BACTERIOLOGICAL INVESTIGATIONS ON FROZEN STUFFED POULTRY[0]

W. B. Esselen and A. S. Levine
Department of Food Technology, University of Massachusetts
Amherst, Massachusetts
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The aerobic, anaerobic, and putrefactive anaerobe populations in the dressing of frozen stuffed chickens showed no significant changes during storage of the chickens for 12 months at —10°F. Packaging material appeared to have no significant effect on the bacteria content of the dressing. During thawing and holding at room temperature, frozen stuffed chickens had a marked increase in aerobic and anaerobic bacteria counts after 20 hours. This was accompanied by an increase in acidity and the development of off-odors. Spores of a putrefactive anaerobe inoculated into the stuffing prior to freezing showed no evidence of germination and growth under this condition. The biological picture seemed to be unaffected by packaging material.

Introduction

A relatively new development in marketing is frozen stuffed ready-to-cook poultry. In the preparation of this product, the eviscerated birds are stuffed prior to being packaged and frozen. It was believed that further information on the bacteriology of frozen stuffed poultry would aid in the achieving of good handling procedures for this product. Specifically, the objects of this study were:

1. To determine whether or not conditions in a mass of bread stuffing, such as those present in stuffed, poultry, are favorable for the growth of anaerobes.

2. To determine the effect of freezing and subsequent storage at 0° F for one year on the survival of aerobic and anaerobic bacteria and the spores of a putrefactive anaerobe in packaged stuffed poultry.

3. To determine the effect of thawing and holding at room temperature on the growth of aerobic and anaerobic bacteria and the spores of a putrefactive anaerobe in frozen stuffed poultry.

Review of Literature

Published reports on the specific subject of frozen stuffed poultry are meager. However, there are such abundant recordings of investigations on the microbiological nature and public health aspects of other frozen foodstuffs that a judicious survey of the literature yields much information that could be applied to frozen stuffed poultry. From a review of the literature associated with this particular study, the following conclusions may be summarized:

1. Certain frozen foods, including poultry and poultry stuffing, provide a good medium for the growth of microorganisms among which are those associated with food poisoning outbreaks. Frozen foods have not been implicated in any case of botulism. However, it has been suggested that improper handling of such foods will cause trouble. On the other hand, some investigators think that the growth of the usual bacterial flora with accompanying acid production would inhibit the growth of *Clostridium botulinum* in spoiled frozen foods. [3, 5, 8, 10, 11, 13, 14, 17, 18, 19, 20, 25, 26, 27, 29, 34, 36, 37, 39]

2. Freezing and low temperature storage are not effective in reducing significantly the numbers of microorganisms present. [4, 6, 15, 23, 24, 31, 33]

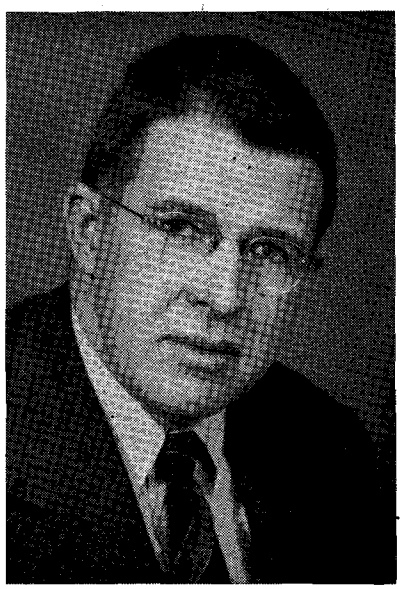
3. Good dressing and eviscerating practices and plant sanitation are essential in keeping bacterial counts to a minimum. [9, 12, 16, 28, 35, 38]

4. The rate of cooling and removal of body heat should be such that the temperature of the product is lowered rapidly enough to prevent any appreciable increase in the number of bacteria present. [22, 30, 32]

5. Prompt packaging of eviscerated poultry provides an effective means of preventing recontamination. [2, 21]

6. Frozen poultry including stuffed poultry is a highly perishable product and should be carefully handled during thawing, cooking, and subsequent holding. A temperature of 165° F should be reached in the center of the stuffing during the roasting period, [1, 7]. This temperature can be reached during the normal cooking of stuffed birds weighing less than 18 pounds. The roasting should be completed at one time.

