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Clostridium botulinum Toxin Inactivation in Selected Soured Soups in South-South Region of Nigeria

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ABSTRACT

The aim of this study was to validate the inactivation of *Clostridium botulinum* toxins in soured soups prepared in the south-south region of Nigeria. In a preliminary study *Clostridium botulinum* neurotoxins were detected in soured Ogbono soup, Egusi soup, Okra soup, Vegetable soup, and Fisherman soup. These soups were cooked and allowed to get spoilt and were used for this study. To ascertain the inactivation of *Clostridium botulinum* neurotoxins in the soured soup these parameters were used: effect of temperature on the toxins and the addition of palm oil. To evaluate the effect of temperature, each of the soured soups was heated to 100ºC and held at this temperature for 1 hour. One hundred milliliter (100 ml) of palm oil was added to each of the soured soups and also heated to a temperature of 100ºC for 1 hour to ascertain the effect of palm oil and heat on the toxins. Tests for the neurotoxins were carried out before and after the experiments. The study revealed that *Clostridium botulinum* neurotoxin was denatured at a temperature of 100ºC. Also, the serological activity of the toxins treated with palm oil was unaffected, whereas, the joint treatment with heat and palm oil remained denatured. It is concluded that heat- treatment at the temperature of 100ºC holding up to 15 minutes can actually inactivate botulinum neurotoxins but the addition of palm oil alone without heat treatment cannot denature botulinum neurotoxin.

Keywords: Soured soup, *Clostridium botulinum*, inactivation, neurotoxins, heat, Palm oil.

Introduction

The main microbiological objective of heating food is to destroy vegetative cells and spores of microorganisms (WHO, 2017). Although very drastic heat treatment (sterilization) can be used to kill all the microorganisms present in a food, most foods are heated to destroy specific pathogens and some spoilage microorganisms, which are important in food (Gideon, 2017). Heating food to a desired temperature for a specific time can help destroy or reduce the activity of microbial cells. Some microorganisms can release toxins in food; also some food can have natural toxins (Tewari and Abdullah, 2015). If a toxin is heat sensitive, sufficient heating will destroy it and consumption of such food will not cause health hazards (Achi, 2005).

It is also important to recognize that microbial (and natural) heat- stable toxins are not completely destroyed even after high heat treatment (EFSA, 2005).

Clostridium botulinum is an anaerobic, spore-forming bacterium that produces a neurotoxin (Gilbert *et al*., 2006). The bacteria can exist as a vegetative cell or a spore. The spore is the dormant state of the bacteria and can exist under conditions where the vegetative cell cannot (Redmond, 2008). When conditions are right, the spore will grow into the vegetative cell. When the vegetative cells grow to high numbers, this bacterium produces the toxin. The vegetative cells of *Clostridium botulinum* are destroyed by heat but the spore is very resistant to heat (Uzuegbu and Eke, 2005).

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Temperatures well above 100°C (212°F) are needed to destroy the spore. The bacteria and the spore are inhibited from growing in acidic environments. *Clostridium botulinum* produces dangerous toxins (botulinum toxins) under low-oxygen conditions, and the toxins are one of the most lethal substances known (Dolan, *et al*., 2010). Botulinum toxins block nerve functions and can lead to respiratory and muscular paralysis. Human botulism may refer to foodborne botulism, infant botulism, wound botulism, and inhalation botulism or other types of intoxication (Oomes *et al.*, 2007).

