

FOOD MICROBIOLOGY

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The problems facing the food bacteriologist are much the same from year to year; he must detect and take steps to prevent contamination of food with undesirable organisms; sterilize canned goods effectively without impairing quality; improve sanitary conditions in food manufacturing plants; control the growth of microorganisms by refrigeration, freezing, desiccation, or by use of inhibitors. In reviewing literature on food bacteriology for any period, articles will be found dealing with each of these problems. The type of food which receives the bulk of attention will vary from time to time, but the overall problems remain the same. In food bacteriology, as in other fields of bacteriology, there has developed a degree of specialization, and in a short review of this kind, it is impossible to cover adequately all the specialities in the field. Dairy bacteriology, although a branch of food bacteriology, is in reality a specialty of its own and should be reviewed as a specific topic. It has, therefore, not been covered in this review. Also for the sake of brevity, the period has to be limited. The author, therefore, has limited the review to publications which have appeared during the past two years.

EGG PRODUCTS

Liquid egg products have become important food items during the past decade and their use has increased considerably. These products are easily contaminated and provide an excellent medium for the growth of microorganisms. It is, therefore, only natural that they should have received considerable attention from food bacteriologists. Winter, Stewart and co-workers (1, 2, 3) have made a series of studies on the extent of contamination to be found in laboratory and commercially prepared liquid egg products, both in the egg yolk and egg white and in mixtures of the two. They also made detailed studies on the types of contaminating organisms present and on the possibility of eliminating some of the undesirable contaminants, such as *Escherichia coli* and *Salmonella* by

pasteurization. Pasteurization at temperatures up to 51°C. for periods of time varying from a few minutes to 20 min. proved effective. This treatment would not bring about coagulation of the albumin nor seriously interfere with the use of egg products in baking and in other uses. Winter (4) investigated a condition called "black rot" in fresh shell eggs, isolated the causative organisms, and indicated ways and means of reducing this type of spoilage. Winter & Wilkin (5) made a study of the effect of freezing, holding, and storage of liquid egg products in order to control the bacterial growth. They reported that during storage in the frozen condition the bacterial count usually decreased. The decrease was most rapid at temperatures close to -18°C.

Solowey, Sutton & Calesnick (6) made a study of the effect of pasteurization on the *Salmonella* found in sprayed dried whole egg powder. Most of these organisms were destroyed in one minute at 58°C. and the most heat resistant ones in 1.2 min. at 61°C. Solowey & Calesnick (7) made a study of *Salmonella* contamination in reconstituted egg powder and showed that the organisms would multiply rapidly after the eggs had been reconstituted. When the egg powder is reconstituted and used for scrambling, most of the *Salmonella* are eliminated but will survive scrambling temperatures of 56°C. for 17 min.

McFarlane *et al.* (8 to 11) and Watson (12) have published a series of papers dealing with the microbiology of sprayed dried whole egg. In these papers they report on the analysis of several thousand samples of sprayed dried whole egg powder received from various manufacturing plants in the United States from the period September 1, 1943 to January 1, 1945. All samples represented lots of powder, manufactured from unpasteurized, liquid whole eggs according to the United States Department of Agricultural and War Food Administration purchase specifications. They were examined by plate and direct microscopic counts, and for *Salmonella* species, *E. coli*, molds, and thermophiles. The monthly average plate count varied from 80,000 to one million organisms per gram. The direct count varied from below ten million to four billion per gram. Egg powder produced from fresh shell egg liquid had a lower count than did the egg powder produced from frozen egg or storage egg liquid. *Salmonella* species were isolated from 35 per cent of the 5,000 samples examined and *E. coli* from 51 per cent. *Salmonella* organisms were found in many samples that gave neg-

