Effect of thermal constraints on enteropathogenic Escherichia coli survival in porridges based on maize (Zea mays) or millet (Pennisetum glaucum) flour traditionally made in Côte d’Ivoire

Enteropathogenic Escherichia coli species are considered as one of the main causes of bacterial gastroenteritis in children. The aim of this study was to determine the survival of enteropathogenic Escherichia coli strains in millet and maize porridges made with traditional flour under the effect of thermal forces. An inoculum of $10^2$ CFU/ml of each strain was obtained by heating in Brain and Heart Infusion. Five strains harboring enteropathogenic Escherichia coli virulence factors were introduced in infant porridges made by these flours. Survival rate was measured under the effects of various thermal forces including 50, 55, 60 and 65°C during 15 minutes. The results reveal that, more than 90% of enteropathogenic Escherichia coli strains survive in the porridges after 15 minutes of treatment at 50°C and 55°C with a rate of 1.2 to 1.75 x log CFU/ml. From 60°C to 65°C, the time required for their thermal death varied from 3 minutes to 6 minutes in the two matrices. The results of the study showed that, the traditional method of porridges cooking temperature for reducing enteropathogenic Escherichia coli infection risk for infant consumers, need an adaptation at least of 60°C.

Key words: Enteropathogenic Escherichia coli, survival, porridge, millet, maize.

ABBREVIATIONS

EPEC: Enteropathogenic Escherichia coli; E. coli: Escherichia coli; Bfp: bundle-forming pilus; AL: localized adherence; Eae: E. coli attaching-effacing factor

INTRODUCTION

According to Monika et al. (2013) cereals belong to the most important food for the majority of mankind. They are a good source of carbohydrates, proteins, lipids, vitamins, and minerals as related by Wrigley (2004); Fletcher (2004); Corsetti and Settanni (2007). Cereals consumed in the form of porridge flours are usually common in developing countries. Preparation and consumption of simple, fermented or enriched porridges with flour from local cereals is alternately used more in rural and urban areas of Africa such as Senegal (Tou, 2007), Burkina Faso (Akaki et al., 2011), Congo (Elenga et al., 2009), Côte d’Ivoire (Soro-Yao et al., 2014), South Africa (Gadaga et al., 2004) and in another country (Trèche, 2002; FAO, 2007; Nout, 2009). Tou (2007) showed that children usually eat cereals at the time of, weaning period during which breast milk is
important to know thousands children per year (Chen and Ivoire and their involvement in infant diarrhea recycled an already prepared porridge and used it again for several days. Moreover, flour is often pre-milled according to local cereals of maize or traditional porridges based on local cereals of maize or millet according a combination of contamination from utensils, hands, infant porridge themselves, storage, inadequate packaging and additives (Olmez and Aran, 2004). One of the pathogenic bacteria widely studied in humans because of their involvement in infantile diarrhea in developing countries is enteropathogenic Escherichia coli (EPEC) (Saito et al., 2005). EPEC are the cause of the death of hundreds of thousands children per year (Chen and Frankel, 2005). They are responsible for severe and persistent diarrhea, especially among children under five (5) years (WHO, 2012). The ability of E. coli strains to survive in food can constitute a favorable circumstance to the explosion of infant infections associated with EPEC.

In Côte d’Ivoire, infant industrial flours are used to feed children; however, some families substitute this one for traditional porridges based on local cereals of maize or millet according Soro-Yao et al., (2013). Porridges became more important in the nutrition of children, but the technology of cooking remains the great problem because it may be source of infections according to the fact that the flour is often pre-boiled, stored and used to feed infants for several days. Moreover, sometimes there is the tendency to recycle an already prepared porridge and used it again for children. In addition, previous studies have revealed the presence of EPEC in certain food products consumed in Côte d’Ivoire and their involvement in infant diarrhea (Dadié et al., 2000; 2010). The risk of contamination of infant food products by EPEC may be a reality in this environment. Although the use of porridges based on local cereals is a good alternative, it is important to know whether the traditional way of heat treatment during the preparation of these porridges which is adapted to inactivate potential EPEC in case of products contamination is adequate enough.