Foodborne botulism is a very severe intoxication, historically caused by consumption of improperly processed food, preserved low acid, low oxygen foods in which *C. botulinum* had grown and produced BoNT (Chander, *et al.*, 2000). It is a potentially fatal disease if not diagnosed rapidly and treated with antitoxin. Common source of foodborne botulism are homemade canned, preserved or fermented foodstuffs (e.g. canned vegetables, meat and fish) and their preparation requires extra caution (Montanan *et al.*, 2004). Foodborne botulism is a serious, potentially fatal disease. However, foodborne botulism is relatively rare (Mead *et al*., 2000). It is an intoxication usually caused by ingestion of potent neurotoxins, the botulinum toxins, formed in contaminated foods. Person to person transmission of botulism does not occur. Spores produced by the bacterium *Clostridium botulinum* are heat-resistant and exist widely in the environment; and in the absence of oxygen they germinate, grow and then excrete toxins (Pelczar *et al*., 2006).

Not all *C. botulinum* strains cause illness in humans. Strains produce one of seven (7) distinct forms of botulinum toxin types of BoNT (A to G). Only those producing four of these (Types A, B, E and rarely F) cause botulism in humans (WHO, 2002). While Types C, D and E cause illness in other mammals, birds and fish. Strains are also separated into two groups based on physiological differences: Group I (can produce A, B or F toxin) are proteolytic and cause food spoilage; Group II (can produce B, E or F toxin) are non-proteolytic and may be present in foods without obvious spoilage (Agatha *et al*., 2002).

Botulinum toxins are ingested through improperly processed food in which the bacteria or the spores survive, then grow and produce the toxins (Jenson and Moir, 2003).

Though mainly foodborne intoxication, human botulism can also be caused by intestinal infection with *C. botulinum* in infants, wound infections, and by inhalation (Clouditz *et al.*, 2006). Infant botulism is an extremely rare toxico-infection that occurs when *C. botulinum* grows and produces toxins in the intestines of babies; symptoms appear in 3-30 days and include constipation, lethargy, floppiness and breathing difficulties (Paulina *et al.*, 2017). Botulinum toxins are neurotoxic and therefore affect the nervous system. Foodborne botulism is characterized by descending, flaccid paralysis that can cause respiratory failure. Early symptoms include marked fatigue, weakness and vertigo, usually followed by blurred vision, dry mouth and difficulty in swallowing and speaking. Vomiting, diarrhoea, constipation and abdominal swelling may also occur (Sanni *et al*., 2000). The disease can progress to weakness in the neck and arms, after which the respiratory muscles and muscles of the lower body are affected (paralysis of the eyes, mouth, throat and, progressively, muscles). There is no fever and no loss of consciousness. The symptoms are not caused by the bacterium itself, but by the toxin produced by the bacterium. Symptoms usually appear within 12 to 36 hours (within a minimum and maximum range of 4 hours to 8 days) after consuming the contaminated food. Incidence of botulism is low, but the mortality rate is high if prompt diagnosis and appropriate, immediate treatment (early administration of antitoxin and intensive respiratory care) are not given (Dinges *et al*., 2000). The disease can be fatal in 5 to 10% of cases. *C. botulinum* is an anaerobic bacterium, meaning it can only grow in the absence of oxygen.

Foodborne botulism occurs when *C. botulinum* grows and produces toxins in food prior to consumption. *C. botulinum* produces spores and they exist widely in the environment including soil, river and sea water. The growth of the bacteria and the formation of toxin occur in products with low oxygen content and certain combinations of storage temperature and preservative parameters (Martin *et al*., 2001). This happens most often in lightly preserved foods and in inadequately processed, home-canned or home-bottled foods. *C. botulinum* will not grow in acidic conditions (pH less than 4.6), and therefore the toxin will not be formed in acidic foods (however, a low pH will not degrade any pre-formed toxin). Combinations of low storage temperature and salt contents and/or pH are also used to prevent the growth of the bacteria or the formation of the toxin (Leloir *et al*., 2003).

