Research Paper

Volume : 4 | Issue : 4 | April 2015 • ISSN No 2277 - 8179

Methods to Control *Clostridium* botulinum in Meat Products

Food Science

KEYWORDS : Clostridium botulinum, botulism, anti-botulinum activity, botulism in meat products.

Roberto Juliatto — Student from the Graduate Program in Food Engineering, Technology Department, Universidade Federal do Paraná

Nina Waszczynskyj — Graduate Program in Food Engineering, Technology Department, Universidade Federal do Paraná

ABSTRACT

In this study, a review about the more effective methods to avoid the growing of Clostridium botulinum spores and the possible production of toxins in meat products was conducted. Several methods are reported that, when used together are more effective to avoid the growth of Clostridium botulinum, than when used separately. As control methods were discussed the use of nitrite, sodium chloride, sodium lactate, pH, water activity, use of nisin and also some natural extracts. The results obtained by several studies were also compared as well as the existing synergism between nitrite and the other components.

Introduction

Clostridium botulinum is an anaerobic bacteria, Gram-positive, sporeforforming, widely distributed in nature. It has the characteristic of producing one of the most powerful known toxins. The spores have high thermal resistance with D value at 120ºC and pH>4.60 being of 30 minutes. The germination of spores is promoted under anaerobic conditions, in pH over 4.5 and water activity over 0.94. Besides, spores type E may germinate in temperatures over 3.3ºC (CERESER et al., 2008). The development of Clostridium botulinum in food is conditioned by several factors of chemical and/or physical nature called barriers. Among the most commonly used ones are: water activity, acidity, use of high processing temperatures, storage temperature, use of sodium chloride and nitrites or other preservatives. Processed meat products have characteristics needed for the development of Clostridium botulinum and for the production of its toxins (HAUSCHILD, 1989). Those products have enough nutrients, pH near neutrality, are usually vacuum packed, and besides that there is a world trend of consuming food with less salt and with a smaller addition of nitrite, which has been increasingly controlled and its use reduced due to its carcinogenic potential. Due to those factors, the risk of occurrence of botulism in industrialized meat products is considerably increasing, making necessary the use of other methods together with the traditional ones in order to guarantee that the food is safe for consumers. In the current context in which consumers are increasingly looking for safe food that use fewer chemical additives, the combination of natural compounds with smaller amounts of chemical preservatives has been gaining adepts in the meat industry.

1. Nitrites

Sodium nitrite has been used in meat products in order to provide pink coloration, developing flavor and aroma, and also in order to preserve, mainly against bacteria of genus Clostridium (JAY, LOESSNER, GOLDEN, 2005). The preservative action of nitrite happens due to the action of nitric oxide, formed from nitrite, over the iron-sulfur enzymes, such as ferredoxin, present in the vegetative cells of C. botulinum preventing the synthesis of adenosine triphosphate (ATP) from pyruvate (WOODS et al., 1981; REDDY et al., 1983; TOMPKIN et al., 1983). The initial amount of nitrite added is the main factor for the safety of meat products during the storage period for controlling botulism. Studies prove that the residual nitrite at the end of shelf life has little or no influence over the growing of C. botulinum (GIBSON et al., 1984; AMSTALDEN et al., 1997). The amount of nitrite to be added for controlling botulism in meat products depends on several factors, such as: pH, water activity, salt concentration, severity of the thermal treatment applied during processing, storage temperature, number of spores present, nature of the competitive microbiota, amount of iron available in the product, quantity of other additives added such as antioxidants, phosphates and nitrates (ROBERTS & GIBSON, 1986).

Roberts et al. (1982) evaluated the influence of pH over the growing of C. botulinum in crushed pork meat. They tested two ranges of ph (5.6 to 6.03 and 6.27 to 6.56) and observed, in all tested situations, that in higher pH the probability of producing botulinum toxin increases.

Pivinick et al. (1969), Roberts et al. (1981), and Hauschild et al. (1982) evaluated the interaction of nitrite with salt for controlling botulism in meat products and concluded that nitrite is more effective the higher the concentration of salt in the product is. For products without nitrite, a level o 6.1% of salt was needed in order to prevent the production of the botulinum toxin.

Amstalden et al. 1997, evaluated the toxigens of C. botulinum ham and mortadella under the same conditions, only varying the humidity of the products and concluded that the greater the humidity is, the greater the probability of the toxin being present.

