Original Research Article

Changes in Acid-pH Resistance of Food Spoilage and Pathogens Spore-Forming Bacteria in Indigenous Acidic Sorghum-Based Beers Before and After Sublethal Thermal Stress

ABSTRACT

Fermented alcoholic beverages play a major role in the socio-cultural lives of people of Northern Cameroon. However, reports of shelf-life and health problems associated with indigenous sorghum-based alcoholic beverages are a major call for concern. This study aimed to highlight the additional effects of sublethal temperatures (no thermal treatment and 10, 45, 50 and 60 °C for 45, 90, 180 min) and acidic pH of beers (2.79 and 2.63 for 45, 90, 180 min) on fate of four food spoilage and pathogens spore-forming bacteria. The experiments were carried out on real food products formed by both indigenous sorghum-based alcoholic beverages. Pasteurized traditional beers were significantly efficient on all unstressed bacterial spores after 180 minutes of treatment. In addition, spores of *Bacillus megapterium* 8174 and *Geobacillus stearothermophilus* CNCH 5781 were more sensitive to white beer pH 2.79 and red beer pH 2.63 respectively. Previous thermal treatments of spores at some sublethal temperatures have significantly (P=0.05) decreased the effect of both beers on stressed spores. We observed that stressed spores of *Bacillus subtilis* NCTC 3610 at 10°C, *Geobacillus stearothermophilus* spores at 45°C and *Bacillus cereus* T at 50°C and 60°C for 45, 90, 180 minutes were significantly (P=0.05) less affected by white beer pH 2.79. Whereas, in red beer pH 2.63, spores of *B. subtilis* sublethally stressed at 10°C and spores of *B. cereus* stressed at 45°C, 50°C and 60°C were more acid resistant and less affected by acidic pH of alcoholic beverage. The study delivers some overview on the potential microbial (stability and safety) consequences of the current tendency towards milder cold and heat treatments which are greatly used in the food-grade liquids industry.

Keywords: Bacillus spores, alcoholic sorghum-based beverages, acid pH, sublethal thermal stresses, food safety

1. INTRODUCTION

Fermented beverages are one of the indispensable components of the dietary culture of every community in the world [1].The traces of the first artisanal fermentation dated about 6000 - 7000 BC, were found by archaeologists in the Blue Nile valley [2,3]. More than any other fermented beverages, beer is one of the oldest and most frequently consumed alcoholic beverages to humankind [4]. In Africa, sorghum is used to produce various kind of traditional beers named indigenous sorghum beers [5] such as *pito, dolo, tchapalo* [6,7] in some western countries of Africa. In Cameroon, especially in northern regions, brewing technology is a basic activity. It is case of people belong to *kapsiki* tribe who are located in the mountainous Mandara range in the Far-North region of Cameroon. For them, beer's brewing technology is on base of the known-how and social organization of the *kapsiki* society. This highlander community is specialized on the processing of two indigenous alcoholic beverages named as red *kapsiki* and white *kapsiki* locally called *te* and *mpedli* respectively [8,9], and sometimes assimilated to *bil-bil* which is a generic name of cereal-based alcoholic beverage brewed in the country. The *kapsiki* beers are opaque with red or white colour, slightly foamy. They have an alcohol content of 2.5% to 4.5%, a total soluble solids from 4°B to 8°B. They are largely consumed at room temperature as refreshing drink and sometimes warm with a bit liquid pepper. The indigenous sorghum beers are usually consumed as a nutritious beverage to quench hunger and thirst. They have a relatively low price compared to manufactured beers and serving as a lucrative business particularly for the poorest rural folks. According to several studies, moderate consumption of indigenous beers might improve the functioning of the digestive and immune systems, could play a key role on prevention of acute diarrhea, malaria, haemorrhoid, cardiovascular diseases and some cancer [10,11]. Potential microbes that can contaminate indigenous beers include bacteria such as *E. coli,* faecal *Streptococcus*, and spore-forming bacteria [9]. It is known that acidic environment is quite effective in

controlling bacterial growth even that of spore-forming bacteria, a good fence against any bacterial proliferation and largely used to prevent contamination. Unfortunately, the acidic character of these kind of beers with pH lower than 4.5 are not sufficient to avoid a high load of bacterial spores [8,9]. The presence of spore-forming bacteria in beverages is not as dangerous but it is their germination and growth to vegetative cells which always remains a serious threat to the health of consumers. In this situation, the presence and persistence of spore-forming bacteria in indigenous beers becomes really problematic. In spite of rich literature on spore-forming bacteria in cereals, little or no information is available with respect to bacterial spore presence in some indigenous beers (red kapsiki beer and white kapsiki beer) produced in Cameroon. To date, research to determine the key role of sublethal thermal stress on the ability of spore-forming bacteria to persist in foods has not been well documented in acidic indigenous beers brewed in Africa. Considering the large population of rural residents who consume one or both of these drinks, giving the health risks posed by spore-forming bacteria to man due to the potential for bacterial spores to cause foodborne diseases and at the time where the adaptation of bacteria to environmental stresses have received significant interest because of its implications in the food safety, the aim of this work was to describe the fate of spore-forming bacteria in two indigenous beers with different acid pH, before and after sublethal thermal conditions, simulating environmental stresses occurred during the processing and storage of these beers. The acid pH values tested were in the range of those reported in our previous studies [8,9] on the quality of some indigenous sorghum-based beers produced in Cameroon.

2. MATERIALS AND METHODS

2.1 Raw material

Two local “Mouskwari” sorghum (*S. bicolor* L. Moench) varieties, red and white ones suitable for the production of *red* and white *kapsiki* beers were purchased from Maroua central market in Far north region, Cameroon. However, in the rainy season, “Djigari” sorghum variety can also be chosen. The plant material was taken to the laboratory in cellophane bag and subsequently verified. Water was fetched from local tap water facilities.