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The botulinum toxin has been found in a variety of foods, including low-acid preserved vegetables, such as green beans, spinach, mushrooms, and beets; fish, including canned tuna, fermented, salted and smoked fish; and meat products, such as ham and sausage (Chander *et al*., 2000). The food implicated differs between countries and reflects local eating habits and food preservation procedures. Occasionally, commercially prepared foods are involved. Though spores of *C. botulinum* are heat-resistant, the toxin produced by bacteria growing out of the spores under anaerobic conditions is destroyed by boiling (for example, at internal temperature greater than 85°C for 5 minutes or longer). Therefore, ready-to-eat foods in low oxygen-packaging are more frequently involved in cases of foodborne botulism (Dolan *et al*., 2010). Prevention of foodborne botulism is based on good practice in food preparation particularly during heating/sterilization and hygiene. Foodborne botulism may be prevented by the inactivation of the bacterium and its spores in heat-sterilized (for example, retorted) or canned products or by inhibiting bacterial growth and toxin production in other products (Cheesbrough, 2005). The vegetative forms of bacteria can be destroyed by boiling but the spores can remain viable after boiling even for several hours. However, the spores can be killed by very high temperature treatments such as commercial canning.

In the South-South region of Nigeria, the weather is very humid with typical tropical climate and temperatures which encourages rapid proliferation of microorganisms. This situation coupled with very inadequate supply of electricity for preservation of cooked soups results in ready spoilage of cooked soups. The aim of this study therefore, was to validate the inactivation of *Clostridium botulinum* toxins in soured soups prepared in the south-south region of Nigeria.

Materials and Methods

Type of Soup Samples

Soup ingredients for five different types of soups; Ogbono soup, Egusi soup, Okra soup, Vegetable soup, and Fisherman soup - Odu-fulo: Kalabari, Nigeria) which are common or popular delicacies in the South-South region of Nigeria were bought at Creek Road Market, Port Harcourt and used for the study.

Preparation of Soups Used for the Study

The different types of soups were cooked as is done in the Kalabari traditional setting and left for 3-5 days at room temperature to allow to get spoilt for toxin production before being used. For the preparation of soup samples, five hundred milliliter (500 ml) of each sample (sour soup) was dispensed into a sauce pan separately for analysis. One hundred milliliter (100 ml) of palm oil and 250g of washed/sliced onion bulb was added to each of the sample for further analysis.

Media employed for detection of the bacterial toxins

The media employed for detection of the bacterial toxins is Biothreat Alert BONT/A/B test strip (Gaithersburg) for *Clostridium botulinum* neurotoxin. All media were used according to the manufacturer's instructions.

Detection of Toxins in Soured Soups

Rapid Identification of *Clostridium botulinum* Neurotoxin: Biothreat Alert test strip for the detection of BONT/A/B was purchased from the National Institute of Standard and Technology (Gaithersburg, U.S). Fifty milliliters (50 ml) of sour soup sample was mixed with 10 ml of sample buffer and homogenized with a bench top stomacher to make a homogenous suspension. The soup /buffer mixture was centrifuged at 7,000 x g for 30 minutes at 4^0C to remove solid particles, and was filtered through membrane filter. Five hundred microliters (500 µl) of soured soup supernatant was thoroughly mixed with five hundred microliters (500 µl) of sample buffer in a glass test tube that was used for the experiment. Each test device was removed from the protective pouch and placed on a flat surface. One hundred and fifty microliters (150 µl) of sample was placed into the round sample port according to the manufacturer's instructions and results were recorded visually after 15 minutes.

Determination of the Effect of Addition of Palm Oil on Toxins in Sour Soup

The determination of the effect of addition of palm oil on toxins in sour soup was carried out by the addition of one hundred milliliters (100 ml) of palm oil into each soured soup sample and allowed to stand for 1 hour, and analysed.

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Results

The result of the effect of heat (100ºC) on *Clostridium botulinum* neurotoxin is shown in Table 1. *Clostridium botulinum* neurotoxin that was previously detected in the soured soups was found to be absent after heating the soup at 100ºC for 1 hour.

The result of the effect of palm oil on the stability of *Clostridium botulinum* toxins is shown in Table 2. Data obtained showed that addition of palm oil to the sour soup did not denature or destroy the toxins of *Clostridium botulinum*.

