Original Research Article

EFFET OF LACTIC ACID ON INACTIVATION OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) ISOLATED FROM TUNA LOINS PRODUCED IN CÔTE D'IVOIRE

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 Agrobusiness (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.
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 / 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. MATERIAL 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.

2.2Inoculum 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.

2.3Kinetics 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 [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 collectedwere 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.

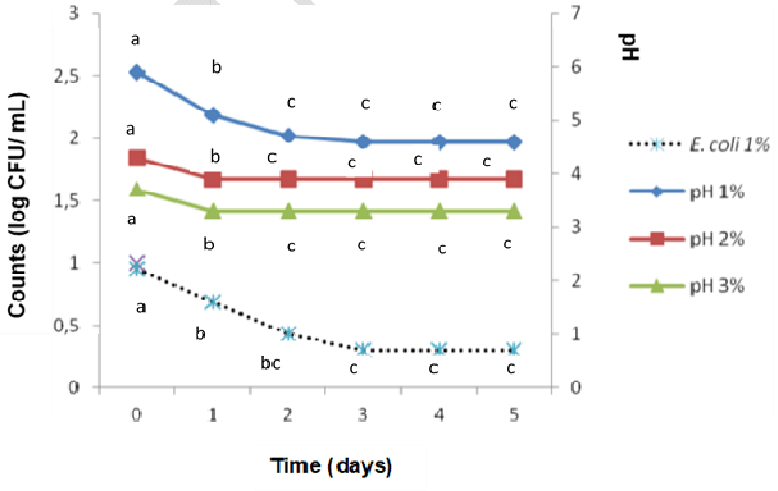

Fig.1a.Effect of lactic acid on the growth of strains of *Escherichia coli* ATCC 25922 in BHI(Values with the same letter on a curve are not significantly different for p>0.05**)**

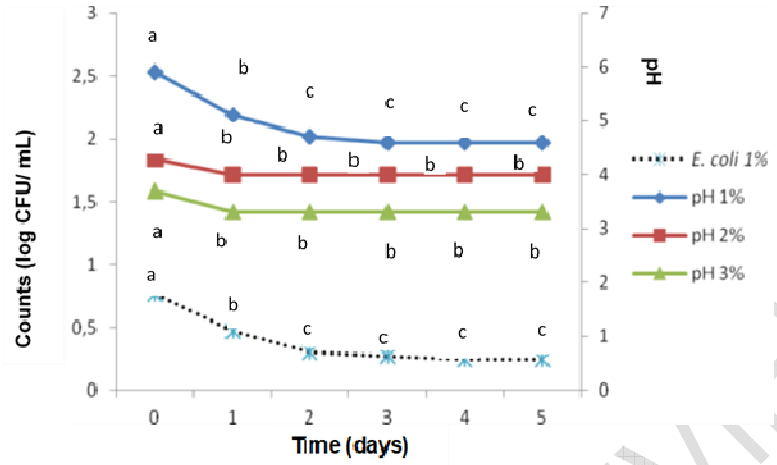

Fig. 1b.Effect of lactic acid on the growth of strains of *Escherichia coli* KO 13 in BHI(Values with the same letter on a curve are not significantly different for p>0.05**)**

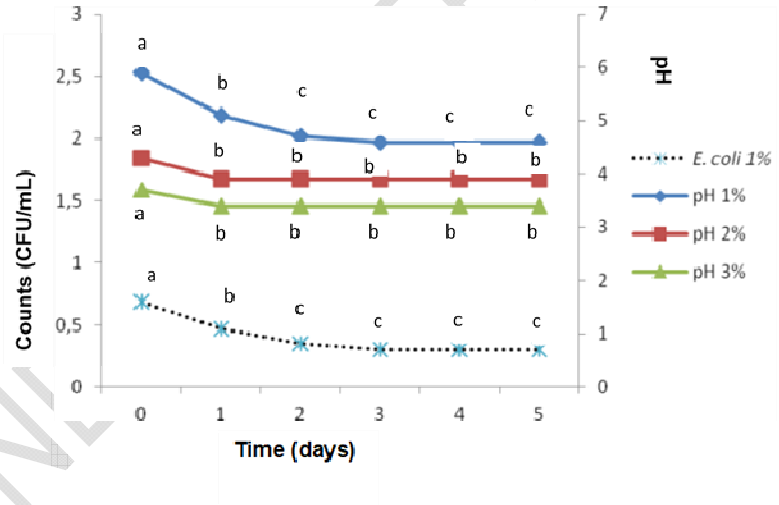

Fig.1c.Effect of lactic acid on the growth of pathogenic *Escherichia coli* from tuna loins in BHI(Values with the same letter on a curve are not significantly different for p>0.05**)**