ative results for *E. coli* and likewise *E. coli* was found in many samples from which *Salmonella* could not be isolated. About 36 per cent of the egg powder samples had mold counts of 100 or more per gm. and less than 2 per cent had counts of 1,000 or more per gm. Several aerobic sporeformers were found that would grow at 55°C. but these were not strictly thermophilic organisms—strict thermophiles were encountered very infrequently. Similar studies have been made by Hirschamann & Lightbody (13). Cantor & McFarlane (14) report on the occurrence of *Salmonella* organisms in and on chicken eggs and state that the occurrence of these organisms in eggs correlates with the amount of dirt found on the eggs. Solowey (15) and McFarlane & Calesnick (16) published further notes concerning the microbiology of egg powder. Wilson (17) made a study of the occurrence of *Salmonella* organisms in stored egg powder. He studied the survival of the organisms in egg powder that has been stored at temperatures varying from -24° up to 45°C. and reports that if powder is stored at 45°C. it becomes free from *Salmonella* in from 30 to 40 weeks of storage. Similar findings are reported in a special bulletin from the Medical Research Council of Great Britain (18).

Australian workers (19) report the development of a method of pasteurizing whole eggs so as to free them from surface contamination. This is done by passing eggs rapidly through a water bath that is kept at 63.5°C. Stewart & Ayres (20) report that in order to produce a stable egg white powder the sugar should be removed. This can be done by inoculating the egg whites with yeast cells which will remove the sugar completely in three to seven days.

HEAT RESISTANCE OF SPORES

During the past two years a considerable amount of attention has been given to a study of the heat resistance of spores and the ease with which spores will germinate following heat treatment. Stumbo (21) reports on the development of a special apparatus for the study of the heat resistance of spores that will make possible a large series of studies with a saving of time and will also permit the use of higher temperatures, with the correspondingly shorter exposure times, than is possible with conventional techniques. Using high temperatures up to 132°C. he found a straight line relationship to exist when the logarithm of death time was plotted against the temperature.

Curran & Evans (22) and Curran, Evans & Bell (23) report on the effect of heat shock upon the rate of germination and upon the viability of spores following heat shock. They believe that following heat shock, the spores are stimulated to intense metabolic activity. The authors suggest a two-stage method of sterilization; the first being a heat shock, followed by an incubation at 37° to 66°C. for a period not to exceed 5 hr., and then a final pasteurization treatment. Davis & Williams (24) and Davis, Williams & Wyss (25) claim that bacterial spores with a high heat resistance can be developed by selecting those which survive a moderate heat treatment. They also find that the most heat resistant spores have the greatest resistance to disinfectants.

Wynne & Foster (26 to 29) published a series of articles on the physiology of spore germination with special reference to *Clostridium botulinum*. They believe that dormancy is a property of the medium in which the spores are suspended rather than a property of the spores themselves. Some of the factors which may prolong dormancy are the absence of carbon dioxide and the presence of certain types of fatty acids, particularly oleic or linoleic acids; but they also believe that there are other substances, not yet identified, that can prevent spores from germinating for relatively long periods of time.

Gross *et al.* (30 to 36) have published a series of papers on bacteriological studies relating to thermal processing of canned meats. They have used a special technique in which meat paste is processed in standard thermal death time tubes. With this technique they have investigated the heat characteristics of a putrefactive anaerobe which is commonly used as an indicator organism in process evaluations. Thermal death time curves follow the monomolecular law. However, the thermal resistance of this organism, as well as other sporeformers which have been isolated from meats, is influenced by the nature of the medium. If the organisms are grown in raw meat, the thermal resistance may be low; whereas if cooked meat is used, the heat resistance may be very high. In a separate paper Gross & Vinton (37) made a study of the thermal death time of various strains of staphylococci in meat. Here again they have found that the heat resistance can be influenced by the medium in which the organisms have grown. In pasteurized meat some strains survived 100 min. at 66°C. while in sterilized meat the heat resistance found was 13 min. at 66°C. Knaysi (38) published an extensive review on the cytology,

biological nature, chemical composition, and the effect of the environment on the germination of spores. Evans & Curran (39) report that isobutyl vanilate is an effective inhibitor for spore-forming bacteria.