Table 3 shows the result of the combined effect of the addition of palm oil, and heat treatment taken together on the toxins. Results showed that palm oil treatment alone had no effect on *Clostridium botulinum* toxin but heat treatment affected *Clostridium botulinum* neurotoxin.

Table 1: Effect of heat treatment on *Clostridium botulinum* toxins

Type of Soured Soup	Temperature (ºC)	Time allowed (Mins)	Observation	
			Before	After
Okra	100	60	+	-
Ogbono	100	60	+	-
Fisherman (Native)	100	60	+	-
Vegetable	100	60	+	-
Egusi	100	60	+	-

Key: Positive (+): Toxin detected; Negative (-): No toxin detected

Table 2: Effect of palm oil on *Clostridium botulinum* toxins

Type of Soured Soup	Temperature (ºC)	Time allowed (Mins)	Observation	
			Before	After
Okra	100	60	+	+
Ogbono	100	60	+	+
Fisherman (Native)	100	60	+	+
Vegetable	100	60	+	+
Egusi	100	60	+	+

Key: Positive (+): Toxin detected; Negative (-): No toxin detected

Table 3: Effect of heat treatment on soured soup and palm oil on *Clostridium botulinum* toxins

Type of Soured Soup	Palm Oil (ml)	Temperature (ºC)	Time allowed (Mins)	Observation	
				Before	After
Okra	100	100	60	+	-
Ogbono	100	100	60	+	-
Fisherman (Native)	100	100	60	+	-
Vegetable	100	100	60	+	-
Egusi	100	100	60	+	-

Key: Positive (+): Toxin detected; Negative (-): No toxin detected

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Discussion

This research studied the effect of heat, and palm oil, on the stability and the inactivation of botulinum toxins in soured soup. This toxin (*Clostridium botulinum* neurotoxin) was previously detected in the soured soups. *C. botulinum* was found to be absent after heating the soup at 100ºC for 1 hour (Table 1). This is supported by the findings of Pat-Harkins (2015) who noted that heat may thermally denatured *Clostridium botulinum* toxins.

At ambient temperature it was observed that *Clostridium botulinum* neurotoxin that was completely inactivated. This is in agreement with the findings of Paulina *et al*. (2017), which noted that heating of sour soup only kills the bacterial vegetative cells but does not affect the spores or the toxins but the toxins of *C. botulinum*. Bacterial spores and toxins according to Pat-Harkins (2015), can survive temperatures up to 100ºC or more. So heating of sour soup up to serving temperatures of 150ºC and above will not destroy or denature some bacterial toxins (especially *Staphylococcus aureus* enterotoxins and *Bacillus cereus* emetic toxins) but can destroy that of *C. botulinum*. This study also agrees with the findings of other workers (Balaban and Rasooly, 2000; Stewart, 2003; Rajkovic *et al*., 2008; Wang *et al*., 2014; Tarek and Mansel, 2013), who observed that Heat stability is one of the most important properties of staphylococcal enterotoxins (SEs) in terms of food safety. Normal cooking times and temperatures are unlikely to completely inactivate staphylococcal enterotoxins (SEs) (Balaban and Rasooly, 2000). The potency of the toxins of *Staphylococcus aureus* is not destroyed by the normal cooking times and temperatures. Cooking at temperatures below 60°C will not fully inactivate Staphylococcus aureus enterotoxin already present in the food (Dinges, *et al*., 2000).

According to Jenson and Moir, (2003), *Bacillus cereus* toxins are highly heat-resistant; they are able to survive at 126°C for 90 minutes, (resistant to all normal food processing and food preparation temperatures). Activity of *B. cereus* emetic toxin persisted at 100ºC for 2 hours before it was inactivated, but *Clostridium botulinum* toxins were more readily inactivated by heat. This is supported by the findings of Szabo and Gibson (2003) and Oliver *et al*. (2010), who found a 99% reduction in toxin concentration in haddock heated at 60°C.

