Combined Irradiation–Heat Processing of Canned Foods : Green Peas Inoculated with Anaerobic Bacterial Spores

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Summary. Combined irradiation-heat processing is shown to be synergistic for killing anaerobic bacterial spores inoculated into canned green peas. Following 1.2 megarad of gamma irradiation an F_0 of 0.5 sterilized green peas inoculated with either 5,000,000 *Clostridium botulinum* 213 B or 300 PA 3679 spores per can. This processing schedule must be considered a minimum since it is based on a limited number of cans. Because sterilization by either irradiation or heat alone can damage the organoleptic properties of canned foods, the lowered schedules possible with combined processing may prove to be of value for preserving vegetables as well as meats.

Introduction

Combined irradiation-heat processing is shown to be synergistic for killing anaerobic bacterial spores inoculated into canned peas. This had previously been demonstrated with canned meat (Kempe *et al.*, 1957; 1959). It had also been shown that sterilizing doses of either heat or ionizing radiations often lower the organoleptic qualities of foods, but that smaller dosages may be acceptable (Schultz *et al.*, 1956). Since reduced quantities of gamma radiation and heat are required when their synergistic effect on bacterial spores is utilized, combined processing could become useful for food preservation. For this reason, it was considered desirable to learn whether combined irradiation-heat processing was also effective in canned vegetables. Green peas were selected for study because they are packed commercially and are a good growth medium for anaerobic bacterial spores (Reed *et al.*, 1951).

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Materials and Methods

A. Packing

Commercially frozen green peas were obtained in 2.5 lb boxes from the stock supply of the University of Michigan Food Service. Labels on the packages indicated that the peas contained a 'slight amount' of added salt.

In preparation for a run, 15 lb of the frozen peas were placed in a stock pot and these were covered with a brine containing 1.8per cent sodium chloride and 2.2 per cent sucrose. This brine was previously described for peas by Reed *et al.* (1951). The stock pot was then placed in a boiling water bath for about 1.5 h during which time the frozen peas melted and were brought to a temperature of 205° F.

Number 1 picnic tin cans were filled with peas to within $\frac{1}{4}$ in. of the top; care was taken to cover the peas with brine. Covers were placed loosely on the cans that were then set in an autoclave which was filled with flowing steam. After the canned peas had been exhausted for a few minutes, individual cans were removed from the autoclave, inoculated at the geometrical centre with 1 ml of a spore suspension, sealed in a commercial-type closing machine, immersed in cold, running water for 20 min, and then refrigerated until they were either irradiated, heat-processed, or incubated at $85^{\circ}F$ as required.

B. Irradiation

The canned peas were irradiated in the centre-well of the large cobalt-60 gamma radiation source in the Fission Products Laboratory at the University of Michigan (Lewis *et al.*, 1954). The quantity of irradiation delivered at the centre of the cans was measured by ferrous-ferric sulphate dosimetry as previously described (Kempe *et al.*, 1954). At the time of this investigation, a dosage of 1 megarad* of gamma radiation required approximately 8 h exposure. When the temperature of the cave was above 40°F, the cans were refrigerated with dry ice to keep the peas below this temperature during irradiation. Following irradiation the cans were placed in a refrigerator at 35°F from which they were removed within 2 days for heat processing.

 \ast One rad is a dose of ionizing radiation capable of producing energy absorption of 100 erg per g of tissue.

C. Heat Processing

Six cans of meat, two of which contained thermocouples, were placed in an autoclave where they were heat processed to the desired F_0 values as previously described (Kempe *et al.*, 1957). The F_0 value is defined as the number of minutes required to sterilize the can of peas at 250°F when the Z value equals 18. These values were calculated from time-temperature curves obtained from the cans containing thermocouples. For this purpose Schultz's graphical modification of Ball's General Method (Schultz and Olson, 1940) was used, and a Z value of 18 was assumed. Following heat processing, the cans were incubated at 85°F.

D. Spores

The spores of anaerobic bacteria used in these studies were prepared and used as previously described (Kempe *et al.*, 1954).

E. Controls

Eight control cans were used. Four of these were not inoculated and four were selected at random from the experimental cans. All eight were then incubated at 85° F. Generally the noninoculated cans swelled within three weeks while gas usually developed within one week in the inoculated cans. This indicated that conditions suitable for microbiological growth were present. However, this did not conclusively demonstrate viability of the inoculum in the peas, since the non-inoculated peas contained bacteria.

When Clostridium botulinum 213 B was used for the inoculum, mouse inoculation tests established the presence or absence of B type toxin in the peas (Kempe *et al.*, 1954). Occasionally this type of toxin was also recovered from non-inoculated control cans which indicated that the peas contained C. botulinum type B spores when originally frozen. For this reason, the F_0 required to sterilize non-inoculated canned peas was established by control runs.

