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Full Length Research Paper

Anti-bacterial profile of some beers and their effect on some selected pathogens

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The investigation of the therapeutic properties of some beers, allow us to study their anti-bacterial profile against food born diseases caused by bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Staphilococcus aureus, Bacillus cereus*, *Bacillus anthracis* and *Bacillus subtilis*. The results obtained show that beers B2 and B3 had no anti-bacterial activity on the tested bacteria, whereas, beer B1 had bactericidal activity on gram positive and gram negative bacteria. The anti-spore activity of beer B1 on *B. anthracis*, *B. cereus* and *B. subtilis* exhibited a microcycle (sporulation-germination-sporulation), which could permit the application of soft preservation methods during the first 5 h for *B. cereus* and *B. subtilis* and during the first 10 h for *B. anthracis*. Finally, the results seem to confirm some popular practices. The beer B1 presents some potentialities for the treatment of gastro-enteritis.

Keys words: Food contaminations, beer, anti-bacterial substance, gastro-enteritis.

INTRODUCTION

Generally, in many countries in Africa and Cameroon in particular, a number of people living in modern areas almost exclusively use either traditional medicine or casual therapy like consuming some beers in the treatment of diseases such as gastroenteritis (Syder and Merson, 1982; Kouitcheu et al., 2006). That behaviour is linked to various reasons such as higher cost of treatment, fear of drug resistance in both human and plant pathogenic microorganism (Boda et al., 2006; Pieme et al., 2008).

As far as beer is concerned, it is well known that a huge research work has been focused on the improvement of its brewing process and its organoleptic properties (Murroungh-I, 1995; Bardi et al., 1996). To our knowledge, therapeutic aspects of beer remains unknown until explored. The present study therefore was designed to investigate the antibacterial profile of some Cameroonian beers commonly used as medicine against gastroenteritis. Thus, the spectrum of activity of beers, the minimal bactericidal concentration (MBC) of vegetative and spores forms and destruction kinetic were determined.

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Abbreviations: MBC, Minimal bactericidal concentration; **TSB,** soy broth with tryptone; **D_T,** decimal reduction time; **OD,** optical density.

MATERIALS AND METHODS

Preparation of the biologic material

The bacteria used for this work were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Bacillus anthracis*, *Bacillus subtilis* BGA (kindly donated by Service de Microbiologie Immunologie Pathologie Infectieuse de L'EISMV de Dakar), and *Bacillus cereus T* (kindly donated by the Institute of Food Research, England). These strains were revived, and then purified on soy broth with tryptone (TSB)-agar. The obtained pure strains were preserved in TSB broth at 4°C.

Spores' production

Tests tubes were incubated without agitation for 7 days at 37°C.

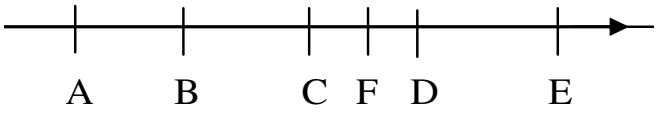

Figure 1. Determination of the MBC of the vegetative forms.

The purification of spores was done according to Bruch et al. (1968) method. After recuperation and suspension in distilled sterile water, spores were centrifuged four times at 500 g for 20 min to eliminate the soluble substances, weak density particles and to separate the vegetative from spores form. After every centrifugation, the cell pellet was washed with distilled sterile water. The remaining vegetative forms were eliminated by adding enzymes into the solution (100 µg/ml of trypsine and 200 µg/ml of lysozyme in a tampon phosphate 0.1 M, pH 7.0) (Hawirko et al., 1976). The suspension was then incubated for 4 h at 37°C with continuous agitation, followed by 3 centrifugations at 1000 g for 20 min; 4000 g for 10 min; 10000 g for 5 min. The obtained spores were suspended in the distilled sterile water and preserved at 4°C, until their utilization, in order to test the sporicide activity of beer.