The amount of iron available in the product also influences the growing of Clostridium botulinum, the more iron there is, the more increases the chance of spore germination and more nitrite is needed to control the botulism. Hauschild et al. (1982) inoculated spores of C. botulinum in liver pate, with addition of 50, 100 and 150mg/kg of sodium nitrite and stored the product at 27ºC. At the end of one week the samples with 50 and 100mg/kg became toxic and the ones with 150 mg/kg became toxic at the end of the second week. This happens because the iron present in the medium reacts with the NO reduced from nitrite, reducing the nitric oxide of the medium, which is responsible by controlling the botulism. Due to this, a higher presence of iron demands a greater addition of nitrite TOMPKIN et al., 1983).

Addition of antioxidants such as ascorbate or erythorbate also helps in the control of botulism. They act as reducers, reducing nitrite to NO, which destroys the iron-sulfur enzymes of C. botulinum avoiding its growth. Antioxidants also act reducing the state of oxidation of metals decreasing their catalytic activity. This characteristic helps when there is presence of much iron in the medium, avoiding its reaction with NO TOMPKIN et al., 1983; BORENSTEIN, 1987).

2. Sodium lactate

Sodium lactate is a sodium salt from natural lactic acid, produced by the fermentation of sugars, mainly of sugarcane. It has a smooth saline flavor, antimicrobial properties and neutral pH. Its molecular formula is CH3CHOHCOONa, with molecular weight equal to 112.07 and is sold in aqueous solution at 60%. It is a product widely used in the food industry as an acidity regulator and bactericide (SAKAMOTO, 2009).

According to Shelef (1994) there are two mechanisms more accepted for the bacteriostatic action of sodium lactate which are: the formation of weak lipophilic acids that have the capacity of crossing the cell membrane in their undissociated form, and dissociating inside the cell, acidifying the medium. The second mechanism is based in the capacity of the lactate to reduce the water activity in food.

Miller, Call, Whiting (1993) demonstrated that the addition of sodium lactate at 2% in turkey meat was responsible for inhibiting the growing of C. botulinum, as well as to avoid the production of toxin. Meyer et al. (2003) demonstrated that 2.5% of sodium lactate added in cooked turkey breast, without the addition of nitrite, preserved at room temperature, were enough to increase from 8 to 22 days the time for the germination of spores of C. sporogenes added to the product. Unda Molins, Walker (1991) proved in their studies that 2% of lactate inhibited the growing of C. sporogenes.

Houtsma et al. (1994) inoculated spores of C. botulinum in culture medium with peptone and yeast extract. On incubation at 15ºC the production of toxin happened in 14 days for 0% and 1% of lactate; for 2% of lactate, this time increased to 21 days and, in the medium containing 3% of lactate there was no formation of toxin at the end of 49 days of incubation. For incubation at 20ºC the formation of toxin happened in less than 5 days in the medium without lactate, 11 days for 1.5% of lactate, 15 days for 2.5% of lactate and there was no growing at the end of 32 days for the medium containing 4% of lactate. On incubation at 30ºC, the production of toxin in the medium was detectedat 11 days of incubation for a concentration of 4% of lactate.

Maas, Glass, Doyle (1989) in their studies demonstrated that the addition of sodium lactate delayed the formation of toxin by C. botulinumin vacuum packed cooked turkey breast, stored at 27ºC. Toxicity happened in less than 3 days for the product without lactate, for 2% of addition it happened within 4 to 5 days, for 2.5% within 4 to 6 days, for 3% within 7 days and for 3.5% within 7 to 8 days. In this study, the only preservative added was sodium lactate.

3. Nisin

Nisin is a bacteriocin produced by some strains of Lactococus lactis, discovered more than six decades ago. Nisin is a polypeptide characterized by the presence of hydrophilic and hydrophobic regions in the same molecule. It has molecular weight of 3,500 being formed by 34 amino acids. In its chain it has some uncommon sulfur amino acids such as lanthionine and β-methyl lanthionine. The main properties of nisin for its use as preservative in food are (JAY, LOESSNER, GOLDEN, 2005):

- Non toxic
- Produced naturally by strains of Lactococcus lactis
- Thermally stable
- Destroyed by digestive enzymes
- Does not change the taste or odor of food
- Has wide microbial spectrum

Nisin has a bactericide activity against a wide spectrum of Gram-positive bacteria, including the sporogenic of Bacillus and Clostridium genera. It has no effectiveness against Gram-negative fungi and bacteria. Basically the mechanism of action of nisin consists in the permeabilization of the cytoplasmic membrane of bacteria, creating pores in the cell wall, causing an ionic imbalance and flow of phosphate ions. The consequence of this formation of pores is the dissipation of the proton force which is directly involved with the ATP synthesis, protein phosphorylation, synthesis and rotation of flagella, transport of proteins and others. (BRUNO, MONTVILLE, 1994).