Results

Data for a typical run are shown in Table I. Two series of runs were conducted. In the first series, shown in Table II and Fig. 1,

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Table I. F_0 values required to sterilize canned green peas packed in No. 1 picnic tin cans, inoculated with PA 3679 spores, and irradiated with gamma rays from cobalt-60 before heat processing

Run No. CP 6	
Can Size	No. 1 Pienic (211×400)
Product	Green Peas
Inoculum	300 PA 3679 spores per can
Irradiation	0.279 megarad
Processing temperature	230°F
Incubation temperature	$85^{\circ}F$

F_0	Can No.	Days to gas formation
0.77	13	4
0.77	14	4
0.77	15	4
0.77	16	4
1.47	17	õ
1.47	18	4
1.47	19	4
1.47	20	5
2.19	21	
$2 \cdot 19$	22	õ
$2 \cdot 19$	23	
2.19	24	
3 ·16	9	_
3.16	10	
3.16	11	
3.16	12	
Controls :	1	3
Not inoculated	2	
	3	23
	4	6
Controls :		
Inoculated	5	3
	6	3
	7	6
	8	7

Conclusions: Following 0.279 megarad of gamma radiation from cobalt-60, canned peas were sterilized by an F_0 between 2.2 and 3.2.

Table 11. Combined irradiation heat processing treatments required to sterilize
canned green peas packed in No. 1 picnic tin cans and inoculated with anaerobic
bacterial spores

	Run No.	Pre-irradiation megarad	F_0 range min
1) Se	ries 1 : 5,000.00	00 C. botulinum 213 B spores per	can
,	PB 6	2.33-2.79	None
	PB10	> 2.79	None
	PB19	2.79 - 3.29	None
	PB11	None	< 1.54
	PB 4	None	$1 \cdot 39 - 1 \cdot 82$
	PB 1	None	> 1.44
	PB 3	0-465	1.03 - 1.53
	PB14	0.465	0.60 - 0.75
	PB15	0.650	0.80-0.92
	PB 5	0.930	0.36 - 0.50
	PB 8	1.40	0.33 - 0.48
	PB18	1.63	< 0.30
	PB17	1.96	< 0.06
b) S	eries 2: 300 PA	. 3679 spores per can	
	CP 2	1.77 - 2.05	None
	CP 1	None	$4 \cdot 1 - 4 \cdot 9$
	CP 6	0.279	$2 \cdot 2 - 3 \cdot 2$
	CP 3	0.465	1.39 - 2.25
	CP 5	0.697	0.33-0.69
	CP 4	0.930	0.25 - 0.49
Son i	noculated conti	rols	
	PB 7	None	> 0.06
	PB 9	None	> 0.054
	PB12	None	0.35 - 0.52
	PB13	None	> 0.20
	PB15	None	0.10-1.10
	PB 7	> 0.46.5	None
	PB15	> 0.620	None

5,000,000 C. botulinum 213 B spores were used per No. 1 can of peas. In the second series, shown in Table II and Fig. 2, 300 PA 3679 spores were similarly used. These data show that gamma radiation and heat are synergistic when used together for processing canned peas. Less of either form of energy was needed for sterilization when combined with the other than when used alone.

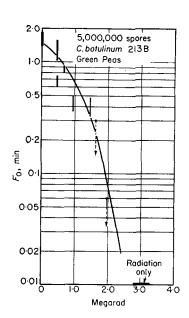


Fig. 1. F_0 required to sterilize green peas packed in No. 1 picnic tin cans, inoculated with 5,000,000 *C. botulinum* 213 B spores per can and irradiated with gamma rays from cobalt-60 before heat processing

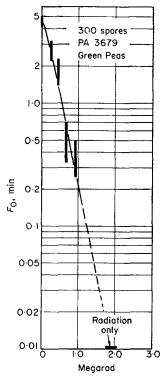


Fig. 2. F_0 required to sterilize green peas packed in No. 1 picnic tin cans, inoculated with 300 PA 3679 spores per can, and irradiated with gamma rays from cobalt-60 before heat processing

Following 1.2 megarad of gamma radiation an F_0 of 0.5 sterilized the peas whether they were inoculated with 5,000,000 C. botulinum 213 B or 300 PA 3679 spores. Below 1 megarad pre-irradiation, more heat processing was required to sterilize cans of peas inoculated with 300 PA 3679 spores than was necessary when 5,000,000 C. botulinum 213 B spores were used; above 1 megarad preirradiation, the reverse was true.

Discussion

The synergistic lethal action of gamma radiation is probably a

general phenomenon since it is shown to be essentially as pronounced for spores suspended in canned green peas as it has previously been reported to be for such spores suspended in canned beef and in phosphate buffer (Kempe *et al.*, 1957; 1955). It would therefore appear reasonable from a microbiological viewpoint that this synergistic lethal property could be expected to be applied to the sterilization of most canned foods. It also appears that the synergistic action results from sensitization of the spores to heat as a result of irradiation. This effect varies in degree according to the medium in which the spores are suspended so wherever utilization of the effect is considered, the actual application should be studied.

It must be pointed out that the combined irradiation-heat processing treatments reported here are minimum values because they are based on a limited number of runs and upon four cans at each level as shown in Table I.

Relevant to the possible improvement in organoleptic values to be derived from combined irradiation-heat processing, Gillies (1959) has recently reported on studies with canned peas. He found that thermally processed peas were superior to those processed either by gamma radiation alone or by combined irradiation-heat processing. However, he also stated that combination processed peas were significantly better, in most cases, than those processed with radiation alone.

Acknowledgement. This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 936 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of Defence.

The authors also wish to acknowledge the assistance of Ronald E. West and George T. Tsao for assistance in conducting the heat processing work.

Antitoxin was obtained through the courtesy of Dr. Jesse L. Henry of the New York State Department of Health.

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