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Influence of Sublethal Temperatures on Some Spore-forming and Vegetative Foodborne Bacteria and Impact on Hygienic Quality of the "Foléré" (*Hibiscus sabdariffa*) Beverage

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Authors' contributions

This work was carried out in collaboration among all authors. Author JRB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RDD and FEE managed the analyses and interpretation of data from the study. Authors RDD and FXE managed the literature searches, read and corrected the first draft of the article. All authors read and approved the final manuscript.

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ABSTRACT

Aims: "Foléré" beverage is a refreshing locally made drink much appreciated by the populations of the hottest parts of Cameroon. This paper aims at investigating microbial contamination of "Foléré" beverage and highlights impact of the sublethal temperatures on the hygienic quality the beverage.
Study Design: Design used for describing physicochemical and microbial profile is a random sampling and for impact of sublethal temperature on hygienic quality, we used food matrix simulations.

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Place and Duration of Study: Samples: Local markets, between August 2018 and June 2019.
Methodology: The sample pH and soluble solids content were recorded using portables devices. Microbial analysis focused on total aerobic mesophilic bacteria, *Escherichia coli*, and spore-forming bacteria were determined according to accredited culture methods. Acidic "Foléré" beverage produced by an artisanal processing was sterilized and inoculated by *Bacillus cereus* T spores and *Escherichia coli* ATCC 25922 cells thermally stressed by pre-incubation at 10°C, 45°C, 50°C or 60°C for 45 min, 90 min and 180 min. The recovery method was used to determine acid pH resistance of both bacteria before and after sublethal thermal processing.
Results: "Foléré" beverage is of poor hygienic quality according to standards, despite its very low pH (2.01). Beverages at pH 2.01 and inoculated by each one of referenced bacteria indicated that the samples which contained thermally stressed bacteria were worse hygienic quality than the same ones which contaminated with unstressed bacteria. Some of recovery percentages of bacteria thermally stressed were significantly higher (P =.05) than those of same bacteria thermally unstressed after acid treatments. That phenomenon was called thermal-induced bacterial acid resistance.
Conclusion: it appeared that some sublethal cold and heat shock treatments (10°C/45 min, 45°C/45 min, 50°C/90 min and 180 min) could negatively affect food quality. So, the control of emergence and evolution of stress-resistant bacteria in food could help to improve food safety.

Keywords: Handicraft beverages; sublethal temperatures; cross protection; quality; Foléré.

1. INTRODUCTION

Beverages are food-grade liquids mainly processed from animal or plant resources which play a predominant role in the diet of African people. Depending on the processing steps, beverages are classified as either alcoholic or non-alcoholic. Amongst the non-alcoholic beverages produced in Cameroon, "Foléré" also named "oyoro" is one of the most popular refreshing homemade drinks, highly consumed in this country [1]. The "Foléré" beverage is an aqueous nectar from dried calyces of roselle (*Hibiscus sabdariffa* L), a plant from the family of *Malvaceae*. It is an annual bushy branched herb found in tropical and semi-tropical regions of the world mainly in West Africa and the East Indies [2]. This plant can grow up to 3.5 metres high, and presents a cylindrical stem, tap root system and a green to red coloured leaves [3]. Therefore, two botanical varieties are recognised, the calyces of the red variety which are mostly used in making drink as "Foléré" and soup, while the calyces of the green one are used to cook soup, stew, and sauces [4]. In most Cameroonian cities, the sales and consumption of the "Foléré" beverage are important due to the high cost of other non-alcoholic manufactured drinks. The non-alcoholic nature of this handicraft beverage makes it to be readily consumed by both Muslims and Christian alike as a substitute for alcoholic ones. "Foléré" is most especially served in plastic bottles or polyethylene films once it is cool or in glasses for home-brewed ones. The beverage is the source of income for the producers and it is usually sold in the motor parks, school premises, market places and even during social gatherings.

