EFFECT OF SELECTED COATING MATERIALS ON THE BACTERIAL PENETRATION OF THE AVIAN EGG SHELL[1]

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Abstract

Whole shell eggs were coated with the following materials: Zein (corn prolamine), Polidene 930-H (polyvinylidene chloride), Epolene Wax E-45 (epolene wax emulsion), and 974-1 (hydrolyzed sugar derivative plus shellac). Surfaces of coated and uncoated eggs were inoculated with *Salmonella typhimurium* and *Pseudomonas fluorescens*. Contents of each egg were then replaced aseptically with a sterile agar medium containing triphenyl tetrazolium chloride. Eggs were sealed, incubated, and examined for shell penetration and growth of *P. fluorescens* and S. *typhimurium*. The ability of the two microorganisms to decompose films of the dry coatings was also tested. All coatings greatly retarded penetration by both microorganisms, although *P. fluorescens* was retarded more than S. *typhimurium*. When incubated for 7 days, heavy suspensions of *P. fluorescens* and S. *typhimurium* did not decompose films of dried coatings. After 48 hr incubation, growth of either organism was not obtained in media containing only dried coating films as added substrates. The dry coatings did not inhibit growth of either organism.

Application of various coatings to whole shell eggs for such purposes as increasing the shell strength and preserving the internal quality has been studied *(4, 9)*. Recently, Meyer and Spencer *(8)* coated eggs with acrylic resin, casein, polyvinyl acetate, polyvinylidene chloride, prolamine, and epolene wax emulsion. They found that these coatings strengthened the shell, reduced moisture loss, and retarded loss of internal quality and increase in albumen pH.

The rate of bacterial contamination of eggs has been shown to be related to the porosity of the shell *(3)*. The blunt end of the egg, where the shell porosity is maximal, has been shown to be the most vulnerable to infection by *Pseudomonas aeruginosa (11)*. Correspondingly, regions of lesser porosity, i.e. the equatorial region and the narrow end of the egg, were found to be less vulnerable to infection. Lifshitz et al. *(7)* demonstrated that common egg-invading organisms, *Pseudomonas fluorescens, Salmonella paratyphi*, and *Alcaligenes bookeri* could pass through the barrier of a clean egg shell with no additional source of nutrients except those found naturally in the cuticle and shell. Reproduction of the microorganisms was demonstrated to be essential for the penetration process. The shell of an egg has been shown to be a less effective barrier to bacterial penetration than the inner shell membrane *(6)*.

Penetration through the shell is the most common way by which salmonella organisms enter and infect the egg, with the exception of *Salmonella pullorum* and *Salmonella gallinarum* which usually enter through ovule infection *(1)*. Solowey et al. *(10)* in an investigation of the source and mode of entry of salmonella in spray-dried whole egg powder indicated that 16% of dirty eggs and at least 2% of clean eggs contained the organism on the shell and in the pores of the shell.

A method to prevent multiplication on egg shell surfaces and penetration of egg shells by such organisms could be of public health importance. For this reason, this study was undertaken to determine the effect of selected coating materials on the microbial penetration of egg shells.

Methods and Materials

Nest clean, nonfertile eggs were obtained from one strain of White Leghorn nens one day post oviposition. The eggs were candled and those having cracked or checked shells were discarded. The remaining eggs were wiped with a wet cloth and then sanitized by dipping in 70% ethanol and flaming.

Three sets of ten sanitized eggs each were used for the uncoated, uninoculated controls and the uncoated inoculated controls.

Coating of eggs

Triplicate sets of eggs were also coated with each of the following materials: Zein[3] (a prolamine from corn gluten), Polidene 930-H[4] (polyvinylidene chloride), Epolene Wax E-45[5] (a wax emulsion), and 974-1[6] (hydrolyzed sugar derivative plus shellac). Characteristics of the coatings used, with the exception of the 974-1 were described by Meyer and Spencer *(8)*. The material 974-1 consisted of approximately 10% hydrolyzed sugar derivative, 12 to 15% shellac, a small amount of ammonium oleate, and alcohol. This coating was used undiluted as supplied by the manufacturer. Sanitized

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[3]Supplied by Nutrilite Products, Inc., Buena Park, California.
[4]Supplied by the Stanley Chemical Co., Kearny, New Jersey.
[5]Supplied by Eastman Chemical Products, Inc., Kingsport, Tennessee.
[6]Supplied by Pacific Chemical, Seattle, Washington.

