Effect of Lactic Acid on Inactivation of Enterotoxigenic Escherichia Coli (ETEC) Isolated from Tuna Loins Produced in Côte D’Ivoire

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AES designed the work, wrote protocol, carried out the experiments and wrote the first draft of manuscript. Authors YAA and TA analyzed data obtained. Author OK carried out literature searches. Authors RKN and KMD read and approved the final manuscript. All authors read and approved the final manuscript.

ABSTRACT

Aims: The aim of this work was to study the effect of lactic acid on the growth of pathogenic strains of Escherichia coli (ETEC) isolated from tuna loins.

Study Design: Bacteriological study.

Place and Duration of Study: Laboratory of Microbiology of the Central Laboratory of Food hygiene and Agribusiness (LCHAI), Abidjan, Côte d’Ivoire between September 2014 and December 2014.

Methodology: Enterotoxigenic Escherichia coli (ETEC) strains were isolated from tuna loins. Lactic acid (LA) 1%, 2% and 3% were tested in pathogenic strains in liquid medium (brain heart infusion broth, BHI) and in tuna loins.

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Results: At lactic acid 1%, the bacterial loads decreased during the first two days and then stabilized. *E. coli* strains in tuna loins were higher (1.25 to 0.9 log CFU/g) than *E. coli* in liquid medium (0.69 to 0.3 log CFU/g). No bacterial growth was observed in the tuna loins and in BHI for concentrations of 2% and 3% of lactic acid.

Conclusion: Lactic acid has an inhibitory effect at 1% and bactericidal effect at 2% and 3% on the growth of *E. coli*. The use of lactic acid as a preservative could be a solution for the preservation of these products.

Keywords: Enterotoxigenic Escherichia coli (ETEC); lactic acid (LA); tuna loins; brain heart infusion broth (BHI).

1. INTRODUCTION

Microorganisms of varying types and numbers can be found on food of animal and plant origin. The types and number of microorganisms on food can be changed due to food processing, inappropriate purchasing, storing, preparing, cooking or serving [1]. Increase in the number of these microorganisms due to the abovementioned changes may lead to spoiling of the food, causing a pathogenic effect on humans. The most important of foodborne pathogenic bacteria is *Escherichia coli* [2].

The Enterotoxigenic *Escherichia coli* (ETEC) strains are mainly associated with two important clinical syndromes, choleriform watery diarrhea in children called infant diarrhea and traveler's diarrhea (or "turista") in developing countries [3]. The pathogenic power of ETEC is mainly explained by the secretion of thermostable (ST) and l or thermolabile (LT) toxins [4]. People living in developing countries have often been reported to have this pathotype in their feces and shown to have developed immunity against this microorganism. Being a cause of mortality in children under 5, the most frequently observed microorganism in childhood diarrhea is ETEC and it is also responsible for 30–60% of travelers' diarrhea. Infection is characterized by watery diarrhea and, depending on the person, its course may range from a normal course to cholera-like defecation with the addition of symptoms such as vomiting and high fever [5,6,7]. Diarrhea is the most common causes of mortality in society and among young children, especially those living in Asia and sub-Saharan Africa with inadequate healthcare systems and limited access to clean drinking water [8,9].

Côte d'Ivoire through the processors and exporters of fish products, has become one of the largest exporters of tuna products to the global level [10]. There are 2 types of tuna products exported: Tuna finished products (canned) and tuna semi-finished products (tuna loins, tuna flakes, tuna skin and tuna pulp). The tuna loins are portions of the tuna flesh usually skinless and boneless and ready to use. However, industries have difficulties to export tuna loins because they don't satisfy the criteria for hygienic quality and existing standards always. ETEC has been found in these products [11], which poses a major health and public health problem and causes economic losses for companies producing tuna products.

The aim of this work was to study the effect of lactic acid on the growth of pathogenic strains of *Escherichia coli* (ETEC) isolated from tuna loins.

2. MATERIALS AND METHODS

2.1 Sample preparation

Each sample of tuna loins was crushed and aseptically distributed in Pyrex bottles then sterilized at 121°C for 15 min. Each sample was approximately 100 g in each bottle.