"Foléré" beverage is sometimes considered by most consumers as being medicinal. According to the population, frequent consumption of this beverage helps to increase blood rate, prevent and cure many diseases. Indeed, several studies have shown that roselle (*H. sabdariffa*) possesses some positive effects on human health such as prevention and management of anaemia, liver and cardiovascular diseases, malaria, high blood pressure, inflammatory skin disorders, most notably eczema [5-7] and remarkable antimicrobial activity against some pathogens [8,9]. Despite these beneficial effects of roselle to humans, *H. sabdariffa* base-beverages remain nevertheless highly contaminated, and a source of foodborne pathogens and food spoilage bacteria [10,11]. Consequently, the beverage of this kind has a short shelf-life (just one or two days at most) and potentially a source of illness. Some researchers have reported that the consumption of beverages related to "Foléré" drink contaminated by microorganisms caused enteric fever, gastrointestinal tract diseases, diarrhoea, bacillary dysentery, and food poisoning [10]. Several studies conducted so far in similar drinks to "Foléré" beverage like "zobo" drink have shown the presence of many bacteria and fungi [10]. Amongst these bacteria, we have spore-forming bacteria such as *Bacillus cereus* and vegetative cells such as *E. coli* which represent

one of the major cause of foodborne poisoning and health concern in many African countries. Moreover, due to their ubiquitous characters and resistances, bacterial spores as those of *B. cereus*, can easily contaminate, persist and spoil food. Despite the acidic character (very sour taste) of these handicraft base-beverages, the poor hygienic nature result from the cross-contamination of used water, raw and packaging materials and the rich phytonutrients and minerals [1,12]. Indeed, acidic environment is often quite effective in controlling microbial growth and largely used to prevent contamination in food [13] but these acid taste beverages are very prone to contamination and sometimes a good room for proliferation of microorganism.

Environmental stresses (heating, chilling, acidity, storage) are known to induce response within the bacterial cell. According to the general principle of stress adaptation of Hill et al. [14], bacteria that are withstand to a sublethal stress often become more resistant to the subsequent applications of the same stress (homologous resistance) or to other unrelated stresses (cross-protection). Amongst these, we have the sublethal temperatures that can expose spoilage bacteria and pathogens to similar conditions of cold shock or heat shock. These temperatures which occurred during processing, storage or cooling are able to increase heat resistance and/or acid resistance which results in greater survival of *E. coli* in some commercial manufactured food products [15,16]. The same observations have been done with bacterial spores in experimental conditions but these observations have not yet been extended in food [17-19]. So far, no study has yet reported about the role of sublethal temperatures on the ability of spore-forming and vegetative foodborne pathogens to persist in some locally made African soft and refreshing acid taste drinks as "Foléré" beverage. At the time that the adaptation to environmental stresses have received recent attention because of its implications in the food safety, the purpose of this work was to evaluate the effect of sublethal temperatures that may be encountered in food processing, storage and chilling environments on *B. cereus* spores and *E. coli* cells into the locally made "Foléré" beverage in order to check the key role of that specific sublethal stress on the hygienic quality of this valuable refreshing drink. Furthermore, microbiological and physicochemical properties of the beverage samples collected in the study field were also explored.

2. MATERIALS AND METHODS

2.1 Collection of Beverage Samples and Plant Material

A Hundred and ninety-five samples were randomly collected from sellers of popular markets in far north region of Cameroon. Sampling lasted for ten months, with each market sampled four times on different dates. The samples were collected using capped sterile bottles and transferred to the Microbiology Laboratory for analysis. Dried calyces of roselle (*H. sabdariffa*) used for production of the test beverage samples requisite for simulations of the behaviour of bacteria on acid pH drinks after sublethal thermal treatments were purchased from local markets and taken to the laboratory in sterile cellophane. Dried calyces were subsequently verified, and the test beverage samples were prepared by maintaining the appropriate hygienic standards.

2.2 Physicochemical Analysis

Soluble solids content (°Brix) and pH of "Foléré" beverage were measured by a standardized portable refractometer (RHW-25ATC) calibrated at 20°C and a pH-meter (EQUIP-TRONIC EQ-610) respectively [20]. The pH meter has been pre-calibrated using commercial test solutions (PALINTEST©). The total sugar content was determined according to the refractometric method as describe by AOAC [21] converting the refractive index at 20°C from a reference table. The total titratable acidity (expressed as percentage of malic acid) was evaluated by the alkali-potentiometric method using a 0.1 N sodium hydroxide solution [21]. All measurements were performed three times for each sample.