The explanation for this observation is that despite its extreme potency, botulinum toxin is easily destroyed. Heating to an internal temperature of 85°C for at least 5 minutes will, therefore, decontaminate affected food or drink (Johnson, 2007; Szabo and Gidson, 2003).

The result of the effect of palm oil on the stability of the toxins showed that addition of palm oil to the sour soup did not denature or destroy the toxins of *Clostridium botulinum* (Table 2). According to Gaze (2013), addition of palm oil resulted in the development of heat resistance by *C. botulinum* due to encapsulation of the bacterium in the oil, which protected the cell from thermal injury. It is possible; therefore, that palm oil cannot destroy the bacterial toxins.

Result of the combined effect of the addition of palm oil, and heat treatment taken together on the toxins showed that palm oil treatment alone had no effect on *Clostridium botulinum* toxin but heat treatment affected *Clostridium botulinum* neurotoxin (Table 3). This meant that *Clostridium botulinum* toxin was inactivated at 100°C after 60 minutes in the presence of palm oil and onion bulb, which still showed that temperature denatures and destroys the toxins, not the presence of the palm oil.

In conclusion, Palm oil treatment did not destroy the toxins produced by *Clostridium botulinum*. *C. botulinum* neurotoxin was destroyed by heating the sour soup to a temperature of 100ºC for 5-15 minutes. It is, therefore, unsafe to consume soured soup to which palm oil is added as a means to treat the soup or with the hope of repairing or restoring the soup. Although heat treatment destroyed the toxins of *C. botulinum*, one can never be sure of the kind of toxins present, whether heat-resistant or not.

References

Achi, O.K. (2005). Spoilage of egusi soup, *Academic Journals. 4(7)*: 56-59. Org/ AJB. Retrieved June 26, 2018 from http://WWW.

Ajiboye, A., Adebola, O., Owoseni, A. and Olugbenga, O. (2013). Effects of palm oil on microbial load and water quality parameters. *Int. J. of Lakes and Rivers. 6(1)*: 19-20.

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Agata, N., Ohta, M. and Yokoma, K. (2002). Production of *Bacillus cereus* emetic toxin (cereulide) in various foods. *Int..J. Food Microbiology 73 (6)*: 23-27.

Balaban, N. and Rasooly, A. (2000), Staphylococcal enterotoxins. *Int. J. Food Microbiol. 61(8)*: 1-10

Chandler, B., Beller, M., Jenkerson, S., Middough, J., Roberts, C., Residorf, E., Rausch, M., Savage, R. and Davis, J. (2000). Outbreaks of Norwalk-like Viral gastroenteritis, Alaska and Wisconsis, 1999. *Morbidity and Mortality report*. *49(10)*: 207-211.

Cheesbrough, M. (2005). *District laboratory practice in tropical countries 2*. Cambridge University Press, London. 112 115.

Dinges, M.M., Orwin, P.M., and Schlievert, P.M. (2000). Exotoxins of *Staphylococcus aureus*. *Clinical Microbiology Reviews*, Minneapolis *13(1)*: 16-34.

Clouditz, A., Resch, A., Weiland, K.P., Peschel, A. and Gotz, F. (2006). Staphylococcus plays a role in the fitness of *Staphylococcus aureus* and its ability to cope with oxidative stress. *Infection and Immunity*. *74(8)*: 4950-4953.

Dogan, B. and Boor, K.J. (2003). Genetic Diversity of Spoilage Potential among Pseudomonas spp. Isolated from fluid milk products and dairy processing plants. *Appl. Environ. Microbiol*. *69(1)*:130-138.

Dolan, L.C., Matulka, A.R. and Burdock, G.A. (2010). Naturally occurring Food toxins. *Toxins J. 2(9)*: 2289-2332.

