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EFFECT OF SODIUM PROPIONATE ON TOXIN PRODUCTION BY *Clostridium botulinum* TYPE A IN BEEF TREATED BY COMBINED PROCESSES INVOLVING IRRADIATION

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BACKGROUND

Microbiologically stable and safe food products are increasingly being recognized to be the consequence of preservative factors acting in combination, often at levels at which they singly would not be inhibitory (Roberts, 1989). In this sense, alternatives to the conventional way of treating meat and meat products to achieve safety against *Clostridium botulinum* have been studied and reported (Rowley et al, 1983). Rodríguez et al.(1992) reported that a shelf-stable beef product could be obtained by combined treatments involving curing, cooking, vacuum packaging and gamma irradiation.

Specific antimicrobial agents and additives are increasingly being studied for potential antibotulinal effect (Miller et al., 1993). They reported a delay in *C. botulinum* toxigenesis with added 2 and 6% of sodium propionate in an uncured turkey product. Moreover, they stated that samples containing 2% of sodium propionate became toxic after 5 days of incubation at 28°C while samples containing 6% remained toxin free after 18 days of incubation at the same temperature.

Therefore, the objective of this research was to study the effect of sodium propionate on *C. botulinum* toxin production in shelf-stable beef treated by combined processes involving irradiation.

METHODS

Spore composite for challenging studies, product formulation and processing, as well as, neurotoxin bioassay were done according to the procedures reported elsewhere (Rodríguez et. al, 1992).

Experimental Design: Four experiments were arranged in a factorial design, each including one concentration of sodium propionate, four irradiation doses (2.5, 5, 7.5, and 10 kGy), five inoculation levels (10^1-10^5 *C. botulinum* spores per package) and three replicates. This gave a total number of 240 inoculated samples. In addition, non-inoculated samples were prepared for using in sensory analysis.

Curing solutions: Three curing solutions were prepared as follow: Brine A) 7.7% NaCl(wt/vol), 0.064% $NaNO_2$ (wt/vol). Sodium propionate was added in concentrations of 0, 10, 30 and 60% (wt/vol) to obtain after injection (10%) concentrations of 0, 0.8, 2.0 and 3.3% respectively of sodium propionate in the sample. Brine B) 7.7% NaCl(wt/vol), 0.064% $NaNO_2$ (wt/vol). Brine C) 5.0% NaCl(wt/vol), 0.064% $NaNO_2$ (wt/vol).

Inoculation and Packaging: Bags of 58μ thickness, impermeable to oxygen and composed of EVA-polyethylene-EVA-SARAN-EVA (BB_4L, Grace Argentina, S.A.) were used. Once samples were inside the bag, they were inoculated in 5-fold increasing concentrations of the inoculum of *C. botulinum* composite. Samples were then vacuum packaged, distributed into expanded polystyrene boxes according to required irradiation doses, and frozen in dry ice.

Irradiation: Samples were irradiated with 2.5, 5, 7.5 and 10 kGy using a source of ^{60}Co at the irradiation facility of the National Commission for Atomic Energy (CNEA, Ezeiza, Argentina). Samples were kept at -20°C during irradiation and proper dosimetry was carried out while irradiation processing took place.

Storage and monitoring: All samples were stored at 28°C up to 4 months. Twice a week, they were examined for evidence of off-odors (pungent, putrid) and textural changes (mushiness, friability). Samples showing evidence of spoilage were analyzed immediately.

Number of surviving spores and probability of toxin production: The Most Probable Number of spores capable of outgrowth with toxigenesis was determined according to the analytical method described by Thomas (Peeler et al., 1992): $MPN/g = p/\sqrt{NT}$; where p is the number of positive samples, N is the total quantity of sample (in grams) in all negative packages and T is the total quantity of sample (in grams) in all packages. The probability (P) of individual spores to successfully survive the process, overcome the inhibition, grow out and produce toxin was calculated as: $P = MPN/s$; where s is the number of challenged spores per sample (Hauschild, 1982).

Sensory evaluation: Non contaminated (non-challenged) samples were sensory evaluated by an eight-member trained panel.

RESULTS AND DISCUSSION

Botulinal toxin was detected on samples containing 0% of sodium propionate subjected to all irradiation doses and challenged with the highest spore level (Table 1). No toxin was found in samples inoculated with 10^1, 10^1 and 10^2 and 10^1 to 10^4 spores of *C. botulinum* and irradiated with 5, 7.5 and 10 kGy, respectively. For samples containing 0.8% of sodium propionate toxin was detected at high spore inoculation levels when irradiated with 2.5, 5 and 7.5 kGy. Samples were not toxic at inoculation levels of 10^1 and 10^2 (2.5 kGy), 10^1 to 10^3 (5 kGy), 10^1 to 10^4 (7.5 kGy) spores of *C. botulinum*. Samples containing 0.8% of sodium propionate and irradiated with 10 kGy were not toxic even at the highest inoculation level (Table 1). No toxin was detected in samples containing 2 and 3.3% of sodium propionate at any of the irradiation doses used. Hence, the inhibitory effect of sodium propionate on *C. botulinum* outgrowth and toxin production is clearly related to the amount added to the samples.