2.3 Microbiological Analysis

The "Foléré" beverage samples were serially diluted in sterile distilled physiological water (NaCl 8.5‰) and appropriate dilutions were placed on nutrient agar supplemented with 0.5% cycloheximide, potato dextrose agar (PDA) added with 0.05% chloramphenicol and adjusted at pH 5.2, bubble lactose bile with brilliant green (BLBVB-Difco), eosin methylene blue (EMB) agar + Kovac's reagent, and bromocresol purple glucose agar, for respectively counts of total aerobic bacteria, total fungi, Coliform, *E. coli* and spore-forming bacteria after 10 minutes

preheating at 100°C [22]. The nutrient agar, PDA, eosin methylene blue agar and glucose agar plates were incubated respectively at 30°C for 48h, 25°C for 72h, 37°C for 24 h, 44°C for 48h, 35°C and 55°C for 48 h.

2.4 Artisanal Processing of "Foléré" Beverage

The "Foléré" beverage was prepared using the results of the survey carried out on sixty-five producers distributed in the main town of the country. Dried calyces of *H. sabdariffa* were manually cleaned by handpicking stones and other unwanted debris. They were then thoroughly washed separately using sterile deionized water. Fifty (50) grams of already cleaned calyces of *H. sabdariffa* was added to 500 ml hot boiling distilled water and was left to stand for 15-45 min to extract the nectar. The hot, red-coloured aqueous nectar was filtered using a sieve or cotton wool into a plastic bowl and tightly covered. After cooling, 30 g of grounded sugar was added to the red filtrate obtained as described above and homogenized to make a complete "Foléré" beverage [23].

2.5 Microorganism and Spore Production

Two bacterial genera were used to the testing effect of sublethal temperatures on acidic conditions of "Foléré" beverage: *E. coli* O157: H7 obtained from ATCC reference strains collection and *B. cereus* T originating from the collection of the Microbiology Laboratory, Institute of Food Research of Reading, UK. For spore production, an active culture 100°C preheated for 10 min was introduced in the freshly prepared thioglycolate broth during 24 h at 35°C, and sporulation was carried out in the fortified nutrient agar as previously described [22]. After the spore crop was washed twice and re-suspended in sterile distilled water. The spore suspensions were stored at 4°C for 3 months to ensure their stability before use.

2.6 Sublethal Thermal Treatments

Using the method describe by Bayoï et al. [22], the thermal treatments are performed at 10°C, 45°C, and 50°C for *E. coli* vegetative cells, and at 10°C, 45°C, 50°C and 60°C for *B. cereus* spores suspension, using a cooling system (SUPERSER) and a water bath equipped with a stirring and circulation system (MEMMERT). For each temperature, submission time was 45, 90 and 180 min. A volume of 10 ml of cells suspension ($3.2x10^9$ cells/ml) was filled in a pyrex tube covered with a cap and then introduced into the cooling system or a water bath previously set.

2.7 Simulation of Behaviour of Microorganisms in Acid "Foléré" Beverage

To perform this simulation, the adapted method described earlier by Bayoï et al. [22] was used. Firstly, fifty microliters of thermally unstressed bacteria ($3.2x10^9$ cells/ml) of each bacteria was used to contaminate 4.65 ml of pasteurized "Foléré" beverage stabilized at the mean pH 2.01 of sampled drink (pH < 4.5). Then, homogenize acid mixture preparation was neutralized with 0.3 ml of 0.1N NaOH solution after 45, 90 and 180 min of incubation at laboratory temperature. Secondly, fifty microliters of thermally stressed bacteria (10, 45, 50 and 60°C) was then used to contaminate another group of 4.65 ml of pasteurized "Foléré" samples, and the set was put at the same conditions as above. One hundred microliters of appropriate decimal dilution of each contaminated preparation were spread on Eosin Methylene Blue (EMB) agar + Kovac's reagent, and bromocresol purple glucose agar respectively for *E. coli* cells and *B. cereus* heat-activated spores. The plates were incubated for 24 h at the optimal growth temperature of each species. Each experiment was performed three times and the seeding is done in triplicate. The number of colony-forming units (cfu) were counted and expressed in terms of recovery percentage using the formula below:

Recovery percentage (%) = (number of cfu from contaminated acidic samples/number of cfu from contaminated control sample) x 100

2.8 Statistical Analysis

The results of physicochemical parameters, microbial counts and acid pH resistance by recovery percentage were expressed in terms of mean ± standard deviation. The comparison of means was made using ANOVA (analysis of variance) one-way with the STATGRAPHICS software Centurion version 16.1 for Windows. When analysis of variance revealed a significant effect (P =.05), the data means were compared by Tukey's multiple HSD comparison test.

