

Effect of Post-Lethality Thermal Treatment on Log Reduction of *Staphylococcus aureus* in *Balangu* Ready-To-Eat Meat Product

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Abstract - An experiment was conducted to evaluate the effect of post-lethality thermal treatments on the survival of *Staphylococcus aureus* under three thermal regimes: Low Temperature Long Time-LTLT (52°C at 20 minutes), Moderate Temperature Moderate Time-MTMT (62°C at 12 minutes) and High Temperature Short Time-HTSH (72°C at 4 minutes) administered as pre-package surface pasteurization. Twenty *balangu* samples were produced under good manufacturing practice (GMP) and inoculated with a pre-formulated pure culture of *S. aureus* earlier isolated from vended *balangu* samples. One liter of the *S. aureus* was prepared aseptically by transferring 2.5 ml of pure culture isolate to 1000 ml of presterilized TSB and then incubated at 37°C for 24 h to obtain 1 liter of the *S. aureus* containing about log 6-7×10⁶*S. aureus* population. Samples were inoculated by dip method for approximately one minute, removed and equilibrated for three hours. Results showed that a mean *S. aureus* population was 5.5×10⁶ for the control samples. However, pasteurization had a significant effect (P<0.05) in reducing the population. There was a reduction in *S. aureus* by 2.0, 3.5 and 4.25 ×10⁶ for LTLT, MTMT and HTST, respectively. It was concluded that prepackage surface pasteurization of *balangu* at High Temperature Short Time (72°C at 4 minutes) had engendered higher (4.25 ×10⁶) log reduction of *S. aureus* in *balangu*. Therefore, it is recommended that *balangu* should be pasteurized at high temperature for a short time so as to ensure food safety and protect public health.

Keywords: Post-Lethality, Thermal treatment, Survival of *S. aureus*, *Balangu*, Ready-To-Eat Meat Product

I. INTRODUCTION

Food borne diseases remain an important public health problem worldwide and one of the most significant food safety hazards associated with foods from animals [1]. Meat is the most perishable of all staple foods since it contains sufficient nutrients needed to support the growth of microorganisms [2]. In the last years, global surveillance data indicated that the incidences of foodborne diseases has increased in developing countries mainly associated with the consumption of raw or undercooked meat and dairy products

[3][4]. It was also reported that the surveillance of potential contaminant bacteria in different kinds of meat is crucial to safeguard public health is practically limited in the developing countries and constant control work and research has to be done to maintain a high level of meat hygiene and safety [5]

Pathogenic bacteria associated with RTE foods have been implicated in major outbreaks and sporadic cases of *listeriosis*, *gastroenteritis*, *celebrospinalmeningitis*, *cholera*, *salmonellosis* and many other cases with symptoms ranging from diarrhoea, loss of appetite, vomiting, nausea and death in different countries [6]. *Staphylococcus aureus* is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. *S. aureus* is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans [7][8].

The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, water activity (a_w), pH, the presence of oxygen and composition of the food. These physical growth parameters vary for different *S. aureus* strains (Stewart 2003). The temperature range for growth of *S. aureus* is 7–48°C, with an optimum of 37°C. *S. aureus* is resistant to freezing and survives well in food stored below -20°C; however, viability is reduced at temperatures of -10 to 0°C. *S. aureus* is readily killed during pasteurisation or cooking. Growth of *S. aureus* occurs over the pH range of 4.0–10.0, with an optimum of 6–7 [9][10].

S. aureus is uniquely resistant to adverse conditions such as low aw, high salt content and osmotic stress. In response to low aw, several compounds accumulate in the bacterial cell, which lowers the intracellular aw to match the external aw (Montville and Matthews, 2008). As such, most *S. aureus* strains can grow over a aw range of 0.83 to >0.99 [8].

S. aureus is reported to be a poor competitor, but its ability to grow under osmotic and pH stress means that it is capable of thriving in a wide variety of foods, including cured

meats that do not support the growth of other foodborne pathogens [7].

