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Research Article
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Effect of Sodium Chloride (NaCl) on the Growth of *Pediococcus acidilactici* Used for the Improvement of Nutritional and Microbial Quality of Tsire: A Nigerian Grilled Meat Product

Aina AT*

Department of Microbiology, University of Ibadan, Ibadan, Nigeria

***Corresponding author:** Aina AT, Department of Microbiology, University of Ibadan, Ibadan, Nigeria, Tel: +234 1 280 2439; E-mail: ainaadetinuke@gmail.com

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Abstract

This finding was aimed at investigating the effect of varying sodium chloride concentration on the growth of *Pediococcus acidilactici* used for improving the nutritional and microbial quality of Nigerian grilled meat product; tsire. *P. acidilactici* used in the study was isolated from raw beef. *P. acidilactici* was cultured in 5.5, 6.5, 7.5, 8.5 and 9.5% NaCl concentration and incubation time. Tsire was prepared using traditional method and *P. acidilactici* was inoculated on the product and stored for 5 days at 28 ± 2ºC. The samples were analysed for microbial and nutritional content after 5 days. *P. acidilactici* and *Lactobacillus confusus* (23%) each, *Lactobacillus kandleri, Lactobacillus plantarum* and *Lactobacillus fermentum* (12% each) and *Lactobacillus rhamnosus, Lactobacillus viridiscences* and *Lactobacillus vaccinostercus* (6% each) were isolated from raw beef samples. Growth of *P. acidilactici* decreased with increase in NaCl concentration and steady growth was observed with 5.5-7.5% NaCl concentration. LAB counts were high in tsire treated with *P. acidilactici* cultured in 6.5 and 7.5% NaCl (10.75 and 10.10 log cfu/g respectively) and corresponding low coliform counts was also observed (1.17 and 1.05 log cfu/g). Crude protein content was higher in tsire treated with *P. acidilactici* cultured in 5.5, 6.5 and 7.5% NaCl (24.49, 21.17 and 22.54% respectively) with corresponding low FFA values (18.39, 16.13 and 17.00% respectively). Data obtained in this research is being applied in harmonized in further research to obtain tsire of acceptable quality in terms of microbial content, nutritional value and sensory attributes.

Keywords: Tsire; *Pediococcus acidilactici*; Lactic acid bacteria; Sodium chloride; Crude protein; Free fatty acid

Introduction

Lactic Acid Bacteria (LAB) have been implicated in various studies on meat and meat products [1-3]. Lactic acid bacteria plays a very important role in food fermentation as products obtained through them are characterized by hygienic safety, storage stability and attractive sensory properties [4]. Their microbial antagonism results from the lone or combined production of organic acids, ethanol, diacetyl, hydrogen peroxide, and carbon dioxide [5]. Lactic acid bacteria that have been reportedly used in meat production include Pediococcus species *(P. cerevisae* and *P. acidilactici)* and *Lactobacillus* species (*L. plantarum*, *L. brevis* and *L. buchneri)* and *Lactococcus* species [1,6,7].

Meat is a major source of protein and vitamins for most people in many parts of the world, thus they are essential for the growth repair and maintenance of body cells and necessary for our everyday activities [8]. Meats are highly perishable foods due to their chemical and physiological composition thus they support the growth of a wide range of microorganisms [9]. Several methods have been used to preserve meat including cooking, fermenting, salting, smoking and drying. **Salting** meat is an ancient preservation technique in which the salt draws out moisture and creates an environment inhospitable to bacteria [10]. Other functions of salts include microbial growth suppression, water reduction, salt-soluble protein release, and pro-oxidant effects [11]. NaCl and $NaNO_2$ are the common curing salts used in meat production with NaCl making the bulk of the mixture.

Tsire is a Nigerian grilled meat product consumed as evening delicacies. Tsire is synonymous to suya. Tsire vendors often have left over from their daily sales thus the chances of contamination as storage facilities are often unavailable or inadequate. Tsire is prepared using de-boned meat (often beef) spiced with ingredients such as common salt (NaCl), garlic, pepper, groundnut cake etc. Studies by Onilude et al. [12], established that lactic cultures influence the quality attributes of tsire and Olaoye [13] also found that organisms such as *P. acidilactici* could be used as starter cultures for extending the shelf-life of the product. This research is thus aimed at investigating the effect of NaCl on the growth of *P. acidilactici* used for the improvement of nutritional and microbial quality of tsire.

This study is aimed at studying the effect of varying sodium chloride concentration on the growth of *P. acidilactici* used for improving the nutritional and microbial quality of Nigerian grilled meat product; tsire.

Materials and Methods

Raw meat samples were purchased from local vendors at retail outlets in Ibadan, Oyo State. The samples were incubated at 37C for 24 h to allow bacterial colonization. Ten grams of the meat samples was homogenized in 90 mL sterile normal saline solution, pour-plated on de Mann Rogosa and Sharpe (MRS) agar and incubated at 37C for 48 h in an anaerobic jar. [14]. Isolates were identified using API 50CH and CHL medium (API system, Montalieu, Vericeu, France).

