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Effect of Vacuum Packaging on Growth of *Clostridium botulinum* and *Staphylococcus aureus* in Cured Meats[1]

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ABSTRACT

CHRISTIANSEN, LEE N. (The University of Wisconsin, Madison), AND E. M. FOSTER. Effect of vacuum packaging on growth of *Clostridium botulinum* and *Staphylococcus aureus* in cured meats. Appl. Microbiol. **13**:1023–1025. 1965.—Incrimination of vacuum-packaged smoked fish in outbreaks of botulism has raised questions about the safety of this process in comparison with other methods of packaging foods. It has been suggested, for example, that *Clostridium botulinum* may grow better in a vacuum-packaged product than in one that is packaged without vacuum. To evaluate this possibility, sliced bologna was inoculated with spores of *C. botulinum* type A, packaged in transparent plastic film with and without vacuum, and stored at temperatures within the growth range of the organism. There was no detectable difference in the rate of toxin development in the two types of packages. In contrast, vacuum packaging markedly inhibited the growth of *Staphylococcus aureus* on sliced ham. The results indicate that vacuum packaging has little if any effect on the ability of *C. botulinum* to grow in cured meats, but it may reduce the likelihood of staphylococcal food poisoning.

The incrimination of vacuum-packaged smoked fish in outbreaks of botulism (Anonymous, 1964; Kautter, 1964) has led both scientists and laymen to wonder if the anaerobic conditions established by this process may increase the opportunity for growth of *Clostridium botulinum*. It is easy to reason that (i) *C. botulinum* is an anaerobe; (ii) vacuum packaging produces anaerobic conditions; therefore, (iii) vacuum packaging may encourage the growth of *C. botulinum*.

By the same reasoning, it may be adduced that vacuum packaging should inhibit the growth of food-poisoning staphylococci. Though facultative, these organisms grow better under aerobic than under anaerobic conditions.

Huge amounts of cured meat products are marketed in vacuum packages, and many of them will support the growth of food-poisoning organisms if inoculated and held at a suitable temperature. This study was designed to see whether vacuum packaging does, in fact, affect the likelihood of toxigenesis if products are mishandled. For this purpose, suitable cured meat products were inoculated with food-poisoning organisms, packaged with and without vacuum, and stored at temperatures above that of normal refrigeration. The rates of development of the test organisms were compared in the two types of packages.

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MATERIALS AND METHODS

Packaging with vacuum. Slices of bologna or chopped ham were inserted into 6 by 7 inch Flexar 12 pouches made of 0.0005-inch Mylar polyester film/0.0001-inch Saran coating/0.002-inch polyethylene (Standard Packaging Corp., Clifton, N.J.). The pouches were evacuated to 15 mm of Hg and heat-sealed with a Goglio packaging machine.

Packaging without vacuum. Two or more slices of meat were positioned on a piece of waxed cardboard of appropriate size and wrapped in a single thickness of 60-gauge Saran film. The edges of the film were heat-sealed to each other against the cardboard backing, but the packages were not air tight.

Botulinum toxin formation in bologna. Slices of large bologna obtained directly from the producer were smeared on one side with a cotton swab that had been dipped in a suspension containing 10^7 spores of *C. botulinum* type A, strain 109, per milliliter. The spores had been heated to 80 C for 10 min after washing, and then suspended in 4% NaCl solution before use. The inoculum was applied in two ways in separate trials.

Trial 1. One side of each of five slices was smeared with spores; then the slices were stacked, and an uninoculated one was placed on top. Thus, the inoculum was located within the stack between adjacent slices.

Trial 2. Six slices again were used, but the

inoculum was smeared only on the top slice next to the packaging material.

In both trials, samples were packaged with and without vacuum and stored with uninoculated controls at 37, 30, and 22 C. At intervals thereafter, two packages of each type were selected at random to test for botulinum toxin as follows. The two center slices (trial 1) or the two top slices (trial 2) were blended for 1 min with three times their weight of physiological saline in a Waring Blendor. The slurry was centrifuged, and 0.5 ml of clear supernatant liquid was injected intraperitoneally into each of two mice. Control mice received (i) supernatant liquid plus type A antitoxin, (ii) boiled supernatant liquid, or (iii) unheated supernatant liquid from incubated but uninoculated samples. Type A botulinum toxin was judged to be present if both test mice died within 3 days and the control mice survived.

Growth of Staphylococcus aureus in chopped ham. Sliced chopped ham (a loaf-type sandwich meat) obtained directly from the producer was packaged with and without vacuum, two slices per package. The outer surface of the top slice was smeared with a cotton swab that had been dipped in 4% NaCl solution containing 10^7 cells of *S. aureus* strain S 6 per milliliter. Samples of each type were stored at 22 and 15 C. Daily thereafter three packages of each type were selected at random and the entire contents of each were blended with physiological saline. Staphylococci were counted by plating in triplicate on the surface of mannitol-salt-agar (Chapman, 1945).