Thermal treatment is one of the most reliable and commonly used methods to ensure food preservation and safety. However, thermal treatment can alter the quality of muscle food by affecting the liquid loss and thereby influencing texture and content of water soluble nutrients [11]. Thermal treatment may also modify amino acids making them less available as nutrients [12]. Thermal resistance of a pathogen is influenced by many factors such as strain variations, growth phase, growth conditions, exposure to thermal shock, acid and the composition of the heating medium. After the thermal treatment, the number of surviving cells detected depends on the ability to recover, recovery method, recovery medium and incubation conditions used.

Thermal treatment has been reported to be among the food technologies that are intended to improve the safety of food commodities and is critical in controlling foodborne pathogens in RTE-MPs [13]. For a non-spore forming mesophilic bacterium, *S. aureus* has a relatively high heat resistance [10]. The observed average decimal reduction value (D-value, the value at which the initial concentration of bacterial cells would be reduced by 1 log₁₀ unit) was 4.8–6.6 min at 60°C when heated in broth [14]. Adequate thermal treatment could improve food safety by either inactivating or destroying the microorganisms that causes foodborne illness [15][16]. The objective of this study is to evaluate three post-lethality thermal treatments for determining *S. aureus* survival in *balangu* RTE-MP.

II. MATERIALS AND METHODS

Survival of *S. aureus* was investigated in three modes of post-lethality thermal treatments on the *balangu* RTE meat product to determine the survivability of the pathogen at various temperatures.

A. Preparation of Inoculum

The preparation of cell suspensions was adapted from the procedure described by [17]. Tryptone soya broth (TSB, Oxoid, Basingstoke, England) was inoculated with the *S. aureus* and incubated at 37°C for 24 hours. The culture was diluted in 0.1% peptone to produce a concentration of about 10 cfu/ml and 1 ml was transferred to 100 ml of TSB, which was incubated at 37°C between 16-19 hours. The 100 ml culture was centrifuged (IEC Centra® MP4R, IEC International Equipment Company, Massachusetts, USA) at 1400 xg for 20 minutes at 21°C and re-suspended in 40 ml TSB. One liter of the *S. aureus* was prepared aseptically by transferring 2.5 ml of pure culture isolate to 1000 ml of pre-sterilized TSB and then incubated at 37°C for 24 h in a sterile 2-liter beaker to obtain 1 liter of the *S. aureus*. The inoculum suspension was enumerated on plate count agar (PCA, Oxoid, Basingstoke, England) plates after incubating at 37°C for 24 hours.

B. Preparation of Samples

Three kilograms of fresh beef was purchased from Birninkebbi central market and trimmed of all external fats and connective tissues. The lump of the beef was partially sliced to about 1-2 cm thick and divided into three groups weighing about 600-650 grams and stored at 4°C for further processing. Prior to the processing of the product, pre-lethality examination of the fresh beef for the presence of *S. aureus* was conducted. *Balangu* was then prepared from the sliced beef in the manner of [18] in a portable drum kiln specifically designed to process the product under optimal hygienic conditions. The sliced beef was placed on a brown paper sheet that has been spread over the wire mesh two feet above glowing fire from firewood. After 3 hr with regular turning interval of 30 mins, *balangu* was produced. After gradual cooling for 30 mins in the kiln, a clean and sterile knife was used to cut pieces of samples with a dimension of 3×3cm². A total of 20 samples were prepared, wrapped in sterile aluminum foil and ready for post-lethality examination (Table 1).

TABLE 1.