PLANT SANITATION

Wolford & Berry (40, 41) made a study of the source of contamination in a plant making orange juice and found that high bacterial counts are common in the slime, wash water, and debris accumulating on various pieces of equipment. In some of this material large numbers of coliform organisms may be present. They also observed that contamination was extensive if the oranges themselves were not carefully selected so as to avoid oranges that were partly spoiled from "soft rot." If unsound oranges are excluded and if the plant is kept perfectly clean, orange juice can be produced with a very low bacterial count. Teunisson & Hall (42) made a study of the bacterial flora of citrus juice and found that most of the organisms isolated can not survive for any long period of time in the juice produced. They also claimed that none of the bacteria produced any noticeable changes in quality, color, or flavor.

Sognefest & Jackson (43) developed a method of sterilizing tomato juice before it is canned by continuously running it through a tubular heat exchanger under pressure. The juice is heated to 121°C. and held for 0.7 of a minute or heated to 138°C. and cooled immediately. A good quality tomato juice with an excellent keeping quality was produced even though it was inoculated beforehand with heat resistant organisms. Wildman & Clark (44) found that *Oidium lactis* can be a common contaminant in tomato juice where slimes are allowed to accumulate on the machinery. Smith (46) made a study of the mold count in tomato products.

Howard & Pederson (45) report that the growth of naturally occurring contaminating bacteria in maple sap causes an increase in alkalinity which in turn may be responsible for a darkening of the color of the resulting maple syrup. To obtain light colored syrup, it is important to maintain equipment in a sanitary condition and to handle the sap rapidly so as not to allow excessive bacterial growth.

Anderson (47) recommends the use of a direct microscopic count as a control of sanitation in a dairy plant, suggesting 200,000 organisms per cc. as the upper limit to be tolerated. Stumbo (48)

stresses the importance of not keeping food at an elevated temperature any longer than the lag phase of the growth curve of the contaminating bacteria.

Niven (49) discusses the effect of bacterial contamination on sausage discolorations and suggests preventive measures. Olson (50) discusses the type of organisms that are apt to be found as contaminants in food plants, depending upon the sources of contamination and the environmental factors. Nagy (51) and Ohart (52) report on experiences with ultraviolet light when used as aid in sanitation. Nagy finds that colored mold spores have such a high resistance to ultraviolet light that it is impractical to depend on ultraviolet for the destruction of molds in food manufacturing plants. Baren (53) emphasizes that good sanitation can be obtained only when this responsibility is put in the hands of a separate department that is responsible directly to top executives. Feiner (54) reports on the development of a new type of kettle to be used in food manufacturing plants that can be more easily cleaned and kept free from contamination. Lipske & Hubbard (55) discuss the sources of thermophilic contamination in a canning plant and point out how it can be avoided. The authors emphasize that the important thing is to keep the temperature in the plant either below or above a temperature at which the thermophiles can grow, the optimum of which is around 54°C. The best method of reducing the thermophilic count, according to them, is to flood the machinery continuously for 24 hr. with clean water while the machinery is in motion so as to wash out the thermophilic contaminants. Gunderson, Rose & Henn (56) and Gunderson (57, 58) made a thorough study of a chicken boning plant to find out where contamination occurs. They call attention to the very heavy contamination that can result in a plant handling this type of product. Counts of several million per gram of meat were frequently encountered. A number of articles have appeared in the literature giving the results of bacteriological surveys in various food manufacturing plants; thus Bohrer (59) reports on such a survey in a corn canning plant. Vaughn, Winter & Smith (60) made such studies in a plant handling dried fruit.

FROZEN FOODS

Pederson (61) made a study of the extent of contamination encountered in frozen vegetables and on the effect of various

methods of handling upon this contamination. The greatest danger is to allow the vegetables to become contaminated by the organisms which are in the active growth phase and to hold the food at a temperature at which they continue to grow for any length of time. He states that counts of 10,000 to 100,000 per gram may be expected in frozen vegetables that have been properly handled, but if the counts go above a million per gram, it is an indication of careless handling.

