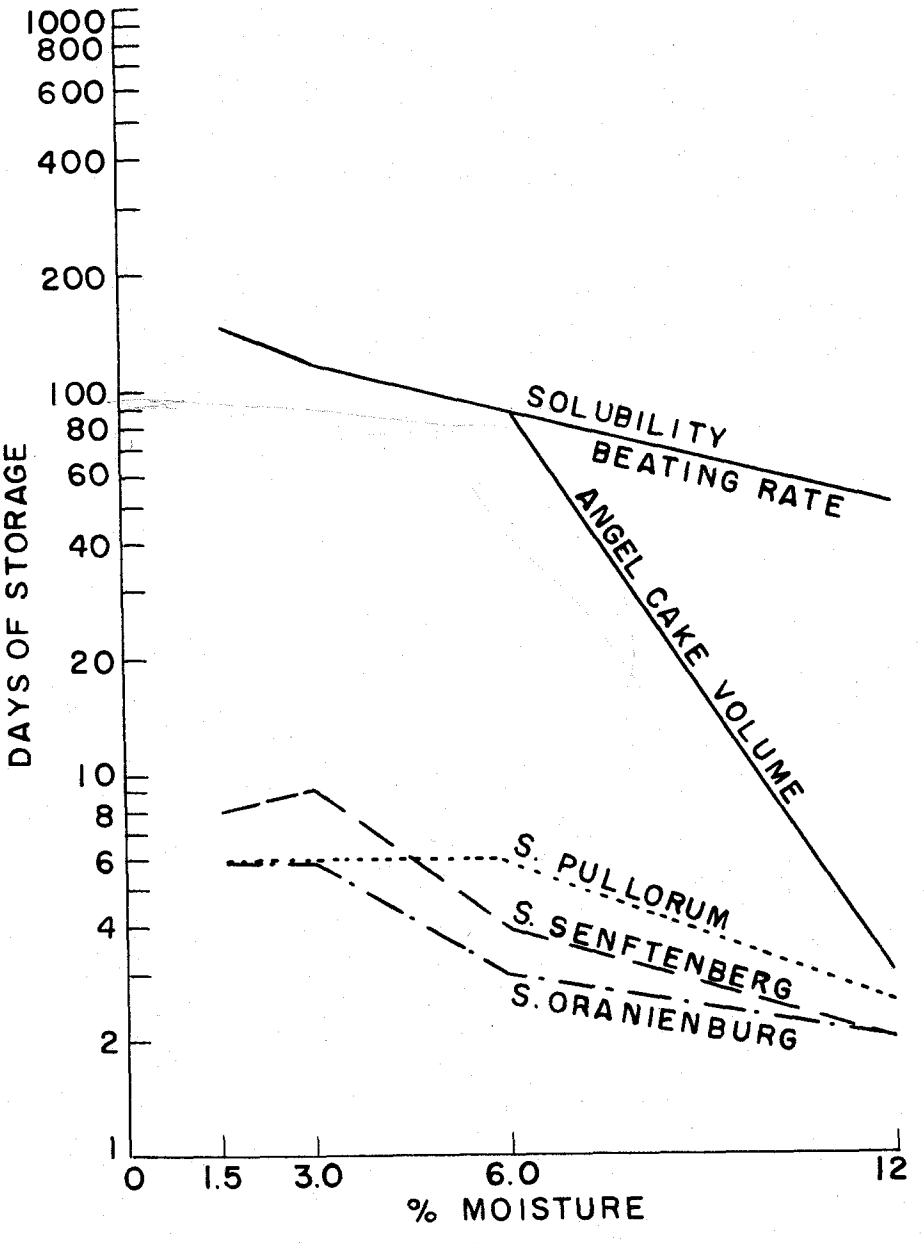


Figure 20. Limiting conditions for storing spray dried albumen at 50° C. (Areas above the solid lines indicate times of storage for each of the moisture levels where there were observable losses in functional properties. Areas above the broken lines indicate time-moisture relationships where populations of the Salmonella species were reduced to less than 0.18 cells per gram.)

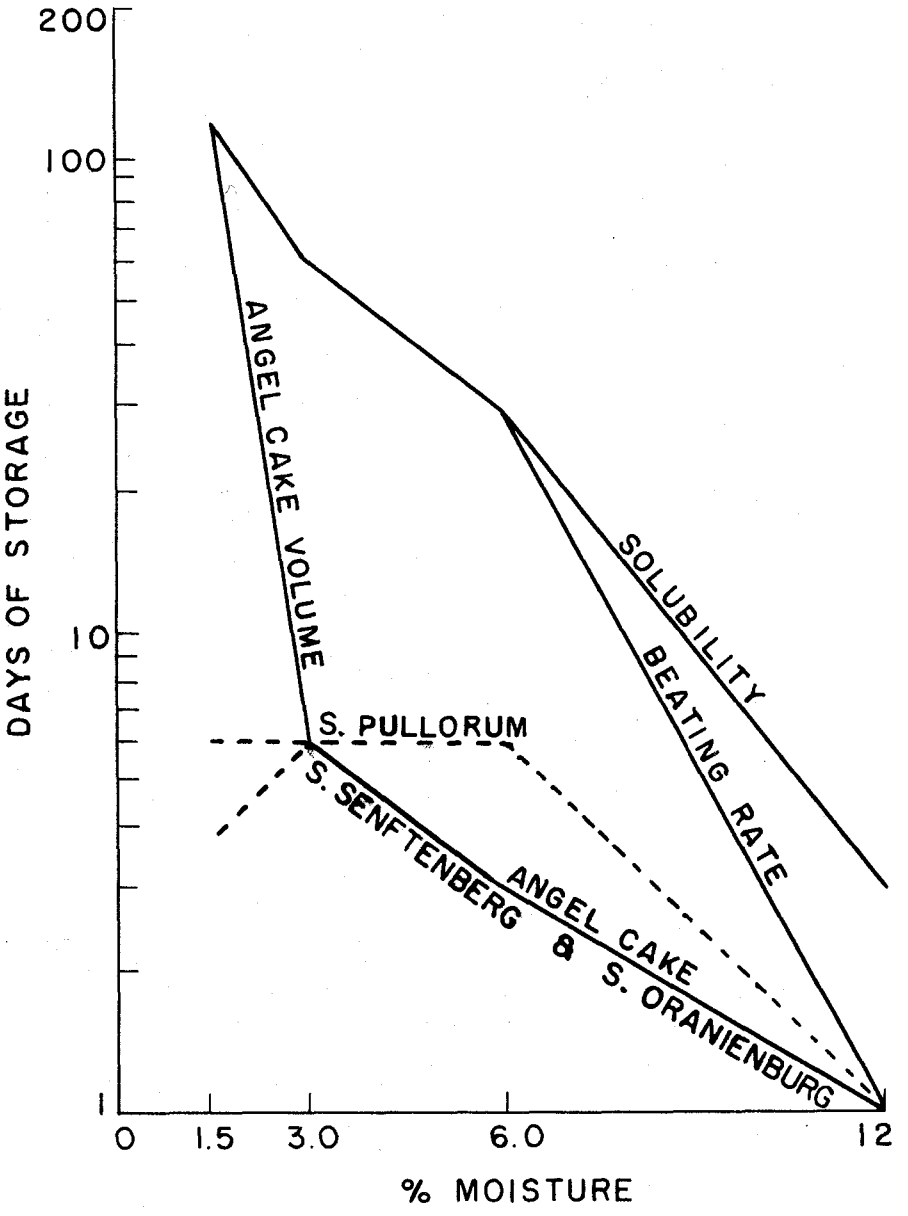


Figure 21. Limiting conditions for storing spray dried albumen at 60° C. (Areas above the solid lines indicate times of storage for each of the moisture levels where there were observable losses in functional properties. Areas above the broken lines indicate time-moisture relationships where populations of the Salmonella species were reduced to less than 0.18 cells per gram.)