3. RESULTS

3.1 Physicochemical Parameters of "Foléré" Beverage

Table 1 shows the physicochemical profile of the "Foléré" samples. We noticed a mean pH value of 2.01, this gives the samples analyzed, like beverages with good quality relative to pH and according to CODEX-STAN 243. According to the same table, the drink samples showed an average total acidity value from 1.34% which can be considered as too low. All the samples recorded different soluble solids content whose mean value was 8.27 °B and the sugar content average was 143.7 g/l.

3.2 Microbiology of "Foléré" Beverage

Table 2 shows the microbiological profile of "Foléré" beverage samples sold in the local markets of the far-north region, Cameroon. The results revealed average counts of $4.2x10^6$ cfu/ml and $5.1x10^5$ cfu/ml of aerobic plate count (APC) and total fungi (yeast and moulds) respectively. This shows high levels of contamination which are beyond the permissible limits for liquids ready for consumption, based on the APC (10^5 cfu/ml) and fungi (10^4 cfu/ml) from Food Quality Check Programme Microbiological Recommendations [24]. The total coliforms, thermo-tolerant coliforms and *E. coli* counts showed respectively mean values of $1.53x10^4$ cfu/ml, $1.3x10^4$ cfu/ml and $7.2x10^3$ cfu/ml above the acceptability limits. Total aerobic spore-forming bacteria counts are very beyond the permissive limits ($<10^3$ cfu/ml) with respective values of $2x10^5$ and $3.7x10^6$ cfu/ml for thermophilic and mesophilic bacteria.

3.3 Behaviour of Microorganism in Acid "Foléré" Beverage before and after Sublethal Treatment

Fig. 1 showed the effect of "folére" beverage pH 2.01 on thermally unstressed *B. cereus* spores and *E. coli* cells. We found that "Foléré" beverage at pH 2.01 is significantly effective (P = .05) both on spore-forming bacteria (*B. cereus*) and vegetative bacteria (*E. coli*), and this from 45 minutes of treatment with the acidic beverage. At that time, we obtained with reference to control the reduction of recovery percentage to 57% and 20.5% respectively for unstressed *B.cereus* spores and *E. coli* cells. The decreasing of recovery continued to 45% and 9% at 180 minutes with the same unstressed bacteria. These results clearly showed that the acidic beverage pH 2.01 is more significantly effective (P = .05) on *E. coli* cells compared to *B. cereus* spores. Indeed, spore-forming bacteria are more resistant to physical and chemical agents than vegetative cells.

The histograms of Fig. 2 show recovery of colonies from thermally unstressed (TA45, TA90 or TA180) and stressed (10°C, 45°C, 50°C, and 60°C) *B. cereus* spores introduced and left into "Foléré" beverage pH 2.01 during 45 min, 90 min and 180 min. We found that the recovery percentages obtained from *B. cereus* spores thermally stressed at 10°C for 45 min (91.7%, 83%, 73%) and 180 min (76.8%, 61%), 45°C for 180 min (61%, 65.5%), 50°C for 45 min (79%, 72%, 74%), 90 min (91%, 71%) and 180 min (94.8%, 91%, 87%), 60°C for 45 min (69%, 74%, 56.5%), before their introduction into the pasteurized "Foléré" drink were significantly

Table 1. Physicochemical profile of "Foléré" beverage samples sold in the local markets

pH	Total titratable acidity (%)	Soluble solids (°B)	Total sugar (g/l)
2.01 ± 0.14*	1.34 ± 0.07	8.27 ± 0.15	147.3 ± 7.2

**: This mean value was used to stabilize the pH value of the laboratory produced beverages samples. Values are presented as mean ± standard deviation*

Table 2. Microbiological profile of "Foléré" beverage samples sold in the local markets

Flora	Load (cfu/ml)	Norms
Total aerobic plate count	$(4.2 \pm 0.7)\ 10^6$	$< 10^5$
Total fungi	$(5.1 \pm 0.6)\ 10^5$	$< 10^4$
Total coliforms	$(1.53 \pm 0.02)\ 10^4$	0
Thermo-tolerant coliforms	$(1.3 \pm 0.2)\ 10^4$	0
E. coli	$(7.2 \pm 1.3)\ 10^3$	0
Aerobic mesophilic spore-forming bacteria	$(3.7 \pm 0.08)\ 10^6$	$< 10^3$
Aerobic thermophilic spore-forming bacteria	$(2.0 \pm 0.1)\ 10^5$	$< 10^3$