Samples containing 0% of sodium propionate became toxic at 17, 24, 24, 38 days when irradiated with 5, 2.5, 7.5, and 10 kGy respectively, while samples containing 0.8% of sodium propionate became toxic at 35, 58, 58 days when irradiated with 5, 2.5, 7.5 kGy respectively. Samples treated with 0.8% of sodium propionate and irradiated with 10 kGy remained toxin free after 4 months of incubation at 28°C. Addition of 0.8% of sodium propionate resulted in a delay in toxigenesis of 18, 34 and 34 days at irradiation doses of 5, 2.5, 7.5 kGy respectively when compared to control samples (0% of sodium propionate) (Table 2). The delay in botulinal toxin formation observed in this study is in agreement with the one observed by Miller et al.(1993), who reported delays in toxin formation of 5 and more than 18 days with added 2 and 6% of sodium propionate in an uncured turkey product.

Sodium propionate has been widely used as mold inhibitor and its inhibitory effect against bacteria was limited to inhibit the bacteria that causes rope in bread (Wagner and Moberg, 1989). In the current work it is demonstrated that sodium propionate is effective in preventing botulinal toxin formation in beef treated by combined processes.

When the product was evaluated by trained panelists, results showed that samples containing 2% of sodium propionate were the more acceptable among the others, while samples containing 3.3% of sodium propionate were the more rejectable. Samples with concentrations of 0 and 0.8% were in between giving more acceptance to the 0.8% ones.

The calculated MPN of surviving spores and probability (P) of toxin production in samples subjected to each irradiation dose and different concentration of sodium propionate are listed in Table 3. Probability of one spore to survive, outgrowth, and produce toxin decreased with increments in the irradiation doses applied (5, 7.5, 10 kGy). This shows the lethal effect of irradiation at each sodium propionate concentration used. Samples irradiated with 5 kGy had less inhibitory effect than those irradiated with 2.5 kGy. This could be attributed to a better synergistic effect among treatments in samples treated with 2.5 kGy, although both irradiation doses (2.5, 5 kGy) resulted in a poor inhibition of *C. botulinum* spores.

When comparing samples without added sodium propionate to those containing 0.8% of sodium propionate, the probability of toxin production decreased in approximately 1 log unit for the same irradiation dose (Table N°3). This denotes a marked antibotulinal effect when adding sodium propionate. The fact that no toxin was detected in samples containing 2 and 3.3% of sodium propionate indicates that the corresponding probabilities are below $3.5x10^{-5}$. Taking the log 1/P as the number of Decimal Reductions (DR) applied (Hauschild, 1982), the safety level of the product would be >4.45 D. Higher inoculation levels, for instance, could allow to test the effectiveness of the combination of treatments in preventing *C. botulinum* toxin production by achieving a higher safety level. This is particularly important when related to a shelf-stable meat product as it is necessary to achieve complete safety against *C. botulinum*.

CONCLUSIONS

Sodium propionate showed to be a very effective antibotulinal agent when coupled with curing, vacuum packaging and gamma irradiation. Moreover, the use of this GRAS substance in developing shelf-stable meat items shows a lot of potential from both sensory and safety standpoints.

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TABLE N°1: TOXIN ASSAY IN CHALLENGED SHELF-STABLE BEEF SAMPLES

Inoculation level[a] (log)	Irradiation dose (kGy)							
	2.5		5.0		7.5		10.0	
	% sodium propionate							
	0	0.8	0	0.8	0	0.8	0	0.8
5	3/3[b]	1/3	3/3	3/3	3/3	1/3	1/3	0/3
4	3/3	1/3	3/3	2/3	2/3	0/3	0/3	0/3
3	1/3	1/3	3/3	0/3	1/3	0/3	0/3	0/3
2	1/3	0/3	3/3	0/3	0/3	0/3	0/3	0/3
1	2/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

a: spores/package.
b: # positive samples/# analyzed samples.

TABLE N°3: MPN OF *Clostridium botulinum* SURVIVING SPORES AND PROBABILITY (P) OF TOXIN PRODUCTION

Irradiation dose (kGy)	MPN[a]		P[b]	
	% sodium propionate			
	0	0.8	0	0.8
2.5				
5.0	3684	110	$3.7x10^{-3}$	$1.1x10^{-4}$
7.5	37947	750	$3.8x10^{-2}$	$7.5x10^{-4}$
10.0	936	36	$9.3x10^{-4}$	$3.5x10^{-5}$
	36	<36	$3.5x10^{-5}$	$<3.5x10^{-5}$

a: Most Probable Number of surviving spores/gram.
b: Probability of toxin production= MPN/inoculation level.

TABLE N°2: TOXIN PRODUCTION IN SHELF-STABLE BEEF SAMPLES CHALLENGED WITH 10^5 TO 10^4 SPORES OF *C.botulinum* AND STORED AT 28°C FOR 4 MONTHS.

Irradiation dose[a]	sodium propionate[b]	Storage time (days)						
		1	17	24	35	38	58	130
2.5	0	0	0	1[c]	6	7	7	10
	0.8	0	0	0	0	0	1	3
5	0	0	1	9	10	11	12	12
	0.8	0	0	0	2	2	3	5
7.5	0	0	0	2	3	3	4	4
	0.8	0	0	0	0	0	1	1
10	0	0	0	0	0	1	1	1
	0.8	0	0	0	0	0	0	0

a: kGy; b: % in the sample (wt/wt); c: cumulative n° of positive samples.
Total number of samples per combination (irradiation dose/concentration of sodium propionate) = 15