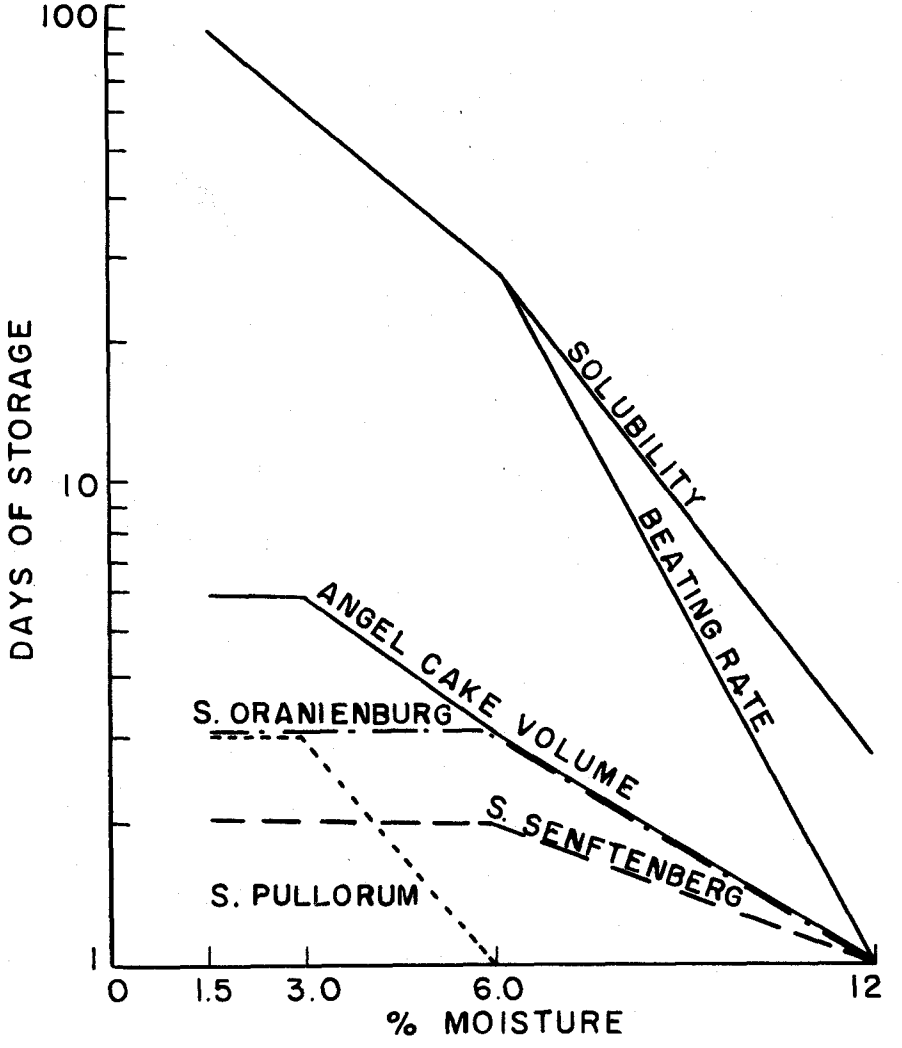


Figure 22. Limiting conditions for storing spray dried albumen at 70° C. (Areas above the solid lines indicate times of storage for each of the moisture levels where there were observable losses in functional properties. Areas above the broken lines indicate time-moisture relationships where populations of the Salmonella species were reduced to less than 0.18 cells per gram.)

from which no Salmonella could be isolated. The angel cake-making properties of albumen with 12 per cent moisture deteriorated at a rate that was greater than the reduction in numbers of Salmonella. The beating rate and solubility of the albumen were retained sufficiently long for the Salmonella to be eliminated.

When a storage temperature of 60° C. was used (Figure 21), the number of Salmonella was reduced to less than 0.18 per gram before the solubility of the albumen at any of the moisture levels tested was significantly ($P = .01$) reduced; however, the beating rate was significantly lower with albumen containing 12 per cent moisture and angel cake-making properties were impaired in albumen with either 6 or 12 per cent moisture.

At a storage temperature of 70° C. (Figure 22) the solubility and beating rate of albumens were retained in those powders containing 6 per cent moisture or less; on the other hand, at any of the four moisture levels, the angel cake-making properties of the albumen were significantly decreased by the time the number of Salmonella was reduced to less than 0.18 per gram.

It was evident from these curves that in the case of albumen with a moisture content of 12 per cent, storage at elevated temperatures was not too desirable as a means of eliminating Salmonella, since the functional properties were

seriously impaired. In the time period required to eliminate Salmonella from albumen containing 6 per cent moisture, a storage temperature of 60° C. was detrimental to the angel cake-making properties; however, if the albumen was not intended for use in angel cakes, a storage temperature of 70° C. could be maintained for as long as 60 days without significant losses of solubility or beating rate. In the case of albumens with 1.5 or 3 per cent moisture and a storage temperature of 50° or 60° C., functional properties of the albumen were retained until the Salmonella were eliminated. The angel cake-making ability of the albumen was impaired at 70° C., but if not intended for this use, the albumens could be stored at this temperature for 60 days without significant loss of beating rate or solubility; this time period was definitely adequate to secure albumen from which no Salmonella could be recovered.

V. CONCLUSIONS

1. Selenite-F, tetrathionate, Ruys' medium or liquid egg white do not support the growth of Salmonella as well as does nutrient broth. The organisms tested reproduce equally well in tetrathionate or in Selenite-F.

2. Liquid albumen adjusted to an alkaline pH represses the rate of reproduction of S. senftenberg, S. oranienburg and S. pullorum.

3. According to the results of the experiments reported herein, the addition of liquid whole egg to enrichment broths results in less retardation of growth of Salmonella than occurs in enrichment broths containing no whole egg. The effect of whole egg addition is especially evident in the case of Ruys' medium.

4. The number of cells of three species of Salmonella present in egg white is lowered by more than 90 per cent during the first 12 hours of incubation when chlortetracycline, oxytetracycline or streptomycin is added at a level of 0.1 ppm.

5. Spray drying of albumen results in a lower per cent of survivors of Salmonella than does pan drying.

6. Comparing the rate of death of S. senftenberg, S. oranienburg and S. pullorum shows that S. oranienburg is the

most resistant while S. pullorum is the least resistant to death during storage of dried albumen at elevated temperatures of 50°, 60° or 70° C.

