CASE REPORT

Clean Label Solution for the Control of *Clostridium botulinum* in Cooked Meats – A Case Study

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Abstract

Clostridium botulinum bacteria may be of concern in raw and cooked refrigerated meat products with a shelf-life greater than 10 days, for which strict cold-chain management cannot be guaranteed. This case study describes the testing of a new proprietary clean-label system from Kerry Taste & Nutrition, Rosal XB, for the inhibition of *C. botulinum* spore germination in a number of cooked poultry products. Products were inoculated with non-proteolytic strains of *C. botulinum* under modified atmosphere packaging (MAP) conditions. They were stored under simulated cold-chain conditions and assayed for *C. botulinum* growth at appropriate intervals. Research results demonstrated that under the test conditions, products can achieve a shelf-life of 25 days, without the risk of *C. botulinum* growth.

Key words: clean label, *Clostridium botulinum,* cooked meat products

Introduction

Botulism is a rare but potentially fatal neuroparalytic illness caused by a neurotoxin produced by the bacterium *Clostridium botulinum* (Schiavo i Montecucco, 1997). Foodborne botulism is rare but it has the potential to kill rapidly, and contaminated products can cause foodborne outbreaks (CDC, 1998; ECDC, 2015; 2016; WHO, 2018). Foodborne botulism has a moderate to high outbreak potential if transmitted through food and therefore, represents a significant public health risk (CDC, 1998; WHO 2018).

There are generally considered to be five forms of botulism, conditional on the mode of acquisition. Foodborne botulism results from the ingestion of food containing preformed toxin (Smith, 1993). Wound botulism can occur when spores of the bacteria infect and produce toxin in a contaminated wound (CDC, 1998; WHO, 2018). Infant botulism is due to the endogenous production of toxin by germinating spores of *C. botulinum* in the intestine of the infant. Adult intestinal botulism is a very rare form of botulism, similar to infant botulism. This can occur if bacterial spores get into an adult's intestines, grow, and produce the toxin (CDC, 1998; WHO, 2018). Iatrogenic botulism can happen if excess botulinum toxin is injected for cosmetic or medical reasons (Ghasemi, 2012).

The incidence of food-borne botulism in the EU/EEA is low (ECDC, 2015, 2016). Between

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2010 and 2015 a total of 51 foodborne outbreaks were caused by *C. botulinum* toxins in the EU (Table 1). Of the 51 confirmed cases, 10 were linked to pork meat and related products with a 6 further outbreaks linked to other meat and meat products, including poultry. In the Czech Republic, botulism is very rare. Since 1960, a total of 155 cases have been reported, while between 2010 and 2017, there have been a total of 6 confirmed cases, 4 in 2013, 1 in 2014 and 1 in 2017. Three of the four cases that occurred in 2013 was a domiciliary occurrence of botulism following consumption of home-made pork and liver pâté in three family members admitted to the Department of Infectious, Tropical and Parasitic Diseases, Na Bulovce Hospital in Prague in May 2013 (ECDC, 2015).

Table 1 Reported strong-evidence foodborne outbreaks caused by *Clostridium botulinum* toxins, EU Member States, 2010–2015 (ECDC, 2016)

Implicated food vehicle	No. of outbreaks in 2010	No. of outbreaks in 2011	No. of outbreaks in 2012	No. of outbreaks in 2013	No. of outbreaks in 2014	No. of outbreaks in 2015	Total outbreaks 2010-2015
Canned food products		1	1	2	2	3	**9**
Pig meat and products thereof	3	2	1			4	**10**
Vegetables and juices and other products thereof	1	3		2	2		**8**
Other meat and meat products(a)		1		1		4	**6**
Fish and fishery products	1						**1**
Cereal products including rice and seeds/pulses (nuts, almonds)						1	**1**
Mixed food						1	**1**
Other foods	2	5	3	2	1	1	**14**
Unknown						1	**1**
Total Outbreaks	**7**	**12**	**5**	**7**	**5**	**15**	**51**

Note: information summarised in this table is based on Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC.
(a): Other meat and meat products include information on 'other meat and meat products' and 'other, mixed or unspecified poultry meat and products thereof'.

C. botulinum is a Gram positive, spore forming, obligate anaerobe bacterium that produces the Botulinum neurotoxin (BoNT) under anaerobic conditions (Schiavo and Montecucco, 1997). They have the ability to survive for extended periods under harsh conditions, and may survive some heat processes applied by the food industry (Roberts and Ingram, 1965; Smith, 1993; Peck, 2006). *C. botulinum* are widely distributed in nature, particularly soil, aquatic and marine sediment. They are also found in the gastrointestinal tract of most mammals (Smith, 1993).