Values are presented as mean ± standard deviation

higher (*P* =.05) than the recovery percentages of colonies from spores thermally unstressed and subjected to the action of the same acidic beverage for 45 min (57%), 90 min (50%) and 180 min (45%). The histograms of figure 3 present recovery of colonies from thermally unstressed (TA45, TA90 or TA180) and stressed (10°C, 45°C and 50°C) *E. coli* cells put into pasteurized acidic "Foléré" beverage for 45, 90 and 180 minutes at pH 2.01. We observed that recovery percentages of colonies obtained from thermally stressed *E. coli* cells at 10°C for 45 min (32.5%, 20.1%), 45°C for 90 min (25.5%, 34%) and 180 min (16.2%, 20.2%) and 50°C for 90 min (28.1%, 36.3%) prior bring to the pasteurized acidic beverages samples were significantly higher (*P* =.05) than recovery percentages of colonies obtained from thermally unstressed *E. coli* subjected to the action of the same acid beverage pH 2.01 during 45 min (20.5%), 90 min (12%) and 180 min (9%). We also noticed that recovery percentages of two bacteria species automatically decreased when the sublethal thermal pre-incubation at 10°C were performed at least for 90 min. Which means that low temperatures are more effective on raising of recovery percentages of bacteria in acid conditions for the short-time pre-incubations.

4. DISCUSSION

The average pH value of 2.01 of our beverage samples is somewhat similar to that obtained by Agassounon et al. [20] in Benin on a similar juice. Indeed, these authors revealed that the pH of the samples of similar beverage submitted for analysis ranged from 2.41 ± 1.1 to 4.08 ± 0.15. The same findings were observed with other similar local drinks [7,10]. The low pH value of the beverages was due to the acidic nature of dried roselle calyces used in the production of the beverage [7,25]. This plant is characterized as highly acidic plant rich in organic acids like oxalic, tartaric, malic and succinic acids [26]. The low values of pH can be correlated to the low total acidity values of the beverages samples. With similar drinks, Bolade et al. [25] obtained total acidity values ranging between 1.5-2.3% which are very close to total acidity mean value of "Foléré" beverage (1.34%). The variation of total soluble solids and sugar content can be explained by the quantity of sugar added to reduce the sour taste of the roselle calyces nectar. The addition of the sucrose sugar depended on the vendor's preference as there is no yet stated and controlled recipe for the production of the "Foléré" beverage [7].

The high level of yeast and moulds can be explained by their ability to grow in an acidic environment and the rich nutrient composition milieu like roselle drink [27] and high water activity in this beverage. The origin of fungi can be associated with *Hibiscus* calyces. Adebayo and Samuel [28] showed that *Aspergillus, Fusarium, Penicillium* and *Rhizopus* were the main fungal genera associated with dried *Hibiscus sabdariffa* calyces. Indeed, in the open market, *Hibiscus sabdariffa* calyces are displayed in large bowls and polyethylene bags for prospective consumers, and in the process, exposed to microbial contamination [12]. We noticed remarkable loads of Coliforms and *E. coli* in our "Foléré" beverage samples beyond the permissible limits which probably confirm a highly contamination of beverage. These microorganisms are faecal indicators, and their presence in the "Foléré" beverage indicates the presence of faecal or sewage contaminants introduced into the food by the use of contaminated water or contamination from the unsanitary environment and equipment or via human handler or operators [29]. Their presence also provides evidence of the very poor hygienic quality of this homemade beverage. The sampled "Foléré" beverage indicated a quite important aerobic spore-forming bacteria which can be from the *Bacillus* genus [11,12]. Agbobatinkpo et al. [30] showed that six species of the *Bacillus* genus amongst which *Bacillus cereus* predominated in *Hibiscus sabdariffa.* Lücking et al. [31] suggested that the contamination by spore-forming bacteria could have come from raw materials. Consequently, it appeared that poor quality of dried calyces used is targeted to explain the presence of spore-forming in sampled "Foléré" beverage. Their presence can also be explained both by the extraordinary resistance to environmental stress (acidity, heat, chemical disinfectant) and the ubiquist nature of these biological contaminants [32]. Omemu et al. [33] suggested that the presence of bacteria in homemade non-alcoholic beverages produced with a boiling method is indicative of post-production contamination during the addition of sugar and other additives. Contamination of the "Foléré" beverage can occur during cooling of the hot extract, addition of flavours and sweeteners, or dispensing of the nectar into nylons and bottles wrapping. Utensils and water used during the post-heating stages can also serve as a source of contamination [10]. Maïworé et al. [1] have identified water used during the processing as the major source of contamination of locally made beverages.