Effect of sodium chloride concentration on the growth of the isolate

P. acidilactici was aseptically inoculated into 15 mL peptone water in test tubes and incubated at 32 ± 5°C for 18 h. 1 mL aliquot of *P. acidilactici* was inoculated into sterile MRS broth with sodium chloride concentration of the media was supplemented with 5.5, 6.5, 7.5, 8.5 and 9.5% sodium chloride. The bottles were incubated at 32 ± 5C for 12, 24, 36 and 48 h. Growth measurement was determined as Optical density (OD) at 620 nm using Spectronic 20 D.

Tsire sample preparation

Tsire was produced from beef using a traditional method described by Onilude et al. [12].

Fresh beef and tsire spices including onion, ginger and garlic were purchased from a local market in Lagos, Nigeria.

The meat(fat-free) was washed in 5% sterile saline water and sliced into layers to about 10 × 6 × 0.5 cm^3, not exceeding a weight of 20 g each. The meat pieces cured with ground pepper and all other spices were hung on stick and allowed to marinate for 10-15 min after which they were grilled on hot coals at a temperature of 130-150°C for 15-20 min with intermittent sprinkling of groundnut oil to stimulate the traditional processing method and to prevent burning of the stick meat samples.

The samples were allowed to cool to ambient temperature and inoculated *P. acidilactici* cultured at different NaCl concentration. Inoculated tsire samples were incubated in a sterile environment at 29 ± 2°C for 48 h before further analysis. Un-inoculated tsire served as the control.

Proximate analyses of tsire samples inoculated with the stressed isolate

The Protein, Moisture, Ash and Free Fatty Acid (FFA) content of the tsire samples were analysed as described by AOAC [15].

Microbiological analyses of tsire samples inoculated with the stressed isolate

10-15 g of each of tsire samples were cut into small pieces and blended in 90 mL sterile distilled water in a disinfected blender to make the initial dilution. Serial dilutions of the samples were then prepared and 1 mL of the appropriate dilutions was plated out using the pour plate method described by Totora et al. [16]. MRS agar was used to enumerate for Lactic acid bacteria, MacConkey agar for Coliforms and Yeast Extract agar for Yeasts. Samples were incubated for 48 h in Gallenkomp 9052 laboratory incubator.

Results

Figure 1 shows the frequency of occurrence of Lactic Acid Bacteria isolated from raw beef sample.

The result of the isolation shows that the highest occurring microorganisms were *P. acidilactici* and L. confusus (23%) each followed by *L. kandleri*, *L. plantarum* and *L. fermentum* (12% each) and the least occurring were *L. rhamnosus, L. viridiscences* and *L. vaccinostercus* (6% each).

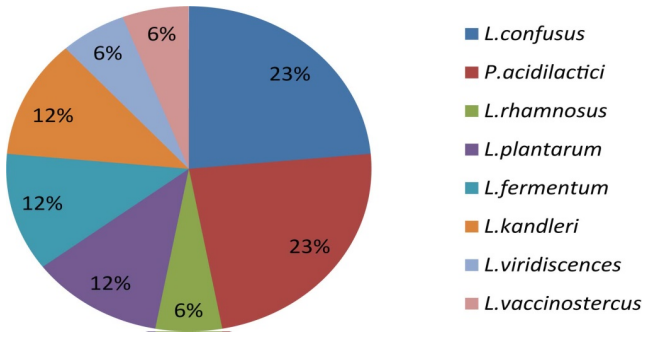

Figure 1: Percentage occurrence of Lactic acid bacteria isolated from raw beef samples.

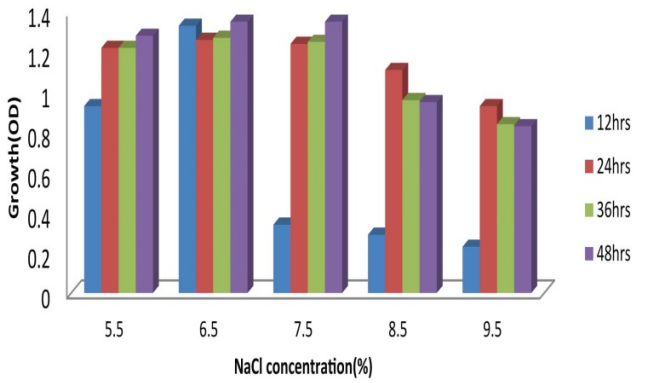

Figure 2: Growth of *Pediococcus acidilactici* at different concentrations of NaCl and incubation time.

NaCl Concentration (%)	LAB	Yeast	Coliforms	Mold
5.5	7.95	2.06	2.53	1.5
6.5	10.75	3.11	1.17	1.71
7.5	10.1	3.5	1.05	2.55
8.5	8.35	2.14	2.02	1.09
9.5	5.15	2.98	3.41	1.12
*Values are mean of three replicates				

Table 1: Microbial load (log cfu/g) of tsire samples inoculated with *Pediococcus acidilactici* (cultured at different NaCl concentration) after storing for 5 days at 28 ± 2°C.