Beers

The beers used in this study were B1, a stout, B2 and B3, ale beer. B1 is a "stout" dark brown or reddish brown in colour. It has an alcohol content of 7.5% and its pH range between 3.5 and 4. It is a top fermentation beer, dense and characterized by a pronounced bitterness. It differs from B2 and B3 in that an additive was included during the fermentation process. B2 and B3 are "ales" of pale amber or blond colour. They contain 5.2% of alcohol and their pH range between 3.5 and 4. They are rich in moss with light bitter taste. Samples of beer were bought from a local market in Ngaoundéré (Cameroon).

Determination of the specter of activity of the beer on the vegetative forms

The specter of activity was obtained by the plate count method (Leclerc, 1995). After introducing bacteria (10^8 cfu/mL) in tubes containing increasing concentrations of beer (from 1 to 5 ml), the tubes were incubated for 24 h at 37°C. Obtained cultures were then submitted to decimal dilutions. 1 ml of every dilution was sowed in the mass of TSB-agar medium. Petri ditches were then incubated for 24 h at 37°C and number of colonies counted thereafter.

From the data gotten the following parameters were obtained. For every volume of beer added, the percentage inhibition (% I) of the bacteria and its percentage of resistance (% R) were calculated using the following formulae:

$$\text{Percentage of inhibition (\% I)} = N_0 - N_n / N_0 \times 100$$

N_0 = initial number of bacteria; N_n = remaining bacteria after the action of n mL of beer.

$$\text{Percentage of resistance (\% R)} = 100 - \% \text{ I}$$

Determination of the MBC of the vegetative forms

The determination of the MBC was done following the experimental result of optimization method (Porte et al., 1988). The initial simplex consist of two measures: A is the quantity of beer that gives the bad answer and D the volume of beer that gives the better of it. Optimization consists of determining the small quantity of beer that gives the best answer.

The answer A is the worst, we determined its symmetrical C with regard to B, the answer in B being bad, we determined its symmetrical C with regard to D. This answer is better than the one gotten in D, we did an interns contraction with regard to D, while reducing the step of variation of 0.5 and we do a measure on point F (Figure 1). This answer would imply an intern's contraction but it has no interest considering the precision of the pipette. The point F is considered therefore as optimum. The percentage of inhibition (% I), the percentage of resistance (% R) and the decimal reduction time were then calculated.

Determination of the kinetics of destruction of the vegetative forms

The kinetic parameters are obtained by the method of numbering on petri dishes (Leclerc, 1995). A concentration of bacterial (10^8 cfu/ml) was introduced into test tubes containing the respective MBC of each beer. These tube were incubated for 0, 1.30, 3, 6, 12, 18 and 24 h. After every incubation time, the decimal dilutions are achieved in a physiological solution and 1 ml of every dilution is sown in agar medium (Table 1).

Determination of the anti-spore activity

After introducing spores in tests tubes containing 10 ml of beer and incubated without agitation at 37°C for 5, 10, 15 and 20 h, the readings of the optical density (O. D) at 620 nm after every incubation time allowed the destruction or the germination of spores in comparison with the control sample in the TSB.

RESULTS AND DISCUSSION

Determination of the specter of activity of the three beers on the vegetative form

The results of the spectrum of activity of the three beers are presented in Table 1. B2 and B3 showed no bactericidal activity on the studied bacteria. It appears that these two beers did not have any anti-bacterial activity. These results may explain why these beers were not used locally in the treatment of gastro-enteritis. On the con-trary, B1 presented bactericidal activity on *E. coli*, *S. aureus* and *S. typhimurium*, but not against *B. anthracis*, *B. cereus* and B. *subtilis*. These results confirmed the fact that bacteria might react differently when in contact with an anti-bacterial substance (Simonet, 1995). Langezaal et al. (1992) have shown that, Humulone and lupulone, the essential oil components in hops have an anti-bacterial effect against gram positive bacteria such as *B. subtilis* and *S. aureus* and against fungi such as *Trichophyton mentagrophytes var. interdigitale*. Our results showed that B1 destroyed gram negative bacteria *S. typhimurium* and *E. coli*, but had no effect on gram positive bacteria *B. subtilis*. The main ingredients of beer with known therapeutic virtues are the hops (Schmitz and Jackel, 1998; Miranda and Helmrich, 1999; Miranda et al., 2000; Vonderheid-Guth et al., 2000). In the present

Table 1. Determination of the spectrum activity of beers B1, B2 and B3. After an incubation of 24 h at 37°C, the cultures were sown on solid medium for colonies counting.