Rayman et al. (1981), in their works with pork meat inoculated with spores of Clostridium sporogenes per gram and submitted to thermal process similar to the industrial process of ham cooking and stored at 35ºC concluded that: 75ppm of nisin inhibited the growing of spores at pH below 6.5, and also proved to be more efficient that the addition of 150ppm of sodium nitrite on the samples. On this same research the authors concluded that 40ppm of nitrite with 75ppm of nisin was more efficient than only 150ppm of nitrite.

Scott e Taylor (1981), observed in their studies using cooked meat as culture medium than the addition of 5000 U.I./ml (125ppm) of nisin were not enough to prevent the growing of type A C. botulinum in the medium. They also verified that 2000 U.I./ml (50ppm) did not inhibit the growing of type B strains.

Rogers e Montville (1994), concluded in their experiments that the action of nisin for controlling the growth of C. botulinum depends mainly on the temperature, in which the higher the temperature is, the lesser the nisin activity. Nisin activity is only dependent on the food constituents, proving that some components of food may interact with nisin reducing its activity. According to Delves-Broughton, (2005) the use of nisin in meat products is restrict due to the pH near neutrality, the presence of proteolytic enzymes, the presence of fat and also dependent of the storage temperature. The greater activity of nisin happens in pH around 2 and 3, because it is more soluble, in pH around 6 to 7 its solubility decreases making it less effective. The presence of proteolytic enzymes in meat, specifically glutathione S-transferase, inactivate the action of nisin, because they combine with it. Due to this fact, nisin is only effective in cooked products because the heat inactivates the action of the glutathione enzyme. The presence of high concentrations of fat in the product also reduces the efficiency of nisin, because they change its solubility.

4. Essential oils and natural extracts

In the last decade, the use of natural products aiming to better conserve food has been researched. This fact occurred due to the incorrect or abusive use of chemical products, which have been causing many side effects in consumers (OLIVEIRA, 2010).

Essential oils are also defined as a part of the metabolism (combination of metabolites) of a vegetal, normally composed by terpenes which are associated, or not, to other components, most of which are volatile and generate together the characteristic odor of the vegetable (BANDONI e CZEPAK, 2008).

A large number of researches have been reporting the antibacterial and antifungal properties of in natura spices, of their essential oils (EO's) and their extracts. Several plants used to aromatize food have antimicrobial activity, such as laurel, marjoram, basil, clove, cinnamon, garlic, oregano, coriander, rosemary, and others (BEUCHAT e GOLDEN, 1989).

According to Vigil, Palou, Alzamora (2005) some EO's of spices may have a wide antimicrobial spectrum, others may be specific against some groups of microorganisms, such as Gram-positive or Gram-negative bacteria, or only reaching bacteria but having no effectiveness against fungi or yeast. Due to the existence of a huge number of different groups of chemical components in the EO's, it is more probable that antimicrobial activity is not assigned to a specific mechanism of action, but to several targets in the microbial cell (SKANDAMIS e NYCHAS, 2001; CAESON, MEE, RILEY, 2002). Normally the mechanisms of action of the natural compounds are the disintegration of the cytoplasmic

Volume : 4 | Issue : 4 | April 2015 • ISSN No 2277 - 8179

membrane, destabilization of the Proton-Motive Force (PMF), flow of electrons, active transportation and coagulation of the cell content. Not all action mechanisms act in specific targets, some sites may be affected in consequence of other mechanisms (BURT, 2004). An important characteristic responsible by the antimicrobial action that the essential oils have is their hydrophobicity, which allows the partitioning of lipids of the bacterial cell membrane disintegrating its structure and making it more permeable (SIKKEMA, BONT, POOLMAN, 1994).

Ismaiel e Pierson (1990) studied the effectiveness of EO's of oregano against C. botulinum spores in crushed pork meat. They used the concentration of 0.4μl/g of essential oil of oregano and verified that it did not have a significant influence on the number of spores, or in their growth. However, when a small quantity of sodium nitrite was added together with the same concentration of oregano extract, a decrease in the growth of bacteria was observed compared to the use of nitrite only. This reduction depended upon the number of inoculated spores, being greater for the addition of 300 spores/g than for 3000 spores/g.