7. In large stuffed birds, heat transfer during cooking is too slow to provide temperatures adequate to destroy potentially harmful bacteria at the center of the stuffing.[1, 7]



Professor Esselen received his B. S. in Food Technology at the University of Massachusetts in 1934; M.S., 1935; and Ph. D., 1938. He was a food technologist with the Owens-Illinois Glass Co., Toledo, Ohio, 1939-41. Since 1941, he has been on the Experiment Station staff in the Department of Food Technology, University of Massachusetts. Consultant, War Food Administration, U. S. Department of Agriculture, 1942-45. He has carried on research work on nutritive value of fruits, effects of processing on nutritive value and quality of foods, processing glass packed and home canned foods, thermal destruction of enzymes in fruit juice, apple products and pickles, and thermal destruction of bacterial spores.

Experimental Procedure

The experimental procedures in these investigations may be summarized as follows:

1. *Bacteriological Methods*

a. *Sampling*: Packages of poultry stuffing and packaged frozen stuffed poultry were opened aseptically with a sterile knife and spatula. A plug of stuffing from the outside to the center of the mass was removed by means of a sterile trier, and 10-gram samples were weighed into sterile wide-mouth dilution bottles containing 90 ml of water and glass beads. The bottles (which provided an initial 1 to 10

[0]Contribution No. 931 Massachusetts Agricultural Experiment Station

TABLE 1—GROWTH OF BACTERIA AND CHANGES IN pH OF POULTRY STUFFING PACKAGED IN CRYOVAC, PLIOFILM, AND CELLOPHANE DURING STORAGE AT ROOM TEMPERATURE

Time (hrs.)	Log of bacteria count per gram of stuffing					
	Control			Inoculated with P.A. 3679		
	Cryovac	Pliofilm	Cellophane	Cryovac	Pliofilm	Cellophane
			Aerobes			
0	6.02	6.02	6.02	5.96	5.96	5.96
24	8.11	8.32	8.24	8.08	8.17	8.10
48	8.00	8.16	8.07	8.18	8.04	7.94
72	8.25	8.21	8.31	8.17	8.34	8.12
96	8.40	8.26	8.45	8.32	8.30	8.26
118	8.19	8.15	8.21	8.11	8.17	8.10
142	8.43	8.39	8.14	8.17	8.11	8.16
166	8.13	8.14	8.17	8.14	8.43	8.10
			Anaerobes			
0	5.82	5.82	5.82	5.57	5.57	5.57
24	7.53	7.53	7.52	7.53	7.53	7.57
48	7.84	8.00	7.84	8.00	8.00	7.84
72	8.60	8.00	8.57	8.57	8.00	8.57
96	8.00	7.60	8.57	8.00	9.53	8.60
118	7.84	8.84	7.84	8.60	7.84	9.53
142	8.00	8.74	7.74	8.00	8.00	8.00
166	8.00	8.00	8.60	8.60	8.60	7.84
			pH			
0	5.45	5.45	5.45	5.45	5.45	5.45
24	5.14	5.17	5.20	5.16	5.18	5.17
48	4.86	4.95	4.93	4.87	5.00	4.89
72	4.61	4.56	4.60	4.55	4.53	4.55
96	4.46	4.39	4.36	4.39	4.50	4.41
118	4.33	4.30	4.29	4.33	4.32	4.29
142	4.30	4.30	4.29	4.29	4.34	4.37
166	4.22	4.07	4.12	4.11	4.08	4.08

dilution) were shaken 50 times to disintegrate the stuffing thoroughly. Serial dilutions up to 1 to 10 billion were then prepared for plating.