2.2 Brewing of indigenous alcoholic beverages

Processing of indigenous beers from fermented red and white sorghum were followed in fifteen production sites of Mayo-Tsanaga division (Cameroon) distributed into four areas of Mogode sub-division.

2.3 Processing of white beer

The production process involves three main stages: malting, brewing and fermentation. The smallest quantity approximatively 1/6 of white sorghum “mouskwari” grain was soaked and left to settle for 24 h. Supernatant and suspended matter were removed and the hydrated grains was drained. The grains was germinated in dark at room temperature for 48 h, sun-dried for 6 to 10 h and ground into a coarse malt sorghum flour which was kept for further use. The greatest quantity about 5/6 of white sorghum “mouskwari” grain was cleaned and ground in raw sorghum flour. Sorghum flour was soaked for 72 h after wet floury pellet was separated from the supernatant. The pellet was mixed with tap water and cooked for approximately 3 h and roasted dough was obtained. After cooling, the stored sorghum malt flour was added to the raw dough and the mixture was hand-kneaded until a must dough was obtained. After pouring into earthenware pots a spontaneous alcoholic fermentation occurs and lasts 48 h. The final product was strained using a special cloth and the opaque liquid obtained constitutes white beer or *mpedli* [8].

2.4 Processing of red beer

The processing starts with the quenching of red “mouskwari” sorghum grain for 12-24 h, followed by germination for 2-3 days at room temperature and sun-drying to bring moisture of the germinated grain at 15–20%. After milling of the dried grains, we obtained a malted flour which kept in dark for 2 days before brewing. This flour was mixed out with tap water containing a sticky substance as okra or sap. The mixture obtained was decanted for 3 h, then supernatant was separated from a mealy bottom. This bottom was pre-cooked during 3-5 h, later mixed with the previous supernatant to give a sour must after a night spontaneous fermentation. The sour must was cooked during 5-10 h to give sweet must. After cooling, it is poured into earthenware pots and inoculated with dried starter culture harvested from previous brew for alcoholic fermentation during 12-24 h. The liquid obtained after alcoholic fermentation is called *té* or red beer [9].

2.5 Spore-forming bacteria strains

Four bacteria species grouped in *Bacillus* and *Geobacillus* genera were used to simulate the effect of environmental sublethal stresses on the fate of bacterial spores in acid locally brewed beers. *B. cereus* T, *B. megaterium* 8174 and *B. subtilis* NCTC 3610 spores came from the collection of the Microbiology Laboratory Institute of Food Research of Reading, Reading, United Kingdom (UK). *G. stearothermophilus* CNCH 5781 spores was obtained from the Institut Appert, Paris, France.

2.6 Culture of mature spores

The mature spores used in this work were produced from the stored spore collection. Culture was carried out in two stages according to the protocol earlier described by Bayoï et al. [12]. Firstly, the vegetative cells were produced from the stored spores. They were heat-activated at 100°C for 10 min and spread on trypticase soya broth for a night. Cultures were incubated for 24 h at 35°C for *B. cereus*, *B. megaterium, B. subtilis* and 65°C for *G. stearothermophilus*. At the end of the incubation, the vegetative cells were obtained. Secondly spores were produced from freshly grown vegetative cells. The vegetative cells of each bacterial species were spread on sporulation fortified agar and plates were incubated for 5-7 days at the optimal growth temperature previously used for each bacteria. The spore crop obtained after incubation was washed twice and re-suspended in sterile distilled water. The mature bacterial spores were obtained after keeping of the purified spores at 4°C for 3 months at least to ensure their stability before use.

2.7 Fate of the unstressed spores into acid sorghum-based beers

To perform this test, the method described earlier was used [12]. A hundred (100) microliter of thermally unstressed spores ($1.6x10^7$ cells/ml) of each bacteria test was used, introduced into 9.4 ml of heat-pasteurized red kapsiki and white kapsiki beers. The white beer was stabillized at pH 2.79 and red one at 2.63. The different preparations were neutralized using 0.5 ml of a solution of sodium hydroxide 10% after 45, 90 and 180 min of treatment at laboratory temperature. Then, a hundred (100) milliliter of appropriate dilution heat-activated of each preparation were spread on nutrient agar medium. The plates were incubated at optimal growth temperature of each bacteria specie. Each experiment was performed three times and the seeding is done in triplicate. After incubation, the number of colonies from spores treated by acid beer were enumerated and expressed in terms of recovery percentage as compared to control spores which were unstressed and added to the same volume of sterile distilled water. Recovery percentage was calculated according to the formula below:

Recovery percentage (%) = (number of cfu from spores unstressed and acid beer treated samples/number of cfu from control spores no thermally stressed and no acid beer treated samples) x 100.

2.8 Fate of the thermally stressed spores in acidic pH indigenous sorghum beers

The adapted method to that described by Bayoï et al. [13] was used to perform this experiment. Five (5) milliliters of spores ($1.6x10^7$ cells/ml) suspension of each bacteria specie were filled in a pyrex tube covered with a cap and then introduced into a cooling system (Superser) or a water bath (Memmert). The thermal shocks were carried out at various sublethal temperatures (10°C, 45°C, 50°C, 60°C) during 45 min, 90 min and 180 min according to the method at constant temperature as described by bayoï et al. [12]. Then, one hundred (100) microliter of each bacterial spore specie thermally stressed was inoculated into 9.4 ml of heat-pasteurized red beer (pH 2.63) and white beer (pH 2.79). Preparations were incubated with slight stirring for 180 min. A group of unstressed and untreated spores with both indigenous beers was used as control samples. At the end of each treatment, spore preparations were neutralized by adding 0.5 ml of sodium hydroxide 10% and the suspension spores were heat-activated at 100°C for 10 min. Triplicate determinations were carried out. A hundred microliter of appropriated dilution was spread on nutrient agar medium and culture was done as described above. After incubation, the number of colonies from spores stressed and treated with acid indigenous beer was counted and expressed in terms of recovery percentage as compared to unstressed and no beer-treated control spores, according to formula given below:

Recovery percentage (%) = (number of cfu from spores thermally stressed and acid beer treated samples/number of cfu from control spores unstressed and no acid beer treated samples) x 100.