Cui, Gabriel, Nakano (2010) evaluated the anti-botulinic activity of several extracts of plants used isolated or in combination with sodium nitrite. Extracts soluble in ethanol or water were used, obtained from 90 types of herbs or fresh or dehydrated spices, in model using agar medium TPGY and in model using meat. In this study they verified that the aqueous extract of clove, the alcoholic extracts of eucalyptus, lemon, apple and licorice have higher anti-botulinic activity in agar medium TPGY in the minimum inhibitory concentration varying from 0.05% to 0.2%. When the combination of extracts with sodium nitrite in agar medium TPGY was used, the synergistic effect was observed only with aqueous extract of Coptis rhizome, inhibitory concentration of nitrite from 6-8 ppm until 2 ppm combined with 0.05% of Coptis extract. On the model using meat was verified synergistic action of alcoholic extract of nutmeg (0.05%) and sage (0.02%) and aqueous extract of clove (0.05%) with 10ppm of nitrite for the control of C. botulinum.

Conclusion

Nitrite remains the best option for preventing botulism in meat products, however the ideal for this control is to use the "theory of obstacles", where several methods are used together to avoid the occurrence of this disease, thus making it possible to reduce the use of chemical preservatives. In this context products such as sodium lactate, nisin and, mainly, natural preservatives are options that have a good synergism with nitrite and may be used together aiming to provide barriers to botulism in meat products, thus allowing to reduce the use of nitrite.