European Food Safety Authority (EFSA). (2005). Opinion of the scientific panel on biological hazards on *Bacillus cereus* and other *Bacillus* spp. in food stuffs. *EFSA Journal*. *175(12)*: 1-48. http://www. efsa. europa.ev/en/efsajournal/pub/175.

Gaze, J. (2013). The effect of palm oil on the heat resistance of *Staphylococcus aureus. Int. J. of Food Microbiol*. *2(4)*: 277-283.

Gideon, W.T. (2017). *Infectious Diseases in India*, Gideon Informatics, Online books.

Gilbert, S., Lake, R., Hudson, A. and Cressey, P. (2006). Risk profile: *Clostridium botulinum* in honey. *Report FW 05115 for the New Zealand Food Safety Authority. Institute of Environmental Science and Research (ESR) Ltd*. *Available at: http/www.nzfsa. govt.nz/science/risk-profile/oct-2006-c-bot-honey.pdf.* Accessed 24 Aug. 2018.

International Commission on Microbiological Specifications for Foods. (2008). *Bacillus cereus*. *In*: Roberts, T.A., Baird-Parker, A.C. and Tompkins, R.B. eds., *Microorganisms in Foods, Characteristics of Microbial Pathogens. Published by Blackie Academic and Professional, London*. *Pp*. 20-35.

Jenson, I., and Moir, C.J. (2003). *Bacillus cereus and other Bacillus species*, Chapter 14, *In*: Hocking A.D. (ed.) *Foodborne Microorganisms of Public Health Significance. 6th ed.; Australian Institute of Food Science and Technology (NSW Branch) Sydney*. *Pp*.445-478.

Johnson, E.A. (2007). *Clostridium botulinum*. *In*: Doyle, M.P., Beuchat, L.R. and Montville, T.J. (Ed) *Food Microbiology, Fundamental and Frontiers*. *3rd edition, ASM press, Washington DC*. 401-421.

Ladyatiey, G. (2011). Food Spoilage Microorganisms. *Int. Journal of Microbiol*. *20(1)*: 1497-1483.

Leloir, M., Baron, F. and Gautier, M. (2003) *Staphylococcus aureus* and Food poisoning. *Genetics and Molecular Research, Rabeirao Preto*. *2(1)*: 63-76.

Martins, S. E., Myers, E. R. and Landolo, J. J. (2001). *Staphylococcus aureus. In*: Hul, Y.H., Pierson, M.D., Gorltan, J.R. (Ed). *Foodborne disease handbook-bacterial pathogens 2nd ed. New York; Marcel Dekker*. *Pp* 345-381.

Mead, P.S., Slutsker, L., Dietz, V., McCarg, L., Bresee, J., Shaprio, C., Griffin, P. and Tauxe, R. (2000). Food related illness and death in the United States. *Center for disease control and prevention, Atlanta, USA*. *3(9)*: 278-287.

Montanari, G., Borosari, A. I., Chiavar, C., Ferri,G., Zambonelli,C. and Gazia, L.,(2004). Morphological and Phenotypical Characterization of *Bacillus sporothermodurans*. *J. Appl.Microbiol*. *97*: 802-809.

Microsoft Encarta Premium. (2009). *Encarta Encyclopedia* on food safety.

Nemeth, K. and Piskula, M.K. (2007). Food content, processing, absorption and metabolism of onion flavonoids. *Critical reviews in food science and nutrition. 47(4):397-409.*

Citation: Odike, O. (2023a). *Clostridium botulinum* Toxin Inactivation in Selected Soured Soups in South-South Region of Nigeria. *International Journal of Microbiology and Applied Sciences*. *2*: 76 - 82.

Nweze, E.A. (2010). Aetiology of Diarrhoea and Virulence Properties of Diarrhoeagenic *E. coli* among Patients and Healthy Subjects in Southeast, Nigeria. *Journal of Health Popul. Nutri*. *28(3)*: 245-252.