7. As the temperature of storage of dried albumen is increased from 50° to 70° C., the rate of death of Salmonella is increased proportionally.

8. Although there is no significant difference in the rate of death of three species of Salmonella in dried albumen containing 1.5 to 3 per cent moisture, the rate of death increases significantly as the moisture content of the powder is increased from 3 per cent to 12 per cent.

9. The use of carbon dioxide in the storage atmosphere of dried albumen does not increase the rate of death of the Salmonella present.

10. Although the use of ethylene oxide in the storage atmosphere aids in reducing the numbers of the organisms tested, the functional properties of the albumen are impaired by the presence of this gas.

11. The functional properties of albumens containing 12 per cent moisture are impaired during storage at 50°, 60° or 70° C. Therefore, storage of albumen with a 12 per cent moisture level is not desirable at any of these temperatures.

12. It is possible to store dried albumen containing 1.5, 3 or 6 per cent moisture at 50° C. or albumen

containing 1.5 or 3 per cent moisture at 60° or 70° C. and eliminate large numbers of Salmonella without significantly impairing the functional properties of the product.

13. High initial counts of Salmonella can be eliminated in dried albumen containing 1.5, 3 or 6 per cent moisture and stored at 50°, 60° or 70° C. without noticeably affecting either beating rate or solubility.

VI. SUMMARY

The growth of eight species of Salmonella in Selenite-F, tetrathionate, Ruys' or nutrient broth with and without the addition of whole egg was studied. Selective agars (BG, WB, DCLS and SS) were compared for their ability to support the growth of six species of Salmonella.

The reproduction of three species of Salmonella (S. senftenberg, S. oranienburg and S. pullorum) was observed in liquid albumen adjusted to pH 6.0, 7.0, 8.0, 9.0 or 10.0, and in liquid albumen containing 0.1, 1 or 10 ppm. of the antibiotics chlortetracycline, oxytetracycline or streptomycin.

Liquid albumens were inoculated with S. senftenberg, S. oranienburg or S. pullorum and subsequently spray dried or pan dried. After drying, the albumens were adjusted to 1.5, 3, 6 or 12 per cent moisture and stored at 50°, 60° or 70° C. During storage of the albumens, Salmonella, coliform and total counts were determined; also the solubility of the dried product and the pH, beating rate and angel cake-making ability of the reconstituted product were measured.

The results of the tests of enrichment broths indicated that none of the broths supported the growth of Salmonella

as well as did nutrient broth; however, Selenite-F or tetrathionate proved to support the growth of the eight species of Salmonella better than did Ruys' medium. Liquid whole egg seemed to mask the property or properties of the broths that were inhibitory to these organisms; the effect of adding whole egg was especially evident in the case of Ruys' medium.

Growth of six species of Salmonella on four differential agars showed that BG was less inhibitory than were the other three selective agars (DCLS, SS, WB). DCLS and SS were found to be inhibitory to all of the organisms when tested at the 1 per cent level of significance, while WB significantly inhibited four of the six species of Salmonella used in this phase of the study.

The growth of three species of Salmonella was retarded in egg white at an alkaline pH; this was particularly noticeable at pH 9.0 and pH 10.0.

The antibiotics chlortetracycline, oxytetracycline or streptomycin incorporated into egg white or nutrient broth retarded the growth of S. senftenberg, S. oranienburg and S. pullorum. These antibiotics (0.1 ppm.) retarded the growth of Salmonella in egg white for 12 hours, but were ineffective when incubation was prolonged for 24 hours.

As the temperature of storage of dried albumen was increased from 50° to 70° C., the rate of death of Salmonella

was increased proportionally. In general, as the moisture content of albumen was increased, the death rate of Salmonella was increased. There was no apparent difference in the rate of death of Salmonella in albumen containing either 1.5 or 3 per cent moisture.

Under the conditions of this study, there was less loss of viability of S. oranienburg during storage of dried albumen than was observed in the case of either S. senftenberg or S. pullorum. These species of Salmonella survived storage better in pan dried albumens than they did in spray dried albumens.

Volume of resultant angel cakes was more affected by storage of dried albumen than were either solubility or beating rate of the albumens. However, it was possible to store dried albumen containing 1.5 or 3 per cent moisture at 60° or 70° C. and eliminate large loads of Salmonella without significantly impairing the angel cake-making properties of the reconstituted albumen. High counts of Salmonella were eliminated in dried albumen containing 1.5, 3 or 6 per cent moisture and stored at 50°, 60° or 70° C. without noticeably affecting either solubility of the dried albumen or beating rate of the reconstituted product.

VII. LITERATURE CITED

Adams, A. T., Ayres, J. C. and Tischer, R. G.
1951. The effect of subtilin and heat in preventing spoilage in comminuted beef. Food Technol. 5: 82.

_____, _____, _____ and Ostle, B.
1952. Effect of subtilin on spoilage of thermal processed beef. Food Technol. 6: 421-424.

Alderton, G., Ward, W. H. and Fevold, H. L.
1946. Identification of the bacteria-inhibiting, iron-binding protein of egg white as conalbumin. Arch. Biochem. 11: 9-13.

Allen, F. W. and Smock, R. M.
1937. Carbon dioxide storage of apples, peas, plums and peaches. Proc. Am. Soc. Hort. Sci. 35: 193-199.

Andersen, A. A. and Michener, A. D.
1950. Preservation of foods with antibiotics. I. The complementary action of subtilin and mild heat. Food Technol. 4: 188-189.

Anellis, A., Lubas, J. and Rayman, M. M.
1954. Heat resistance in liquid eggs of some strains of the genus Salmonella. Food Research. 19: 377-395.

Association of Official Agricultural Chemists.
1950. Official Methods of Analysis of the Association of Official Agricultural Chemists. 7th edition. Washington, D. C.

Ayres, J. C.
1949. A procedure for the quantitative estimation of Salmonella in dried egg products. Food Technol. 3: 172-176.

_____ and Slosberg, H. M.
1949. Destruction of Salmonella in egg albumen. Food Technol. 3: 180-183.

_____ and Stewart, G. F.
1947. Removal of sugar from raw egg white by yeast before drying. Food Technol. 1: 519-526.

Bainbridge, F. A.
1911. The action of certain bacteria on proteins. J. Hyg. 11: 341-355.

Baldwin, R. B., Campbell, H. A., Thiessen, R. J. and Lorant, G. J.
1953. The use of glucose oxidase in the processing of foods with special emphasis on the desugaring of egg white. Food Technol. 7: 275-282.

Balls, A. K. and Swenson, T. L.
1934. The anti-trypsin of egg white. J. Biol. Chem. 106: 409-419.

_____ and _____.
1936. Dried egg-white. Food Research. 1: 319-324.

Barker, H. A.
1933. The effects of water content upon the rate of heat denaturation of crystallizable egg albumin. J. Gen. Physiol. 17: 21-34.

Barmore, M. A.
1934. The influence of chemical and physical factors on egg white foams. Colo. Agr. Expt. Sta. Tech. Bull. 9.