Botulinum neurotoxin is one of the most powerful neurotoxins known with approximately 30-100 ng of the toxin being fatal to humans (Thirunavukkarasu et al., 2018). There are currently seven confirmed serotypes of BoNT designated A to H (Peck, 2009). A proposed eighth serotype, type H, has been shown to be a chimeric toxin composed of parts of types F and A (FA) (Jason et al., 2014). Types A, B, E, rarely F cause illness in humans. Types C, D and E cause illness in other mammals, birds and fish (Peck, 2009; Thirunavukkarasu et al., 2018). *C. botulinum* itself

is broken down into two main types; psychrotrophic (also known as non-proteolytic), is capable of neurotoxin formation during cold storage, and mesophilic (also known as proteolytic) that require higher temperatures for neurotoxin formation (Peck, 2009). Strains of non-proteolytic *C. botulinum* form BoNT types B, E or F, while strains of proteolytic *C. botulinum* form BoNT types A, B, and/or F. Foodborne botulism is most commonly associated with botulinum neurotoxin of types A, B, or E (Thirunavukkarasu et al., 2018).

Botulinum neurotoxin is produced when conditions are favourable for spore germination. The minimum temperature at which non-proteolytic *C. botulinum* forms BoNT is 3 °C, while for proteolytic *C. botulinum* this is 10-12 °C. Thus, in correctly stored chilled foods (maximum of 8 °C), there is the potential for non-proteolytic *C. botulinum* but not proteolytic *C. botulinum* to form BoNT (Lindström et al., 2006; Peck, 2006, 2009). Once ingested, the toxin enters the circulatory system and makes its way to the peripheral nerve synapses (PNS) where it exerts its effect by cleaving key proteins required for nerve activation. At the PNS it blocks the release of neurotransmitter acetylcholine (ACh). Without acetylcholine neural signaling skeletal muscles fail to contract and thus paralysis is induced (Schiavo and Montecucco, 1997). Symptoms include vision disturbances, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, and muscle weakness. In severe cases BTX can block nerves controlling the respiratory system or heart, resulting in death. Symptoms generally begin 12 to 36 hours after eating a contaminated food (range 6 hours - 30 days). Associated foods include home canned foods with low acid content (e.g. asparagus, green beans, corn), baked potatoes in foil, honey (infants), fermented fish, herb infused oil etc.

For all raw and ready-to-eat (RTE) chilled products stored under anaerobic conditions i.e. low oxygen modified atmosphere packaging (MAP) or vacuum packed (VP), *C. botulinum* is still considered a risk. In such chilled meat products with a shelf life of more than 10 days, non-proteolytic *C. botulinum*, which is the type of C. botulinum of most concern, is in the main, controlled by inhibition rather than destruction. This inhibition involves one or more controlling factors (Smith, 1993; Peck, 2006). The safety of any meat product will depend on the number and value of each controlling factor that is acting in combination to exert a combined effect on *C. botulinum* (Peck, 2006; Food Standards Agency, 2007). These conditions include:

- Storage Temperature below 3 ˚C (it is assumed that products in distribution cannot be controlled below this temperature),
- Heat treatment/cook of 90 ˚C for 10 minutes (or equivalent),
- Water activity (aw) below 0.97 throughout the food,
- pH less than 5 throughout the food,
- Minimum salt level of 3.5 % in the aqueous phase throughout the food,
- Preservative factors, traditionally nitrite salt (normally ≥2 %) that can be demonstrated to prevent growth and toxin production by non-proteolytic *C. botulinum*.

All of these conditions have inherent problems, whether it be the lack of 'cold-chain' control, the drive for salt reduction and its subsequent effect on water activity, or consumer demands for E-number-free products. With this backdrop, in the summer of 2017, Kerry were approached by a large poultry processor who required a clean-label (E-number free) solution for their cooked poultry products that would maintain their current shelf-life of 16 – 25 days, but in a reduced salt environment. In addition, the solution was required to give their products validated protection against non-proteolytic C. botulinum in order to comply with the 10-day shelf-life rule for such products in the UK. Utilizing Kerry's proprietary technologies and expertise within the Freshness Collection, a proprietary clean-label system was developed, *Rosal XB*, to deliver on the customers needs.

