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 ± 2°C. 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 vacinostercus* (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 NaNO2 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 Oloaye [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 37°C 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 37°C 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 ± 5°C 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 cm³, 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).

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.

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

<table>
<thead>
<tr>
<th>NaCl (%</th>
<th>Ash</th>
<th>Crude Protein</th>
<th>Free Fatty Acid (FFA)</th>
<th>TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>9.4</td>
<td>24.49</td>
<td>18.39</td>
<td>1.25</td>
</tr>
<tr>
<td>6.5</td>
<td>9.87</td>
<td>21.17</td>
<td>16.13</td>
<td>1.04</td>
</tr>
<tr>
<td>7.5</td>
<td>8.92</td>
<td>22.54</td>
<td>17</td>
<td>1.15</td>
</tr>
<tr>
<td>8.5</td>
<td>11.27</td>
<td>18.44</td>
<td>20.98</td>
<td>1.05</td>
</tr>
<tr>
<td>9.5</td>
<td>10.47</td>
<td>15.2</td>
<td>21.06</td>
<td>1.27</td>
</tr>
</tbody>
</table>

*Values are mean of three replicates.

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.

References