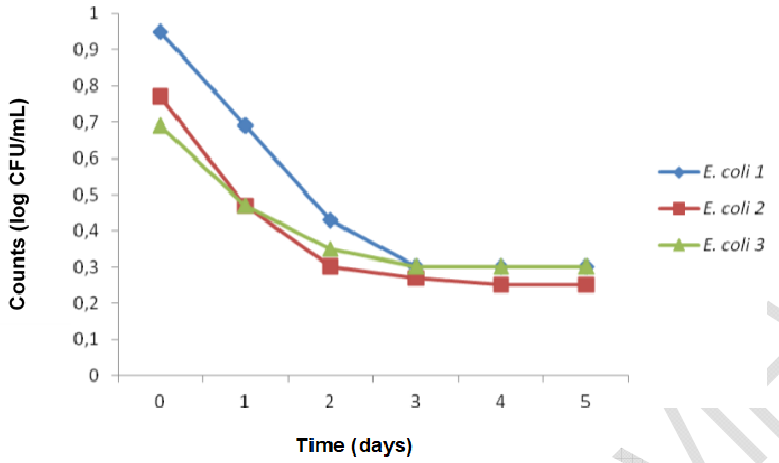

Fig.1d.Comparative evolution of the effect of lactic acid (1%) on the growth of three *Escherichia coli* strains in BHI

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

3.2Effect 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.

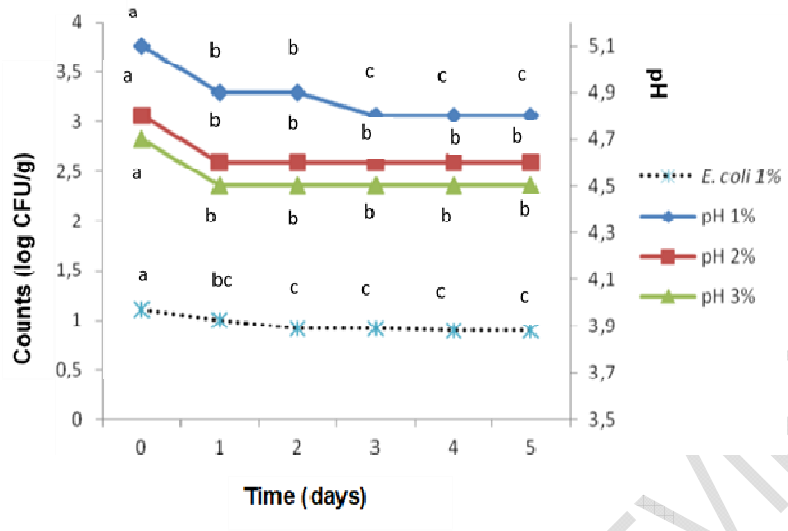

Fig. 2a. Effect of lactic acid on the growth of strains of *Escherichia coli* ATCC 25922 in tuna loins(Values with the same letter on a curve are not significantly different for p>0.05**)**

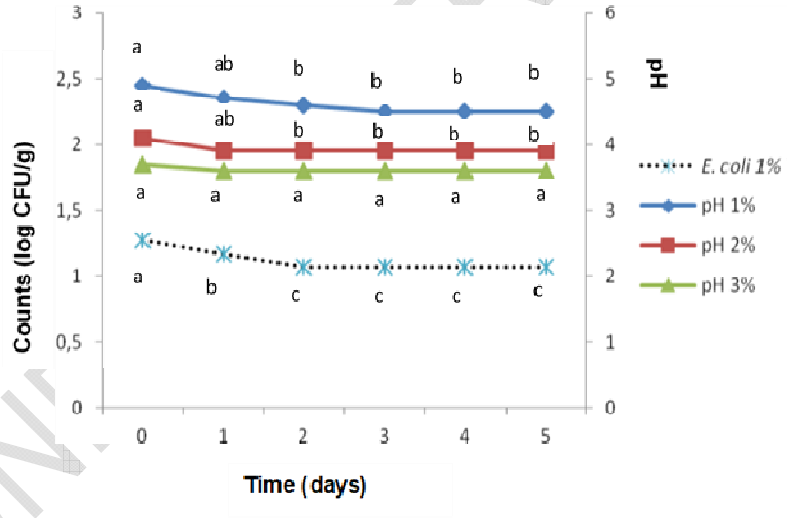

Fig. 2b. Effect of lactic acid on the growth of strains of *Escherichia coli* KO 13 in tuna loins(Values with the same letter on a curve are not significantly different for p>0.05**)**