2.9 Data analysis

The results were expressed as recovery percentage and represented as mean ± sd which were used to draw the plots with EXCEL program (Microsoft Office Professional 2013, Windows, USA). Analysis of variance (ANOVA) one-way and Tukey's HSD tests were performed with STAGRAPHICS software centurion version 16.1 (Technologies Inc., Virginia, USA) to compare the means. Statistical differences with *P*=.05 were considered significant. The multivariate analysis especially Principal Component Analysis (PCA) based on the correlation matrix and Hierarchical Clustering Analysis (HCA) were performed to compare the responses of the stressed spores samples obtained from different conditions. PCA allowed the measured variables to be grouped into new variables called 'components' or 'factors.' HCA allowed to draw dendrogram used to analyze relationship between the different species of spores after thermal stresses and beer treatments. The SPSS Statistical program (SPSS20, IBM Inc., Armonk, New York, USA) was used for the multivariate analysis.

3. RESULTS

3.1 Recovery evolution of the unstressed spores in acid pH indigenous beers

Figure 1 shows the effect of white beer pH 2.79 on the recovery of colonies from germinated unstressed spores. Regardless to the nature of the non-thermally stressed bacterial spore, we observe a significant decrease (P = 0.05) in the recovery percentages, depending on stay time of the spore. Until a stay of 90 minutes, the spores of *B. cereus* were more sensitive to the action of the white beer pH 2.79 than those of *G. stearothermophilus*. But after 90 minutes and till 180 minutes, we observed a reverse sensitivity between both spores' species. At that time, we have obtained the recovery percentages of 31.8% for *B. megapterium* spores, 31.75% for *B. subtilis* spores, 28.3% for *B. cereus* spores and only 15.9% for *G. stearothermophilus* spores. With the red beer at pH 2.63 (Figure 2), we noticed significant effectiveness (P=.05) of the beer pH on the bacterial spores after an action time of 180 minutes. This was more pronounced on the spores of *B. megapterium* compared to the other bacterial spores' species. The effectiveness was shown by the recovery percentages of 90.5%, 68.4%, 41.25% and 17.5% respectively with the spores of *B. subtilis*, *B. cereus*, *G. stearothermophilus* and *B. megapterium*.

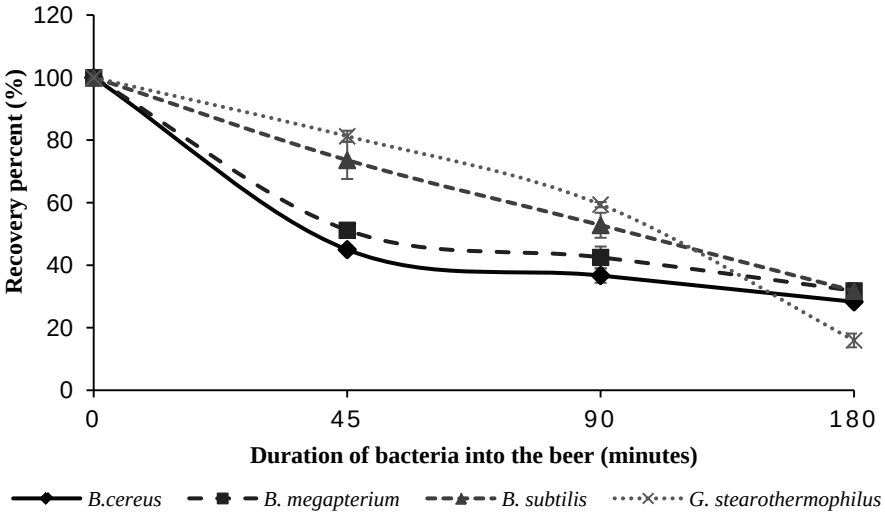


Fig. 1. Evolution of unstressed spores dropped in the white beer at pH 2.79

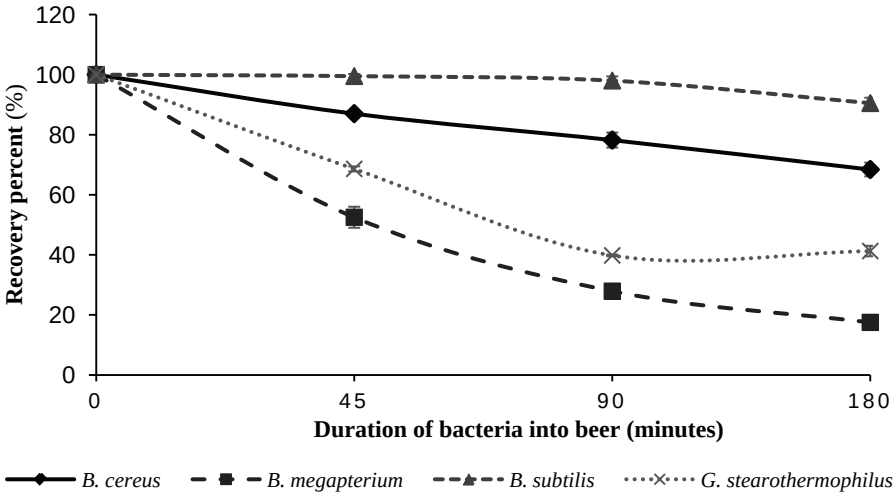
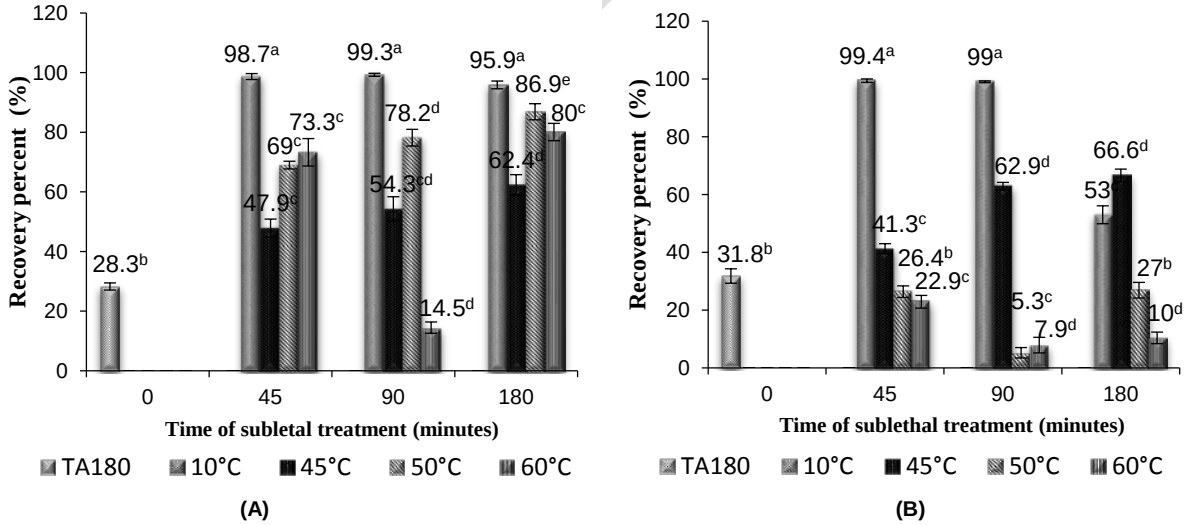