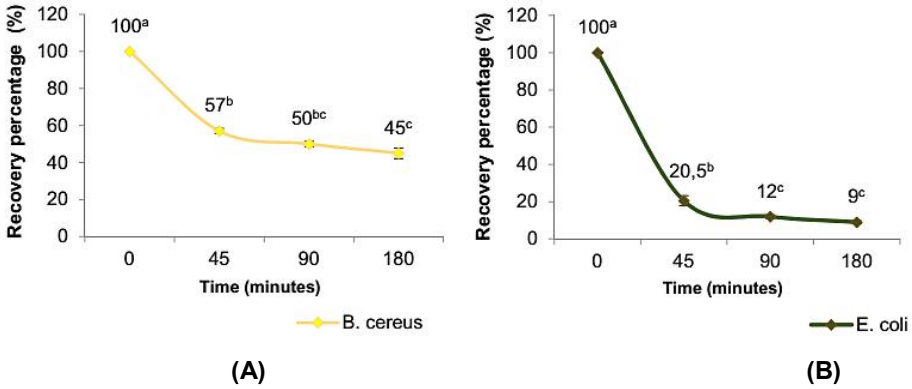

Fig. 1. Effect of acidic "Foléré" beverage at pH 2.01 on thermally unstressed *B. cereus* spores (A) and E. coli cells (B)

Mean values preceded by at least one common letter (a, b, or c) are not significantly different (P = .05) according to ANOVA anlysis and Tukey's multiple HSD comparison test. Errors bars below different means values represent standard deviation

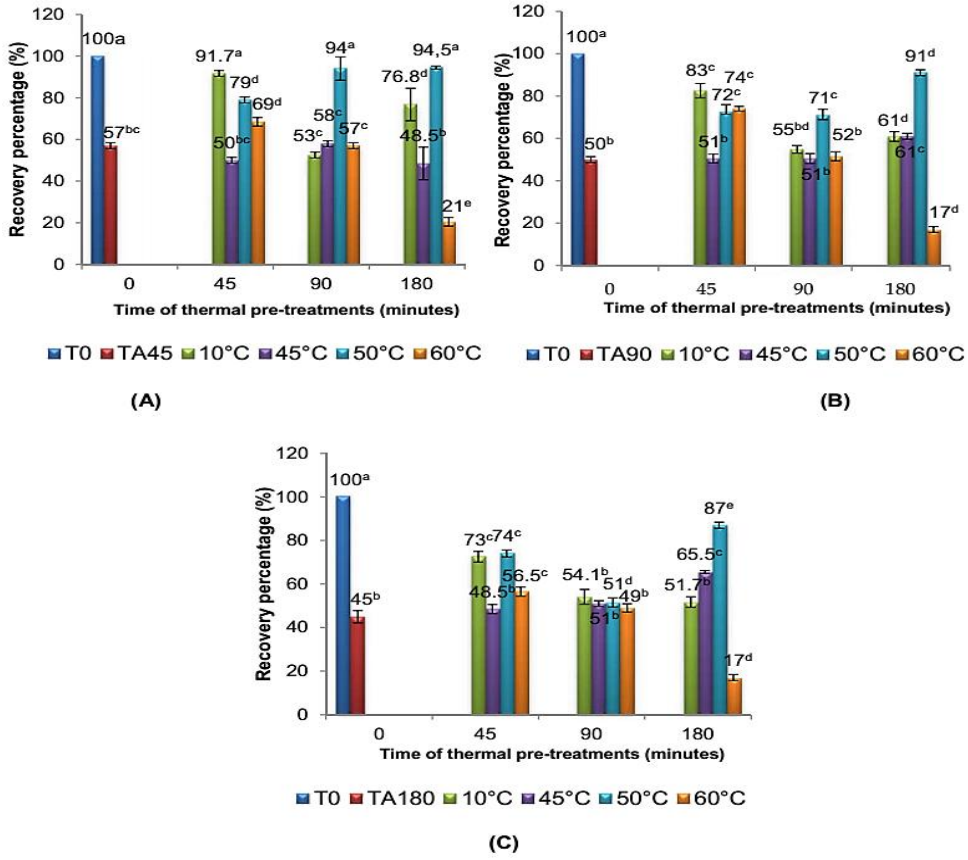

Fig. 2. Recovery of colonies from thermally unstressed (TA45, TA90 or TA180) and stressed (10°C, 45°C, 50°C, and 60°C) *B. cereus* spores introduced in *"Foléré"* beverage pH 2.01 for 45 minutes (A); 90 minutes (B); and 180 minutes(C). T0: Control; TA45, TA90, TA 180: acidic beverage contaminated with unstressed bacteria for 45, 90 and 180 min

