

Application of Bio-Preservation Technique for Food Preservation in Yobe State, Nigeria

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Abstract: Non-thermal treatments are attracting interest of the food industry due to their capability of assuring the quality and safety of food. Among them, bacteriocins from lactic acid bacteria, such as nisin, pediocin and enterocins, are potentially useful for the food industry. In this study the effects of the bacteriocins on local foods in Yobe State were tested against different bacteria including *Brochothrixthermosphacta*, *Listeria monocytogenes* and *Staphylococcus aureus* the treatments with bacteriocins of lactic acid bacteria (LAB) produced in situ on the survival of this three pathogens in food was investigated. The food were collected from local market in Zone A, B and C of Yobe State at random inoculated with the bacterium respective at approximately 105CFU/ml. Three different bacteriocin-producing LAB were added at approximately 106CFU/ml as adjuncts to the starter. The foods were treated adequately on day 2 or 50 at 300 MPa for 10 min or 500 MPa for 5 min, at 10°C in both cases. After 60 days, the bacterial count in foods was carried out. A higher inactivation was achieved in the Camel meat treated without bacteriocin-producing LAB with a unit reduction of 6.5-log and Nisin the chosen bacteriocin. Results from the treatment of boiled cow meat inoculated with Enterocin and *Listeria monocytogenes* shows a unit reduction of 2.0-log while shawarma and cheese inoculated with *Staphylococcus aureus* and treated with Nisin raised a unit reduction of the microbes to 6.3-log and 3.0-log reduction respectively.

Keywords: Bio-preservation, Bacteriocins, Food, pathogens, Yobe State

I. INTRODUCTION

Food preservation is described as the science of extending the shelf-life of food as well as maintaining its nutritional value and avoiding growth of unwanted microbial population (Abdulhussain *et al.*, 2020). Various novel techniques of food preservation are readily available this include, super chilling, ultrasonic preservation, radio frequencies, high pressure processing, cool plasma and natural antimicrobials (bio preservation). Rose *et al.*, (2002) defined bio-preservation as the use of micro-organisms or their products to extend the shelf-life and at the same time improve the safety of available foods. Presently consumers are more conscious on their health concerning the type of additives used in foods. The market value of food processed without the addition of chemical preservatives is becoming more attractive in the food market; therefore, the only alternative to improve the process and to satisfy this demand is the use of Bacteriocins as natural food ingredients (Eduardo *et al.*, 2013; da Costa *et al.*, 2019)..

Bacteriocins are known to be natural compounds capable for improving the safety and quality of food (Stracket *et al.*, 2020).

Pilaret *et al.*, (2010) and Meng *et al.*, (2020) further explain that, Bacteriocins are basically classified into four main classes; class I is lantibiotic, class II are heat stable and non-modified peptides and is the biggest in all gram-positive Bacteriocins, class III are high labile proteins and class IV are characterized by a bonding peptides between the C- and N-terminus. Matinez *et al.*, (2008) confirmed class I, II and IV as the most LAB Bacteriocins which has been approved for use in food biopreservation. Daba *et al.*, (2020) confirmed that, nisin has known to be a broad spectrum against inhibiting the outgrowth of many pathogenic microorganisms inhabiting in canned food and many other dairy products. Eduardo *et al.*, (2013) renowned that, nisin forms pores design to disrupt proton motive force and pH equilibrium of the cell and causes leakage of ions and hydrolysis of ATP leading to cell death. However, Lucy *et al.*, (2006) confirmed this phenomenon which is mediated by the potency of nisin to bind lipid II (peptidoglycan precursor) and hence inhibit cell wall biosynthesis. The use of bacteriocins as preservatives has broad spectrum of importance including:

1. Lowering the cost of biopreservation process which may be highly attractive, for small economy and developing countries, where food quality and safety may be promising (Holzapfel, 2002).
2. Collins *et al.*, (2010) noted that, Bacteriocins has advantages over other methods of preservations by providing extra control of pathogenic and undesirable flora, and establishing beneficial population of bacteria.
3. Bacteriocins applied as ingredients the quality and flavor of food as well as preventing the growth of spoilage organism (Lucy *et al.*, 2006). It also gives immunity to the food by producing detectable protein.
4. Hence, this research was designed to assess the efficacy of natural antimicrobials techniques as a novel preservation of food with reference to Bacteriocin as the inhibitory substance produced by lactic acid bacteria (LAB).

Objectives of the Study

- To find out the effectiveness of Lactic Acid Bacteria (L.A.B) on some selected local food in Yobe State
- To test the efficacy of Bacteriocin as the inhibitor substances produce by L.A.B. on the foods consumed in the study area.

Significance of the Study

Currently consumers in Yobe State are conscious on the health concerning food additives. Food processed without the addition of chemical preservatives is becoming more attractive in the food market; therefore, the only alternative to improve the process and to satisfy this demand is the use of Bacteriocin as natural food ingredients.

Study Area

Yobe State has three geopolitical zones (Zone A, B and C) with a population of about 3,294,100. Most of the people in Yobe State are farmers and Business men who's relied on mobile food, such as Boiled Cow milk, Shawarma, Milk and Cheese that contain preservatives to enable them carryout their activities effectively as they spend most of their time outside. This research investigates the role of biological agents' bacteriocins in a view to reduction of contaminant microbes in the food that will increase the shelf-life of food with less effect on public health.