EXPERIMENTAL LAYOUT FOR COMPARATIVE STUDIES OF POST-LETHALITY THERMAL TREATMENT OF *BALANGU* (PHASE III)

Thermal treatment	Number of samples
Pre-lethality examination	4
Post-lethality examination	
Un-Inoculated fresh balangu	4
CONTROL (00°C at 00 minutes)	4
LTLT (52°C at 20 minutes)	4
MTMT (62°C at 12minutes)	4
HTST (72°C at 4 minutes)	4
Total	20

LTLT= Low Temperature Long Time

MTMT= Moderate Temperature Moderate Time

HTST= High Temperature Short Time

C. Post-Lethality Inoculation, Thermal Treatment and Enumeration

Out of the twenty *balangu* samples, 16 samples were carefully removed from the aluminum foil and dipped into the already prepared inoculum, and held for approximately 1 min. Four samples were not inoculated. Samples were removed from the inoculum using a pair of forceps and allowed to equilibrate for approximately 3 hr. The inoculated samples were then randomly allotted to four groups for post-lethality thermal treatment containing four samples each and four uninoculated samples were for control.

Thermal treatment pasteurization was administered using a Kenwood® digital microwave oven that has temperature control. Samples were placed in a preset oven and closed. Holding time was recorded using a digital stop. Four

inoculated samples were not administered any thermal treatment. The remaining samples were for each four samples, pasteurized at: low temperature long time (LTLT - 52°C at 20 mins), moderate temperature moderate time (MTMT - 62°C at 12mins) and high temperature short time (HTST - 72°C at 4 mins), respectively (Table 1).

For all treatments including 00 hours (initial *S. aureus* level), four *balangu* samples were used for enumeration. After each holding period, 5g of *balangu* sample was placed into a beaker, and 50 ml of 0.1% peptone water (Difco, Detroit, MI, U.S.A.) was added to the beakers. The samples and diluent were then thoroughly shaken for 1 minute. Serial dilutions were prepared. *S. aureus* populations were enumerated after spread plating 0.1-mL of diluent onto quadruplicate mannitol salt agar plates (Difco BD and Company, Sparks, MD, U.S.A.) and incubating for 48 h at 35°C.

III. RESULTS AND DISCUSSION

A. Assessment of Post-Lethality Thermal Treatment on Balangu

Results for assessment of post-lethality thermal treatment of balangu are shown in Table 2. Fresh beef had a total mean *Staphylococcus aureus* (*S. aureus*) population of 11.25×10^1 cfu/g after three trials. The *S. aureus* population significantly reduced to 0.25×10^1 cfu/g in freshly produced balangu. For the inoculated samples, after inoculation, the total mean *S. aureus* population of three trials was 5.5×10^1 cfu/g for the control samples. However, pasteurization had significantly reduced ($P < 0.05$) the *S. aureus* population from $\log 5.5 \times 10^1$ cfu/g to between $\log 2.0$ and 4.25×10^1 cfu/g across the treatments.

TABLE 2

EFFECT OF THERMAL TREATMENT ON *S. AUREUS* POPULATIONS (LOG CFU/G) IN LABORATORY PREPARED BALANGU

Treatments	<i>Staphylococcus aureus</i> population				
	First trial Mean	Second trial Mean	Third trial Mean	Total Mean	Log Reduction
**Fresh beef	10.25	11.5	12.5	11.25	0.0
**fresh balangu	0.0	0.5	0.25	0.25	11.0
Inoculated samples					
Control (0°C at 0 min)	6.0	5.75	4.75	5.5	0.0 ^d
LTLT (52°C/20 min)	3.25	3.75	3.5	3.5	2.0 ^e
MTMT (62°C/12min)	2.0	1.75	2.25	2.0	3.5 ^b
HTST (72°C/4 min)	1.0	1.25	1.5	1.25	4.25 ^a
SE					0.22

a,b,c,d= means with different superscript along the same column differ significantly ($P < 0.05$)

LTLT (Low temperature long time)

MTMT (Moderate temperature moderate time)

HTST (High temperature short time)