Error bars below different mean values represent standard deviation. Mean values not followed by at least one same letter (a, b, c, d or e) differ at 5% level of significance according to the ANOVA analysis and Tukey's multiple HSD comparison test

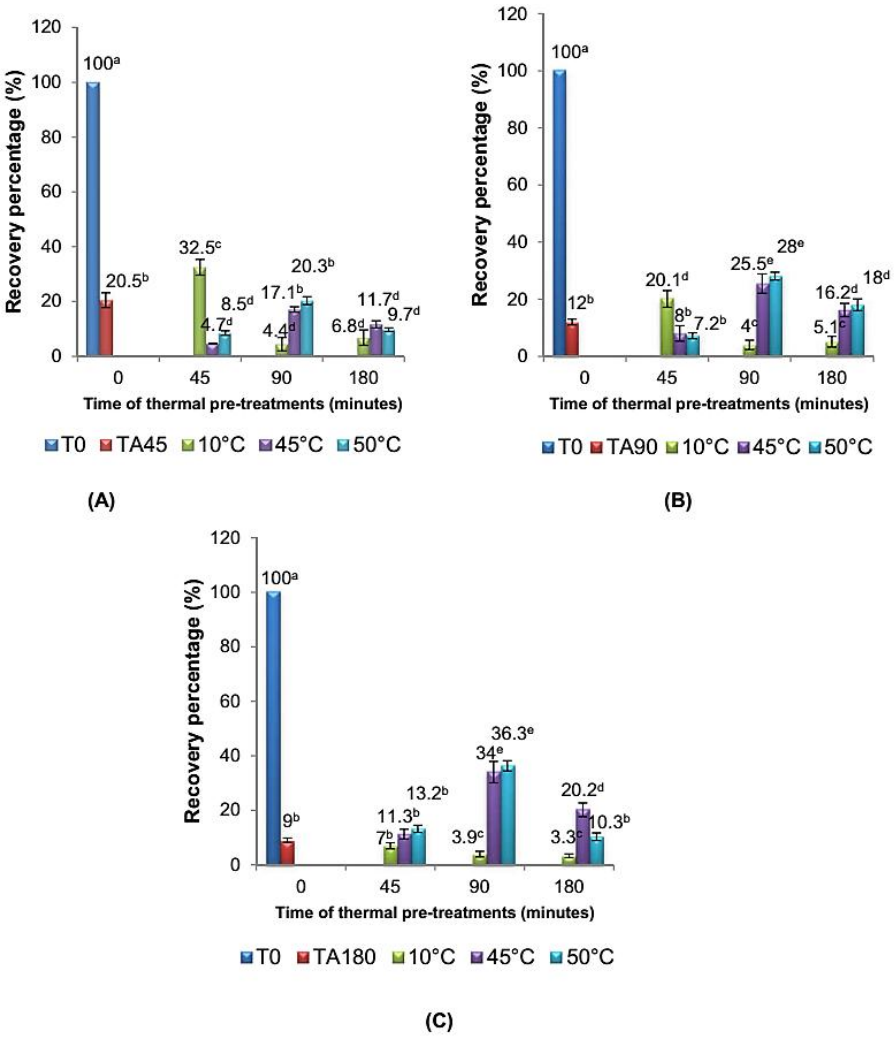

Fig. 3. Recovery of colonies from thermally unstressed (TA45, TA90 or TA180) and stressed (10°C and 45°C) *E. coli* cells introduced in *Foléré* beverage at pH 2.01 for 45 minutes (A); 90 minutes (B) and 180 minutes (C). T0: Control; TA45, TA90, TA 180: acidic samples at pH 2.01 contaminated with unstressed bacteria for 45, 90 and 180 min respectively