The aim of this study is to determine the survival of enteropathogenic Escherichia coli strains under the effect of thermal forces in order to contributed to the food safety of traditionally manufactured porridges

### MATERIALS AND METHODS

#### Selection of strains

The experimental material comprised three E. coli strains possessing virulence factors identified in a previous study (Dadie et al., 2010; 2014) and two others isolated during this study were used as reference strains in all the tests (Table 1). All of them were isolated in Abidjan, economic capital of Côte d’Ivoire.

#### Preparation of Escherichia coli starter (inoculum)

The cultures were activated by plating on RAPID’ E. coli 2 agar (Bio-Rad, France). A pure colony was inoculated in 25 ml of Brain and Heart Infusion (BHI) (Biokar, France) for 2 h ± 20 minutes at 37°C. After 2 h, 1 ml of BHI was taken every 5 minutes, heated in a water bath at 55°C during 30-40 minutes and then plated on RAPID’E coli 2 agar (Bio-Rad, France). At each interval of 5 minutes, a dual culture of 0.1 ml of the suspension was carried out on Rapid’E coli 2 agar (Bio-Rad, France). This suspension was incubated at 42°C for 24 hours. Enumeration of colonies was performed

#### Table 1. EPEC strain used in this study

<table>
<thead>
<tr>
<th>EPEC strains</th>
<th>Pathotype</th>
<th>Virulence factor</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb26</td>
<td>EPEC</td>
<td>eae, bfp, AL</td>
<td>Dadie et al., 2010</td>
</tr>
<tr>
<td>Bg31</td>
<td>EPEC</td>
<td>eae, bfp, AL</td>
<td></td>
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<tr>
<td>He33</td>
<td></td>
<td>eaeA</td>
<td>Dadie et al., 2014</td>
</tr>
<tr>
<td>He80</td>
<td>EPEC</td>
<td>eaeA-bfpA, AL</td>
<td></td>
</tr>
<tr>
<td>ABmi027</td>
<td>EPEC</td>
<td>eaeA</td>
<td></td>
</tr>
<tr>
<td>Yoma031</td>
<td>EPEC</td>
<td>eaeA-bfpA</td>
<td>isolated in this study</td>
</tr>
</tbody>
</table>

Pb26: EPEC strain isolated in food at Port-bouët
Bg31: EPEC strain isolated in food at Bingerville
He33: EPEC isolated from human diarrheic stool
ABmi027: EPEC isolated from millet porridge
Yoma031: EPEC isolated from maize porridge
Eae: E. coli attaching-effacing factor
Bfp: bundle-forming pilus
AL: localized adherence
**Table 2.** Survival rate of EPEC in matrix at 50°C to 65°C

<table>
<thead>
<tr>
<th>Matrice</th>
<th>Temperature of treatment</th>
<th>Survival rate (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>He33</td>
</tr>
<tr>
<td>Millet porridge</td>
<td>50°C</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>55°C</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>60°C</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>65°C</td>
<td>0</td>
</tr>
<tr>
<td>Maize porridge</td>
<td>50°C</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>55°C</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>60°C</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>65°C</td>
<td>0</td>
</tr>
</tbody>
</table>

Bg31: EPEC strain isolated in food at Bingerville  
He33, He80: EPEC isolated from human diarrheic stool  
ABmi027: EPEC isolated from millet porridge  
YOma031: EPEC isolated from maize porridge

Preparation of matrices

Two models of traditional porridges were prepared to show the evolution of *Escherichia coli* strains. The first was a porridge made from maize flour and the second from millet flour. Indeed, flour, water for cooking and all materials for the experiment were sterilized by autoclaving at 121°C for 15 minutes to make sure of their hygienic quality, according to Clavero and Beuchat (1996). The experiments were performed in aseptic conditions. Flour was previously roasted and sieved through a sieve of 0.250 millimeter to have instantaneous flour. Porridges from instantaneous flour were cooked according to TPA (1998) method. In this case, approximately 500 g of each flour (instantaneous flour of maize or millet) divided into 20 portions (25 g for each) were used. A quantity of 52 g of flour was mixed with water in proportion of 2:1 to make a paste. This paste, is then poured slowly, stirring in 260 ml of water at temperature neighboring 50 or 55°C (under to 15 minutes) and cooked during 5 to 10 minutes, still stirring (Bruyeron et al., 1998).

After cooking, 14 grams of sugar or milk was added to each portion of porridge according to traditional cooking.