_____.
1936. The influence of various factors, including altitude, in the production of angel food cake. Colo. Agr. Expt. Sta. Tech. Bull. 15.

Bartram, M. T.
1943. Bacteriological changes during dehydration of eggs. J. Bacteriol. 45: 48.

Beigelman, P. M.
1949. In vitro determination of bacterial sensitivity to aureomycin. Proc. Soc. Exptl. Biol. Med. 72: 89-92.

Bergquist, D. H.
1951. Functional properties of egg white as influenced by atomization and drying. Unpublished Ph.D. Thesis. Ames, Iowa, Iowa State College Library.

Bernard, C., Slosberg, H. M., Lowe, B. and Stewart, G. F.
1948. Factors influencing performance of egg white in angel cake. Poultry Sci. 27: 653-654.

Best, L. R.
1944. Some factors affecting the production and storage of low moisture dried eggs. Unpublished M.S. Thesis. Ames, Iowa, Iowa State College Library.

Bier, M., Duke, J. A., Gibbs, R. J. and Nord, F. F.
1952. Composition and fractionation of ovomucoid. Arch. Biochem. Biophys. 37: 491-493.

_____, Terminiello, L., Duke, J. A., Gibbs, R. J. and Nord, F. F.
1953. Investigations on proteins and polymers. X. Composition and fractionation of ovomucoid. Arch. Biochem. Biophys. 47: 465-473.

Blomberg, C. G.
1932. How eggs are dried. Food Ind. 4: 100-102.

Blount, W. P.
1949. The public health aspects of poultry disease. J. Roy. Sanitary Inst. 69: 359-366.

Bohrer, C. W.
1951. National Canners Association experimental program with subtilin. N.C.A. Information Letter No. 1325: 95-98.

Bollenback, C. H.
1949. Removal of glucose from egg albumen by a controlled fermentation. Unpublished Ph.D. Thesis. Ames, Iowa, Iowa State College Library.

Bornstein, S., Saphra, L. and Strauss, L.
1941. Frequency of occurrence of Salmonella species. J. Infect. Dis. 69: 59-64.

Branham, J. R. and Shepherd, M.
1939. Gasometric method and apparatus for the analysis of mixtures of ethylene oxide and carbon dioxide. J. Research Natl. Bur. Standards. 22: 171-189.

Broh-Kahn, R. H.
1946. The laboratory diagnosis of enteric infections caused by the Salmonella-Shigella group. Military Surgeon. 99: 770-776.

Brooks, C., Bratley, C. O. and McColloch, L. P.
1936. Transit and storage diseases of fruits and vegetables as affected by initial carbon dioxide treatments. U.S. Dept. Agr. Tech. Bull. 519.

Brown, H. J. and Gibbons, N. E.
1950. Enterococci as an index of fecal contamination in egg products. Can. J. Research, sect. F. 28: 107-117.

Bumzahnov, A. D.
1944. Dehydration of egg products. U.S. Egg and Poultry Mag. 50: 198-202, 231, 234-236, 259-262, 282, 284-285, 312-315, 327-334, 356-358, 374-375.

Burroughs, J. D. and Wheaton, E.
1951. Results of studies on the preservative action of antibiotics in processed foods. N.C.A. Information Letter No. 1325: 99-101.

Bushnell, L. D. and Maurer, O.
1914. Some factors influencing the bacterial content and keeping quality of eggs. Kansas Agr. Expt. Sta. Bull. 201.

Buxton, A.
1948. Avian Salmonellosis in England and Wales from 1939-1947. 8th World's Poultry Congress, Copenhagen, 1948. Official Report 1: 609-615.

Byrne, A. F.
1954. Incidence and types of Salmonella found in commercial processing of dehydrated egg products. Stability in dehydrated eggs; a symposium sponsored by the Quartermaster Food and Container Institute. p. 68-74.

Cahill, V. R., Kunkle, L. E., Goldberg, H., Weiser, H. H. and Deatherage, F. E.
1952. Exploratory studies on the processing of fresh beef by the infusion of antibiotics. J. An. Science. 11: 747.

Cantor, A. and McFarlane, V. H.
1948. Salmonella organisms on and in chicken eggs. Poultry Sci. 27: 350-355.

Carlin, A. F. and Ayres, J. C.
1951. Storage studies on yeast-fermented dried egg white. Food Technol. 5: 172-175.

Carlin, A. F. and Ayres, J. C.
1953. Effect of the removal of glucose by enzyme treatment on the whipping properties of dried albumen. Food Technol. 7: 268-270.

Cathcart, W. H., Merz, A. and Ryberg, R.
1942. Staphylococci and Salmonella control in foods. IV. Effect of cooking bakery custards. Food Research. 7: 100-103.

Chase, F. E.
1947. Salmonella studies in fowl. Can. J. Public Health. 38: 82-83.

Curran, H. R. and Evans, F. R.
1946. The activity of penicillin in relation to bacterial spores and the preservation of milk. J. Bacteriol. 52: 89-98.

D'Albora, J. B., Ingegno, A. P. and Edson, J. N.
1945. Outbreak of food poisoning due to Salmonella montevideo in an army general hospital. Varied clinical manifestations and demonstrations of Salmonella and heterophile agglutinins. J. Am. Med. Assoc. 129: 10-12.

Darby, C. W. and Stafseth, H. J.
1942. Salmonella infections common to man, animals, and birds. Report of 46th Annual Meeting of the U.S. Livestock Sanitary Assoc. 1942: 189-202.

Dernby, G. K.
1921. La concentration optima in ions hydrogene favorisant le developpement de certains micro-organisms. Annales de L'Institute Pasteur. 35: 277-290.

Doyle, T. M.
1925. Bacillary white diarrhea of chicks. J. Comp. Path. and Thereap. 28: 266-281.

Eakin, R. E., Snell, E. E. and Williams, R. J.
1941. The concentration and assay of avidin, the injury-producing protein in raw egg white. J. Biol. Chem. 140: 535-543.

Edwards, P. R.
1939. Incidence of Salmonella types in fowls in the United States. 7th World's Poultry Congress, Cleveland, 1939. Official Report. 7: 271-274.

_____ and Bruner, D. W.
1938. Two new Salmonella types isolated from fowls. J. Hyg. 38: 716-720.

_____ and _____.
1941. Two new Salmonella types closely related to Salmonella muenchen. Am. J. Hyg. 34B: 121-124.

_____ and _____.
1943. The occurrence and distribution of Salmonella types in the United States. J. Infect. Dis. 72: 58-67.

_____, _____ and Moran, A. B.
1948a. The genus Salmonella. Its occurrence and distribution in the United States. Ky. Agr. Expt. Sta. Bull. 525.

_____, _____ and _____.
1948b. Further studies on the occurrence and distribution of Salmonella types in the United States. J. Infect. Dis. 83: 220-231.

Feeney, R. E. and Nagy, D. A.
1952. The antibacterial activity of the egg white protein conalbumin. J. Bacteriol. 64: 629-643.