Error bars below different mean values represent standard deviation. Mean values not followed by at least one same letter (a, b, c, d or e) differ at 5% level of significance according to the ANOVA analysis and Tukey's multiple HSD comparison test

The efficiency of "Foléré" beverage pasteurized on unstressed bacteria is linked to the very low pH (2.01) of the beverage and could be explained either by acidification of the protoplast. Lambert and Stratford [34] suggested that at acid pH, the organic acids of "Foléré" beverage are in an undissociated form which allows them to easily cross the cell membranes. Once inside the cell, these acids will dissociate and induce proton release, which will result to a decrease of the internal pH of the cell that can induce a targeted inhibition of keys metabolic enzymes, the complexation of bivalent cations cofactors, the inhibition of proton pump H^+/ATPase and cationic permeases. All these events will probably be aimed at slowing down or completely stopping metabolism in non-spore-forming bacteria like *E. coli* cells and reinforcing metabolic inertia and dormancy in spore-forming bacteria such as spores of *B. cereus*.

The increasing of the recovery percentages observed after some sublethal thermal pre-treatments of spores of *B. cereus* and vegetative cells of *E. coli* may imply an enhancement in resistance of both foodborne bacteria to the acid

pH food environments. Many authors [19,22,35] suggested similar findings, but in non-food environments, corroborating a "thermal-induced resistance" phenomenon. In the spore-forming bacteria, this likely raising of the acid pH resistance, translated by a rise of recovery percentages after sublethal thermal pre-treatment could be explained by structural modifications of the spore-forming bacteria molecules, mainly those of the different tunics, and the inner membrane as proteins and lipids. These molecules would undergo conformational changes in the presence of sublethal cold, milder, and warmer temperatures, which could lead to the reduction of spore permeability with respect to chemicals in general and acids in particular [22]. In the vegetative cells of *E. coli*, this probable increasing of the acid pH resistance could be linked with the heat shock proteins synthesis. Wang and Doyle [35] have clearly shown that the induction of acid resistance by thermal stress involves the synthesis of new proteins. According to these authors, two proteins of 15 KDa and 22 KDa were synthesized at the outer membrane during the sublethal heat stress. The sequences of these proteins revealed that the first 10 amino acids of the NH_2-terminal portion of 22 KDa protein were Met-Ser-Lys-Ile-Asn-Thr-Lys-Ile-Lys-Pro while the NH_2-terminal portion of 15 KDa protein corresponded to Met-Ile-Thr-Gly-Ile-Gln-Ile-Thr-Lys-Ala. Indeed, these two proteins are subunits of an alkyl hydroperoxide reductase probably involved in the transport of protons out of the cell [35]. Moreover, it showed that one of the responses of vegetative cells to sublethal cold temperature was the synthesis of 7 kDa cold shock proteins (CSPs). At least 15 different cold shock proteins were induced in *E. coli* [13]. These proteins are involved in a variety of essential functions such as recombination in *E. coli* and may be implied on activation of acid resistance protein system which neutralizes the protons entering in the cell and contributes to increasing of internal pH [36]. The "thermal-induced resistance" phenomenon observed both in foodborne spore-forming and vegetative bacteria used in this study after the sublethal thermal pre-treatment could be called, "thermal-induced bacterial acid resistance" phenomenon.

5. CONCLUSION

This work showed that the physicochemical parameters of "Foléré" beverage are very interesting like the very acidic pH (2.01). However, we observed high contamination of "Foléré" beverage beyond the acceptable limits. This can be a public health hazard, as these microorganisms can be responsible for some diseases, ranging from foodborne illness to food poisoning. Although the contamination of this local beverage is probably a post-production event, it appears that sublethal temperatures may play a major role in the emergence of a mechanism that would allow bacteria to withstand acidic conditions. This mechanism known as "thermal-induced bacterial acid-resistance" may contribute to the spoilage of acidic beverages. We found that "thermal-induced bacterial acid-resistance" phenomenon varies according to the intensity and time of sublethal thermal stress, the nature and biophysical form of the bacteria, but it was more important when spore-forming bacteria were pre-incubated at 10°C for 45 min, 50°C for 180 min and vegetative bacteria were thermally stressed at 45°C for 45 min and 50°C for 90 min prior acid treatment at pH 2.01. Despite the progress of knowledge on the emergence of this phenomenon, it appears necessary in further studies to extend the demonstration of that one in other locally made beverages as alcoholics ones which are potentially recoverable at a semi-industrial scale.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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