b. *Aerobic Bacteria Count*: Fifteen-ml amounts of Difco Nutrient Agar were used with poured Petri plates for determining the aerobic bacteria count. The plates were incubated for 3 days at 92° F.

c. *Anaerobic Bacteria Count*: The anaerobic bacteria count was determined by the decimal dilution procedure wherein 1-ml amounts of the dilutions were inoculated into freshly exhausted tubes of liver broth containing 0.1 percent soluble starch. These liver broth tubes were stratified immediately with a melted mixture of two parts mineral oil to one part paraffin. The tubes were incubated at 92° F for two weeks after the last positive tube appeared.

d. *Putrefactive Anaerobe Spore Count*: A second series of liver broth tubes was prepared and inoculated according to the procedure for anaerobe counts. To destroy vegetative cells, these stratified tubes were held in boiling water for five minutes and immediately chilled in cold water. The tubes were incubated at 92° F for two weeks after the last positive tube appeared. The presence of putrefactive anaerobes was detected by gas formation, digestion of meat, and characteristic odor.

2. *Packaging*

Portions of stuffing were packaged in small Cryovac (also known as Cryorap)† bags, Pliofilm (FF 120 gauge), and Cellophane (300 MSAT 87). The stuffed poultry was packaged in Cryovac bags and Cellophane (300 MSAT 87). The Cryovac packages were evacuated with an aspirator, twisted, clipped, and shrunk in water at 205° F. Sheets of Pliofilm and Cellophane were used with the drug-store wrap and heat-sealed. These packages had only a single thickness of film, except at the folds.

3. *Bacteriological Studies on Commercial Poultry Stuffing*

Three different lots of poultry stuffing prepared in a commercial plant engaged in the packing of frozen stuffed poultry were used. The stuffing was frozen at the plant and maintained in that condition during transportation to the laboratory. Packaged one-half pound portions of the stuffing, with and without inoculation of approximately 10,000 spores per gram of Putrefactive Anaerobe 3679 (a standard test organism), were stored at room temperature (at about 72° F) for approximately one week. The total aerobe and anaerobe counts and pH values were determined at intervals during storage. The results are summarized in Table 1.

The proximate composition of the stuffing was found* to be as follows:

Constituent	*Percent*
Moisture	44.9
Protein (N x 6.25)	7.6
Fat (ether extract)	18.4
Extract matter (carbohydrate)	26.2
Fiber	0.4
Ash	2.5

†Cryovac (sometimes known as Cryo-rap) is an oriented plastic film, copolymer of vinylidene chloride and vinyl chloride. Supplied by Dewey and Almy Chemical Company, Cambridge, Mass.

*Analysis by courtesy of Prof. J. W. Kuzmeski, Mass. Agr. Expt. Sta.

4. *Survival of Aerobic and Anaerobic Bacteria and Inoculated Spores of Putrefactive Anaerobe No. 3679 in Frozen Stuffed Poultry During Freezing and Storage at −10° F for One Year.*

Stuffed broilers, prepared under commercial conditions (at about 50° F) with uninoculated stuffing and with stuffing inoculated with spores of Putrefactive Anaerobe No. 3679 (10,000 spores per gram) were packaged, frozen, and stored at −10° F for one year. Immediately after being frozen and at intervals during storage, the aerobic and anaerobic and putrefactive anaerobe counts and pH values of stuffing from the poultry were determined. A minimum of five birds each were sampled for each test period and variable. The results are presented in Table 2.