Escherichia coli count in matrices

Each porridge sample was inoculated with 25 ml of each inoculum (10^2 CFU/ ml in 25 ml). The porridges were inoculated and maintained at temperatures of 50°C, 55°C, 60°C and 65°C. A quantity of 25 g of porridges samples were taken and mixed in 225 ml of sterile peptone water (Difco) at 0.1% (Clavero and Beuchat, 1996) in a polyethylene bag and mixed with a stomacher 400 (AJSeward, London, UK) for 2 minutes. One (1) ml of sample was taken and added to 9 ml of Trypticase soy broth (TCS) (Bio-Rad, France). Samples were serially diluted with sterile peptone water 0.1% (Difco) and surface plated with 0.1 ml in duplicate on RAPID'E. coli 2 agar (Bio-Rad, France). For temperatures of 50°C to 60°C, 10 g of samples was carried at each 3 minutes during 15 minutes for analyzing. Also for temperature of 65°C, 10 g of samples have been done for analyzing at interval of 1 minute during 5 minutes. For all tests two independent assays were performed for each analyzed. The same procedure was used for the control samples. Petri dishes were incubated at 42°C and colonies were counted after 21 ± 3 hours.

Calculation of D-values and decimal reduction rate (n)

\[ n = \frac{N_t}{N_0} \]  

(Equation 1)

Where n was the rate of decimal reduction, \(N(t)\) was the viable plate count at time t and \(N(0)\) was the initial count before treatment

Statistical Analysis

Different variables, including the number of *E. coli* were compared using ANOVA One Way test. Geometric and log-transformed means were used to calculate bacterial load. The threshold for statistical significance was set at p<0.05.

RESULTS

The Table 2 showed that the rate of thermal death in the temperature range examined was not significantly affected (p> 0.05) by the medium and by the strain. The rate of thermal death was zero in the different matrices after 15 minutes of treatment for the temperatures of 60°C and 65°C. However, it varied from 0 to 1 for temperatures of 50°C and 55°C for the same duration. Gradually, as the temperature increased the survival rate decreased in...
different matrices.

The results showed a higher survival rate of EPEC at temperatures of 50°C. At this temperature, 100% of the strains survived in both porridges made from traditional maize flour and the porridges made from traditional millet flours. In porridges heated at 50°C (Figure 1a, 1b), there were no complete death of the entire cell populations; We noted a reduction of 2 log to 0.9 log CFU/ml in the porridges made from traditional millet flour and 2 log to 0.8 log CFU/ml in the porridges made from traditional maize flour. The effect of heat treatment on the strain was felt after the 3rd minute in the traditional porridge made from millet flour (Figure 1a) but at the 6th minute in the porridges made from traditional maize flour (Figure 1b).
But all strains exhibited the same behavior at the 15th minute.

At 55°C (Figure 2a, 2b), 3 *E. coli* cells, He33, Yoma031 and Bg31 in millet porridge and 2 EPEC cells, He80 and Bg31 in maize porridges underwent reduction at 15 minutes of heating (reduction by 2 log units), whereas *E. coli* ABmi027 and *E. coli* He80 in the millet porridge (reduction respectively by 1.1 x log and 1.7 x log units) (Figure 2a), *E. coli* He33, Yoma031 and ABmi027 strains survived (reduction by approx. 5.5 x log units) (Figure 2b). These results indicated that EPEC strains no ABmi027 was more resistant to heating than others *E. coli* at temperatures fewer than 60°C.

At 60°C (Figure 3) all *E. coli* cells are reduced logarithmically in any porridge after 12 minutes of heating (reduction by 2 log units). In millet porridge, the number of cell reduction in entire population cell took place after 9 minutes (*E. coli* He80, *E. coli* ABmi027, *E. coli* Yoma031 and *E. coli* Bg31) or 12 minutes (*E. coli* He33) (Figure 3a). While, in maize porridges, cell reduction of the entire
population took place after 6 minutes (E. coli Yoma031), 9 minutes (E. coli He80, E. coli ABmi027 and E. coli Bg31) or 12 minutes (E. coli He33) (Figure 3b) at 60°C. The cells reduction of the entire population took place after 2 minutes in both millet and maize porridges at 65°C (Figure 4a, 4b). Cells were reduced logarithmically at 2 log to a reduction < 0.5 log. After 3 minutes, any viable cell in both millet and maize porridge. These results indicated that EPEC strains were more resistant to heating in millet porridge than in the maize porridge.