TABLE 1. PERCENTAGE OF EGGS[1] PENETRATED BY *S. typhimurium* AND BY *P. fluorescens*

	Inoculated, uncoated controls				Uninoculated, uncoated controls				Polidene 930 H			
Penetration score[2]	+++	++	+	0	+++	++	+	0	+++	++	+	0
S. typhimurium	86.7	6.7	6.7	0.0	0.0	0.0	3.3	96.7	7.1	14.2	57.1	21.4
P. fluorescens	46.7	26.7	26.7	0.0	0.0	0.0	3.3	96.7	0.0	13.3	23.3	63.3
	Zein				974-1				Epolene wax E-45			
Penetration score	+++	++	+	0	+++	++	+	0	+++	++	+	0
S. typhimurium	0.0	0.0	16.7	83.3	0.0	3.3	33.3	63.3	6.9	3.4	34.5	55.2
P. fluorescens	0.0	0.0	3.3	96.7	0.0	0.0	0.0	100	0.0	0.0	13.3	86.7

[1]Based on 30 eggs per treatment (3 replicates, 10 eggs each).
[2]+ indicates less than 20 bacterial penetration sites.
++ 20 to 40 bacterial penetration sites.
+++ greater than 40 bacterial penetration sites.

eggs were coated by immersing in the appropriate coating solution, allowing the egg to drain briefly, and then drying by use of forced air. Details of the coating procedure were described by Meyer and Spencer *(8)*.

Inoculation of eggs with test microorganisms

After each coating had thoroughly dried, three separate sets each were inoculated with suspensions of *Salmonella typhimurium* and *Pseudomonas fluorescens*. Organisms were grown 18 to 24 hr on Standard Methods Agar (BBL) slants and harvested by washing the slants with 2-ml quantities of sterile 0.25% saline. Suspensions of each organism were diluted to 100 ml with 0.25% sterile saline and the turbidity determined at 625 mμ using a Bausch and Lomb Spectronic 20 spectrophotometer. The turbidity of each suspension was then adjusted to give approximately 1 to 5 × 10^7 organisms/ml according to a predetermined relationship between turbidity and resultant number of viable cells. One hundred milliliters of each diluted suspension were added to 900 ml of sterile 0.25% saline in a stainless steel beaker in which a teflon-coated stirring bar was placed underneath a wire gauze egg support. The inoculum was kept at a temperature of 5 to 8 C and continuously stirred by placing on a magnetic stirrer.

Eggs that had been held at room temperature were inoculated for 15 min. The eggs were then removed to a sterile, specially constructed drying board consisting of points of nails driven through plywood spaced 2.54 cm apart.

After eggs were completely dried, five uncoated, inoculated eggs were used to determine the resultant surface population using the blending method described by Gunaratne and Spencer *(5)*. The contents of each egg were discarded and the egg shell, membranes and adhering albumen were blended in 100 ml of sterile 0.1% peptone using a Sorvall Omni-mixer. Serial dilutions were made and plated on Standard Methods Agar (BBL).

Detection of bacterial penetration of coated and uncoated eggs

The remaining coated and uncoated eggs were used to determine the extent of bacterial penetration. A method described by Board and Board *(2)* was used with the following modifications. Approximately 1 to 2 cm^2 of the shell at the pointed end of completely dry eggs was removed by first gently cracking the shell with the edge of a sterile knife, then removing shell segments with sterile rat-tooth forceps. The contents of eggs were then aseptically removed using suction. Sterile distilled water was used to rinse the inside shell and membranes and similarly removed. The emptied shells were then filled with a sterile medium similar to that used by Board and Board *(2)* and of the following composition: glycerol, 0.5% (w/v); yeast extract (Difco), 0.05% (w/v); agar (Difco), 2%; triphenyl tetrazolium chloride (Difco), 0.02%; tap water, pH 7.2, 97.43%.

After the agar had solidified, eggs were inverted and the open end sealed with sterile paraffin wax contained in the depressions of aluminum palette trays. Eggs in trays were then incubated at 25 C for growth of *P. fluorescens* and at 37 C for growth of *S. typhimurium*.

After 24 and 48 hr of incubation, eggs were examined by placing the sealed end of the egg into the aperture of an egg candling lamp. The dark red spots of triphenyl formazan were qualitatively estimated according to the following scale: (+) – indicated the appearance of < 20 spots, (++) – indicated 20 to 40 spots, and (+++) – indicated > 40 spots of triphenyl formazan.

