APPLIED MICROBIOLOGY, May 1967, p. 471-475

Vol. 15, No. 3
Printed in U.S.A.

Coconut and *Salmonella* Infection

CARL P. SCHAFFNER, KLAUS MOSBACH, VENUSO C. BIBIT, AND COLIN H. WATSON

Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey 08903, Institute of Biochemistry, University of Lund, Lund, Sweden, and Red V Coconut Products, Ltd., Manila, Republic of the Philippines

Received for publication 23 September 1966

Raw, unprocessed coconut supports the growth of salmonellae as well as that of other enteric bacteria, salmonellae being particularly resistant to subsequent desiccation. Original contamination is not due to carriers or to polluted water supplies, but to contact with bacteria-containing soils followed by dispersion via infected coconut milk and shells. Pasteurization of raw coconut meat in a water bath at 80 C for 8 to 10 min effectively killed such bacteria, did not injure the product, and provided a prophylactic method now widely used by the coconut industry.

So much literature exists documenting the ability of salmonellae to resist desiccation (5, 6, 10) that Joe (7) proposed the dispatch of dried stool specimens to central laboratories for detection of enteric bacteria.

Wilson and Mackenzie (18) first reported processed coconut as a potential carrier of salmonellae; Kovacs (8) also isolated them from dried coconut, as well as from other desiccated products. A notation by Galbraith et al. (4) and an outbreak of salmonellosis in the Liverpool, England, area in 1960–1961, directly traced to coconut by Semple (14), concentrated attention on this particular food. Daniels-Bosman and Huisman (1), Winkle, Rohde, and Adam (19), Ellingsen and Skogsholm (2), and Velaudapillai et al. (16) offered confirmation of such findings, all reporting positive samples from Papuan or Ceylonese coconut, or from both. It would be reasonable to suspect Philippine coconut also, and in 1961 U.S. Federal authorities encountered viable salmonellae, warranting detention of Philippine imports.

We cannot consider salmonellosis as a fully controlled disease; within recent years, van Oye (12) found it a "world problem," Weil and Saphra (17) reported fatalities of 4 to 20% for the invasive salmonellae, and Kovacs (8) referred to the general increase of such infections in man. A 1965 California outbreak received wide press coverage, perhaps, only because of the concentration of immediate sufferers and the fact that many were children.

With the vast improvement in food distribution techniques in the last decade, there is no doubt of the constant potential threat of rather widespread *Salmonella* poisoning due to contaminated foods. Concern over the contamination in such dried food products as eggs, milk, and nuts has in recent years increased. Additionally, salmonelloses mimic any of the other microbial infections (17) and thus avoid proper detection.

Because of the grave economic implications for the firms and countries producing coconut products, we undertook the following study.

MATERIALS AND METHODS

Sampling. From delivery trucks at the processing plant, random samples of coconuts were taken without regard to maturity, surface condition, cleanliness, or freedom from injury. Those leaking milk were rejected. Samples were taken from the insides of shells with cotton swabs; the meat was removed with sterile knives, and the milk with sterile pipettes. Additional studies concerned other, obviously contaminated, milk-free, cracked nuts.

Detection of Salmonella. Taylor's methods (15) were employed for all isolations. Samples were pre-enriched by cultivation in 0.5% mannitol-purple broth base (1-g samples of solid material or 1-ml liquid samples being inoculated per 10 ml of medium). After incubation at 37 C for 24 hr, 0.2 ml of the culture on mannitol broth was transferred to 10 ml of cystine-selenite broth (11) and to 10 ml of brilliant green tetrathionate broth (9) for enrichment culture and held at 37 C for 24 hr. Tubes of mannitol purple and brilliant green tetrathionate media served as controls.

After streaking enrichment broths on brilliant-green agar plates, suspect colonies were transferred to dulcitol-lysine-lactose-iron agar slants above a phenol red butt (15) and to a modified lysine broth (3), for incubation at 37 C for 24 hr.

The last two media were used for generic biochemical identification of suspect colonies. Salmonellae usually ferment dulcitol, produce H_2S, utilize L-lysine, and do not ferment lactose. In addition, they characteristically react upon the slants to produce an alkaline response (red) and H_2S blackening in the center, with a gas-ruptured acid butt (yellow). By