Oliver, G., Weingart, S., Tanja, S., Conny, M., Diana, P. and Martin, B. (2010). The case of botulinum toxin in milk: Experimental Data. *J. Appl. And Eviron. Microbiol. 7(6)*: 3293-3300.

Oomes, S.J., Van Zujilan, A.C., Hehenkamp, J.O., Witsenboer, H., Van dervossen, J.M. and Brul, S. (2007). The characterization of *Bacillus* spores occurring in the manufacturing of (low acid) Canned products. *International Journal of Food Microbiology*. *120(4)*: 85-94

Ossai, O.S. (2012). Bacteriological Quality and Safety of Street Vended Foods in Delta State, Nigeria. *Journal of Biology, Agric. and Health care. 2(5)*: 2224-3208.

Pat-Harkins, O. (2015). The Heat Resistance of Bacterial Spores and Toxins. *Available from http:// www.Spores.Toxins.gov/Bacteria/2015HeatResistance -htm;* Accessed 2018 July 13.

Paulina, R., Jesper, S.H., Ingemar, A. and Karin, L.P. (2017). Thermal Stability and Structural changes in bacterial toxin responsible for food poisoning. *Academic Journal of science. 34(9)*: 23-29.

Pelczar, M.J., Chana, C.S. and Kreig, N.R. (2006). *Food poisoning*: *Microbiology 5th ed. Tata McGraw , Hill Publishing Company Limited*, New Delhi.

Rajkovic, A., Uyltendade, M., Vermeulen, A., Andjelkovic, M., Fitz-James, I.,Int'tveld, P., Denon, Q., Verhe, R. and Debevere, J. (2008). Heat Resistance of *Bacillus cereus* emetic toxin, cereulide. *Lett Appl. Microbiol. 46(5)*: 536-541.

Redmond. W.A., (2008). Food-borne Illness. Microsoft® Encarta® 2009 (DVD) Microsoft corporation.

Sanni, A.L., Ayemo, G.S., Sakyi-Dawson, E., Sefa, B. and Dedeh, S. (2000). "Aerobic spore-forming bacteria and chemical composition of some Nigerian fermented soup condiments". *Plants Foods Human Nutri*. *55(2)*: 111-118.

Stewart, C.M. (2003). *Staphylococcus aureus* and Staphylococcal enterotoxin. *In*: Hocking, A.D. (Ed). Foodborne Microorganisms of Public Health significance. *Australian Institute of Food Science and Technology. 5(3)*: 34-39.

Szabo, E.A., and Gibson, A.M. (2003). *Clostridium botulinum. In*: Hocking, A.D. (Ed). *Foodborne microorganisms of public health significance. Australian Institute of Food Science and Technology*. *57(9)*: 256-267.

Tarek, F. and Mansel, W. (2013). *Foodborne infections and intoxication (4th Ed). Washington, DC.*

Tewari, A. and Abdullah, S. (2015). *Bacillus cereus* Food- Poisoning: *International and Indian Perspective Journal*. *34(10)*: 345-364. DOI: 10.1007/ 513 197-014-1344-4.

Uzuegbu, J. and Eke, O. (2005). "*Basic Food Technology*": principle and practice, Owerri Osprey Publication 102-103.

Wang, J., Ding, T. and Oh, D.H. (2014). "Effect of temperature on the growth, toxin production and heat resistance of *Bacillus cereus* in cooked rice". *Foodborne Pathos. Dis. 11 (2)*: 133-137.

World Health Organization (WHO) (2017). Food Safety Strategies, using high temperature treatment. *World Health Assembly*, Geneva.

Citation: Odike, O. (2023a). *Clostridium botulinum* Toxin Inactivation in Selected Soured Soups in South-South Region of Nigeria. *International Journal of Microbiology and Applied Sciences*. *2*: 76 - 82.