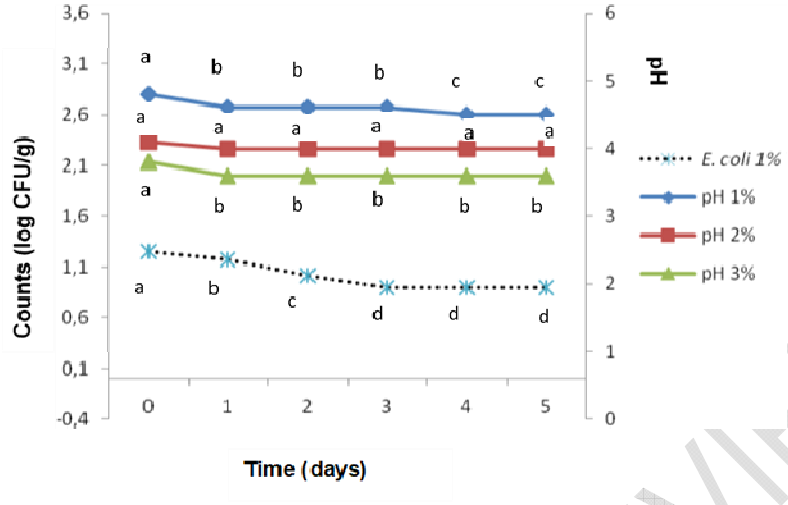

Fig. 2c. Effect of lactic acid on the growth of pathogenic *Escherichia coli* from tuna loins in tuna loins(Values with the same letter on a curve are not significantly different for p>0.05)

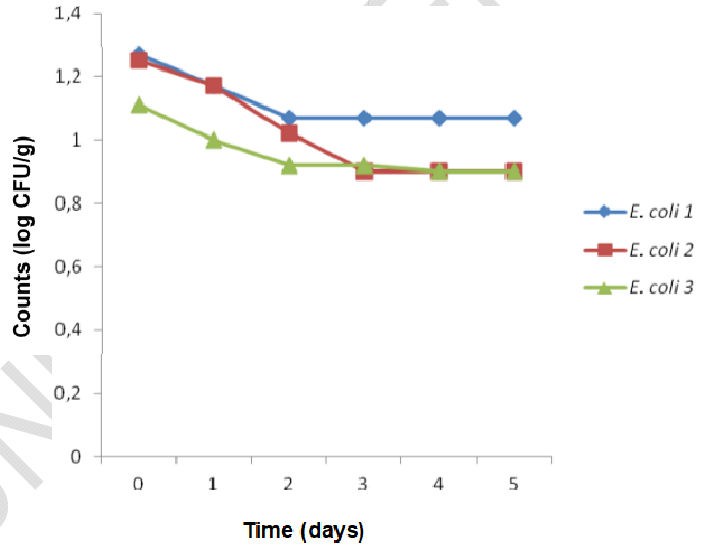

Fig. 2d. Comparative evolution of the effect of lactic acid (1%) on the growth of three *Escherichia coli* strains in tuna loins

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

3.3Comparative 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.