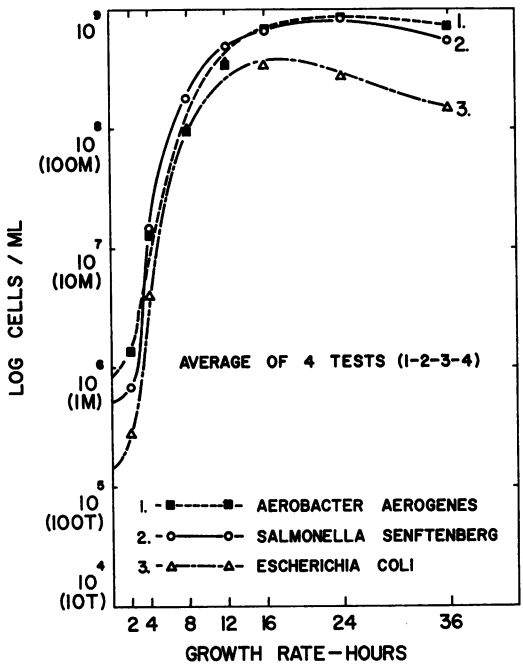

FIG. 1. *Growth curves for Aerobacter aerogenes, Escherichia coli, and Salmonella senftenberg incubated in coconut milk for 48 hr at 37 C.*

serological typing, Taylor (15) found the technique valid for generic identification in more than 98% of 300 cultures submitted to typing.

In doubtful cases, further biochemical identification utilized indole, methyl red, and Voges-Proskauer tests; beyond this point, any result not definitely negative was considered positive.

Decontamination, sterilization. Intact nuts were opened aseptically, inoculated with various strains of salmonellae including *Salmonella senftenberg*, and resealed; after 24 hr at 37 C, samples of meat and milk were removed aseptically for examination. Single piece, 10-g samples of meat, or 10 ml of milk in screw-cap culture test tubes, 20 by 150 mm, were immersed in water baths, with temperatures ranging from 40 to 100 C in 10-C increments; at 1-min intervals, samples were removed and were cooled in ice baths; the number of residual salmonellae was determined by plating directly on brilliant-green agar plates, after pre-enrichment and after enrichment.

Additionally, we used live steam at 215 F (101.7 C) for 1 min, with subsequent techniques identical to those with water baths.

RESULTS AND DISCUSSION

Proof of the ability of desiccated coconut products to harbor salmonellae had not, by 1962, led to determination of the nature and site of contamination, but by 1962 increasing detection of coconut by the U.S. Food and Drug Administration made some solution for the industry imperative. Our investigations were made in the Philippines in two microbiological control laboratories we established, one at Lucena City, Luzon, the other at Oroquieta, Mindanao.

The coconut industry considered the seemingly sudden, certainly increasing reports of contamination statistically skewed and the result of bad luck. Governmental authorities felt it a matter of continuing contamination, most probably from feces but perhaps from carrier infection or polluted water, this last answer seemingly ideal, because production occurred in parts of the world where general sanitary conditions were often less than perfect.

Microbial infection of the growing nut proved nonexistent or negligible except when mechanical injury exposed meat or milk to such infection. In common practice, however, the ripe nut detached from the tree is clustered on the ground for storage on soil itself contaminated by human and animal excreta since manures fertilize the palm fields and oxen often transport the nuts. The very nature of the coconut tends toward injury in husking, as it does in loading, hauling, and unloading; severe damage permits seepage of milk, an ideal carrier for most enteric bacteria. We therefore expected contamination in this order: earth to husk, to shell, to milk, to meat, and to other shells ad infinitum.

Figure 1 illustrates growth curves for *Aerobacter aerogenes*, *Escherichia coli*, and *S. senftenberg* incubated in coconut milk 48 hr at 37 C. With an average volume of 300 ml of milk per coconut, one contaminated nut served as reservoir for as many as 8.6×10^8 viable salmonellae per ml.

Bacterial examination of nuts on delivery vans and in the nut bodega, or storage shed, revealed occasional heavy infections of enteric bacteria, the most prevalent contaminant of whole and cracked nuts being *A. aerogenes*, whose characteristic odor pervaded the bodega and the shelling and paring areas, and whose gas production within intact nuts sometimes caused a dangerous explosion.

Salmonellae, however, produced no discoloration, no meat-softening, slime, or odor; nor was there some obvious signal to alarm even experienced plant personnel, who have always discarded raw material showing spoilage.

Because any delay in moving the exposed coconut to the drying process obviously increased the microbial population, the time between shelling, paring, and desiccating was less than 1 hr. Following shelling and paring, the nuts were cracked and the milk discarded.

After washing, the meat proceeded to grinders and finally to continuous, through-circulation

driers. Random microbiological checks prior to grinding revealed occasional pieces of meat heavily infected with enteric bacteria.

Drying, for 30 min at temperatures between 200 and 250 F (93.3 and 121.1 C), destroyed a great proportion of bacterial population, the numbers of bacteria surviving being directly related to the extent of contamination of the wet, ground meat. Mixed, ground meat was always more uniformly contaminated.