Bacteria	Beer	Volume (ml)	%I	%R
E. coli	B1	1	94.6	5.39
E. coli		2	91.98	8
E. coli		3	99.32	0.,67
E. coli		4	100	0
E. coli	B2 and B3	15	0	100
S. typhimurium	B1	1	99.74	0.26
S. typhimurium		2	93.61	6.39
S. typhimurium		3	100	0
S. typhimurium	B2 and B3	15	0	100
S. aureus	B1	1	99.99	0.01
S. aureus		2	100	0
S. aureus	B2 and B3	15	0	100
B. subtilis	B1, B2 and B3	20	0	100
B. anthracis	B1, B2 and B3	20	0	100
B. cereus	B1, B2 and B3	20	0	100

(%I): percentage of inhibition, (%R): percentage of resistance.

work, it was difficult to assign this anti-bacterial activity towards gram negative bacteria exclusive to hops. However, synergistic effects (hops – alcohol + acid) as well as the action of the food additive incorporated in this beer remained to be verified. Otherwise, *B. anthracis*, *B. cereus* and *B. subtilis* were resistant to the three beers. This resistance may be linked to sporogen character of these bacteria. Indeed, daily observation of cultures showed an acceleration of sporulation, by the presence of spores after 24 h of incubation for *B. cereus*, and 48 h for *B. anthracis* and *B. subtilis* in presence of B1, compared to the control medium, where spores were only observed after 72 h for *B. cereus* and 96 h for *B. anthracis* and *B. subtilis*. B1 could be poor in nutrient thereby depriving the bacteria of some nourishing element, thus leading to premature sporulation. This accelerated sporulation is of dual interest: Firstly, on the cytology plane, the structures of the spore are not yet completely formed, thus the spore remain fragile. Secondly, on the technological plane, a fast, premature sporulation is interesting for food preservation. Given that the sleeping spore is known to be innocuous in terms of pathogenicity (Etoa and Adegoke, 1995; Leclerc, 1995), it would therefore be necessary to determine the composi-tion of this beer, and establish which component could be exploited in food preservation.

Determination of the MBC on the vegetative form

The optimization experimental method allows the determination of the MBC (Figure 2). Independent of the character, gram-positive or negative, these results confirm the following hypothesis: When bacteria are in contact with an anti–bacterial substance, their destruction is not instantaneous but follows a statistical law (Leclerc, 1995; Mackey and Derrick, 1986). Although they are all decreasing, the three curves are not identical.

The destruction curve of *S. typhimurium* shows two phases. A gradual phase from 0 to 1.5 ml, the destruction is slow and a rapid phase from 1.5 ml up to the MBC. These results demonstrate the fact that, in low concentrations, a smaller proportion of the bacteria targets are reached reducing their sensitivity against B1 and therefore, the speed of destruction. At the higher concentrations, the majority of the bacteria targets are attained, with a resultant increase in the speed of destruction. The destruction of *S. aureus* exhibits an exponential curve, suggesting that the destruction of the bacteria is in direct proportion with the increase in the concentration of B1.

The destruction curve of *E. coli* shows 2 phases, at weak concentrations up to 1 ml, where a small proportion of the bacteria targets are reached. At intermediate concentrations, very little change is observed in the number of bacteria destroyed. At higher concentrations of about 3 ml, the bacteria targets are saturated leading to their complete destruction. From these results, we note that B1 act on these bacteria at very weak quantities. Its MBC after 24 h of contact are 1.5 ml for *S. aureus*, 2 ml for *S. typhimuriums* and 3.5 ml for *E. coli*.