II. MATERIAL AND METHODS

Ex-situ and Insitu Production of Bacteriocin: Adapted from Eduardo *et al.*, (2013).

Media preparation and sterilisation:

Nutrient Agar media

Nutrient agar 14g was weighed and dissolve in 1000ml of distilled water, also 10g of agar No.2 was weigh and put in the same bottle containing 1000mls of water. The media was sterilise by autoclaving at 121°C and 15psi for 15minutes. The preparation was allowed to cool approximately 47°C after sterilisation.

III. RESULTS AND DISCUSSION

Table 1.0 Bacteriocins in food biopreservation and shelf-life extension test.

Bacteriocins	Culture producer	Target microorganism	Food	Reduction(log CFU g ⁻¹)
Nisin	<i>Lactococcuslactis</i>	<i>Brochothrixthermosphacta</i>	Camel meat	6.5
Nisin	<i>L. lactis</i>	<i>Listeria monocytogenes</i>	Fermented milk	5.0
pediocin	<i>Lactobacillus plantarum</i>	<i>L. monocytogenes</i>	Local Youghort	3.0
Enterocin	<i>Enterococcus faecalis</i>	<i>L. monocytogenes</i>	Boiled Cow milk	2.0
Enterocin	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	Shawarma	6.3
Nisin z	<i>Lactococcuslactis</i>	<i>S. aureus</i>	Cheese	3.0

Viable count using Spread Plate technique

A loopful of the fermented food was spread evenly on the Nutrient agar plate and incubate at 37 °C for 24hrs.

Gram Staining Technique:

- ❖ Using sterile wire loop, pick a colony from the agar plate
- ❖ Heat fix the colony on the slide
- ❖ Add crystal violet stain for 1 minute
- ❖ Wash off the excess stain with water
- ❖ Add grams iodine stain for 2 minutes
- ❖ Wash off the excess stain with ethanol
- ❖ Wash off the ethanol with water
- ❖ Add safranin stain as decolorizer for 1 minute
- ❖ Wash off the excess decolorizer
- ❖ Allow the stain to air dry
- ❖ Observed the slide under oil immersion objective lens of the microscope

Microscopic examination

- Clip the slide on the stage of the microscope. Make sure the specimen is over the hole in the stage.
- Rotate the nose-piece to bring the objective lens just above the specimen
- Place a lamp in front of the microscope and tilt the microscope mirror so that the light is directed up through the eye-piece
- Look through the eye-piece and adjust the diaphragm of the iris until the light is bright.
- Look from the side of the microscope and turn the coarse adjustment knob and lower the objective lens until it is close to the slide.
- Look through the eye-piece and slowly turn the coarse adjustment knob to raise the objective lens until the specimen comes in view.
- Finally use the coarse adjustment knobs to focus the specimen sharply.

IV. DISCUSSION

O'Connor *et al.*, (2020) noted that, Bacteriocins is a good alternative to ensure complete safety in food system, and is often more positive with reference to gram-negative bacteria which are safe by the presence of an outer membrane. In this study the effects of the bacteriocins on local foods in Yobe State where investigated the tested against different bacteria including *Brochothrixthermosphacta*, *Listeria monocytogenes* and *Staphylococcus aureus* in the local foods obtainable in Yobe State including Boiled Cow milk commonly (called nononshanu) fermented milk, locally made Shawarma and Cheese. The treatment with bacteriocins of lactic acid bacteria (LAB) produced in situ on the survival of this three pathogens in food was investigated. The food were collected from local market in Zone A, B and C of Yobe State at random inoculated with the bacterium respectively at approximately 10^5 CFU/ml. Three different bacteriocin-producing LAB were added at approximately 10^6 CFU/ml as adjuncts to the starter. The foods were treated adequately. After 60 days, the bacterial count in foods was carried out. A higher inactivation was achieved in the Camel meat treated without bacteriocin-producing LAB with a unit reduction of 6.5-log and Nisin the chosen bacteriocin Nisin is a widely used novel bacteriocin that has shown promising efficacy against antimicrobial resistant species such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* etc. Eduardo *et al.*, uses Nisin to treat dairy foods and coming up with positive result and record reduction rate of 3.5 in pork. Results from the treatment of boiled cow meat inoculated with Enterocin and *Listeria monocytogenes* shows a unit reduction of 2.0-log while shawarma and cheese inoculated with *Staphylococcus aureus* and treated with Nisin raised a unit reduction of the microbes to 6.3-log and 3.0-log reduction respectively. The results also shows pediocin inhibiting the growth of *Listeria monocytogenes* with a unit reduction rate of 3.0-log. This result is in agreement with that of Kassa *et al.*, (2019) were pediocin registered a unit reduction rate of 4.2-log in a fermented milk.

V. CONCLUSION

Consumers are fully informed on the health risk regarding food additives. Food process without the addition of chemicals preservatives are becoming more attractive, this call for food production industries to pay attention to the use of lactic acid bacteria-producing Bacteriocins. Bacteriocins produce by LAB are antimicrobial peptides acting against some spoilage organisms and diseases causing gram-positive pathogens. As the use of nisin does not cause any toxic effect to human (Kassaa *et al.*, 2019), therefore this study suggested Bacteriocins as novel biological tools for biopreservation and shelf-life extension of foods, unlike the use of other techniques that tend to support the growth of pathogenic

micro-organisms. Bacteriocins have the efficiency to cover a wide field of application, including food industry and pharmaceutical industries.

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