TABLE 2—BACTERIA COUNTS OF STUFFING OF FROZEN STUFFED CHICKENS DURING STORAGE AT −10° F FOR ONE YEAR

Storage time (Months)	Log of Bacteria Count per Gram of Dressing			
	Control		Inoculated with P.A. 3679	
	Cryovac	Cellophane	Cryovac	Cellophane
		Aerobes		
0	5.43	5.43	5.28	5.28
1	5.32	5.30	5.17	5.34
2	5.56	5.48	5.56	5.49
3	5.14	5.34	5.33	5.36
4	5.55	5.28	5.39	5.44
5	5.50	5.49	5.55	5.57
6	5.72	5.58	5.45	5.68
8	5.75	5.67	5.92	5.65
10	5.59	5.72	5.78	5.76
11	5.65	5.72	5.81	5.71
12	5.59	5.61	5.90	5.92
Average	5.55	5.55	5.63	5.61
		Anaerobes		
0	6.00	6.00	5.00	5.00
1	5.00	4.00	5.84	5.00
2	6.00	5.00	6.00	4.00
3	4.87	4.56	5.00	5.60
4	5.00	4.82	4.00	5.84
5	4.74	5.53	5.00	5.74
6	5.00	3.00	4.70	4.84
8	3.74	4.83	5.84	4.00
10	4.60	4.74	5.70	5.60
11	5.74	4.60	5.57	5.00
12	5.00	4.00	4.00	3.00
Average	5.45	5.19	5.52	5.35
		Putrefactive anaerobes		
0	2.00	2.00	4.00	4.00
1	2.00	2.00	4.60	4.74
2	2.00	0.00	4.00	4.00
3	1.87	.48	3.84	3.78
4	2.00	1.84	4.00	4.60
5	1.74	1.00	3.84	4.60
6	0.00	1.74	4.00	4.00
8	2.00	1.60	4.60	4.74
10	1.84	1.74	3.74	4.60
11	2.00	3.00	4.84	3.74
12	2.00	2.00	4.00	4.00
Average	1.91	2.14	4.30	4.41

5. *Effect of Thawing and Holding Frozen Stuffed Poultry at Room Temperature on the Growth of Aerobic and Anaerobic Bacteria and Putrefactive Anaerobes*

Stuffed broilers, prepared as described above, were frozen and stored at −10° F. After various storage periods, groups of birds were removed from the freezer and held at room temperature (about 72° F). Aerobic and anaerobic bacteria and putrefactive anaerobe counts, and pH values of the stuffing from individual birds were determined at six-time intervals, up to 65 hours, during thawing and holding at room temperature. The birds were also examined organoleptically for evidence of spoilage during these tests. The results of five series are summarized in Table 3. Typical data obtained are also shown in Figures 1 to 4.

DISCUSSION AND RESULTS

Bacteriological Studies on Commercial Poultry Stuffing

There was a rapid increase in the bacteria count of poultry stuffing packaged in Cryovac, Pliofilm, and Cellophane, stored at room temperature during the first 24 to 48 hours. At the end of that time the bacteria counts tended to level off until there was some evidence of a decrease in viable organisms after 100 hours of storage. Both the aerobic and anaerobic bacteria counts showed essentially the same trends. The increase in bacterial population of the stuffing during storage was accompanied by a decrease in pH value (increase in acidity). These changes were to be expected in a predominantly carbohydrate medium such as poultry stuffing. During these tests there was little evidence of growth of putrefactive anaerobes.

The data obtained showed no significant differences in the development of bacteria with the three kinds of packaging materials used. The increase in bacteria counts in the stuffing during storage at room temperature was rapid and essentially the same with all the three packaging materials.

After 3 to 4 days of storage at room temperature the stuffing, which was not in vacuumized packages, sometimes showed considerable surface mold growth; whereas, that which was vacuumized showed no evidence of such mold growth at any time. This absence of mold growth in the latter can be attributed to the maintenance of a low oxygen tension within the package and the absence of "air pockets."

The moisture content of the poultry stuffing tested (in the range of 40 to 50 percent) was conducive to good bacterial growth, and such a medium could also be very favorable to the growth of organisms of public health significance.