Fleming, A.
1922. On a remarkable bacteriolytic element found in tissues and secretions. Proc. Roy. Soc. (London) 93B: 306-317.

Forsythe, R. H., Ayres, J. C. and Radlo, J. L.
1953. Factors affecting the microbiological populations of shell eggs. Food Technol. 7: 49-56.

Fraenkel-Conrat, H. and Feeney, R. E.
1950. The metal binding activity of conalbumin. Arch. Biochem. 29: 101-113.

_____, Snell, N. S. and Ducay, E. D.
1952a. Avidin. I. Isolation and characterixation of the protein and nucleic acid. Arch. Biochem. Biophys. 39: 80-96.

Fraenkel-Conrat, H., Snell, N. S. and Ducay, E. D.
1952b. Avidin. II. Composition and mode of action of avidin. Arch. Biochem. Biophys. 39: 97-107.

Frank, N. A. and Edgington, B. H.
1937. Agglutinin content of eggs from Salmonella pullorum infected hens. Poultry Sci. 16:442-444.

Gage, G. E., Paige, B. H. and Hyland, H. W.
1914. On the diagnosis of infection with Bacterium pullorum in the domestic fowl. Mass. Agr. Expt. Sta. Bull. 148.

Galton, M. M. and Hardy, A. V.
1948. Studies of acute diarrheal diseases. XXI. Salmonellosis in Florida. U.S. Public Health Repts. 63: 847-851.

Gerhardt, F. and Ryall, A. L.
1939. The storage of sweet cherries as influenced by carbon dioxide and volatile fungicides. U.S. Dept. Agr. Tech. Bull. 631.

Gibbons, N. E.
1947. Dried whole egg powder. XXVII. Further observations on the occurrence of Salmonella organisms in Canadian powder. Can. J. Research, sect. F. 25: 291-298.

_____ and Fulton, C. O.
1943. Dried whole egg powder. VII. Effect of temperature and moisture on the bacterial content of liquid and dried egg. Can. J. Research, sect. D. 21: 332-339.

_____, _____ and Reid, M.
1946. Dried whole egg powder. XXI. Pasteurization of liquid egg and its effect on quality of the powder. Can. J. Research, sect. F. 24: 327-337.

_____ and Moore, R. L.
1944a. Dried whole egg powder. XI. Occurrence and distribution of Salmonella organisms in Canadian powder. Can. J. Research, sect. F. 22: 48-57.

_____ and _____.
1944b. Dried whole egg powder. XII. The effect of drying, storage, and cooking on the Salmonella content. Can. J. Research, sect. F. 22: 58-63.

Gillespie, J. M., Scott, W. J. and Vickery, J. R.
1950. Studies in the preservation of shell eggs. II. The incidence of bacterial rotting in unwashed eggs and in eggs washed by hand. Australian J. Applied Sci. 1: 215-223.

Godkin, W. J. and Cathcart, W. H.
1952. Effect of antibiotics in retarding the growth of Micrococcus pyogenes var. aureus in custard filling. Food Technol. 6: 224-229.

Gohar, M. A.
1943. Sodium selenite as a bacteriostatic substance and its use in the isolation of paratyphoid bacilli. J. Trop. Med. Hyg. 46: 29-32.

Goldberg, H. S., Weiser, H. H., and Deatherage, F. E.
1953. Studies on meat. IV. The use of antibiotics in preservation of fresh beef. Food Technol. 7: 165-166.

Goresline, H. E.
1948. The Salmonella problem in egg and poultry products. 8th World's Poultry Congress, Copenhagen, 1948. Official Report 1: 604-608.

_____, Hayes, K. M., Moser, R. E., Howe, M. A. and Drewniak, F. E.
1951. Pasteurization of liquid whole egg under commercial conditions to eliminate Salmonella. U.S. Dept. Agr. Circ. 897.

_____ and McFarlane, V. H.
1944. Handling eggs for drying. U.S. Egg and Poultry Mag. 50: 116-119, 140-144.

_____, Stuart, L. S., Smart, H. F. and Dawson, V. T.
1943. Effect of storage temperature on bacterial counts in spray-dried whole egg powder. J. Bacteriol. 45: 48-49.

Gunther, C. B. and Tuft, L.
1939. A comparative study of media employed in the isolation of typhoid bacilli from feces and urines. J. Lab. Clin. Med. 24: 461-471.

Hadley, R. B. and Caldwell, D. W.
1916. The bacterial infection of fresh eggs. Rhode Island Agr. Expt. Sta. Bull. 164.

Hadley, R. B., Caldwell, D. W., Elkins, M. W. and Lambert, D. J.
1917. Infections caused by Bacterium pullorum in adult fowls. Rhode Island Agr. Expt. Sta. Bull. 172.

Haines, R. B.
1937. Microbiology in the preservation of animal tissues. (Gr. Brit.) Dept. Sci. Ind. Research, Food Invest. Board Special Report 45.

_____.
1938. Observations on the bacterial flora of the hen's egg with a description of a new species of Proteus and Pseudomonas causing rots in eggs. J. Hyg. 38: 338-355.

_____.
1939. Microbiology in the preservation of the hen's egg. (Gr. Brit.) Dept. Sci. Ind. Research, Food Invest. Board Special Report 47.

_____ and Moran, T.
1940. Porosity of and bacterial invasion through, the shell of the hen's egg. J. Hyg. 40: 453-461.

Hajna, A. A. and Perry, C. A.
1938. A comparative study of selective media for the isolation of typhoid bacilli from stool specimens. J. Lab. Clin. Med. 23: 1185-1193.

Hall, L. A.
1938. Sterilized spices: new factor in food quality control. Food Ind. 10: 424-425, 464-467.

_____.
1951. Ethylene oxide process reduces spoilage organisms. Food Packer. 32 (12): 26-28, 41.

Hanson, H. L.
1945. Effect of concentrating egg white on desirability of angel cake. Unpublished Ph.D. Thesis, Ames, Iowa, Iowa State College Library.

_____, Lowe, B. and Stewart, G. F.
1947. Pasteurization of liquid egg products. V. The effect on performance in custards and sponge cakes. Poultry Sci. 26: 277-283.

Harte, R. A.
1945. The in vitro proteolysis of egg white. Science. 102: 563-564.

Hartsell, S. E.
1944. Studies on the bacteriology of stored dried egg powder. Food Research. 9: 505-511.

Hawthorne, J. R.
1950. Dried albumen. Removal of sugar by yeast before drying. J. Sci. Food Agr. 1: 199-201.

_____ and Brooks, J.
1944. Removal of sugar of egg pulp before drying, a method of improving the storage life of spray dried whole egg. J. Soc. Chem. Ind. 63: 232-234.

Healy, D. J. and Peter, A. M.
1925. The hydrogen ion concentrations and basicity of egg yolk and egg white. Am. J. Physiol. 74: 363-368.

Hinshaw, W. R. and McNeil, E.
1948. Avian salmonellosis, its economic and public health significance. 8th World's Poultry Congress, Copenhagen, 1948. 1: 599-604.

_____, _____ and Taylor, T. J.
1944. Avian salmonellosis. Types of Salmonella isolated and their relation to public health. Am. J. Hyg. 40: 264-278.