TABLE 1. *Effect of vacuum packaging on rate of toxin production by Clostridium botulinum type A (strain 109) in sliced bologna*

Storage temp	Presence (+) or absence (−) of toxin					
	Trial 1, inoculum between slices			Trial 2, inoculum between product and package		
	Time	With vacuum	Without vacuum	Time	With vacuum	Without vacuum
C	*days*			*days*		
37	4	−−*	−−	4	−−	−−
	7	++	++	6	++	++
30	8	−−	−−	7	−−	−−
	11	+−	++	10	++	++
	12	++	++			
22	12	−−	−−	11	−−	−−
	14	−−	−−	15	+−	++
	16	+−	+−	17	++	++
	18	++	++			

* Each symbol represents two mice injected with the extract from one package of product.

RESULTS

Vacuum packaging did not influence the rate of botulinum toxin development in bologna (Table 1). In every instance, toxin was detected as quickly in nonevacuated packages as in the evacuated ones, even when the spores were deposited on the outer surface of the product (trial 2).

By contrast, the growth of *S. aureus* was markedly inhibited by vacuum packaging (Table 2). At both temperatures, the test organism increased about six generations and then leveled off at approximately 5 to 15 million per gram. Without vacuum, however, the organism went through 11 to 12 generations and reached numbers in the hundreds of millions, which were about 20-fold greater than the maximal populations developed under vacuum.

DISCUSSION

The results for *C. botulinum* type A in bologna agree with earlier findings for type E in smoked fish (Kautter, 1964; Thatcher, Robinson, and

TABLE 2. *Effect of vacuum packaging on growth of Staphylococcus aureus (strain S 6) in sliced chopped ham*

Time of storage	Count/g after storage at *					
	22 C			15 C		
	With vacuum	Without vacuum	Ratio†	With vacuum	Without vacuum	Ratio†
days						
0	77,000	79,000	1:1	97,000	97,000	1:1
1	630,000	1,100,000	1:2	110,000	140,000	1:1
2	7,700,000	130,000,000	1:17	260,000	350,000	1:1
3	8,600,000	220,000,000	1:26	5,100,000	96,000,000	1:19
4	6,400,000	130,000,000	1:20	7,400,000	150,000,000	1:20
5				15,000,000	260,000,000	1:17
6				5,100,000	77,000,000	1:15

* Each value represents the average of nine individual plates (three plates for each of three samples).
† Ratio of count with vacuum to count without vacuum.

Erdman, 1962). When the composition of the foodstuff and the temperature permit, toxin is formed whether the product is vacuum packaged or not.

There is evidence that vacuum packaging may accelerate toxin production somewhat at first under certain test conditions, but the effect is not great. Abrahamsson, De Silva, and Molin (1965) inoculated raw herring fillets with type E spores and packaged them in plastic bags (i) sealed under vacuum, (ii) sealed without vacuum, and (iii) unsealed. After 48 hr at 20 C, the evacuated packages contained more toxin than the others, but at 72 hr there was no difference between them. Similarly, Kautter (1964) found more toxin in smoked fish inoculated on the surface with type E spores and incubated anaerobically than in similar fish incubated in open plastic bags. There was no difference, however, when the spores were introduced into the flesh.

Thus, it appears that Johannsen (1961) was correct in concluding that vacuum packaging has little effect on growth and toxin production by *C. botulinum*. Apparently the oxidation-reduction potential of most foods is low enough to allow toxin formation regardless of the method of packaging.

Nevertheless, the safety of vacuum packaging has been questioned on two other grounds. (i) It increases the shelf life of perishable foods and thus provides "greater time and opportunity for toxin formation" (Kautter, 1964). Of course this is true—the only reason for vacuum packaging a perishable product is to increase its keeping time. But vacuum packaging does not eliminate the need for refrigeration. Failure to refrigerate a potentially dangerous food such as smoked fish can allow toxin to develop whether the product is vacuum packaged or not (Anonymous, 1964). (ii) Vacuum packaging may minimize "the activities of common spoilage organisms whose offensive changes could give warning of potentially unsafe conditions" (Thatcher et al., 1962). Again, this may be true when referring to the growth of molds and other aerobic microorganisms. But it is obvious that reliance can not be placed on the consumer's ability to detect signs of spoilage as a guarantee against consuming toxic foods. Thousands of deaths from botulism in the past, the great majority of which were caused by foods that were not vacuum packaged, testify to the futility of relying on the consumer's sensory perception of atypical odor or other evidence of spoilage.

With regard to the hazard of staphylococcal poisoning, the figures in Table 2 clearly show that vacuum packaging inhibits (but does not completely prevent) growth of *S. aureus*. Thatcher et al. (1962) obtained similar results with inoculated bacon and found, in addition, less enterotoxin to be produced under anaerobic than under aerobic conditions. As Greenberg (1964) pointed out, vacuum packaging may be especially beneficial with products that have a relatively low water activity, because food-poisoning staphylococci require more available water to grow anaerobically than aerobically (Scott, 1953).

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