Fig. 2. Evolution of unstressed spores contained in the red beer at pH 2.63

3.2 Enhancement of recovery of the spores in acid pH beers after sublethal thermal stress

Figure 3 shows the effect of sublethal temperatures on the behaviour of spores in white beer pH 2.79 for 180 minutes. Compared to non-thermally stressed spores, we observed a significant increase (P = 0.05) in the recovery percentages of *B. cereus* spores after sublethal stresses with all the four temperatures used. Same effect was noticed for the spores of *B. megapterium* stressed at 10°C and 45°C. Spores of *B. subtilis* pretreated at 10°C and 50°C and those of *G. stearothermophilus* stressed at 10 °C, 50°C and 60°C showed similar behaviour. Effect of sublethal thermal stress on the behaviour of spores in the red beer at pH 2.63 is presented in Figure 4. We noticed a significant increase (P = .05) in the recovery percentages for the spores of *B. cereus* stressed at 60°C and for 45 and 90 minutes at 10°C, *B. megapterium* spores stressed at 10°C and to a lesser extent at 45°C, spores of *G. stearothermophilus* stressed at 10°C for 45 minutes, at 50°C for 45 minutes and 180 minutes, and 60°C.



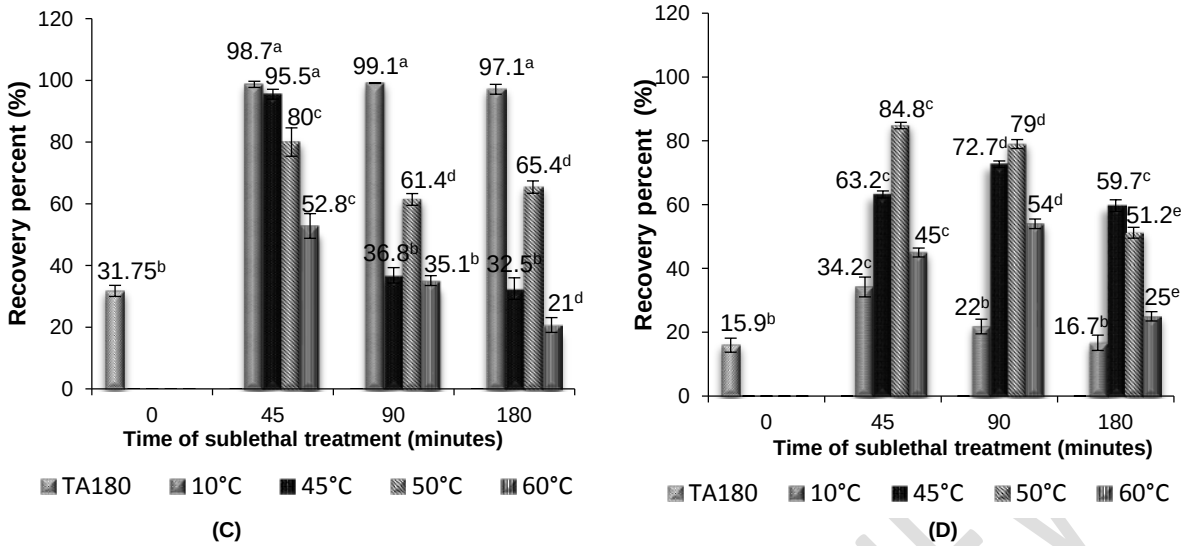


Fig. 3. Evolution of recovery percent from unstressed (TA180) and thermally stressed (10°C, 45°C, 50°C, and 60°C) spores of *B. cereus* (A), *B. megapterium* (B), *B. subtilis* (C), *G. stearothermophilus* (D) dropped in white beer pH 2.79 for 180 minutes. *The values are mean of three independent trials. Error bars under each mean value represent standard deviation. Mean values with the same letter (a, b, c, d or e) are not different at 5% level of significance.*