TABLE 1. *Serotypes isolated by various investigators*

Salmonella serotypes	Daniels-Bosman (1)[a]	Ellingsen (2)	Galbraith (4)	Kovacs (8)	Bartram[b]	Semple (14)	Vallaudapillai (16)	Wilson (18)	Winkle (19)	Totals
S. amager									X	1
S. anatum									X	1
S. angoda			X				X			2
S. arizona									X[c]	1
S. bareilly	X	X	X		X				X	5
S. butantan				X						1
S. chester									X	1
S. cubana		X			X					2
S. daytona								X		1
S. edinburgh				X						1
S. ferlac									X	1
S. hvittingfoss		X	X							2
S. infantis	X									1
S. java	X								X	2
S. kotte	X		X							2
S. lethe									X	1
S. lexington					X					1
S. litchfeld		X	X							2
S. matopeni							X			1
S. mississippi								X		1
S. morbificansbovis								X		1
S. nchanga		X								1
S. newport			X				X		X	3
S. nyborg								X		1
S. orion								X		1
S. oslo		X	X							2
S. paratyphi B		X	X	X		X	X	X	X	7
S. perth	X	X		X			X		X	5
S. potsdam								X		1
S. rubislaw			X							1
S. senftenberg		X	X		X		X	X		5
S. solna			X							1
S. stanley					X				X	2
S. thompson		X						X		2
S. typhi								X		1
S. typhimurium	X		X				X	X		4
S. vancouver			X							1
S. virchow	X									1
S. waycross	X	X	X				X		X	5
S. welikadi							X			1
Unidentified		X[d]						X[e]		2

[a] Figures in parentheses refer to reference in Literature Cited section.
[b] Personal communication.
[c] May be new serotype.
[d] Probably *S. thompson*.
[e] Unidentified, perhaps *S. marylebone*.

After desiccation, the coconut is sifted and sorted in mechanical sieves, a process not conducive to further growth since desiccated coconut has a moisture content of only 2 to 4%, and reinfection is minimal owing to thorough sanitary precautions.

Extensive tests of well water disclosed no positive bacterial pollution; with evidence of overall contamination, the possibility of one, or even several, carriers was discounted.

Sanitary conditions within the plants were excellent; after desiccation, in which bacterial population declined to minimal levels, all possible measures prevented reinfection. During storage at room temperatures, total bacterial counts declined further but not sufficiently, since the Food and Drug Administration still isolated and completely identified salmonellae present in U.S. imports (M. T. Bartram, *personal communication*).

These isolates were confined to five types: *S. senftenberg*, *S. cubana*, *S. lexington*, *S. stanley*, and *S. bareilly*. Over 75% of the isolates were found to be *S. senftenberg*; all were H_2S-negative. Approximately 20% of the isolates were *S. cubana*, the remainder of strains making up the difference. Determination of the pathogenicity of the isolates was of little importance, most authorities considering that any and all of the salmonellae have potential pathogenicity. Table 1 reviews different serotypes isolated from coconut by various investigators, the isolation of even one sample of *S. typhi* suggesting the seriousness of such infection.

With evidence of continuing, residual contamination in every phase of processing, with the lack of any visual or odorous signal of contamination, the necessity of some general sterilization, before or after desiccation, became obligatory.

Galbraith et al. (4) decontaminated desiccated coconut by roasting in a traveling oven; Seiler (13) performed the same decontamination using Reel and Peel ovens, although he found that variation of exposure time, as little as 30 sec, and variation in temperature could be critical. Resultant discoloration of the product often did not appeal to the aesthetic taste of the users. Our own experiments at roasting desiccated coconut gave insufficient decontamination and the same discoloration noted by Seiler, as well as significant general deterioration of the product.

Although Kovacs (8) considered pasteurization of coconut impossible and the only probabilities decontamination by ethylene oxide, radiation by γ-rays of spent fuel rods, cobalt 60, or electron sources from resonant accelerators of van de Graff generators, we felt a more ordinary, more economical, less complex procedure should be first considered. Foodstuffs have most widely been sterilized by wet heat, and the failure of the conveyor-type (and, previously, tray-type) driers during the manufacturing process indicated the low efficiency of dry-heat sterilization.

In a series of pasteurization experiments, those described under Materials and Methods, a 1-min, 100-C contact destroyed salmonellae and related bacteria, but greatly impaired quality of the coconut. Equally effective steam sterilization produced similar serious product degeneration. Exposure in water baths at 90 C for 5 min resulted in complete decontamination, but after desiccation deleterious side effects again prevailed in the product. Treatment at 80 C for 5 min. gave a complete kill and no deterioration of the finished product.