Effect of Freezing and Storage at −10° F on Stuffed Poultry

Examination of stuffed poultry

TABLE 3—CHANGES IN BACTERIA COUNTS AND OF pH STUFFING OF FROZEN STUFFED CHICKEN DURING THAWING AND HOLDING AT ROOM TEMPERATURE

Time (hrs.)	Log of Bacteria Counts: Control		Inoculated with P.A. 3679	
	Cryovac	Cellophane	Cryovac	Cellophane
		Aerobes		
0	5.63	5.55	5.61	5.71
16	5.70	5.74	6.11	5.94
22	6.35	6.06	6.10	5.92
28	6.71	7.52	7.15	7.50
40	8.28	8.15	8.31	8.33
46	8.61	8.61	8.65	8.57
64	9.41	9.45	9.28	9.48
		Anaerobes		
0	5.45	4.79	5.39	5.38
16	6.35	4.80	6.31	5.45
22	6.39	6.35	6.39	7.31
28	7.41	6.39	7.35	7.31
40	7.91	7.66	7.91	8.42
46	8.45	9.35	8.64	8.64
64	8.74	9.00	9.45	8.90
		Putrefactive anaerobes		
0	1.60	1.78	4.00	4.00
16	2.34	0.00	4.45	4.66
22	2.34	1.30	4.79	4.66
28	2.60	0.00	4.42	4.45
40	1.34	1.60	4.45	4.66
46	0.00	2.30	4.45	4.45
64	1.48	1.72	4.45	4.51
		pH		
0	5.56	5.59	5.52	5.42
16	5.54	5.45	5.53	5.40
22	5.46	5.55	5.41	5.45
28	5.46	5.41	5.45	5.45
40	5.13	5.28	4.95	5.10
46	4.86	4.89	4.73	4.87
64	4.52	4.79	4.40	4.44

during storage at −10° F showed no significant changes in the aerobic, anaerobic, and putrefactive anaerobe populations in the stuffing over a period of twelve months. The kind of packaging material had no significant effect on the bacteria content of the stuffing. It would thus appear that the vacuum process employed in the Cryovac package had no significant influence on the stuffing either from a quality or public health standpoint.

It was interesting to note that the poultry packaged in Cryovac showed little or no evidence of freezerburn even at the end of twelve-month cold storage period. This is attributed to the vacuumizing and shrinking process and the gas-tight nature of the film.

Effect of Thawing and Holding Frozen Stuffed Poultry at Room Temperature

When frozen stuffed poultry was thawed and held at room temperature (70 to 80° F), there was a slight increase in the aerobic and anaerobic bacteria counts in the stuffing during the first 20 to 24 hours after removal from the freezer storage (−10° F). Development of bacteria was slow because the temperature of the bird was suboptimal for bacterial growth, and the bacterial cells were in the lag phase of their growth cycle. When the bird had warmed to room temperature, the logarithmic growth phase had been reached, and there was a rapid increase in the aerobic and anaerobic bacteria counts in the stuffing during the period from 20 to 24 hours up to 64 hours after removal from the freezer storage.

During this 64-hour period there was no significant change in the putrefactive anaerobe spore count in the stuffing of the chickens. There was no indication that the spores of Putrefactive Anaerobe No. 3679, previously inoculated into the stuffing, germinated or grew during this period.

The acidity as measured by a drop in pH value showed a progressive increase as the bacteria count increased during holding at room temperature. For example, the pH dropped from an initial value of about 5.5 to about 4.8 in 48 hours. This increase in acidity was to be expected during the fermentation of a predominantly carbohydrate medium such as poultry stuffing.

The failure of the inoculated putrefactive anaerobe spores to germinate and grow is attributed to the development of acid in the medium and also to a rapid overgrowth of other mircoorganisms. This inhibition of spore germination by acid production and overgrowth by other microorganisms has been suggested and recognized by Prescott and Geer [26], Fitzgerald [14], Berry [4], and Perry, *et al.* [25].