Perry *et al.* (62) have concerned themselves with the danger of *C. botulinum* in frozen vegetables. They have found that none of the samples studied showed any production of botulinum toxin even though many of the samples had been inoculated with these organisms before freezing. *C. botulinum* was isolated both from the inoculated samples and uninoculated controls. The authors believe that the mixed flora which is present in frozen vegetables is responsible for the absence of toxin production. Fitzgerald (63) and Proctor & Nickerson (64) discuss the factors which must be watched in order to produce frozen foods with a reasonably low bacterial count. Fitzgerald believes that frozen foods should not contain more than 100 *E. coli* cells per 100 gm. and that the total plate count should not exceed 100,000 per gm. Proctor & Nickerson believe that a direct microscopic count should be used as a control on the operations in plants manufacturing frozen foods. Gunderson & Rose (65) made a study of the survival of bacteria in precooked fresh frozen foods and report that the population of pathogenic enteric bacilli falls rapidly in chicken chow mein stored at -26°C . during the first 5 days. After that, the rate of death decreases until a more or less resistant population remains. They conclude that cold storage cannot be depended upon to pasteurize frozen foods.

FISH PRODUCTS

Aschehoug & Vesterhus (66) report on the type of contamination that may be found in winter herring. Strains of *Pseudomonas* are most common in the fresh fish, while *Achromobacter* are more common in stored fish. Castell (67) has made a survey of the extent of contamination in fish filets, the keeping quality of the filets being correlated directly with the extent of contamination. Again Castell (68) has published the first in a series of articles to deal with the various phases of the fishing industry from

the viewpoint of the bacteriologist. Castell & Anderson (69) and Castell (70) made a survey of the presence of anaerobic spore-formers in fish products. They find that such organisms are not encountered frequently and believe that this is due to high salt content.

GERMICIDES AND SANITIZING AGENTS IN FOOD PLANTS

There is a great deal of literature on the subject of new sanitizing agents and germicides in regard to their effect on sanitation in food plants. Only a few of these articles are covered, those which deal specifically with the effect of these compounds on the sanitary conditions of a plant.

Berstein & Epstein (71) report that they improved the quality of pickles by soaking them in a solution containing a germicidal detergent. This reduced the bacterial count, and furthermore, made the count of the pickles more uniform so that a standard process could be developed that gave consistently good results. A quaternary ammonium compound was found to be the best for the purpose. Mallmann & Zaikowski (72) report good results with the use of a quaternary ammonium compound along with heat in the rinse water in a mechanical dishwashing machine. Johns (73) reports on a glass slide technique for assessing the sanitizing efficiency of quaternary ammonium compounds and hypochlorites. Wolford & Anderson (74) find that propionates are valuable in controlling the growth of microorganisms in fruits and vegetables while they are being prepared for freezing, or while they are in storage prior to preparation for freezing. Penniston & Hedrick (75) were able to reduce the bacterial count substantially in egg products by washing the eggs in a solution containing chlorine or a quaternary ammonium compound. Kivela, Mallmann & Churchill (76) made a study of the mode of action of the surface active agents on spores and vegetative cells. They found that the surface active agents affected the surface primarily and that in many cases the removal of the agent would restore the cells to a viable condition. In many cases where surface active cations are used the spores are actually not killed but simply prevented from germinating. However, vegetative cells could not be revived by removing the germicide.

To prevent contamination in fish it has been advocated that silver be used for the sterilization of sea water. Castell, Ellis &

Anderson (77) show that sodium chloride removed enough of the silver ions to disrupt the germicidal action. In a solution containing 2.5 per cent sodium chloride there was little if any effect of the silver on the bacterial flora.

FOOD PROCESSING METHODS

Stumbo (78), from a theoretical consideration, attempts to ascertain the location in a food container where the probability of bacterial survival is greatest during heat processing. He reaches the rather disturbing conclusion that the center of the can may not be the place where this is most likely to happen. The reviewer is of the opinion, however, that Stumbo's basic assumption needs to be re-examined. Cass (79) has suggested a modification of the Schultz and Olson lethal rate paper for calculating thermal processes for food products in tin containers. Ball (80) has published a series of articles dealing with theoretical considerations concerned with food processing. Martin (81) reports the development of new equipment which allows for the sterilization of fluid products before they are introduced into cans. The process has been successfully carried through a pilot plant stage, and it is claimed that better preservation and high quality can be obtained by the use of this equipment. A sterile, good quality product was produced even when the raw product, the cans, the covers, and the sealing head of the closing machine were heavily inoculated with heat resistant spores.