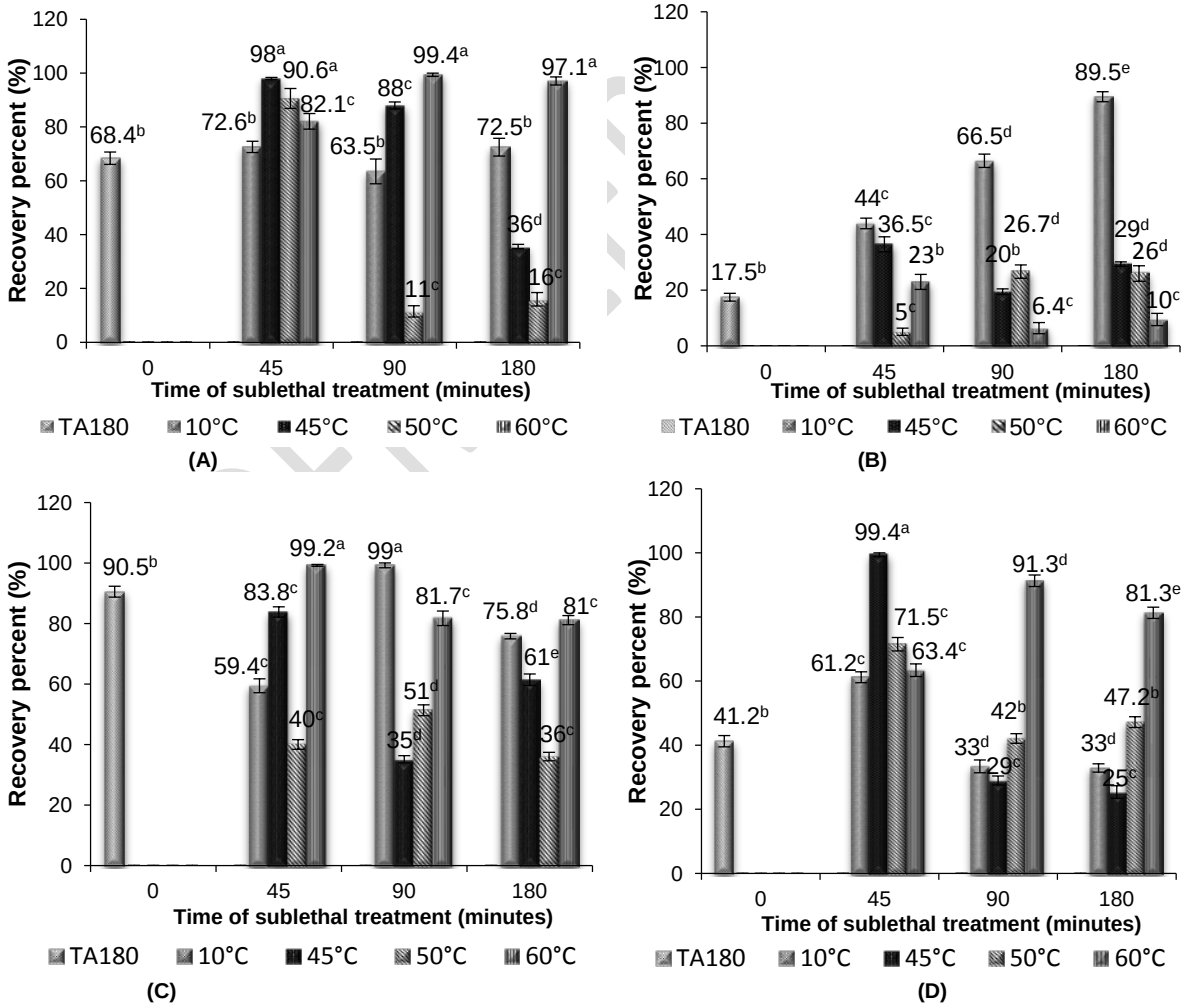


Fig. 4. Evolution of recovery percent from unstressed (TA180) and thermally stressed (10°C, 45°C, 50°C, and 60°C) spores of B. cereus (A), B. megapterium (B), B. subtilis (C), G. stearothermophilus (D) dropped in the red beer pH 2.63 for 180 minutes. *The values are mean of*

three independent trials. Error bars under each mean value represent standard deviation. Mean values with the same letter (a, b, c, d or e) are not different at 5% level of significance.

3.3 Multivariate analysis of indigenous beers containing unstressed and stressed spore-forming bacteria

To simplify the interpretation of the results, the principal component analysis (PCA) was applied to the recovery percentages obtained after action of indigenous beers on unstressed and sublethally stressed spore-forming bacteria. As shown in Figure 5 below, the four biological variables represented by each unstressed spore-forming bacteria specie introduced into the white opaque beer pH 2.79 and red homemade beer pH 2.63 were reduced in two main components (PC1 and PC2). The components PC1 and PC2 explained 99.85% and 99.75% of the total data variance after varimax rotation, respectively, for white beer at pH 2.79 and red beer at pH 2.63. The PCA analysis showed with white beer containing unstressed spores that variables which contributed positively to PC1 were BC (unstressed *B. cereus*) and BM (unstressed *B. megapterium*) whereas the variable GS (unstressed *G. stearothermophilus*) positively loaded to PC2. Moreover, we found a very strong positive correlation (r^2 = 0.996) between the fate of the unstressed spores of *B. cereus* and B. *megapterium* and a positive correlation (r^2 = 0.937) between the fate of the unstressed spores of *B. subtilis* and *G. stearothermophilus* in the white beer at pH 2.79. With the red beer at pH 2.63, the fate of unstressed *B. megapterium* (BM) and unstressed *G. stearothermophilus* (GS) spores were strongly correlated (r^2 = 0.974) and each other are positively loaded to factor PC1 while the behaviour of unstressed *B. subtilis* (BS) spores was highly correlated to the factor PC2. Unstressed spores of *B. subtilis* and *G. stearothermophilus* were very poorly correlated (r^2 = 0.419) in the red beer at pH 2.63. Consequently, it would be likely that fate of unstressed spore-forming bacteria greatly varies with the pH of the indigenous beer and the genus or specie of the bacteria. However, we noticed that all the four variables which contributed positively to the total data variance were on the same side in the space created by the two dimensions of PCA. This could shown that, beers acted the same manner on bacterial spores, and the red indigenous beer has more effectiveness than white indigenous beer on unstressed spores of *G. stearothermophilus*, whereas both traditional beers have approximatively the same effectiveness on the others three unstressed spores.