Hirschmann, D. J. and Lightbody, H. D.
1947. Effect of bacteria on quality of stored lyophilized egg powder. Food Research. 12: 381-392.

Hobby, O. C.
1953. Food and drugs. Statement of general policy or interpretation. Federal Register. 18: 1077.

Hormaeche, E. and Peluffo, C. A.
1941. Salmonelliasis in infancy and its diagnosis. Puerto Rico J. Public Health and Trop. Med. 17: 99-123.

Hoskins, J. K.
1934. Most probable numbers for evaluation of coli-aerogenes tests by fermentation tube method. U.S. Public Health Repts. 49: 393-405.

Johns, C. K.
1944. Microbiological aspects of egg powder. Sci. Agr. 24: 373-382.

Juenker, A. P.
1945. The isolation of four Salmonella types from one carrier. J. Bacteriol. 50: 673-674.

Jungherr, E. and Clancy, C. F.
1939. Serological types of Salmonella isolated from paratyphoid in chicks. J. Infect. Dis. 64: 1-17.

Kaess, G.
1943. Cold storage of eggs in carbon dioxide gas. Z. Ges. Kalte-Ind. 50: 107-113. (Original not available for examination; abstracted in Chem. Abstr. 39: 3084. 1945).

Kaplan, A. M., Solowey, M., Osborne, W. W. and Tubiash, H.
1950. Resting cell fermentation of egg white by streptococci. Food Technol. 4: 316-318.

Karlshoj, J. and Marthedal, H. E.
1948. Om salmonellainfektioner hos fjerkrae i Danmark, specielt med henblik paa infektion med Salmonella niloese. 8th World's Poultry Congress, Copenhagen, 1948. Official Report 1: 615-620.

Kaupp, B. F. and Dearstyne, R. S.
1927. Bacillary white diarrhea. North Carolina Agr. Expt. Sta. Tech. Bull. 29.

Kern, R. A., Kingkade, M. J., Kern, S. F. and Behrens, O. K.
1951. Characterization of the action of lysozyme on Staphylococcus aureus and on Micrococcus lysodeikticus. J. Bacteriol. 61: 171-178.

Kintner, T. C. and Mangel, M.
1953a. Survival of staphylococci and salmonellae experimentally inoculated into salad dressing prepared with dried eggs. Food Research. 18: 6-10.

_____ and _____.
1953b. Survival of staphylococci and salmonellae in puddings and custards prepared with experimentally inoculated dried egg. Food Research. 18: 492-496.

_____ and _____
1953c. Survival of pathogenic bacteria in foods prepared with whole egg solids. Mo. Agr. Expt. Sta. Research Bull. 530.

Kirby, G. W., Atkin, L. and Trey, C. N.
1936. Recent progress in 'rope' and mold control. Food Ind. 8: 450-470, 480.

Kline, R. W.
1945. Dried egg albumen. I. Studies of the non-microbiological changes during storage. Unpublished Ph.D. Thesis. Ames, Iowa, Iowa State College Library.

Knox, R.
1945. The effect of tetrathionate on bacterial growth. Brit. J. Exptl. Pathol. 26: 146-150.

_____, Gell, P. G. H. and Pollock, M. R.
1943. The selective action of tetrathionate in bacteriological media. J. Hyg. 43: 147-158.

Kohler, A. R., Miller, W. H. and Broquist, H. P.
1955. Aureomycin chlortetracycline and the control of poultry spoilage. Food Technol. 9: 151-154.

Lamson, G. H.
1909. Infection and preservation of eggs. Conn. (Storrs) Agr. Expt. Sta. Bull. 55.

Laschtschenko, P.
1909. Uber die Keintotende und entwicklungskemmende Wirkung von Huhnereiweiss. Z. Hyg. Infectk. 64: 419-427.

Leifson, E.
1936. New selenite enrichment media for the isolation of typhoid and paratyphoid (Salmonella) bacilli. Am. J. Hyg. 24: 423-432.

Lerche, M.
1939. Salmonellainfektionen beim geflugel und ihre bedeutung fur die epidemiologie der salmonellabakterien. 7th World's Poultry Congress, Cleveland, 1939. Offical Report 7: 274-278.

Lineweaver, H. and Murray, C. W.
1947. Identification of the tryptic inhibitor of egg white with ovomucoid. J. Biol. Chem. 171: 565-581.

Lorenz, F. W., Starr, P. B. and Bouthilet, R.
1950. Fumigation of shell eggs with ethylene oxide. Poultry Sci. 29: 545-547.

_____, _____, Starr, M. P. and Ogasawara, F. X.
1952. The development of Pseudomonas spoilage in shell eggs. I. Penetration through the shell. Food Research. 17: 351-360.

Mallman, W. L., Ryff, J. F. and Matthews, E.
1942. Studies on the Salmonella group. Methods of isolation and pathogenicity of strains occurring in the intestines of chickens. J. Infect. Dis. 70: 253-262.

Maurer, O.
1911. Bacteriological studies on eggs. Kansas State Agr. Expt. Sta. Bull. 180.

McClellan, P. P.
1950. Manufacture and uses of ethylene oxide and ethylene glycol. Ind. Eng. Chem. 42: 2402-2407.

McCullough, N. B. and Eisele, C. W.
1951a. Experimental human salmonellosis. I. Pathogenicity of strains of Salmonella meleagridis and Salmonella anatum obtained from spray-dried whole egg. J. Infect. Dis. 88: 278-289.

_____ and _____.
1951b. Experimental human salmonellosis. III. Pathogenicity of strains of Salmonella newport, Salmonella derby, and Salmonella bareilly obtained from spray-dried whole egg. J. Infect. Dis. 89: 209-213.

_____ and _____.
1951c. Experimental human salmonellosis. IV. Pathogenicity of strains of Salmonella pullorum obtained from spray-dried whole egg. J. Infect. Dis. 89: 259-265.

McFarlane, V. H. and Calesnick, E. J.
1948. Notes on the microbiology of army low-moisture egg powder. Poultry Sci. 27: 87-90.

_____, Watson, A. J. and Goresline, H. E.
1945. Microbiological control in the production of spray dried whole egg powder. U.S. Egg and Poultry Mag. 51: 250-257, 270-286.

Miller, C. and Winter, A. R.
1951. Pasteurized frozen whole egg and yolk for mayonnaise production. Food Research. 16: 43-49.

Mitchell, R. B., Garlock, F. C. and Broh-Kahn, R. H.
1946. An outbreak of gastroenteritis presumably caused by Salmonella pullorum. J. Infect. Dis. 79: 57-62.

Moore, E. N.
1946. The occurrence of fowl typhoid. Del. Agr. Expt. Sta. Cir. 19.

Moran, T.
1934. The storage of eggs. (Gr. Brit.) Dept. Sci. Ind. Research, Food Invest. Board. 1934: 61-64.

_____.
1937. Gas storage of meat and eggs. Food Ind. 9: 705-706.

_____.
1938. Gas storage of meat and eggs. Food Research. 3: 149-154.

_____ and Pique, J.
1926. The storage of eggs. (Gr. Brit.) Dept. Sci. Ind. Research, Food Invest. Board, Special Report 26.