**DISCUSSION**

Data in the range of 50-65°C showed that the temperature inferior at 60°C were not sufficient to reduce all E. coli strains such as shown by many authors who had studied the thermal destruction of E. coli in foods and in culture,
Death cells were found in the majority of samples with no live cells capable of forming colonies on Rapid’E. coli 2 agar (< 1 CFU/mL). The presence of survival cells was confirmed only when porridges were treated at 50°C to 55°C during 15 minutes. It suggested that E. coli strains are able to resist and growth above optimum conditions. Kaur et al., (1997); Mercer et al., (2015) had been reported that E. coli has harboring several factors which might increase its heat resistance.

The results obtained at 50°C-55°C suggest that temperatures less than 60°C are not able to eliminate EPEC in the matrices. The behaviors of strains were the same at 50°C in both matrices. It should depend of the initial EPEC bacteria (10^2 CFU/g), the density of the porridge and its texture (Guiraud, 1998). However the behavior of strains at 15 minutes would mean that time over of 15 minutes is necessary to destroy strain even if many authors had found a least time (Buchanan and Edelson, 1999; Yang and Chou, 2000; Hara-Kudo et al., 2005; Charimba et al., 2010). At this moment, it is critical to study how this bacteria could be eliminate safely at processing conditions.
time, the temperature had an effect on the strains. That why after 15 minutes there were more deaths cells than survivals cells. Our study showed some difference between others. These differences of results could be explained by effect of heating method. Stringer et al. (2000) showed that to obtain good thermal inactivation data it is important to use a method of heat treatment that avoids local temperature variations, because a subpopulation of cells subjected to a lesser heat treatment will appear to be more heat resistant and tailing will occur. Much of the observed variation is due to differences in test conditions and experimental procedures. The effect of factors such as adverse pH, antimicrobials and the use of different enumeration media has been widely tested.

Some strain such as E. coli ABmi027 showed a resistance at 55°C, many strains also have shown resistance. It might be explained by the fact that it was isolated in porridge previously before using for this experiment. Otherwise, the presence of sugar in the sample had an effect on EPEC behaviors in matrices as showed by (Chu et al., 2001). In fact, there are several ways in which sugar inhibit microbial growth. The most notable is simple osmosis, or dehydration. Sugar, whether in solid or aqueous form, attempts to reach equilibrium with the sugar content of the food product with which it is in contact. This has the effect of drawing available water from within the food to the outside and inserting sugar molecules into the food interior. The result is a reduction of the so-called product water activity (aw), a measure of unbound, free water molecules in the food that is necessary for microbial survival and growth.

Estimated of the infectious dose of EPEC for infant are unknown (Garcia, 2001). Consequently it is vital to eliminate this pathogen from food, rather than merely to prevent its growth. Heat treatment is the method of bacterial destruction most frequently used in food processing. Accurate information on thermal death rates is important to food processors in order to achieve the desired safety margins whilst avoiding over-processing. In this study temperature of 50°C and 55°C were not sufficient to ensure infant protection following to the traditional method of cooking temperature. Also, according to Partnership and Technology in Agro-food (TPA, 1998) it must have less than 10 Escherichia coli in flour to cook and less than 2 in instantaneous flour. Elsewhere, the national laboratory of health (LNS-CDA, 2007) suggest that more than 10 E. coli in flour is a problem of hygiene and represent a risk of infection for children who consume it. In accordance with criteria indications referred to, we can deduce from our results that a thermal treatment mode limited at 50°C or at 55°C from a preparation time not up to 15 minutes, could permit a situation to have porridge with a rate of E. coli sufficiently high to cause infections to children. What's more, most of normative values relate to common E. coli were used to appreciate food quality, however any EPEC strains should not be in infant food ready to eat because children are population at risk. The risk of EPEC infection to children who eat millet and maize porridge could be high in our environment. This risk is also linked to the possibility of contamination of these products and their mode of preparation. The possibility of contamination of these products exists because of previous works done in the same environment which have revealed the failing hygiene of many foods products in informal sector (Dadie et al., 2000; 2010). These authors have shown the presence of coliforms, E. coli and have detected EPEC in both of foods and infectious diarrhea.