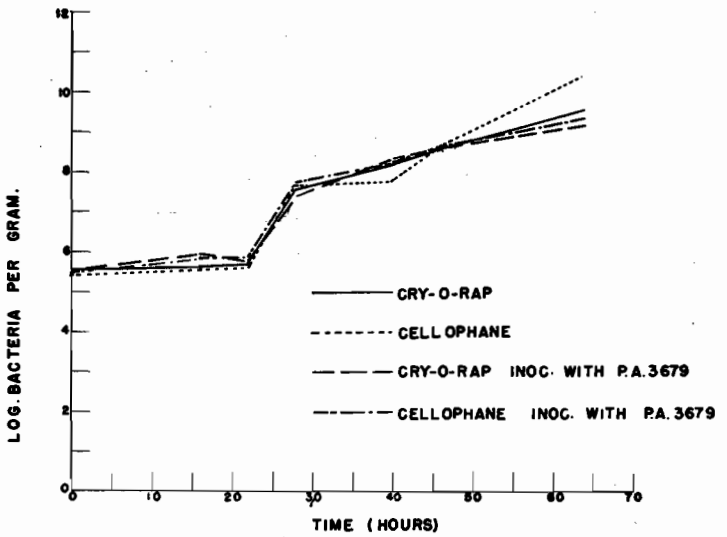
Again the kind of packaging material had no significant effect on the development of bacteria in the stuffing during thawing.

Organoleptic observations were made on these defrosted birds during holding at room temperature. After approximately 16 hours, a slight but noticeable off-odor had developed in all samples. After 24 hours the odor was quite strong and characteristic of spoiled poultry. This odor became progressively more marked and putrid as holding time increased.

CONCLUSIONS

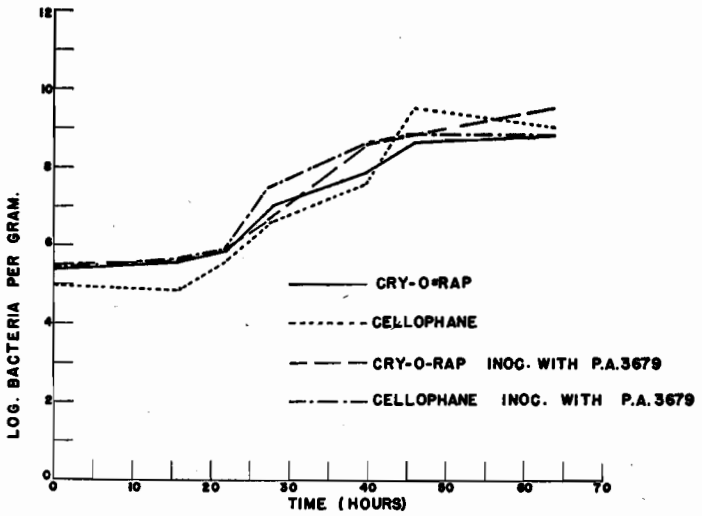
On the basis of the experimental work reported herein, it is concluded that:

1. Commercial poultry stuffing such as that used with frozen prepared poultry is an excellent medium for the growth of bacteria and, therefore, should be handled as a perishable food. The stuffing itself and poultry stuffed with it should be adequately refrigerated or frozen at all times except during the actual preparation and packing operations, when such low temperatures may be impossible to maintain. Under no conditions should the poultry stuffing be permitted to be at room temperature for more than the shortest time consistent with good packing procedures. Not more than



TOTAL BACTERIA COUNT IN DRESSING OF FROZEN STUFFED CHICKENS DURING THAWING AT ROOM TEMPERATURE. (AV. OF THREE TESTS)

Figure 1



TOTAL ANAEROBE COUNT IN DRESSING OF FROZEN STUFFED CHICKENS DURING THAWING AT ROOM TEMPERATURE. (AV. OF THREE TESTS)

Figure 2

four hours should elapse before the product is placed in the freezer.