Determination of the kinetics of destruction on the vegetative form

While in contact with B1, the destruction of bacteria is not instantaneous. It occurs in a progressive manner (Table 2). We observe a destruction of 98.46% for *E. coli,*

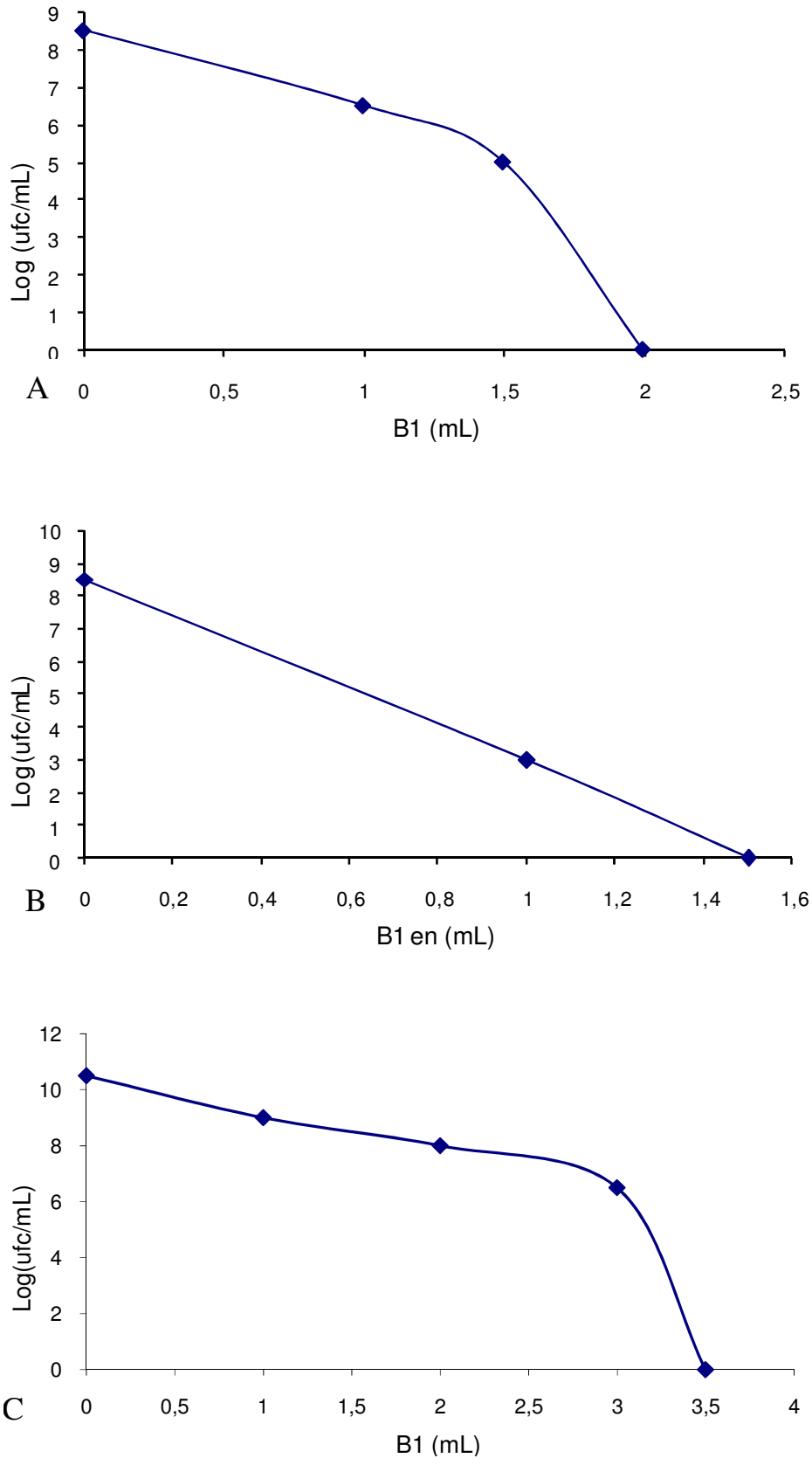

Figure 2. Determination of the smaller quantity of B1 that totally destroyed the bacterium presents in the medium after 24 h of incubation at 37°C. (a): *S. typhimurium*; (b): *S. aureus*; (c): *E. coli.*

Table 2. Evolution of the destruction of bacteria by B1. After incubation at the indicated time at 37°C, the cultures were sown on solid medium for colonies counting.