Merrill (82) had derived constants which may be used for computing sterilization processing times for glass containers. Cathcart, Parker & Beattie (83) and Bartholomew, Harris & Sussex (84) show that loaves of Boston Brown Bread can be treated so as to prevent mold growth by heating with induced current or by the dielectric method. With the dielectric heating a temperature of 150°F. was reached, while with the induced current a surface temperature of 150°F. was attained. Jackson (85) reviews our present state of knowledge concerning the applicability of high frequency electronics for the sterilization of foods. He concludes that the art has not developed as yet to a point where it is practicable. Mickolson (86) claims to have developed equipment that will allow the effective pasteurization of milk with ultraviolet light. The milk is delivered in front of the light in thin films on rotating cylinders.

MICROORGANISMS RESPONSIBLE FOR FOOD POISONING

Dack (87) states that *C. botulinum* and streptococci failed to grow in dried meat with a moisture content less than 30 per cent. *Salmonella* failed to grow with a moisture content less than 50 per cent. Scott & Stewart (88) claimed that *C. botulinum* will grow more readily in canned vegetables in which the cans are lacquered than in those which contain no lacquer. In unlacquered cans, it is claimed enough tin is dissolved to inhibit growth. Anderson & Berry (89) report that certain naturally occurring flavonols found in asparagus inhibit the growth of *C. botulinum*. The most effective compound was quercitrin, which was effective in concentrations of from 80 to 160 p.p.m. Cathcart, Godkin & Barnet (90) made a study of the ability of *Staphylococcus aureus* to grow in various pastry fillings. Chapman (91) reported on a specially improved Stone medium for the isolation and identification of food poisoning staphylococci. Smith & Iba (92) studied the effect of contamination of staphylococci on nut meats and report that at 37°C., or at room temperature, the organisms increase in numbers for several days. This is followed by a drop in population. If the nuts are stored in a refrigerator, the population does not rise but decreases gradually over a long period of time.

MISCELLANEOUS ITEMS

Pederson & Fischer (93) find that there is an antibiotic substance in cabbage tissues that is effective against gram negative bacteria. Hemfeld (94) reports the presence of an antibiotic substance in wheat bran related to a fatty acid which is effective against gram positive organisms, but not against the gram negative bacteria.

Heath (95) made a study of spoilage of dehydrated foods and reports that bacteria are inhibited at a moisture content below 18 per cent, while yeast requires 20 per cent or more, but molds will multiply at 13 per cent moisture.

Ostrolenk *et al.* (96) developed a special medium for the isolation of fecal streptococci which involves incubation at 45°C. in the presence of 0.05 per cent sodium azide. They believe the enterococci are better indices of pollution than *E. coli*. Jensen (97) found that mustiness in foods may be due to the growth of certain varieties of microorganisms. Pederson & Breed (98) have made a study of the fermentation process that goes on in the preparation

of the coffee bean from the harvested berry clusters. The process is a typical lactic acid fermentation such as occurs in other vegetable materials. Rice, Squire & Fried (99) find that the destruction of vitamins in pork may be due to the growth of microorganisms. Epstein (100) reports on a method of testing commercial filter pads that are used to remove microorganisms from liquids in industries. A suspension of *E. coli* is passed through the filters and the filtrate is tested for sterility. Niven (101) has reported the isolation of various types of organisms that can cause green discoloration of sausage. Two organisms belonging to the genera *Lactobacillus* and *Leuconostoc* were particularly active in producing this discoloration. Burrell (102) started publishing a series of articles on various bacteriological problems involved in the manufacture of acid-preserved pickles. Ulrich & Larson (103) have developed a special formula for an indicator to be used in anaerobic jars to determine whether or not anaerobic conditions have been attained.

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