2. Tests with poultry stuffing inoculated with spores of a putrefactive anaerobe of the *Clostridium* genus (P.A. 3679) indicate that at room temperature the rapid growth and acid production by other organisms present prevents or inhibits the growth of the former. Tests by others working with frozen vegetables have shown that this relationship between the growth of different bacteria provides an effective safeguard against the growth of *Cl. botulinum* in thawed frozen foods.

3. The growth and development of bacteria in poultry stuffing held at room temperature was essentially the same with the three different packaging materials used.

4. The observations in the present work indicate that vacuum packaging, by maintaining a low oxygen tension and absence of "air pockets" within the package, is effective in preventing or delaying surface mold growth on packaged products. Such factors in addition to gas-tightness properties and positive sealing properties are effective in inhibiting freezerburn or dehydration. Vacuum packaged frozen stuffed poultry maintained its quality during storage at 0° F for one year.

5. The aerobic, anaerobic, and putrefactive anaerobe populations in the dressing of frozen stuffed chickens showed no significant changes during storage of the chickens for 12 months at −10° F. Packaging material appeared to have no significant effect on the bacteria content of the dressing.

6. During thawing and holding at room temperature, frozen stuffed chickens had a marked increase in aerobic and anaerobic bacteria counts after 20 hours. This was accompanied by an increase in acidity and the development of off-odors. Spores of a putrefactive anaerobe inoculated into the stuffing prior to freezing showed no evidence of germination and growth under these conditions. The biological picture seemed to be unaffected by packaging material.

ACKNOWLEDGMENT

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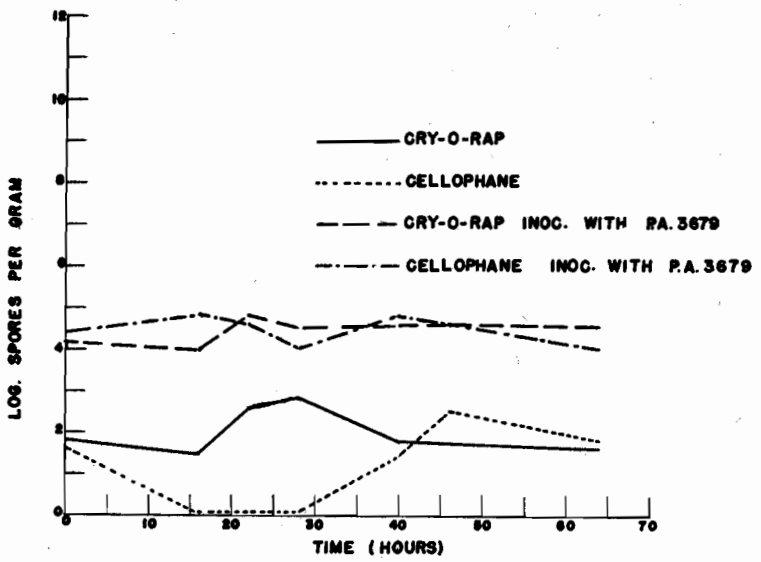
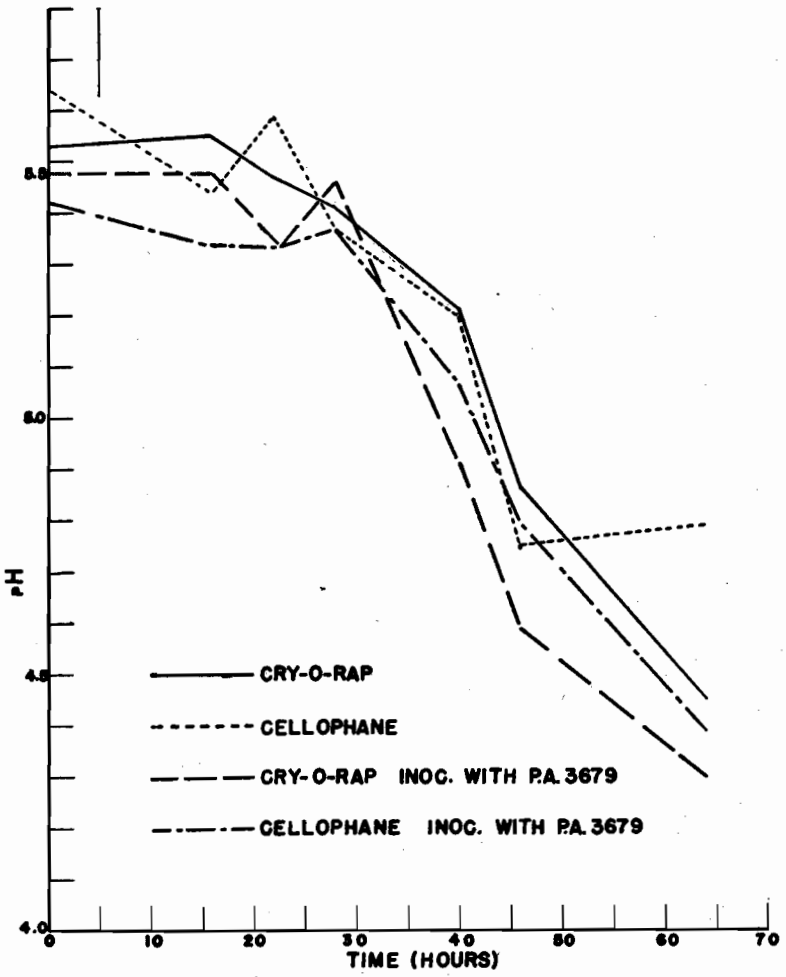