Brain heart infusion broth (BHI) (Biorad, France) was prepared in accordance with the manufacturer's instructions and distributed in Pyrex bottles then sterilized at 121°C for 15 min. The volume of each broth was also 100 mL in each bottle.

2.2 Inoculum Preparation

Three strains of *E. coli* were selected for the various analyzes:

- an enterotoxigenic strain of *E. coli* (ETEC), possessing both the "elt" and "est" genes resistant to amoxicillin, isolated from tuna loins;
- an *E. coli* reference strain (ATCC 25992);
- a strain of *E. coli* (KO 13) from water with the virulence gene "elt".

A colony of each strain was inoculated into 10 mL of Tryptone Soya Broth (TSB) (Mast Diagnostic, France) broth and incubated at 37°C
for 24 hours. The optical density of the inoculated broth was determined using a UV 2700 spectrophotometer (Schimadzu, Germany) at a wavelength of 600 nm. Knowing that the absorbance between 0.5 and 1 corresponds to approximately 10^8 CFU/ml, the different concentrations of E. coli have been determined and the cultures diluted to obtain a final concentration of 10^5 CFU/ml.

### 2.3 Kinetics of Destruction of Escherichia Coli Strains

The study of the kinetics of destruction of the E. coli strains isolated from tuna loins was carried out according to the methods described by Lindqvist and Lindblad [12]. 1 mL of each bacterial culture with a concentration of 10^5 CFU/mL was inoculated into the 100 g of tuna loins and into the 100 mL of BHI. Then, 1 ml of lactic acid (Riedel-De Haën AG Seelze-Hannover, Germany) at 1%, 2% and 3% was added. LA is used as a preservative in the food industry. They were incubated at 30°C for 5 days. At each time interval, 1 mL was taken to determine the pH using pH meter (Milwaukee, USA) and 1 mL was taken to determine E. coli on Tryptone Soya Agar (TSA) (Plasmatec, England). The measurement interval was (days): 0; 1; 2; 3; 4; 5. Samples of tuna loins and BHI that had not been inoculated with E. coli served as negative controls for the various analyzes.

### 2.4 Statistical Analysis

All values were expressed as the mean of three measurements in microbiological analysis. E. coli counts were log transformed (Log10) and the data collected were subjected to one ways Analysis of variance (ANOVA) with the software Statistica 7.1. Duncan test was used in order to determine which means were significantly different from which others (α=0.05).

### 3. RESULTS AND DISCUSSION

#### 3.1 Effect of Lactic Acid on the Growth of Escherichia Coli Strains in Non-Renewed Liquid Medium

Figs. 1a to 1c show effect of different concentrations of lactic acid (1%, 2% and 3%) in BHI on the growth of E. coli ATCC 25922, E. coli KO strains 13 and virulent E. coli isolated from tuna loins respectively. The pH for LA1% concentrations was highest and the E. coli loads decreased considerably from 0.95 to 0.3 log CFU/mL (Fig. 1a); from 0.77 to 0.25 log CFU/mL (Fig. 1b) and from 0.69 to 0.3 log CFU/mL (Fig. 1c). No bacterial growth was observed in liquid medium for concentrations of 2% and 3% of lactic acid. Whatever the curve, the bacterial loads decreased the first two days and then stabilized.

Fig. 1d compares the effect of LA (1%) on the growth of the three strains studied. The three curves have the same appearance: the bacterial loads decreased during the first two days before stabilizing. The E. coli KO 13 strains were the most sensitive to the effect of lactic acid, while the E. coli ATCC 25922 strains were the least sensitive.