Tests for the microbial degradation of coatings

The ability of the test microorganisms to attack or utilize the coatings as growth substrates was examined by the following two methods. *(a)* Sterile glass slides were coated with the materials to be tested and allowed to dry thoroughly for 24 hr at 25 C in sterile petri dishes. Sterile filter paper discs (1 cm diameter) were dipped in suspensions of *S. typhimurium* (2.7 × 10^8 cells/ml) and *P. fluorescens* (1.6 × 10^7 cells/ml) and placed on each coating material. Coated slides with inoculated discs and the coated slides with non-inoculated discs were placed in desiccators containing 400 ml of 4% H_2SO_4 to give a relative humidity of about 97% *(12)*. The desiccators were sealed and incubated at 25 and 37 C for growth of *P. fluorescens* and *S. typhimurium*, respectively. After 7 days, coated slides were removed and examined for evidence of microbial degradation and growth. *(b)* Segments of aseptically prepared dried films of coating materials and dry 1 × 10 cm strips of sterile Whatman No. 4 filter paper impregnated with coating materials were added to separate tubes of sterile saline media (0.5% NaCl in triple distilled water) and sterile saline-peptone media [0.5% NaCl, 0.5% peptone (Difco) in triple distilled water]. One-tenth milliliter of each bacterial suspension used in the first method was added to a set of tubes of both types of media plus coatings and incubated at the approximate temperatures. After 48 hr, the tubes were examined for presence or absence of microbial growth and/or inhibition.

RESULTS AND DISCUSSION

Penetration of coated egg shells

The method described by Board and Board *(2)*

was effective in demonstrating penetration of the egg shell by the microorganisms *P. fluorescens* and *S. typhimurium*. Uncoated inoculated eggs were frequently penetrated, as evidenced by the numerous spots of triphenyl formazan, which resulted from the reduction of triphenyl tetrazolium chloride by the actively growing microorganisms. This penetration was prevented or reduced to a great extent by the application of the various coatings studied.

Microbial growth within the agar was not observed in any of the eggs. Deposition of triphenyl formazan was limited to the inner surface of the shell and the shell membranes, indicating that the actively growing organisms had progressed only this far within the 48 hr incubation period.

The percentage of eggs which were penetrated by *S. typhimurium* and *P. fluorescens* is presented in Table 1. All of the inoculated uncoated controls were penetrated by both organisms. Only 3.3% of the uninoculated uncoated control eggs were penetrated by organisms present on the egg shell.

The average shell population of the uncoated eggs was found to be 1.1×10^7 microorganisms per egg when inoculated with *S. typhimurium* and 2.6×10^7 when inoculated with *P. fluorescens*.

Zein was the most effective coating against the penetration of *S. typhimurium*, preventing penetration in 83.3% of the eggs. Coatings 974-1 and Epolene wax prevented penetration in 63.3% and 55.2% of the eggs, respectively, while Polidene 930-H prevented penetration of *S. typhimurium* in only 21.4% of the eggs inoculated. When viewed with transmitted light, the shell of eggs coated with Polidene 930-H had a mottled appearance, indicating the coating may not have covered the shell surface evenly.

All coatings were more effective in preventing penetration of *P. fluorescens* than *S. typhimurium*, even though the inoculum level was of approximately the same order. Coating material 974-1 prevented penetration by *P. fluorescens* in 100% of the eggs. The success of this coating was unexpected because during the inoculation process the inoculum acquired a slight oily appearance which seemed to indicate that part of the coating had been removed. It is possible that the shellac present in the coating remained in the pores of the egg, preventing the bacterial penetration.

Zein prevented penetration in 96.7% of the eggs inoculated with *P. fluorescens* while Epolene wax and Polidene 930-H prevented penetration in 86.7% and 63.3%, respectively.

Microbial degradation of coating materials

Sterile filter paper discs dipped in the two test microorganisms did not result in any growth of the microbes when placed on the slides coated with the various coating materials, nor was any decomposition of the coating materials detected either microscopically or macroscopically after incubation for 7 days at 97% relative humidity.

Further evidence for the resistance of the coating materials to microbial decomposition was obtained in the tests where the dried coating films or coatings applied to filter paper were incubated in liquid media. No growth of either *P. fluorescens* or *S. typhimurium* was obtained in saline containing only the coating materials as the substrates. Heavy growth of both organisms was obtained in the saline + peptone media; however, no decomposition of the coatings was apparent. Strips of the dried coating 974-1 dissolved in the liquid media, causing it to be slightly milky in appearance. Additional turbidity from microbial growth in the saline was not evident when comparisons were made with the uninoculated control tubes of saline and the coating material.