PUTREFACTIVE ANAEROBE SPORE COUNT IN DRESSING OF FROZEN STUFFED CHICKENS DURING THAWING AT ROOM TEMPERATURE.

Figure 3



pH CHANGE IN DRESSING OF FROZEN STUFFED CHICKENS DURING THAWING AT ROOM TEMPERATURE

Figure 4

of light source, that is, fluorescent or incandescent, is the criterium for determining whether or not meat will be discolored.

Sanitarians must be aware of the fact that although produce in the display case may be spoiled, it may have been caused by improper handling prior to delivery at the retail outlet. Retail men have an interest in getting a good product. Company recommendations should be followed regarding the amount of produce and placement of produce in the open display case. By using proper methods the material will be maintained at the proper temperature and humidity in an open unit and will be attractive so that maximum customer appeal will be obtained.

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THIRD REPORT OF THE RESOLUTIONS COMMITTEE of the Association of Food and Drug Officials of the United States May 21, 1954

RESOLUTION X

WHEREAS, The Association of Food and Drug Officials of the United States assembled in annual convention, Des Moines, Iowa, May 20, 1954, has carefully studied and evaluated House Bill 8368, entitled "A Bill to Amend the Agricultural Marketing Act of 1937, so as to Remove Domestic Trade Barriers Affecting Milk and Milk Products", introduced by Hon. August H. Andressen, referred to the Committee on Agriculture,

WHEREAS, It is believed that the authors of this bill, while concerned with the remedies which they sought, failed to recognize the untoward effect that the principle employed would have in depriving all states of their traditional, inherent and rightful authority in ordering their own affairs and in promoting and protecting the health and welfare of their people;

WHEREAS, It is considered apparent that the principle of this bill is wrong, that it would serve no constructive purpose in promoting the sale of milk nor in promoting the principles of public health.

THEREFORE, BE IT RESOLVED, That because of the defective nature of this Bill, both in principle and detail, this Association is not only unalterably opposed to its passage as law, but also is opposed to this principle in legislation.

(Signed) E. W. Constable
(Chairman)
J. H. McCutchen
Sarah Dugan