Discussion

Figure 1 shows the percentage occurrence of LAB isolated from raw beef samples and they were identified as *L. confusus, P. acidilactici, L. rhamnosus, L. plantarum, L. fermentum, L. kandleri, L. viridiscences* and *L. vaccinostercus*. Oliveira et al. [17] reported the isolation of *L. plantarum*, *P. acidilactici* and *L. fermentum* from vacuum packaged beef. Collins et al. [18] reported the isolation of *L. confusus* in meat, *L. viridiscences* was reported to cause greening in meat due to excessive

production of hydrogen peroxide [19]. Meat supports the growth of a wide range of micro-organisms because of its nutritious nature.

NaCl Concentration (%)	Ash	Crude Protein	Free Fatty Acid (FFA)	TBA
5.5	9.4	24.49	18.39	1.25
6.5	9.87	21.17	16.13	1.04
7.5	8.92	22.54	17	1.15
8.5	11.27	18.44	20.98	1.05
9.5	10.47	15.2	21.06	1.27
*Values are mean of three replicates				

Table 2: Nutritional analysis (%) of tsire samples inoculated with *P. acidilactici* (cultured at different NaCl concentration) after storing for 5 days at room temperature.

Figure 2 shows the growth of *P. acidilactici* at different NaCl concentration and incubation time. Similar to previous reports by Altuntas et al. [20], growth of *P. acidilactici* decreased with increase in NaCl concentration. *P. acidilactici* was observed to have steady growth with 5.5-7.5% NaCl concentration. It is believed that the organisms are at the exponential phase of growth with favorable growth conditions while at higher NaCl concentration of 8.5-9.5% growth decreased with increased contact time.

Microbial contamination can lower the quality and shorten the shelf life of meat and meat products resulting in economic loss or health hazard [3]. As shown in Table 1, the highest LAB count (10.75%) was observed on tsire sample treated with *P. acidilactici* grown in 6.5% NaCl. LAB count was least (5.15 log cfu/g) on sample treated with *P. acidilactici* grown in 9.5% NaCl. Lower coliform value was observed in tsire sample treated with *P. acidilactici* grown in 6.5% NaCl being 1.17 log cfu/g and 7.5% NaCl being 1.05 log cfu/g. The lower coliform values observed on samples with higher LAB proliferation can be due to the production of antimicrobial compounds such as lactic acid, bacteriocin, hydrogen peroxide and diacetyl [7]. Tsire produced with *P. acidilactici* cultured in 6.5 and 7.5% NaCl can be considered safe for consumption based on the low coliform values as coliform have direct impact on health and within limit of hot smoked meat product specified by the Food Administration Manual. Yeasts and moulds invade and grow on almost all food items at any time resulting in deterioration and decomposition of foods. They may be hazardous to human health because of their ability to produce mycotoxins. Gandi [21] reported the isolation of *A. niger, A. flavs*, *Penicillum* spp. and *Fusarium* spp. from suya (Table 2).

The ash content of a food sample is synonymous to the mineral content of the sample. Ash content of tsire samples in this study was between 8.92 and 11.27% which agrees with reports by authors such as Olusola et al. [22] reported 10.31% ash on Kilishi which is a dry meat product and Torres et al. [23]; 9.40-11.27% ash content of tsire samples. Ash content of various samples depends on the meat type. The crude protein of tsire samples in this study was within 15.20-24.49%. Proteins are Polymers of Amino acids and it is the only macro nutrient in food that contains nitrogen. Groundnut cake was not used in the production of tsire in this study. Studies by Abdullahi et al. [24]; 31.88% crude protein on tsire and Gandi [21] reported higher crude protein content (31.88 and 40-45% respectively)

Free Fatty Acid (FFA) content of a sample indicates the extent to which the oils in the sample have been decomposed by Lipase action. FFA formation is often accompanied by rancidity and also heat and light accelerate FFA formation. The lower the FFA content of a sample, the better the sample, the better the sample is. The least FFA in this study was observed on tsire samples treated with *P. acidilactici* cultured at 6.5% NaCl concentration. The meat type and part has a direct relationship to the FFA content of meat products. Thiobabituric Acid (TBA) reactive substances are formed as a byproduct of lipid peroxidation measured as the malonaldehyde per weight of the sample. TBA values of tsire samples in this study ranged between 1.04% and 1.27%. Lipids are susceptible to oxidation and enzymatic oxidation under different conditions usually involving free radicals or oxygen species [25].

Conclusion

P. acidilactici can grow in NaCl concentrations up to 9.5%, the effect of culturing *P. acidilactici* in varying NaCl concentration is not significant with respect to the microbial quality of tsire produced using these organisms. However, higher crude protein and lower free fatty acid content was observed in tsire samples treated with *P. acidilactici* cultured in <7.5% NaCl. Data obtained in this study is being applied in further work to obtain tsire of acceptable quality in terms of microbial content, nutritional value and sensory attributes.

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