COMBINED PROCESS TO INHIBIT *Clostridium botulinum* TOXIN PRODUCTION IN A BEEF PRODUCT STORED AT ROOM TEMPERATURE

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Summary

A shelf-stable beef product was developed by combined treatments involving, curing, cooking, vacuum-packaging and gamma irradiation. Challenge studies with 10^3 and 10^5 spores/g of type A *Clostridium botulinum* were conducted on this product. Samples subjected to a 10^3 spore inoculation and 15 kGy gamma irradiation did not show toxine production up to 4 months of storage. Low TBA numbers, slight off-odours and good overall acceptance were found on non-contaminated samples. A process involving curing, cooking, vacuum-packaging and gamma irradiation, should be safe to prevent botulinal toxine formation in beef inoculated with 10^3 spores and kept at 28°C for up to 4 months.

Introduction

The application of combined food preservation treatments involves the use of two or more moderate treatments rather than the extreme application of a single one, and intends to protect organoleptic and nutritional food characteristics. Food items produced following this approach keep better sensory properties, are safe, and eventually less expensive by cutting down energy expenses during processing, shipping, handling and storage. In this context, the employment of irradiation combined with other food preservation treatments, might be an efficient tool to produce healthy and palatable food items (Wierbicki, 1981).

Development of shelf-stable meat items, however, should be carefully monitored in terms of its safeness. When food are packaged and heated sufficiently to kill naturally occurring microflora that might inhibit *C. botulinum*, but are not heated enough to kill *C. botulinum*, there is a risk of botulinal toxine formation mainly due to a favorable temperature during storage. There is an even greater concern, when products stored under these conditions do not appear spoiled or unacceptable for human consumption (Sperber, 1982). The objective of this study was to evaluate the response of a cured, mild-heated, vacuum-pakaged and gamma irradiated beef product stored at 28°C challenged with type A *C. botulinum*.

Materials and methods

Test organism: Strains of type A *C. botulinum* A-N25, A-NCTC 9837 and A-N23 were obtained from the Faculty of Medicine, University of Mendoza, Argentina. Strains were grown in Cooked Meat (Difco Lab) and spore production was done on TPGY medium (BAM, 1978) incubated under anaerobiosis during 10 days. Cells were harvested by centrifugation (twice at 2,800 g) and washing with distilled water (twice). Cells suspended in distilled water were kept at 5°C up to the moment of their use. Prior to inoculation, cell were heat shocked (80°C-10 min). *Product manufacture:* Pieces of approximately 200 g (non-contaminated samples) and 100 g (challenged samples) beef foreshank were cured by 10% pumping and immersion (overnight-5°C) (7.7% NaCl and 0.064% $NaNO_2$). Immediately after, samples were cooked to an internal temperature of 75°C during 20 min in a bath containing a 5% NaCl and 0.064% $NaNO_2$ brine. Non-contaminated samples were vacuum-packaged, frozen (-20°C) and gamma-irradiated with 7.5 kGy or 15 kGy. Samples for challenge studies were inoculated with a composite of 10^3 or 10^5 spores/g prior to vacuum-packaging, then samples were frozen and irradiated as described above. All samples were kept at 28°C during storage. *Packaging materials:* Two different packaging containers were used. A=(from outside to inside) EVA-polyethilene-EVA-SARAN-EVA and B=Nylon-adhesive-SARAN-adhesive-LDLP. *Microbiological assay and toxine determination:* On non-contaminated samples, anaerobes count on TPGY agar (120hs-35°C) and *Lactobacillus* count on MRS agar (72 hs-30°C) were performed. On challenged samples, toxine formation was determined by mouse intraperitoneal inoculation with a 0.2 ml centrifuged sample homogenate

(gelatin buffer PO_4, pH 6.6). Mice were checked for botulism symptomatology up to 5 days. C. botulinum cell viability, sample homogenates were cultured in Cooked Meat, half of them were heat shocked (80°C-10min) and incubated up to 30 days (32°C). Heat shocked samples which showed turbidity were inoculated to mouse, while non-heated turbid samples were inoculated only if their heated counterparts did not show turbidity. Sensory evaluation: A trained panel evaluated organoleptic characteristic in non-contaminated samples. Lipid deterioration: Oxidation was determined by TBA method (Pensel, 1990). Sulfanilamide was added to aliquot samples which had residual sodium nitrite (Shahidi, 1989). Chemical analysis: NaCl and residual nitrite were determined according to AOAC (1975) and Sen and Donaldson (1978) respectively. A_w was determined in a Novasine thermoconstanter apparatus. Monitoring and sampling: Inoculated samples were examined twice a week for swelling or for any other organoleptic change. Samples were analyzed at 0, 2, and 4 months of storage. Five replicates were considered for each combination of treatments. This gave 20 non-contaminated samples (5 replicates x 4 treatments) and 40 challenged samples (5 replicates x 8 treatments) at each storage time. Data analysis: When appropriate, analysis of variance was carried out on data by the SAS procedure. Differences among means were analyzed by the Tukey's studentized range test.