Bacteria	Time (h)	%	% R
E. coli	1.5	98.46	1.54
	3	86.51	13.49
	6	99.75	0.25
	12	92.94	7.06
	18	95.03	4.97
	24	100	0
S. typhimurium	1.5	91.32	8.68
	3	99.38	0.62
	6	98.68	1.32
	12	90.6	9.4
	18	87.23	12.77
	24	100	0
S. aureus	1.5	99.74	0.25
	3	78.57	21.42
	6	95.6	4.4
	12	99.72	0.27
	18	100	0

(%I): percentage of inhibition, (%R): percentage of resistance.

91.32% for *S. typhimurium* and 99.74% for *S. aureus* after 1.5 h of contact. After 3 h, this percentage of inhibition decrease to 86.51 for *E. coli*, 78.57 for *S. aureus* and *S. typhimurium* decreases to 90.6 after 12 h of contact. These results appear to indicate that after a certain contact time (3 h for *E. coli* and *S. aureus*; 12 h for *S. typhimurium*) the bacteria would have developed defence mechanisms to resist the action of B1. However, the fact that the medium remains hostile, could explain the second increase observed in the percentage inhibition, creating an increase-decrease-increase pattern until complete destruction of the resistant forms (100% of inhibition). This occurs after 24 h for *E. coli* and *S. typhimurium* and 18 h for *S. aureus*, corresponding to their lethal times.

According to the decimal reduction time (D_T), which is the time required at a certain temperature to kill 90% of the bacterial population, D_{T37} (having worked at 37°C) was 2.26 h for *S. aureus*, 2.36 h for *S. typhimurium* and 3.26 h for *E. coli*. We know that, the higher the D_T, the more resistant the bacteria to B1. Consequently, *E. coli* is more resistant to B1 followed by *S. typhimurium*, whereas *S. aureus* appears to be the most sensitive.

According to the kinetic, the destruction of bacteria is done in an exponential manner independently of their gram-negative or positive character (Figure 3). It is characterized by the death rate or number of cells destroyed per unit of time. The destruction of *E. coli* and *S. typhimurium* shows 3 phases: A fast exponential phase during the first three hours of treatment with a kinetic of 6.34×10^5 cells destroyed per minute for *E. coli* and 1.41×10^5 cells per minute for *S. typhimurium*. A transitory phase of destruction of the bacteria is characterized by a rate of 1.6×10^4 cells destroyed per minute for *E. coli* and 7.02×10^2 cells per minute for *S. typhimurium.* A constant phase is very slow with a rate of 0 and 65 cells killed per minute for *S. typhimmurium* and 2 cells per minute for *E. coli*.

The destruction of *S. aureus* exhibits two phases, with a very fast exponential phase during the first 3 h of contact, characterized by a rate of 3×10^3 cells killed per minute, and a slow constant phase of 61 cells per minute.

The higher rate of inhibition observed at the beginning of the reaction, could be attributed to the fact that the bacteria had not yet developed a resistance system. After a certain time of contact, the bacteria begin to develop resistance to the medium, thus reducing their sensitivity and consequently the kinetic of their destruction.

Determination of the anti-spore activity

The evolution of spores, while in contact with B1 shows a cycle phenomenon (Figure 4).

Phase 1

Phase 1 is characterized by an increase in the optical density (OD) indicating an increase in refractivity. This increase is the reflection of an increase of the number of dormant spore. Consequently, B1 would induce dormancy in the spores in the first 10 h of contact for *B. anthracis*, and in the first 5 h for *B. cereus* and *B. subtilis*.

Phase 2

This phase is characterized by the decrease of the absorbance between 10 and 15 h for *B. anthracis*, 5 and 10 h for *B. cereus* and *B. subtilis* pointing to the germination of spores.