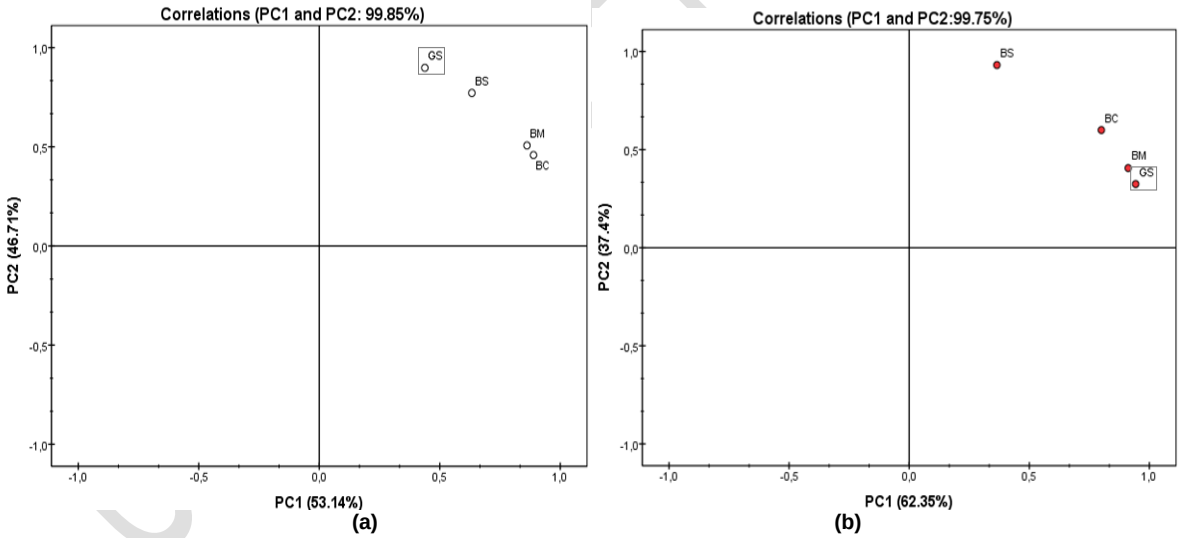


Fig. 5. Loading resulting from PCA of the unstressed spores into the white beer at pH 2.79 (a) and the red beer at pH 2.63 (b). Notes. BC: *Bacillus cereus* spores; BM: *Bacillus megapterium* spores; BS: *Bacillus subtilis* spores; GS: *Geobacillus stearothermophilus* spores. Any spore-forming bacteria species were not submitted to sublethal thermal stress. Only the effect of the acid pH on spore-forming bacteria was followed.

In an attempt to better assess the effect of sublethal thermal stresses on the behaviour of the spores contained in both handicraft beers, four thermal values combined to three treatment times were reduced by two main factors as shown in Table 1. The factors PC1 and PC2 explained 94.5% and 96.5% of the total variance from the beers at pH 2.79 and pH 2.63 containing sublethally stressed spores. The PCA analysis showed that with white beer; the sublethal stresses at 50°C and 60°C for 45 and 90 minutes positively contributed to the main component 1 while the sublethal stresses at 10°C positively contributed to the main component 2 (PC loading > 0.75). On the other hand, sublethal treatments at 45°C were negatively loaded to component 2. This showed that main component PC2

opposes the sublethal stresses at warm temperatures to sublethal stresses at low temperatures, whereas component PC1 only provides information on sublethal stresses at very warm temperatures. With the red beer, component PC1 was mainly described by sublethal stresses at 45°C, 50°C and 60°C which are on the positive side while only sublethal stresses at 10°C positively contributed to the principal component 2. The components PC1 and PC2 respectively describe the sublethal stresses at warm temperatures (heating-stress) and the sublethal stresses at cold temperatures (cooling-stress).

Table 1. Loadings resulting from PCA of the white beer pH 2.79 and red beer pH 2.63 samples containing thermally stressed spores.

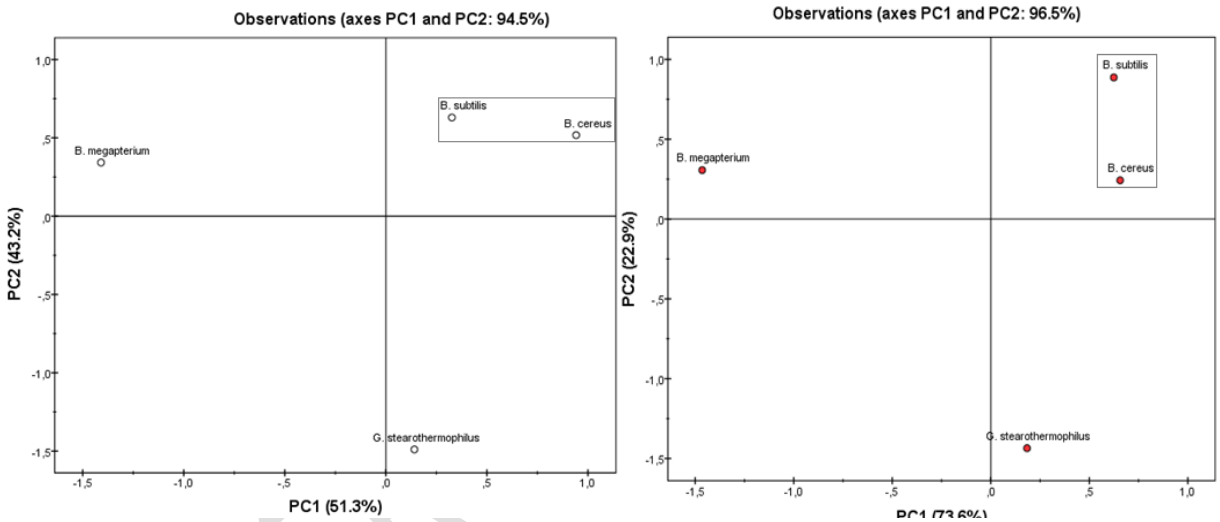
	White beer (pH 2.79)		Red beer (pH 2.63)	
	PC1 (51.3%)	**PC2 (43.2%)**	**PC1 (73.6%)**	**PC2 (22.9%)**
No sublethal treatment	0.247	0.966	0.874	0.451
Sublethal treatment at 10°C	0.089	0.996	0.102	0.994
Sublethal treatment at 45°C	-0.099	- 0.995	0.941	0.109
Sublethal treatment at 50°C	0.972	- 0.201	0.791	- 0.557
Sublethal treatment at 60°C	0.971	- 0.097	0.996	- 0.082
Sublethal treatment for 45 min	0.778	0.446	0.965	- 0.185
Sublethal treatment for 90 min	0.996	- 0.053	0.962	0.270
Sublethal treatment for 180 min	0.734	0.541	0.868	0.449

Note. Rotation method: Varimax with Kaiser normalization (eigenvalue > 1).