Lower temperatures invariably decontaminated incompletely.

Sterilization of coconut milk, opposed to that of meat, occurred at lower temperatures, the difference in kill due indubitably to different rates of heat penetration in milk and meat.

Our findings then added these steps to the manufacture of desiccated coconut. Peeled and cracked nuts, first washed in unheated water to reduce surface contamination, were placed in water immersion tanks maintained at 70 to 80 C, with immersion of the unground meat at that temperature for 8 to 10 min. Grinding followed the manufacturer's norm, as did desiccation in the continuous, through-circulation driers. With these conditions carefully maintained, no viable salmonellae could be detected in the desiccated coconut emerging from the ovens.

Pasteurization offered a simple solution to a most serious problem within the coconut industry; these experiments allowed us to design high-efficiency pasteurization equipment providing a safe foodstuff which hitherto harbored enteric bacteria to varying degrees.

LITERATURE CITED

1. DANIELS-BOSMAN, M. S., AND J. HUISMAN. 1961. Salmonellae in desiccated coconut and coco-products. Trop. Geograp. Med. **13**:281–283.
2. ELLINGSEN, J. K., AND A. SKOGSHOLM. 1962. Salmonellabakterier 1 kokosmasse. Nord. Veterinarmed. **14**:29–38.
3. FALKOW, S. 1958. Activity of lysine decarboxylase as an aid in the identification of salmonellae and shigellae. Am. J. Clin. Pathol. **29**:598–600.
4. GALBRAITH, N. S., B. HOBBS, M. SMITH, AND A. J. H. TOMLINSON. 1960. Salmonellae in desiccated coconut. An interim report. Monthly Bull. Min. Public Health Lab. Serv. **19**:99–106.
5. GALTON, M., M. HARLESS, AND A. HARDY. 1955. Salmonella isolations from dehydrated dog foods. J. Am. Vet. Med. Assoc. **126**:57–58.

6. HOFMANN, P., F. HORCHNER, AND R. WOLLE-JOHN. 1960. Einschleppung seltener Salmonellae durch importierte geflügelfedern. Zentr. Bakteriol. Parasitenk. Abt. I **178**:484–491.
7. JOE, L-K. 1956. The dried filter paper technique for sending stool specimens to a laboratory for bacterial examination. Am. J. Trop. Med. Hyg. **5**(1):133–135.
8. KOVACS, N. 1959. Salmonellae in desiccated coconut, egg pulp, fertilizer, meat-meal, and mesenteric glands: Preliminary report. Med. J. Australia **46**:557–559.
9. MCCULLOUGH, N. B., AND A. F. BYRNE. 1952. Relative efficiency of different culture mediums in isolation of certain members of the Salmonella group. J. Infect. Diseases **90**:71–75.
10. MUELLER, J. 1952. Bacterial examination of imported meat-and-bone meal and the like. Nord. Veterinarmed. **4**:291.
11. NORTH, W. R., AND M. T. BARTRAM. 1953. The efficiency of selenite broth of different compositions in the isolation of *Salmonella*. Appl. Microbiol. **1**:130–134.
12. OYE, E. VAN 1964. The world problem of salmonellosis. W. Junk, The Hague.
13. SEILER, D. A. L. 1960. The effect of time and temperature on the survival of salmonellae in desiccated coconut. Monthly Bull. Med. Res. Council Gt. Brit. **19**:211–212.
14. SEMPLE, A. B. 1962. Annual report of the medical officer of health. Brit. Food J. **64**:173.
15. TAYLOR, W. I. 1961. Isolation of salmonellae from food samples. V. Determination of the method of choice for the enumeration of *Salmonella*. Appl. Microbiol. **9**:487–490.
16. VELAUDAPILLAI, T., K. NITIANANDA, AND K. MEEDENIYA. 1964. Salmonella in desiccated coconut. Z. Hyg. Infektionskrankh. **149**:122–125.
17. WEIL, A. J., AND I. SAPHRA. 1953. Salmonellae and shigellae. Charles C Thomas, Springfield, Ill. 247 p.
18. WILSON, M. M., AND E. F. MACKENZIE. 1955. Typhoid fever and salmonellosis due to consumption of infected desiccated coconut. J. Appl. Bacteriol. **18**:510–521.
19. WINKLE, S., R. ROHDE, AND W. ADAM. 1960. Salmonellabefallene importierte kokosraspeln als eine weitere gefährliche infektionsquelle. Zentr Bakteriol. Parasitenk. Abt. I **179**:583–586.