Phase 3

Phase 3 represents a subsequent increase in the absorbance from the 15th h for *B. anthracis* and from the 10th h for *B. cereus* and *B. subtilis*. At this level, a non-negligible number of bacteria would have become spore, initiating a microcycle (Etoa, 1985). This phenomenon has been observed with the three used strain.

From these results, we can conclude that, the preservative effect of B1 is only valid during the first 5 h of contact for *B. cereus* and *B. subtilis*, while a limit time of 10 h is observed for *B. anthracis*. In all cases, this preservative effect is owed to the profound dormancy of the

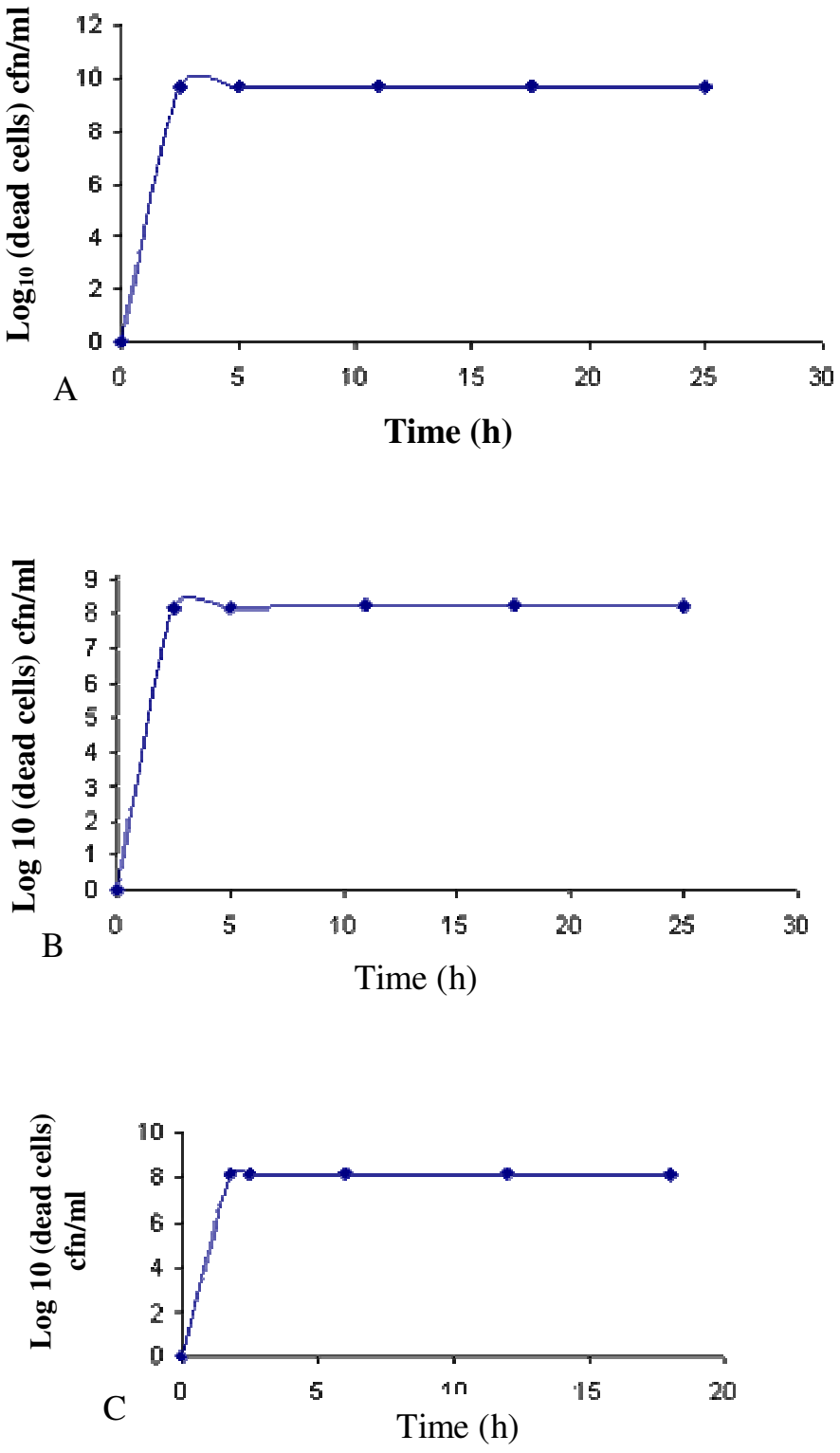