#### 3.2 Effect of Lactic Acid on the Growth of Escherichia Coli Strains in Tuna Loins

Figs. 2a to 2c show the effect of different concentrations of lactic acid (1%, 2% and 3%) in tuna loins on the growth of E. coli ATCC 25922, E. coli KO 13 strains and virulent E. coli from tuna loins respectively. The pH for lactic acid 1% concentrations was highest and the E. coli loads decreased from 1.11 to 0.9 log CFU/g (Fig. 2a); from 1.27 to 1.07 log CFU/g (Fig. 2b) and from 1.25 to 0.9 log CFU/g (Fig. 2c). No bacterial growth was observed in the tuna loins for concentrations of 2% and 3% of lactic acid. Whatever the curve, the bacterial loads decreased the first two days and then stabilized.

Fig. 2d illustrates the effect of LA (1%) on the growth of the three strains studied. The three curves have the same appearance: the bacterial loads decreased during the first two days before stabilizing. The E. coli KO 13 strains were the most sensitive to the effect of lactic acid and the E. coli ATCC 25922 strains were the least sensitive.

#### 3.3 Comparative Effect of Lactic Acid on the Growth of Pathogenic Strains of Escherichia coli from Tuna Loins in Liquid Medium and in Tuna Loins

Fig. 3 shows effect of lactic acid on the growth of pathogenic strains of Escherichia coli from tuna loins in liquid medium and in tuna loins. The bacterial loads decreased during the first two days and then stabilized. E. coli strains in tuna loins were higher (1.25 to 0.9 log CFU/g) than E. coli in liquid medium (0.69 to 0.3 log CFU/g). The strains of E. coli in liquid medium were more sensitive to the effect of lactic acid than those in tuna loins.
Fig. 1a. Effect of lactic acid on the growth of strains of *Escherichia coli* ATCC 25922 in BHI

Values with the same letter on a curve are not significantly different for p>0.05

Fig. 1b. Effect of lactic acid on the growth of strains of *Escherichia coli* KO 13 in BHI

Values with the same letter on a curve are not significantly different for p>0.05

Fig. 1c. Effect of lactic acid on the growth of pathogenic *Escherichia coli* from tuna loins in BHI

Values with the same letter on a curve are not significantly different for p>0.05
Fig. 1d. Comparative evolution of the effect of lactic acid (1%) on the growth of three *Escherichia coli* strains in BHI

*E. coli* 1 = *E. coli* ATCC 25922; *E. coli* 2 = *E. coli* KO 13; *E. coli* 3 = pathogenic *E. coli* from tuna loins

4. DISCUSSION

The results of this work showed that bacterial loads decreased when the concentration of lactic acid at 1% was added to BCC and tuna loins. These results could be explained by the inhibitory effect of lactic acid in the various liquid and solid media. pH values after adding 1% lactic acid in this work varied from 7 to 5. *Escherichia coli* is neutrophilic but able to withstand low pH. Several authors such as [13,14,15,16]; have shown that microbial growth is influenced by physicochemical conditions such as pH.

The results of this study corroborate those of Spyropoulou et al. [17] who showed that at pH values between 4 and 5, the population of *Escherichia coli* was remarkably reduced but not completely inhibited in fermented olives in Spain. Indeed, according to Sutherland et al. [18], *E. coli* strains develop at pH between 4 and 7. Lindqvist and Lindblad [12] have shown that adding 1% lactic acid reduces the growth of *E. coli* in sausages in Sweden.

This work has shown that there is no bacterial growth when 2% and 3% lactic acid is added to the BCC and tuna loins. Indeed, the addition of lactic acid lowers the pH below 4. This very acidic pH prevents the growth of *E. coli* which is not an acidophilic bacterium. The results of this work corroborate those of Cho et al. [19]. Indeed, this author did not observe microbial growth below a pH 4 in "Kimchi" in Korea. Furthermore, Huang and Chen [20] did not observe microbial growth by adding lactic acid 3% in ready-to-eat meals in Japan.