**Not among treatments, a pre-lethality examination that was conducted on fresh beef

The high log reduction of between pre-lethality and post-lethality samples suggested that *S. aureus* population had been significantly reduced by the product processing heat, this is in line with the mechanism of heat effects on thermophiles described by [19]. The high *S. aureus* population found on raw beef samples (11.25×10^1 cfu/g) could be attributed to meat handling, poor practices or as a result of poor quality of raw materials and poor sanitary conditions of Nigerian abattoirs[20] processing, handling and packaging materials [10]. The low *S. aureus* population observed at post-lethality in the prepared *balangu* samples is an indication of heat susceptibility of the organism. Though, some strains of *S. aureus* has been reported to have a high heat resistance for a mesophilic non-spore forming bacterium and its heat resistance was observed to be higher in foods with a lower water activity [10] like *balangu*. It could probably be the reason why some organisms were found after lethality treatment. It could also be as a result of postprocess cross-contamination from utensils, handling or environment as reported by [20] that a drastic decrease in aerobic counts and *Staphylococcus aureus* levels following heat treatment and subsequent increase in counts of these bacteria are suggestive of post-cooking contamination.

Prepackage pasteurization of *balangu* had also shown that temperature and time were important in reducing *S. aureus* populations in post-lethality exposed balangu. The administered thermal treatment indicated that the HTST (72°C at 4 minutes) pasteurization having higher log reduction of *S. aureus* (4.25) could be due to the effect of high temperature on cellular activities of the organism which could result in spontaneous shock leading to instant death. [21] reported that Microorganisms are quickly killed when they are exposed to relatively high temperatures. Pasteurization using MTMT (62°C at 12minutes) also significantly reduced the *S. aureus* population by log 3.5 which still destroyed far more than 50% of the inoculated population. The lower log reduction (2.5) of *S. aureus* in LTLT (52°C at 20 minutes) pasteurization could be as a result of gradual acclimatization of the organism to the temperature. It might also be due to the shape of the sample which could provide a shield for the organism against gradual rise in temperature. Also, there could be a strain that contain protein disulfide oxidoreductase (PDO) which could have protected the protein from degradation and death of the organism in a mechanism described by [19]. The log reduction could also be attributed to the long-time applied at low temperature leading to the death of the organisms. It was reported by [21] that microorganisms can be killed at relatively low hot temperatures, but longer heat treatment periods are necessary. Previous publications relating to administration of thermal treatment in reducing pathogens were scanty on *S. aureus*. However, a considerable work has

been done on *Listeria monocytogenes* which is equally an important pathogen of human concern. Levels of thermal treatment parameters (temperature and time) used to achieve optimal log reduction have been reported. In a study by [22] reported that pasteurizing *kunuzaki* at 70°C for 20 minutes had eliminated all microfloral isolates (including *S. aureus*). A study by [23] achieved 1.25 to 3.5-log reductions of *L. monocytogenes* with treatment times of 60 to 120 seconds (s) and air temperatures of 475 to 750°F (246 to 399°C) using a radiant oven. Muriana, *et al.* (2004) [24] achieved 2.0 to 2.8 log reductions when processed for 60s and 2.8 to 3.8 log reductions when processed for 75s, and also, an improved radiant oven provided 3.53 (60s) and 4.76 (75 s) log reductions of *L. monocytogenes*. Also, [25] estimated that the lethal effect of the cooking process for chicken meat at an internal temperature of 70°C for 2.1 minutes would provide a 7-log reduction of all cell suspensions tested. In a study by [26] it was reported that thermal processing of ham, summer sausage, and frankfurters to 71°C is sufficient to reduce the risk of methicillin resistant *S. aureus* (MRSA) with 7 log cfu/g.

IV. CONCLUSION

The study concluded that prepackage surface pasteurization of *balangu* before consumption at high temperature short time (72°C for 4 mins) could be adequate enough to reduce the risk of *S. aureus* with between 2 and 4.25 log cfu/g as a potential food safety hazard. It is therefore recommended that processors and consumers could use this thermal treatment to ensure public health safety.

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