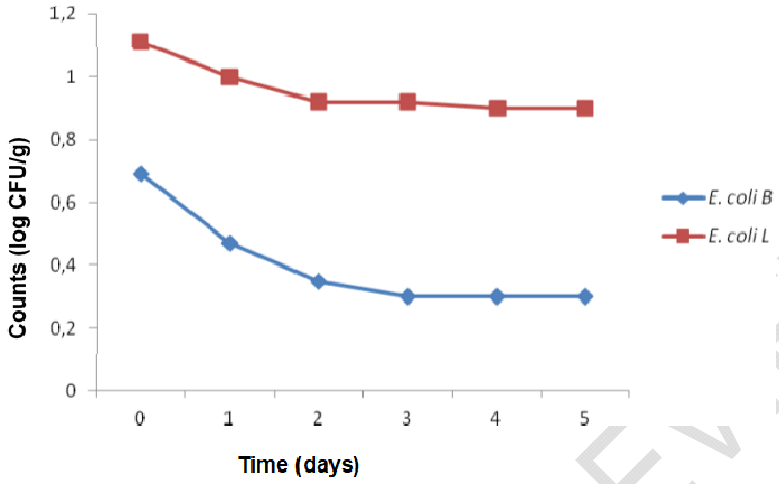

Fig. 3. Effect of lactic acid 1% on the growth of pathogenic strains of *Escherichia coli* from tuna loins in BCC and in tuna loins
E. coli L= E. coli inoculated from tuna loins; E. coli B= E. coli inoculated in liquid medium

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 [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 [18], *E. coli* strains develop at pH between 4 and 7. [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 [19]. Indeed, this author did not observe microbial growth below a pH 4 in "Kimchi" in Korea. Furthermore, [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 antimicrobialactivity and has been used as antimicrobial agent infoods. 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].

LA in the undissociated form can penetrate the cytoplasmic membrane, which results in reduced intracellularpH and disruption of the transmembrane protonmotive force, which accounts for a significant part of itsantibacterial action [22]. LA is also astrong outer membrane disintegrating agent. LA permeabilizesthe outer membrane of Gram-negative bacteria, aproperty 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 a 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). [24]haveshowed that LA at 0.2% imparted a bacteriostatic effect on the growth of Cronobacter andin 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 [29], acidity is the most important characteristic for determining the growth and survival of pathogenic bacteria. However, [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.*[30] found that lactic acid is able to reduce microbial growth in food. Other authors such as [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.

REFERENCES

1. Gözde Ekici and Emek Dümen Escherichia coli and Food Safety, The Universe of *Escherichia coli*, Marjanca Starčič Erjavec, IntechOpen,2019DOI: 10.5772/intechopen.82375. Available from:
2. EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control) The European Union summary report on trends and

sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA Journal 2016;14:4634–4865.
3. Qadri, F., Svennerholm, A., Faruque, A., Sack R.Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clinical Microbiology Reviews,2005b; 18: 465-484.DOI: 10.1128/CMR.18.3.465-483.2005
4. Levine M.*Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. Journal of Infectious Diseases. 1987;155: 377-389.
5. Uçar G, Yörük NG, Güner A.*Escherichia coli* infections. TurkiyeKlinikleri Journals Food HygieneTechnology. 2015;1(3):22-29
6. Zhang W, Sack DA. Currentprogress in developing subunitvaccines against enterotoxigenic*Escherichia coli*-associated diarrhea.Clinical and Vaccine Immunology.2015;22(9):983-991
7. Donnenberg MS. *Escherichia coli* Pathotypes and Principles ofPathogenesis. Baltimore, Maryland,USA: International Encyclopedia ofPublic Health. 2017; pp. 585-593
8. Dutta P., Dutta S. Acute diarrhoea in children. In: Banerjee S., editor. Textbook of Community and Social Paediatrics. 2nd ed. Jaypee Brothers Medical Publishers LTD; New Delhi, India: 2008.
9. B. Dadonaite, H. Ritchie, M. Roser. "Diarrheal diseases". (2020) Published online at OurWorldInData.org. Retrieved from: 'https://ourworldindata.org/diarrheal-diseases' [Online Resource]
10. DPH (Direction of Halieutic Production). Annuaire des statistiques des pêches et de l'aquaculture. Service des études, des statistiques et de la documentation. Document technique. 2009 ;25p.
11. Sika A.E., Kambire O., Zamblé Bi, I. A. B., Aké-Assi Y., Koffi-Nevry R. Virulence Genes and Antibiotic Resistance Profile of *Escherichia coli* Strains Isolated From Tuna Loins and Flakes Produced in Côte d'Ivoire. International Journal of Current Microbiology and Applied Sciences. 2018; 7(09): 3329-3338
12. Lindqvist R., Lindblad M.. Inactivation of *Escherichia coli*, *Listeria monocytogenes* and *Yersinia enterocolitica* in fermented sausages during maturation/storage. International Journal of Food Microbiology. 2009; 129: 59-67.
13. Buchanan R., Edelson S. pH-dependent stationary-phase acid resistance response of enterohemorrhagic *Escherichia coli* in the presence of various acidulants. Journal of Food Protection. 1999; 62:211-218.
14. Juneja V., Marmer B., Eblen B.. Predictive model for the combined effect of temperature, pH, sodium chloride, and sodium pyrophosphate on the heat resistance of *Escherichia coli* O157:H7. Journal of Food Safety. 1999; 19: 147-160.
15. Sanaa M. Microbiologie prévisionnelle : Principaux modèles de croissance utilisés en appréciation quantitative des risques. Epidémiologie et Santé Animale. 2002 ; 41: 169-177.