Materials and methods

As part of the development and applications work for this project, a validated challenge test against non-proteolytic *C. botulinum* was required. For this, Campden BRI Limited (Chipping Campden, UK) was contracted to carry out the trial work. The products challenged tested with non-proteolytic *C. botulinum* along with their characteristics are shown in table 2. In total, four model products were chosen for the challenge testing, two cooked poultry chunk-type products

and two cooked sliced poultry products (turkey and poultry meat). The salt content in each product was set to comply with the 2017 UK targets for these types of products (Table 2). The Kerry Freshness solution, *Rosal XB*, was added directly in the brine prior to injection. The poultry chunks were injected at 10 % (+/-1 %), while the poultry for slicing was injected at 20 % (+/-1 %).

The challenge tests carried out at Campden BRI, were performed according to the United Kingdom Accreditation Service (UKAS) standard method (TES-MB-196). The four products were

Table 2 Product details, characteristics and dosage rates

Product	a_w	% Salt	% Rosal XB*
Turkey Breast Chunks	0.989	0.68	1
Chicken Breast Chunks	0.974	0.68	1
Turkey Breast Sliced	0.978	1.6	1.2
Chicken Breast Sliced	0.974	1.6	1.2

**Rosal XB was added to the brine to achieve the desired dosage in the final product.*

produced and packaged at the clients manufacturing site and shipped to Campden BRI where they were checked upon receipt. The samples were stored frozen prior to testing. A cocktail of three in-house non-proteolytic *C. botulinum* strains were used to inoculate the samples. Samples were inoculated in accordance with the UKAS standard method TES-MB-196. A water and glycerol mix at water-activity (a_w) 0.97 was used as the inoculation medium, with 0.1ml of inoculum inoculated into each pack through the pack using a self-sealing foam pad. The product was inoculated at a target level of 10^2 to 10^3 CFU/g. Uninoculated (negative) controls were tested in parallel to ensure no existing contamination of the products being tested. A positive control to demonstrate the viability of the cultures used under optimal test conditions was also used in this study (data not shown).

After inoculation the samples were stored for 5 days at 3 °C (day 0-5), 12 days at 5 °C (days 6-17), 8 days at 8°C (days 18-25). Triplicate samples of inoculated and negative control samples for non-proteolytic *C. botulinum* were tested at days 0, 11, 17, 21 and 25. Triplicate samples were also tested for a_w and pH at the start of the trial. The following microbiological/chemical analysis was carried out: Aerobic plate count, Anaerobic plate count, Non Proteolytic Sulphite Reducing Clostridia (including *C. botulinum*), a_w and pH.

Results and discussion

This challenge test relies on the detection of *C. botulinum* using Sulphite Reducing Clostridia (SRC) media. *Clostridia* species other than *C. botulinum* can grow on this media, however these are not routinely found in manufactured products. Provided there are no SRC in the control samples then any levels of SRC in the inoculated samples can be taken to be a count of *C. botulinum*. Limited conclusions can be drawn from a trial of this type if there are naturally occurring sulphite reducing *Clostridia* in the products. If high levels of SRC are found in control samples then the test is terminated. No SRCs were detected in the control samples therefore the results could be analysed and an interpretation made as to whether *C. botulinum* is able to grow in the products tested.

All samples tested on Day 0 had an initial inoculum within tolerance (10^2-10^3 CFU/g). The microbiological data for *C. botulinum* Sulphite Reducing Clostridia (SRC) media are shown in figure 1. An analysis was made to determine whether there had been an increase of greater than 0.5log cfu/g in levels of *C. botulinum* (mean value from the triplicate packs of product) at any of the time points when compared to mean levels present at day zero. An increase of >0.5 log is greater than the natural variation in levels that may be expected for biological systems and thus is indicative of growth. When the data from this study was compared against the pre-determined criteria it was shown that there were no increases in

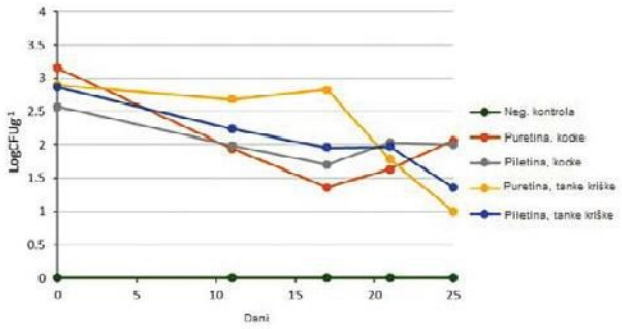

Figure 1 Plot of the microbiological results (log $CFUg^{-1}$) *C. botulinum* inoculated samples

mean microbial levels above 0.5 log and therefore *C. botulinum* was considered not to have grown in the products tested. In conclusion, a shelf life of 25 days was deemed to be achievable for the 4 products tested. Tests to evaluate organoleptic properties was carried out by the client in-house (data not shown).