The plot obtained in space created by the two main components (PC1 and PC2) shows that the sublethal thermal stresses oppose on dimension 1 behaviour of *B. cereus* and *B. megapterium* stressed spores and dimension 2 the behaviour of stressed spores of *B. subtilis* and *G. stearothermophilus* contained in the indigenous beers samples. In the white beer, the stressed spores of *B. cereus* at 50°C and 60°C during 45, 90, 180 minutes were no longer affected by very low acid pH (2.79) of beer for 180 minutes while the spores of *B megapterium* thermally stressed under the same conditions were more affected by the white beer at pH 2.79. The sublethal stresses at 10°C and 45°C also strongly affected behaviour of *B.subtilis* and *G. stearothermophilus* spores respectively in the white beer pH 2.79 (Figure 6A). Independently of the time, the sublethal stresses at 45°C, 50°C and 60°C positively impacted the behaviour of *B. cereus* spores and negatively affected the behaviour

of *B. megapterium* spores dropped on the artisanal red beer pH 2.63. Whereas sublethal stresses at 10°C negatively acted on the spores of *G. stearothermophilus* and positively on the spores of *B. subtilis* (Figure 6B). Moreover, the two plots confirm that white and red beers are more effective on unstressed spores of *G. stearothermophilus* and *B. megapterium* respectively. Despite the similarity of dispersion of the observations in the score plot obtained with the white and red beers, we noticed slight modification on the position of stressed spores of *B. cereus* and *B. subtilis* on the positive side of the space described by the two components. Therefore, the type of beer could influence the behaviour of stressed spores of *B. cereus* and *B. subtilis*.

Cluster analysis was applied to the response of bacterial spores after thermal stresses and treatment to acidic fermented indigenous beers by Euclidean distance measurement. Three and two distinct clusters were separated out in the resulting dendrogram after performing cluster analysis on the white beer pH 2.79 and the red beer pH 2.63. Figure 7 shows dendrogram of four bacterial spores with the eight thermal conditions as variables. In the white beer, *B. cereus* and *B. subtilis* were grouped in the same cluster, which suggested that they were closely related. *B. megapterium* and *G. stearothermophilus* formed the second and third cluster respectively. In the red indigenous sorghum beer, *B. cereus, B. subtilis* and *G. stearothermophilus* were in the same cluster with a close relationship between *B. cereus* and *B. subtilis*. Whereas *B. megapterium* was in separated cluster in the red beer samples. These distinct observations may be caused by composition and pH of both indigenous beers.



(a) **(b)**

Fig. 6. Score plots of stressed *Bacillus* and *Geobacillus* spores dropped in the white beer at pH 2.79 (a) and the red beer at pH 2.63 (b).

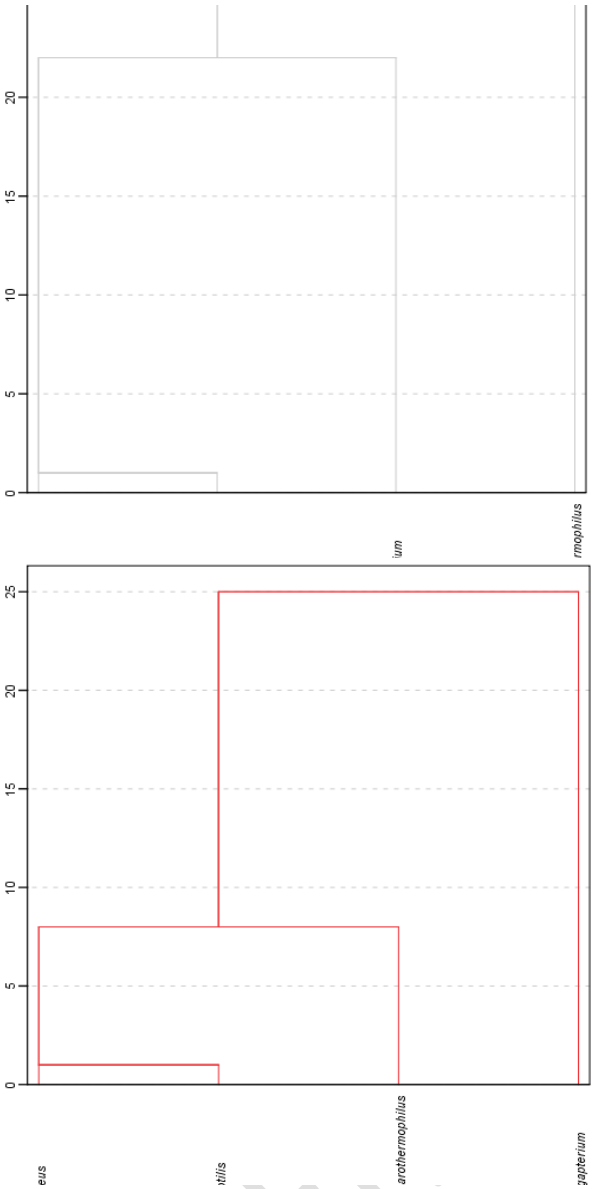


Fig. 7. Dendrogram of *Bacillus* and *Geobacillus* spores thermally stressed into white beer pH 2.79 (a) and red beer pH 2.63 (b) samples from the hierarchical cluster analysis using Euclidean distance and minimal jump linkage.