16. Skandamis P., Stopforth J., Kendall P., Belk K., Scanga J., Smith G., Sofos J. Modeling the effect of inoculum size and acid adaptation on growth/no growth interface of *Escherichia coli* O157:H7. International Journal of Food Microbiology. 2007; 120: 237–249.

17. Spyropoulou K., Chorianopoulos N., Skandamis P., Nychas G. Survival of *Escherichia coli* O157:H7 during the fermentation of Spanish-style green table olives (conservolea variety) supplemented with different carbon sources. International Journal of Food Microbiology. 2001; 66: 3-11.

18. Sutherland J., Bayliss A., Braxton D., Beumont A. Predictive modelling of *Escherichia coli* O157:H7: Inclusion of carbon dioxide as a fourth factor in a pre-existing model. International Journal of Microbiology. 1997; 37: 113–120.

19. Cho G., Lee M., Choi C. Survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during kimchi fermentation supplemented with raw pork meat. Food Control. 2011; 22: 1253-1260

20. Y. Huang, H. Chen. Effect of organic acids, hydrogen peroxide and mild heat on inactivation of *Escherichia coli* O157:H7 on baby spinach. Food Control. 2011; 22: 1178 —1183

21. Official Journal of the European Union. COMMISSION REGULATION (EU) No 101/2013 of 4 February 2013 concerning the use of lactic acid to reduce microbiological surface contamination on bovine carcases. 2013: 3p

22. Alakomi, H.L., Skytta, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., Helander, I.M. Lactic acid permeabilizes Gram-negative bacteria by disrupting the outer membrane. Applied Environmental Microbiology. 2000; 66: 2001–2005.

23. FORSYTHE, SJ. Microbiologia da segurança dos alimentos. 2. ed. São Paulo: Artmed, 2013, 607 p.

24. M.A. Al-Holy, L.F. Castro, H.M. Al-Qadiri. Inactivation of *Cronobacter* spp. (*Enterobacter sakazakii*) in infant formula using lactic acid, copper sulfate and monolaurin. Letters in Applied Microbiology. 2010; 50: 246–251

25. Salam A. Ibrahima, HongYang, Chung W. Seo. Antimicrobial activity of lactic acid and copper on growth of *Salmonella* and *Escherichia coli* O157:H7 in laboratory medium and carrot juice. Food Chemistry. 2008; 109(1): 137-143.

26. Nascimento, EPS. Efeito do ácido lático sobre as características microbiológicas, físico-químicas e sensoriais na carne do sol. 2011. Dissertação (Mestrado em Engenharia Química). Universidade Federal do Rio Grande do Norte, Natal, 2011.

27. Beyaz D; Tayar M. The Effect of Lactic Acid Spray Application on the Microbiological Quality of Sheep Carcasses. Journal of Animal and Veterinary Advances. 2010; 9 (13): 1858-1863

28. K.M.P. Soares, J.B.A. Silva, V.A. Góis. Uso de ácido lático e seu sal sódico em carnes e derivados: uma revisão. Higiene Alimentar. 2017; 31: 67-72

29. Smittle R. Microbiological safety of Mayonnaise, salad dressings and sauces reduced in the United States: a review. Journal of Food Protection. 2000; 63: 1144–1153.

30. Presser K., Ross T., Ratkowsky D. Modelling the growth limits (growth/no growth interface) of Escherichia coli as a function of temperature, pH, lactic acid concentration, and water activity. Applied and Environmental Microbiology. 1999; 64: 1773–1779.

31. Niksic M., Niebuhr S., Dickson J., Mendonca A., Koziczkowski J., Ellingson J.Survival of Listeria monocytogenes and Escherichia coli O157:H7 during Sauerkraut fermentation. Journal of Food Protection. 2005; 68 (7): 1367-1374.