Conclusion

A number challenges needed to be addressed as part of this development. The clear technical challenge was to develop a validated clean-label system for ready-to-eat poultry products that would deliver the required shelf-life of 16-25 days as well as provide the obligatory protection against *C. botulinum*, as a system of this type was not available in the market at the time. In addition, the system was required to have no impact on of the products. The main commercial challenge was to provide an effective solution at an acceptable price for the customer. In summary, Kerry Taste & Nutrition were able to deliver a complete solution that met both the technical and commercial requirements of the customer.

References

[1] **Centres for Disease Control Prevention – CDC (1998):** Botulism in the United States, 1899-1996. Handbook for Epidemiologists, Clinicians, and Laboratory Workers (Atlanta, GA). Available online: https://www.cdc.gov/botulism/pdf/bot-manual.pdf

[2] **European Centre for Disease Prevention and Control – ECDC (2015):** Botulism - Annual Epidemiological Report for 2015. Available online: https://ecdc.europa.eu/sites/portal/files/documents/AER_for_2015-botulism.pdf

[3] **European Centre for Disease Prevention and Control – ECDC (2016):** Type E botulism associated with fish product consumption – Germany and Spain, 21 December 2016. Available online: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/01-12-2016-RRA-Botulism-Germany %2C %20Spain.pdf

[4] **Food Standards Agency (2017):** The safety and shelf-life of vacuum and modified atmosphere packed chilled foods with respect to nonproteolytic Clostridium botulinum. Available online: https://www.foodstandards.gov.scot/downloads/vacpacguide.pdf

[5] **Food Standards Agency:** Salt Reduction Targets. Available online: https://www.food.gov.uk/business-guidance/salt

[6] **Ghasemi, M., R. Norouzi, M. Salari, B. Asadi (2012):** Iatrogenic botulism after the therapeutic use of botulinum toxin-A: a case report and review of the literature. Clin Neuropharmacol. 35 (5), 254-257. doi: 10.1097/WNF.0b013e31826248b8.

[7] **Jason R., S. Barash Stephen, A. Arnon (2014):** A Novel Strain of Clostridium botulinum That Produces Type B and Type H Botulinum Toxins. The Journal of Infectious Diseases 209;2.183–191. DOI: 10.1093/infdis/jit449

[8] **Lindström, M., K. Kiviniemi, H. Korkeala (2006):** Hazard and control of group II (non-proteolytic) Clostridium botulinum in modern food processing. Int J Food Microbiol. 108 (1), 92-104.

[9] **Peck, M.W. (2006):** Clostridium botulinum and the safety of minimally heated chilled foods: an emerging issue? J. Appl. Microbiol. 101, pp. 556-570.

[10] **Peck, M.W. (2009):** Biology and genomic analysis of Clostridium botulinum. Adv Microb Physiol. 55,183-265, 320. https://doi.org/10.1016/S0065-2911(09)05503-9

[11] **Roberts T.A., M. Ingram (1965):** The resistance of spores of Clostridiumbotulinum type E to heat and radiation. J Appl Bacteriol 28, 125-137.

[12] **Schiavo, G., C. Montecucco (1997.):** The structure and mode of botulinum and tetanus toxins. In: The Clostridia. Molecular Biology and pathogenesis. Eds. Rood J, McClane B.A., Songer J.G., Titball R.W. San Diego, California: Academic Press; Pp 295-322.

[13] **Smith, J.P. (1993):** Clostridium botulinum-Ecology and control in foods. Haushchild H.W. and Dodds K.M. (Eds). Marcel Dekker, New York.

[14] **World Health Organization – WHO (2018):** Botulism Fact sheet. Available online: http://www.who.int/en/news-room/fact-sheets/detail/botulism

[15] **Thirunavukkarasu, N., E. Johnson, S. Pillai, D. Hodge, L. Stanker, T. Wentz, B. Singh, K. Venkateswaran, P. McNutt, M. Adler, E. Brown, T. Hammack, D. Burr, S. Sharma (2018):** Botulinum Neurotoxin Detection Methods for Public Health Response and Surveillance. Front Bioeng Biotechnol. 6, 80. doi: 10.3389/fbioe.2018.00080

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