Dead cells were found in the porridges which contain no live cells capable of forming colonies on Rapid'E. coli 2 agar (< 1 CFU/mL) immediately after heating at 60 and 65°C.

The temperature of 65°C could be considered as a security temperature during 10 minutes of traditional food preparation. The decimal reduction time is ≤ 8 minutes and the survival rate is ≥ 2. At this temperature all the strains are destroyed. Infant foods are safe and can’t be contaminated. This temperature is death temperature for all strains in the matrices.

The populations that survived heating at 60 and 65°C temperatures were dominated by damaged cells, unable to grow on selective agar. A similar phenomenon was observed by Yang and Chou, (2000); Hara-Kudo et al., (2005); Charimba et al., (2010).

Peng et al. (2012) had showed the thermal effect at 60 to 67.5°C with a strain reduction in milk at a time inferior at our time. The difference of their result with ours could be related to the texture of the matrices and the time applied. In other hand this observation showed that some E. coli strains could survive to treatment of 60 or 65°C.

The decimal reduction time D_{65°C} and the survival rate (n) are respectively less at 3 minutes and 6 minutes. Even if the time is different to other studies, temperatures of 60°C and 65°C are pasteurizing temperatures and are better for the treatment of infant foods. Several studies such as those led by Charimba et al., (2010) increased the temperature further and found that the strain E. coli undergoes heat inactivation for, 80 seconds at 65°C and 60 seconds at 70°C. Considering the results of studies of these authors, the temperature of 60°C and 65°C which were found in our results are the ideal temperatures for E. coli strains destruction in porridges. Usajewicz and Nalep (2006) also working on the survival of E. coli in milk at high temperatures showed that strains were progressively destroyed when the temperature was increased.

However, the exponential law in this study is in accordance with that described by Guiraud (1998). It means that, when the number of survivors decreased the time of treatment increased. This law depends on the quantity of the initial inoculums of the strains. Indeed, the effectiveness of treatment was even greater (shorter time) than the initial load in EPEC is small. This is consistent with our results for temperatures of 55°C, 60°C and 65°C for 15 minutes.

The results from different behaviors of strain in this study guide on the fact that, the parameters such us temperature (of treatment) and contact time when preparing food were determining for growth or death of E.
coli and consequently, the risk of infection when EPEC contaminate food.

The study showed that EPEC strains are more resistant to heating in millet porridge than in the maize porridge. According to the characteristics of nutritional composition of millet and maize, maize porridge could overheat quickly than millet. Several studies have shown that traditional porridges were poor in nutrients. The density of these must be supply by sugar, salt or others (Treche, 2002). Furthermore, other studies have shown that millets gruels had very low energy density, even after addition of sugar, and low lipid, protein and mineral contents, well below recommendations for complementary foods (Mouquet-Rivier et al., 2008).

Olsen and Nottingham (1980) added that the measured thermal resistance of a species can be influenced by many factors, including the growth conditions, such as the growth phase of the cells, composition, pH and water activity of the growth medium, growth temperature, holding period before heat treatment, heat shock, the heating method, for example use of open heating system and rate of heating, the heating menstruum, including its composition, pH, water activity and the recovery conditions. Heat resistance may also be affected by physical interactions such as attachment to solid surfaces.

The increasing of temperature inactivated and damaged cells. There are no anabolisms and catabolisms reactions, so all protein molecules with metabolic activity are denatured. The enzymes involved in the expression of its genome are inactivated; it died because these phenomena are often irreversible (Guiraud, 1998).

The Table 1 showed that typical EPEC (with bfp gene) and atypical EPEC (without bfp) were used for the study. The destruction of EPEC was not significantly related to the type of strain. In both millet porridge as in maize porridge, destruction of the strains are identical and linked to the increase in temperature.

The above data indicate that Gram-negative bacteria, considered sensitive to high temperatures, do not always undergo complete inactivation during heating at temperature < 60°C during 15 minutes, times used to prepare infant food following traditional method. To avoid risk of contamination, it is better to apply hygiene rules and to prepare infant food with temperatures up to 60°C under high pressure.

ACKNOWLEDGMENTS

We sincerely thank the sellers of traditional flours, the management team and staff of laboratory of Pathology and Molecular Biology at the CNRA for their support during the study.

Conflict of interests

The authors have declared that there is no conflict of interest concerning this manuscript.

REFERENCES