_____ and Smith, E. C. B.
1936. Physics and chemistry of the hen egg. (Gr. Brit.) Dept. Sci. Ind. Research, Food Invest. Board. 1936: 35-40.

Mulvaney, A. A.
1941. How eggs are dried... methods and standards. Food Ind. 13(12): 50-53.

Ogilvy, W. S. and Ayres, J. C.
1951a. Post-mortem changes in stored meats. II. The effect of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken. Food Technol. 5: 97-102.

_____ and _____.
1951b. Post-mortem changes in stored meats. III. The effect of atmospheres containing carbon dioxide in prolonging the storage life of frankfurters. Food Technol. 5: 300-303.

_____ and _____.
1953. Post-mortem changes in stored meats. V. Effect of carbon dioxide on microbial growth on stored frankfurters and characteristics of some microorganisms isolated from them. Food Research. 18: 121-130.

Olcott, H. S.
1951. Problems in the application of antibiotics to food processing. N.C.A. Information Letter No. 1325: 102.

Orr, B. B. and Moore, E. N.
1953. Longevity of Salmonella gallinarum. Poultry Sci. 32: 800-805.

Osborne, W. W., Straka, R. P. and Lineweaver, H.
1954. Heat resistance of strains of Salmonella in liquid whole egg, egg yolk and egg white. Food Research. 19: 451-463.

Pappas, H. J. and Hull, L. A.
1952. The control of thermophilic bacteria. Food Technol. 6: 456-458.

Parsons, C. H. and Mink, L. D.
1945. Concentrating egg white. U.S. Patent 2,377,645. June 5, 1945. Abstracted in Chem. Abst. 39: 36036.

Payawal, S. R., Lowe, B. and Stewart, G. F.
1946. Pasteurization of liquid-egg products. II. Effect of heat treatments on appearance and viscosity. Food Research. 11: 245-260.

Pennington, M. E.
1912. Practical suggestions for the preparation of frozen and dried eggs. U.S. Bureau of Chemistry Cir. 98.

Pfizer, C.
1953. Glucatase. An enzyme system for desugaring eggs. Pfizer Tech. Bull. 54.

Rahn, O.
1945. Physical methods of sterilization of micro-organisms. Bacteriol. Rev. 9: 1-47.

Rettger, L. F.
1913. The bacteriology of the hen's egg with special reference to its freedom from microbic invasion. Conn. (Storrs) Agr. Expt. Sta. Bull. 75.

_____, Berman, N. and Sturges, W. S.
1916. Further studies on bacterial nutrition: the utilization of proteid and non proteid nitrogen. J. Bacteriol. 1: 15-33.

Rettger, L. F., Slanetz, C. A. and McAlpine, J. G.
1926. Animal feeding experiments with Bacterium pullorum. The toxicity of infected eggs. Conn. (Storrs) Agr. Expt. Sta. Bull. 140: 99-110.

_____ and Stoneburn, F. H.
1909. Bacillary white diarrhea of young chickens. Conn. (Storrs) Agr. Expt. Sta. Bull. 60.

Rievel, H.
1939. Die durchlassigkeit der eischale fur die bakterien und ihre bedeutung fur die kuhlhauslagerung. 7th World's Poultry Congress, Cleveland, 1939. Official Report. 7: 478-483.

Rochaix, A., Tapernous, A. and Couture, E.
1933. Sur le presence des microbes des groupe Bacterium pullorum dans les oeufs de poule. Bull. Soc. Sci. Vet. Lyon. 38 (5): 297-307.

Romanoff, A. L. and Romanoff, A. J.
1949. The avian egg. New York, N. Y. John Wiley and Sons, Inc.

Rosser, F. T., White, W. H., Woodcock, A. H. and Fletcher, D. A.
1942. Preservation of eggs. I. Treatments for maintaining quality in shell eggs at ordinary temperatures. Can. J. Research, sect. D. 20: 57-70.

Runnells, R. A. and Van Roekel, H.
1927. Further observations on the occurrence of white diarrhea infection in eggs laid by hens reacting to the agglutination test. Poultry Sci. 6: 229-232.

Ruys, A. C.
1934. Ein brillantgrun-medium fur die isolierung von paratyphus B-bakterien aus stuhl und urin. Zentr. Bakteriol., Parasitenk., 132: 349-351.

_____.
1940. The isolation of typhoid, paratyphoid, and dysentery bacteria from feces and urine. A comparative study of some culture media. Brit. Med. J. 1: 606-607.

Schade, A. L. and Caroline, L.
1944. Raw hen egg white and the role of iron in growth inhibition of Shigella dysenteriae, Staphylococcus aureus, Escherichia coli, and Saccharomyces cerevisiae. Science. 100: 14-15.

Schaible, P. J. and Bandemer, S. L.
1946. Composition of fresh and storage eggs from hens fed cottonseed and non-cottonseed rations. V. Cause of discoloration. Poultry Sci. 25: 456-459.

Schiff, F.
1938. Salmonella panama. Occurrence in serious infections of infants in New York City. J. Am. Med. Assoc. 111: 2458-2460.

Schneider, M. D.
1946a. Investigation of Salmonella content of powdered whole egg with not more than two percent moisture content. II. General survey on occurrence of species of Salmonella in high quality egg powder. Food Research. 11: 313-318.

———.
1946b. Investigation of Salmonella content of powdered whole egg with not more than two percent moisture content. III. Effect of preheater on survival of species of Salmonella during commercial spray drying. Food Research. 11: 521-524.

———.
1951. Investigation of Salmonella content of powdered whole egg with not more than two percent moisture content. IV. Bactericidal action of the preheater on Salmonella in liquid whole egg. Food Technol. 5: 349-352.

Scott, D.
1953. Glucose conversion in preparation of albumin solids by glucose-oxidase-catalase system. J. Agr. Food Chem. 1: 727-730.

Seligmann, E., Saphra, I. and Wassermann, M.
1943. Salmonella infections in man. An analysis of 1,000 cases bacteriologically identified by the New York Salmonella center. Am. J. Hyg. 38: 226-249.

Seligmann, E., Saphra, I. and Wassermann, M.
1946. Salmonella infections in the U.S.A. A second series of 2,000 human infections recorded by the New York Salmonella center. J. Immunol. 54: 69-87.

Sharp, P. F. and Powell, C. K.
1931. Increase in the pH of the white and yolk of hen's eggs. Ind. Eng. Chem. 23: 196-199.

_____ and Stewart, G. F.
1931. Carbon dioxide and the keeping quality of eggs. U.S. Egg and Poultry Mag. 37: 30-32, 63-68.

_____ and Whitaker, R.
1927. The relation of the hydrogen-ion concentration of egg white to its germicidal action. J. Bacteriol. 14: 17-46.

Shillinglaw, C. A.
1941. Effect of carbon dioxide and acids on survival of microorganisms. Iowa State College J. Sci. 16: 132-133.