Results and discussion

Non-contaminated samples. Anaerobes and lactobacillus counts were below the limit of detection irrespective of the treatment at zero time (Table 1). They increased at month 2 of storage on samples irradiated with 7.5 kGy being Lactobacillus the dominant flora. Lactobacilli, usually, become the dominant spoilage flora in products packaged under modified atmosphere. Nonetheless, counts were too low to produce sensory detectable changes. No organisms were found up to month 4 of storage on samples subjected to 15 kGy. NaCl content was near the expected value (3%), since this was the maximum NaCl content that panelists accepted during preliminary assays (result no shown) and which, should have an effect in lowering a_w. NaCl content had no differences ($p>0.05$) for both 7.5 and 15 kGy irradiated samples. NaCl content was relatively constant up to 4 months of storage. Residual nitrite on samples subjected to 7.5 kGy were different ($p<0.05$) from those subjected to 15 kGy at zero time. Irradiation produce a decreasing in $NaNO_2$ (Artuso, 1988). Residual NO_2 significantly decreased ($p<0.05$) at month 2 of storage. Nitrite is a reactive chemical compound and when used for curing purpose, less than 50% of the NO_2 added can be analyzed after completion of processing (Cassens et al 1979). Flavor and aroma improved as storage time increased regardless the radiation dose, while tenderness and juiciness were kept constant. Nonetheless, a trend suggest that samples from packaging A showed better aroma and flavor than samples from packaging B, and among them 7.5 kGy samples were better than the 15 kGy ones. The TBA numbers found were lower than those from other reports (Chang, 1961); however, other different test condition were used and this might explain that difference. No differences on TBA were found between the two packaging containers used. TBA numbers for samples with 2 months of storage were higher ($p<0.05$) than the ones from the other two times tested.

Challenged samples. Results of monitoring and toxine evaluation are shown on Table 2. Toxin production occurred in samples either irradiated with 7.5 kGy (both inoculum levels) or irradiated with 15 kGY and challenged with 10^5 spores/g. Most of samples (77.55%) had viable C. botulinum cells at zero time; however, only 20% of samples (visually spoiled and with toxine formation) had viable cells. This finding is partially different from that of Rowley et al (1983) who found a greater proportion of viable cells, whether toxine was produced or not, in inoculated bacon. Reports on C. botulinum occurrence in raw meat are scarce. However, it has been found at levels of 1 spore/1-7.1 lb in products such as cooked ham, smoked bacon, franks and raw chicken (Genigeorgis, 1986). In our study a high inoculum level was used, and irrespective of treatment visible organoleptic changes (severe tissue disruption, "mushy-like" texture and offensive odor) preceded toxine formation. This finding

is somewhat in agreement with that reported by Hauschild et al (1985) who showed that spoilage occurred prior to vacuum-packaged raw beef became toxic. Anellis et al (1965) reported that canned bacon challenged with 10^6 spores was sterilized by a dose of 4.5 Mrad. They calculated a D12 value of 2.65-2.87 Mrad. Rowley et al (1983) in turn, prevented swelling of bacon challenged with 2 spores/g and irradiated with 1.5 Mrad regardless of nitrite input (0, 40 or 120 ug/g). However, when the product was inoculated with 160 spores/g, 1.5 Mrad did not accomplished a complete protection against temperature abuse (27°C-60ds). In our study, 15 kGy has proved to be effective in preventing toxine formation in a beef product challenged with 10^3 C. botulinum spores/g., so far up to 4 month storage at 28°C; it is stressed however, that viable cells remained in these samples. Further studies combining different levels of $NaNO_2$ and irradiation are currently under way at our laboratory.

Conclusions

A process involving curing, cooking, vacuum-packaging and irradiation to produce a shelf-stable beef product should be suitable to prevent C. botulinum toxine formation up to 4 months of storage at 28°C.

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TABLE 1
Non Contaminated Samples of Beef Shelf-stable Product Microbial Counts, Lipid Stability, NaCl Content, Residual $NaNO_2$ and Aw

Storage time (months)		7.5 kGy PACKAGING [α] A	7.5 kGy PACKAGING [α] B	15 kGy PACKAGING A	15 kGy PACKAGING B
0	Anaerobes (CFU/g)	<1.69	<1.69	<1.69	<1.69
	Lactobacillus (CFU/g)	<1.69	<1.69	<1.69	<1.69
	TBA number	0.0406	0.0383	0.0653	0.0719
	NaCl (%)	2.77	2.80	2.65	2.60
	$NaNO_2$(ppm)	123	122	85.6	81.6
	Aw	0.989	0.988	0.987	0.978
2	Anaerobes (CFU/g)	3.81	2.18	<1.69	<1.69
	Lactobacillus (CFU/g)	3.289	2.22	<1.69	<1.69
	TBA number	0.0960	0.1025	0.1208	0.1199
	NaCl (%)	2.91	3.30	3.15	2.77
	$NaNO_2$(ppm)	30.6	31	19.6	18.8
	Aw	0.975	0.965	0.975	0.972
4	Anaerobes (CFU/g)	NA	NA	<1.69	<1.69
	Lactobacillus (CFU/g)	NA	NA	<1.69	<1.69
	TBA number	NA	NA	0.0756	0.0833
	NaCl (%)	NA	NA	2.76	2.94
	$NaNO_2$(ppm)	NA	NA	17.2	15.0
	Aw	NA	NA	0.968	0.967

α: See text for composition. NA: Not analized.

TABLE 2
Challenged Samples of Beef Shelf-stable Product

Irradiation Dose (KGy)	Inoculum Level (spores/g)	Packaging [α]	Rejection Time (weeks) [b]	Toxine Detection (months) [c]	Cell Viability
0	10^3	A	3 (days)	3 (days) 5/5	NA [d]
		B	3 (days)	3 (days) 5/5	NA
7.5	10^3	A	7	2 (1/5)	+
		B	7	2 (1/5)	+
	10^5	A	7	2 (2/5)	+
		B	7	2 (1/5)	+
15	10^3	A	–	4 (0/5)	+
		B	–	4 (0/5)	+
	10^5	A	7	2 (3/5)	+
		B	7	2 (2/5)	+

α: See text for packaging composition

b: First sample (n=5) showing organoleptic change/s

c: Number of toxic samples/Total number of analyzed samples

d: Not analyzed.