REFERENCE

1. Amstalden, V. C. J., Serrano, A. M. & Manhani, M. R. (1997). Avaliação da toxigênese de C. botulinum em mortadela e presunto. Ciência Tecnologia de Alimentos, v. 17 (2), p. 154-159. | 2. Bandoni, A. L. &Czepack, M. P. (2008). Os Recursos Vegetais Aromáticos no Brasil: seu aproveitamento industrial para produção de aromas e sabores. EDUFES, p. 623. | 3. Beuchat, L.R. & Golden, D. A. (1989). Antimicrobials occurring naturally in foods. Food Technology, v. 43, p. 134-142. | 4. Borenstein, B. (1987). The role of ascorbic acid in foods. Food Technology. v. 41 (11), p. 98-99. | 5. Bruno, M. E. C. & Montville, T. J. (1993). Common mechanistic action of bacteriocins from lactic acid bacteria. Applied Environmental Microbiology, v. 59, (9), p. 3003-3010. | 6. Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods – a review.International Journal of Food Microbiology, v. 94, p. 223-253. | 7. Carson, C. F.; Mee, B. J. & Riley, T. V. (2002). Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. Antimicrob. Agents Chemother, v. 46, p. 1914-1920. | 8. Cereser, N. D.; Costa, R. F. M.; Júnior, O. D. R.; Silva, D. A. R. &Sperotto, V. R. (2008, jan-febr) Botulismo de Origem Alimentar. Ciência Rural, v. 38 (1), p. 280-287. | 9. Cui, H.; Gabriel, A. A. & Nakano, H. (2010). Antimicrobial efficacies of plant extracts and sodium nitrite against Clostridium botulinum. Food Control, v. 21, p. 1030-1036. | 10. Delves-Broughton, J. (2005).Nisin as food preservative. Food Australia. V. 57, p. 525-527. | 11. Gibson, A. M.; Roberts, T.A. & Robinson, A. (1984). Factors controlling the growth of Clostridium botulinum types A and B in pasteurized cured meats VI. Nitrite monitoring during storage of pasteurized pork slurries. Journal of Food Technology, v. 19, p. 29-44. | 12. Hauschild, A. H. W.; Jarvis, G. & Raymond, D. P. (1982). Contribution of nitrite to the control of Clostridium botulinum in liver sausage. Journal of Food Protection, v. 45, p. 500-506. | 13. Houtsma, P. C.; Heuvelink, A.; Dufrenne, J. &Notermans, S. (1994). Effect of sodium lactate on toxin production, spore germination and heat resistance of proteolytic Clostridium botulinum strains.Journal Food Protect, v. 57, p. 327-330. | 14. Ismaiel, A. A. & Pierson, M. D. (1990). Effect of sodium nitrite an origanum oil o growth and toxin production of Clostridium botulinum in TYG both and ground pork.Journal of Food Protection, v. 53, p. 958-960. | 15. Jay, M. J.; Loessner, J. M. & Golden A. D. (2005).Protección de los alimentos com sustâncias químicas y mediante biocontrol. In:______. Microbiologia Moderna de Los Alimentos (5rd ed.). Zaragoza, MA: Acribia, p. 305-345. | 16. Maas, M. R.; Glass, K. A. & Doyle, M. P. (1989). Sodium lactate delays toxin production by Clostridium botulinum in cook-in-bag turkey products. Applied and Environmental Microbiology, Washington, v. 55, p. 2226-2229. | 17. Meyer, J. D.; Cerveny, J. G. &Luchansky, J. B. (2003). Inhibition of nonproteolytic, psychrotrophic clostridia and anaerobic sporeformers by sodium diacetate and sodium lactate in cook-in-bag turkey breast. Journal Food Protect, v. 66, n. 8, p. 1474-1478. | 18. Miller, A.J.; Call, J.E. & Whiting, R.C. (1993). Comparison of organic acid salts for Clostridium botulinum control in an uncured turkey product. Journal Food Protect, v. 56, p. 958-962. | 19. Oliveira, M. J., Araújo, W. M. C. &Borgo, L. A. (2005). Quantificação de nitrato e nitrito em linguiças do tipo frescal, Ciência e Tecnologia dos Alimentos, n. 25 (4), p 736-742. | 20. Pivnick, H.; Barnett, H. W.; Nordin, H.R. & Rubin, L. J. (1969). Factors affecting the safety of canned, cured, shelf-stable luncheon meat inoculated with Clostridium botulinum. Canadian Institute of Food Science and Technology Journal, v.2, p. 141-148. | 21. Rayman, M. K., Aris, B. & Hurst, A. (1981). Nisin: a possible alternative or adjunct to nitrite in the preservation of meats. Appl. Environ. Microbiol. v. 41, p.375-380. | 22. Reddy, D., Lancaster, J. R. & Cornforth, D. P. (1983). Nitrite inhibition of Clostridium botulinum: Electron spin resonance detection of iron-nitric oxide complexes. Science, n. 221, p 769-770. | 23.Robert, T. A. & Gibson, A. M. (1986). Chemical methods for controlling Clostridium botulinum in processed meats. Food Technology, v. 40, p. 163-176. | 24. Roberts, T. A. Gibson, A. M. & Robinson, A. (1981). Factors controlling the growth of Clostridium botulinum types A and B in pasteurized, cured meas. I. Growth in pork slurries prepared from "low" pH meat (pH ranges 5.5-6.3). Journal Food Technology, v. 16, p.239. | 25. Robinson, A.; Gibson, A. M. & Roberts, T. A. (1982). Factors controlling the growth of Clostridium botulinum types A and B in pasteurized cured meats. V. Prediction of toxin production: nom-linear effects of storage temperature and salt concentration. Journal of Food Technology, v. 17, p. 727-744. | 26. Rogers, A. M. & Montville, T. J. (1994). Quantification of factors, which influence nisin's inhibition of Clostridium botulinum 56A in a model food system. JournalFood Science, v. 59: p. 663-668, 686. | 27. Sakamoto, P. (2009, mar-abr). Desafios na carne não-curada.Aditivos & Ingredientes, v. 61, p. 79-83. | 28. Shelef, L. A. (1994). Antimicrobial effects of lactates: a review. Journal Food Protect,v. 57, p. 445-450. | 29. Sikkema, J.; De Bont, J. A. M. &Poolman, B. (1994). Interactions of cyclic hydrocarbons with biological membranes. . Journal Biological Chemistry,v. 269, p. 8022-8028. | 30. Skandamis, P. N., &Nychas, G. J. E. (2001). Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. Journal of Applied Microbiology, v. 91, p. 1011-1022. | 31. Thomas, L. V. & Delves-Broughton (2005). Nisin. In Davidson P. M., Sofos, J. N. &Branen, A. L. (Eds.), Antimicrobials in food (pp. 237-274). New York, Unit States: CRC Press, Taylor & Francis Group. | 32. Tompkin B. R. (2005). Nitrite. In Davidson P. M., Sofos, J. N. &Branen, A. L. (Eds.), Antimicrobials in food (pp. 169-236). New York, Unit States: CRC Press, Taylor & Francis Group. | 33. Tompkin, R. B., Davison, P. M. &Branen, A. L. (1983). Antimicrobials in Food. (3rd. ed.). New York, MA: Marcel Dekker. | 34. Unda, J. R., Molins, R.A. & Walker, H. W. (1991). Clostridium sporogenes and listeria monocytogenes: Survival and inhibition in microwave-ready beef roasts containing selected antimicrobials. Journal of Food Science, v. 56, p 198. | 35. Vigil, A. L., Palou, E. &Alzamora, S. M. (2005). Naturally occurring compounds – plant sources. In Davidson P. M., Sofos, J. N. &Branen, A. L. (Eds.), Antimicrobials in food (pp. 492-451). New York, Unit States: CRC Press, Taylor & Francis Group. | 36. Woods, L. F. J., Wood, J. M. & Gibbs, P. A. (1981). The involvement of nitric oxide in the inhibition of phosphoroclastic system in Clostridium sporogenes by sodium nitrite. Journal of General Microbiology, v. 125, p. 399-406. |