Slosberg, H. M.
1946. Some factors affecting the functional properties of liquid albumen. Unpublished Ph.D. Thesis. Ames, Iowa. Iowa State College Library.

_____, Hanson, H. L., Stewart, G. F. and Lowe, B.
1948. Factors influencing the effect of heat treatment on the leavening power of egg white. Poultry Sci. 27: 294-301.

Smith, A. J. M.
1935. Viscosity of the white and of the yolk. (Gr. Brit.) Dept. Sci. Ind. Research. Food Invest. Board Report 1934: 53-56.

Snedecor, G. W.
1946. Statistical methods. 4th ed. Ames, Iowa. Iowa State College Press.

Snyder, E. G.
1953. New enzymes "open new doors". Food Eng. 25(6): 89-92.

Solowey, M.
1947. Paracolon organisms in spray-dried whole egg powder. J. Bacteriol. 53:667.

Solowey, M. and Calesnick, E. J.
1948. Survival of Salmonella in reconstituted egg powder subjected to holding and scrambling. Food Research. 13: 216-226.

_____, McFarlane, V. H., Spaulding, E. H. and Chemerda, C.
1947. Microbiology of spray-dried whole egg. II. Incidence and types of Salmonella. Am. J. Publ. Health. 37: 971-982.

_____, Spaulding, E. H. and Goresline, H. E.
1946. An investigation of a source and mode of entry of Salmonella organisms in spray dried whole egg powder. Food Research. 11: 380-390.

_____, Sutton, R. R. and Calesnick, E. J.
1948. Heat resistance of Salmonella organisms isolated from spray-dried whole-egg powder. Food Technol. 2: 9-14.

_____ and Watson, A. J.
1951. The presence of enterococci in spray-dried whole egg powder. Food Research. 16: 187-191.

Somogyi, M.
1945. Determination of blood sugar. J. Biol. Chem. 160: 69-73.

Stark, C. N. and Herrington, B. L.
1931. The drying of bacteria and the viability of dry bacterial cells. J. Bacteriol. 21: 13-14.

Stewart, G. F., Best, L. R. and Lowe, B.
1943. A study of some factors affecting the storage changes in spray-dried egg products. Proc. Institute Food Technologists. p. 77-89.

_____ and Kline, R. W.
1941. Dried egg albumen. I. Solubility and color denaturation. Proc. Institute Food Technologists. p. 48-56.

Stiles, G. W. and Bates, S. L.
1912. A bacteriological study of shell, frozen and desiccated eggs. U.S. Dept. Agr. Bull. 158.

Stuart, L. S. and Goresline, H. E.
1942a. Bacteriological studies on the "natural" fermentation process of preparing egg white for drying. J. Bacteriol. 44: 541-549.

_____ and _____.
1942b. Studies of bacteria from fermenting egg white and the production of pure culture fermentations. J. Bacteriol. 44: 625-632.

_____, Hall, H. H. and Dicks, E. E.
1942. Storage changes in spray-dried whole egg powder. U.S. Egg and Poultry Mag. 48: 629-633, 658.

_____ and McNally, E. H.
1943. Bacteriological studies on the egg shell. U.S. Egg and Poultry Mag. 49: 28-31, 45-47.

Sutton, R. R. and McFarlane, V. H.
1947. Microbiology of spray-dried whole egg. III. Escherichia coli. Food Research. 12: 474-483.

Tarr, H. L. A. and Deas, C. P.
1948. Action of sulfa compounds, antibiotics and nitrite on growth of bacteria in fish flesh. (Can.) J. Fisheries Research Board. 7: 221-223.

_____, Southcott, B. A. and Bissett, E. M.
1952. Experimental preservation of flesh foods with antibiotics. Food Technol. 6: 363-366.

Thistle, M. W., White, W. H., Reid, M. and Woodcock, A. H.
1944. Dried while egg powder. XIV. Effects of low temperature, low moisture content, carbon dioxide pack, and copper contamination on keeping quality. Can. J. Research, sect. F. 22: 80-86.

Tittsler, R. P., Hewang, B. W. and Charles, T. B.
1928. The occurrence and significance of Salmonella pullorum in eggs. Pa. Agr. Expt. Sta. Bull. 235.

Van Oijen, C. F.
1940. Salmonella infectie in endeneiren. Tijdschr. v. Diergeneesk. 67: 280-300.

Vernon, H. M.
1904. The protective value of proteids and their decomposition products on trypsin. J. Physiol. 31: 346-358.

Watson, A. J. and McFarlane, V. H.
1948. Microbiology of spray-dried whole egg. IV. Molds. Food Technol. 2: 15-22.

Watt, J.
1945. An outbreak of Salmonella infection in man from infected chicken eggs. U.S. Public Health Repts. 60: 835-839.

_____ and Carlton, E.
1945. Studies of the acute diarrheal diseases. XVI. An outbreak of Salmonella typhimurium infection among newborn premature infants. U.S. Public Health Repts. 60: 734-740.

Watts, B. M. and Elliot, C.
1941. Utilization of dried egg white in bakery products. Cereal Chem. 18: 1-9.

Weaver, H. J. and Weldin, J. C.
1931. Incidence of Salmonella pullorum in eggs from reactor hens. Poultry Sci. 10(3): 118-119.

Weiser, H. H., Goldberg, H. S., Cahill, V. R., Kunkle, L. E. and Deatherage, F. E.
1953. Studies on fresh meat processed by the infusion of antibiotics. Food Technol. 7: 495-499.

Whelton, R., Phaff, H. J., Mrak, E. M. and Fisher, C. D.
1946. Control of microbiological food spoilage by fumigation with epoxides. Food Ind. 18: 23-25, 174-176, 318, 320.

Wilkin, M. and Winter, A. R.
1947. Pasteurization of egg yolk and white. Poultry Sci. 26: 136-142.

Wilson, M. E.
1948. Survival of Salmonella organisms in stored egg powder. Food Ind. 20: 873-875.

Winter, A. R., Stewart, G. F., McFarlane, V. H. and Solowey, M.
1946. Pasteurization of liquid-egg products. III. Destruction of Salmonella in liquid whole egg. Am. J. Publ. Health. 36: 451-460.

Wolk, J., McNally, E. H. and Spicknall, N. H.
1950. The effect of temperature on the bacterial infection of shell eggs. Food Technol. 4: 316-318.

Yesair, J. and Cameron, E. J.
1938. Present status of the sterilization of spices. The Canner. 86(2248): 108-110.

Zagaevsky, J. S. and Lutikova, P. O.
1944a. Sanitary measures in the egg breaking plant. U.S. Egg and Poultry Mag. 50: 17-20, 43-46.

_____ and _____.
1944b. Bacterial contamination in eggs. U.S. Egg and Poultry Mag. 50: 75-77, 88-90.

VIII. ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. John C. Ayres for his encouragement and guidance throughout this investigation.

The writer is grateful to Dr. Richard Forsythe for his aid in procuring spray dried albumen and to Dr. Allen Kraft, Alonzo Adams and Mary Jeanne Fanelli for their interest and assistance in the laboratory. The technical assistance of Mrs. Betty Wymore in the preparation of angel cakes is gratefully acknowledged.