4. DISCUSSION

The positive action of both traditional pasteurized beers on unstressed spores was materialized by an important decrease in recovery percentages, after the germination of the remaining spores in the both drinks at the end of the treatment. This effectiveness of beers is probably due to composition and the acidic character of these fermented beverages. Indeed, both indigenous beers have a very low pH, which would be responsible in rising of the spore's coat permeability following solubilization of some acid proteins of the inner and outer tunics. In most cases, the susceptibility of the unstressed spores was greater in the white beer than in the red beer. This would be related to the difference in biochemical composition of the handicraft beers. Indeed, previous studies carried out on these alcoholic homemade beverages showed that the white beer has higher total acid content than the red beer. This great total acid content is linked to lactic acid bacteria which are strongly involved in the heterofermentative process of white beer and responsible for the production of organic acids (lactic acid, acetic acid, formic acid). These differences in sensitivity also can be explained by the chemical nature and content of the organic acids in beer. Some authors reported that lactic and acetic acids are more effective on spores than citric and malic acids [14]. In addition, accumulation of carbon dioxide into the coat of spore and oxidation of membrane lipids by hydrogen peroxide produced by lactic acid

bacteria can cause a dysfunction in the permeability of the spore [15,16]. Moreover, Low molecular organic compounds produced by lactobacilli such as reuterin could react irreversibly with the sulfur-rich proteins of the spore's coat layers which could induce inactivation of the spore [17]. Some bacteriocins produced by lactobacilli, endowed with bactericidal activity, can be active against some spoilage spores (*B. subtilis, B. megapterium, G. stearothermophilus)* and pathogenic spores (*B. cereus*). The Principal Component Analysis (PCA) below carried out confirmed that the spores of *B. megapterium* were sensitive to both craft beers whereas the red beer pH 2.63 had more effect on the spores of *G. stearothermophilus* than the white beer pH 2.79. These results agreed with those of Berendsen et al. [18] and Marshall et al. [19] who showed that resistance of bacterial spores varies from one specie to another and is linked to the sporulation conditions (temperature, composition of the medium). Indeed, these conditions influence the composition, structure, morphology that strongly impact the resistance of the spore.

Some spores of the *Bacillus* and *Geobacillus* species sublethally stressed at temperatures of 10 °C 45°C, 50°C and 60°C before their introduction into the indigenous pasteurized beers at pH of 2.79 and 2.63, presented very high recovery percentages compared to unstressed spores which showed significantly low recovery. Indeed, the sensitivity of spores to the acidity of beers drastically decreased after they were kept at one of the sublethal temperatures. This drop in the spore sensitivity, reflected by the raising of the recovery, is potentially linked to an increase in the resistance of stressed spores to acidity and some organic substances produced by craft beers. This behaviour, known as thermal-induced bacterial acid resistance, has been already reported by Bayoï et al, [12,13]. These authors reported that sublethal heat stresses at 45°C, 50°C and 60 °C would increase the resistance of *B. subtilis* and *G. stearothermophilus* spores to acetic acid at pH 4.5. Most recently, they have shown that spores of *B. cereus* previously maintained at sublethal temperatures of 10°C, 45°C, 50°C and 60°C are more resistant to the high acidity (pH 2.01) of flash pasteurized "foléré" beverage, a homemade refreshing drink from Cameroon.

This phenomenon, translated as thermal-induced bacterial acid resistance, is still unknown and not well biochemically elucidated. However, it was suggested that some structural changes of proteins and lipids which make up the spore may be responsible for the appearance of the thermal-induced acid resistance phenomenon. One of the potential mechanisms suggests that sublethal stresses at warm temperatures (45°C, 50°C and 60°C) lead to modifications of the three-dimensional structure of sulfur-rich proteins in tunics and cortex, the activation of the "heat-shock" proteins and small acid soluble proteins also called SASPs synthesized during sporulation and kept respectively in the coat and protoplast of spore. Changes of lipids of the inner and outer membranes of spore would be essentially translate by saturation of the fatty acids or transition of the unsaturated fatty acids from the normal "cis" configuration to the abnormal "trans" configuration. The sublethal stresses at low temperatures (10°C) could induce a rigidity of the proteins and lipids coat causing the vitrification of the bacterial spore. Incidence of all these modifications will be to drastically reduce the permeability of the spore, preventing the entrance of protons into the protoplast. However, the protons and organic acids which have reached protoplast will be neutralized by the small acid soluble proteins activated before their interaction with DNA or other keys enzymes in the spore. The changes in conformation of the spore molecules can also reduce contact between active organic compounds in indigenous beers and the spore membranes.

5. CONCLUSION

The changes in the behaviour of spore-forming bacteria in some acid and fermented African indigenous beers at thermal sublethal conditions were investigated in this study. Traditional fermented sorghum beers produced at the household level in the northern part of Cameroon were very efficient on unstressed spoilage and pathogens spore-forming bacteria. These results reported could serve as starting point to well understand the key antimicrobial role of our local cereal-based alcoholic beverages if they are produced at the hygienic standards. Therefore, thermal sublethal conditions which occurred during processing and storage of beers have significantly reduced antimicrobial activity of pasteurized white beer pH 2.79 and red beer pH 2.69 on spoilage and pathogens spore-forming bacteria. Indeed, some thermal sublethal treatments at 10°C, 45°C, 50°C and 60°C have significantly decreased efficiency of both indigenous beers on some stressed spores of *Bacillus* and *Geobacillus*. Independently of the nature of both fermented beers, stressed spores of *B. cereus* and *B. subtilis* had the same behaviour and were closely related. As known, this phenomenon has not been biochemically elucidated, it appears that a rush-study should be carried out to investigate the real changes at cellular and molecular levels which occurred during the emergency of this phenomenon.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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