Some inhibitory effect on the growth of *P. fluorescens*, *Salmonella derby*, and *Aerobacter aerogenes* has been shown by the application of wet filter paper discs soaked in the coating solutions to inoculated agar plates *(8)*. In their tests, Meyer and Spencer showed that Epolene wax inhibited growth of all three microorganisms, Polidene 930-H inhibited the growth of *P. fluorescens*, and Zein had no inhibitory effect. It is possible, therefore, that application of certain coating materials would have a sanitizing effect or at least prevent further growth of the microbes present. Inhibitory properties of the coatings tested in this study were less than those observed by Meyer and Spencer *(8)*.

In the event that any one of these coatings might be used commercially to increase egg shell strength, they would have the added advantage of reducing bacterial penetration through the egg shell. For maximum resistance to bacterial penetration, it appears that the coatings Zein and 974-1 would be the best choices. Although shelf-life studies of coated inoculated intact eggs were not done, from data obtained in these experiments, it seems reasonable to hypothesize that coating of shell eggs would markedly increase their shelf-life.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT

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Commercial Powered Food Preparation Equipment, were reviewed by the Joint Committee. Following completion of the review, the proposed amendments, as revised, were recommended for adoption. Copies of this and all other Standards or Criteria are available from the National Sanitation Foundation, NSF Building, Ann Arbor, Michigan, 48105.

Standard No. 12—Automatic ice making equipment

Following the 3-year review of the proposed revisions in NSF Standard No. 12, Automatic Ice-Making Equipment, the amendments, as revised, were recommended for adoption. During the course of reviewing the Standard, the need for further study of Item 4.302 relating to the cleanability of cold plates including their appurtenances and the containers for the storage of edible ice where such plates are installed in the edible ice compartment.

Standard No. 29—Detergent and chemical feeders for commercial spray-type dishwashing machines

The current provisions of NSF Standard No. 29, Detergent and Chemical Feeders for Commercial Spray-Type Dishwashing Machines, were discussed as they related to other than detergent feeders. The Joint Committee agreed that it was its intent to include performance requirements for all types of chemical feeders. To this end, the Foundation staff was requested to prepare a proposed revision in the Standard to provide such requirements.

Covers and doors over the food zone (Applicable to almost all standards)

The following specifications for covers and doors over the food zone have been approved by the Joint Committee following a 2-year study to provide a feasible plan and uniformity between the applicable Standards:

(a) Where under anticipated use condition, surfaces adjacent to top openings to food zone may permit contaminated drainage to enter the food zone, such openings shall be protected by a raised rim which shall extend at least 3/16 inch above the level to which liquids may accumulate.

(b) Where under anticipated use conditions, top openings to food zones are required to be covered to protect against the entrance of contaminants. Such openings shall be covered to effectively preclude the entrance of contamination. Covers shall meet the following requirements: *(i)* The cover or door shall be provided with a flange which overlaps the opening and shall be designed to prevent spillage or other foreign materials from entering the food zone in the closed position or when being opened. *(ii)* The cover or door shall be sloped to provide drainage from the door or cover surface. *(iii)* Doors and covers shall be designed with sufficient clearance to avoid contact with the foods which they cover. *(iv)* Hinges or pivots shall be designed to be easily cleanable and of simple, take-apart design and construction. Piano hinges are not acceptable in the food zone. Covers shall be readily removable or easily cleanable in place.

When under anticipated use condition, top openings to food zones do not require protective covers or where the cover is provided as a functional part of the equipment such as covers for pressurized vessels, steam retaining enclosures, as exampled by bun warmers, or in units where a cover is provided but not required for protection against contamination, the cover or door need not comply with the requirements of Item 2(a) through 2(c). The covers, however, should comply with Item 2(d). Covers designed to permit stacking of units need not comply with Item 2(b).

Future plans

The scheduled 3-year review of Standards Nos. 1, 2, and 20 should be ready for study and comment by this Committee before 1973. In addition, plans for Standards for cart washers, retail food store refrigeration equipment, high pressure spray cleaning equipment, carpeting for food service areas, compactors, and beverage cases are being considered.

Furthermore, the Foundation staff reported that a number of comments had been received suggesting that the Foundation include in its Standards appropriate references to the metric system. Following a brief discussion, the Joint Committee agreed that the Foundation Standards should include dual references to the English and metric systems.

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