Organic acids (lactic acid, acetic acid) and mineral acids (sulfuric acid, nitric acid) are widely used in the food industry as antimicrobial agents in order to inhibit microbial load and microbial contaminants. Lactic acid is a weak-organic acid, which presents an antimicrobial activity and has been used as antimicrobial agent in foods. LA is produced as the result of the oxidation of glucose to two molecules of pyruvate then pyruvate to lactate. It can spoil food but also can increase the shelf life of foods. It is used in the food industry as an additive (E270) as an antioxidant, acidifier or flavor enhancer. Lactic acid is also present in the form of salts: sodium salt (E325), potassium salt (E326) and calcium (E327). These salts are in powder form and are also soluble in water. The lactic acid provide protection against spoilage by producing natural bacteriocins (substances that kill bacteria). In its Opinion, EFSA concludes that the treatments using lactic acid for decontamination are of no safety concern, provided that the substance used complies with Union specifications for food additives. In addition, EFSA concludes that treatments with lactic acid provide a significant reduction of microbiological contamination compared to no treatment or to treatment with potable water and that it is unlikely that such treatments would contribute to the development of microbial resistance [21].
Fig. 2a. Effect of lactic acid on the growth of strains of *Escherichia coli* ATCC 25922 in tuna loins

Values with the same letter on a curve are not significantly different for $p > 0.05$

Fig. 2b. Effect of lactic acid on the growth of strains of *Escherichia coli* KO 13 in tuna loins

Values with the same letter on a curve are not significantly different for $p > 0.05$

Fig. 2c. Effect of lactic acid on the growth of pathogenic *Escherichia coli* from tuna loins in tuna loins

Values with the same letter on a curve are not significantly different for $p > 0.05$
LA in the undissociated form can penetrate the cytoplasmic membrane, which results in reduced intracellular pH and disruption of the transmembrane proton motive force, which accounts for a significant part of its antibacterial action [22]. LA is also a strong outer membrane disintegrating agent. LA permeabilizes the outer membrane of Gram-negative bacteria, a property that could help other antimicrobials penetrate bacterial cells and produce a toxic effect [22]. In solution, the weak types, such as lactic acid, present twice a year: one dissociated and one not dissociated, the latter being a solution in the plasma membrane of microorganisms. Thus, lactic acid, in its non-dissociated form, crosses a membrane of microbial cells and, upon reaching the cell cytoplasm, undergoes dissociation, deviating the pH close to the neutral point in the intracellular space, resulting in the formation of relationships and anions [23]. The antimicrobial effect of these effects is due to several factors, such as acidification promoted by the volume of H+, or impaired transport of essential elements for microbial development, disruption of...
membrane function and inhibition of essential metabolic reactions, which leads to the death of the micro-organism or the delay of its development (Baird Parker). Al-Holy et al. [24] have showed that LA at 0.2% imparted a bacteriostatic effect on the growth of Cronobacter and in contrast, LA at 0.3% elicited the most pronounced bactericidal effect against Cronobacter in infant formula; LA at 0.2% reduced the bacterial load of Salmonella spp and Escherichia coli 0157:H7 in BHI and carrot juice [25]. LA at 1% and 2% on fresh meat and its derivatives greatly reduced the bacterial load of aerobic mesophilic germs, coliforms and E. coli [26,27,28]. According to Smittle [29], acidity is the most important characteristic for determining the growth and survival of pathogenic bacteria. However, Buchanan and Edelson [13] have shown that organic acids are more effective than mineral acids in inhibiting the growth of E. coli. Lactic acid and acetic acid have been described as the most effective molecules for inhibiting the growth of E. coli. Presser et al. [30] found that lactic acid is able to reduce microbial growth in food. Other authors such as Niksic et al. [31] showed that low pH and high acidity were associated with the reduction of the E. coli population.

5. CONCLUSION

The aim of this work was to study the effect of lactic acid on the growth of pathogenic strains of Escherichia coli (ETEC) isolated from tuna loins. Lactic acid had bacteriostatic effect at low concentrations (1%), and bactericidal effect at higher concentrations (2 and 3%) on pathogenic strains of E. coli. Pathovars of E. coli isolated from tuna loins are a hazard to be considered in the microbiological risk assessment of the consumption of these tuna products. However, the use of more than 1% lactic acid as a preservative could be a solution for the preservation of tuna loins produced in Côte d’Ivoire.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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