Figure 3. Kinetic of destruction of the vegetative forms while incubating 10^8 cfu/ml cells with minimal bactericidal concentration of B1 at 37°C. (a): *E. coli*, (b): *S. typhimurium* (c): S. *aureus*

spore. We can also speculate that, B1 would contribute to the preservation of food for about 5 h. After this period, it would be necessary to apply moderate preservation thermal treatments like pasteurisation in order to preserve

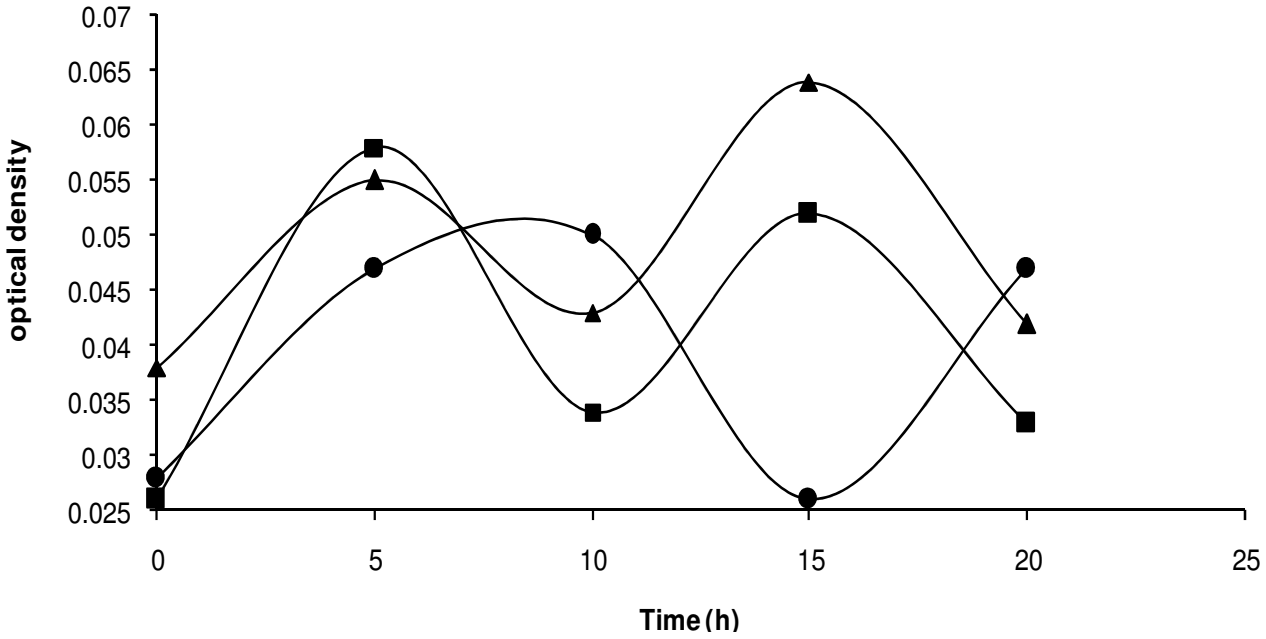

Figure 4. Evolution of three bacteria's spore incubated with 10 ml of B1 at 37°C without agitation, by reading the optical density at 620 nm. (■): Spore of *B. cereus*; (●): spore of *B. anthracis*; (▲): spore of *B. subtilis.*

nutritional properties of food.

Conclusion

From this work, we can conclude that beers B2 and B3 do not have an anti-bacterial activity on the tested bacteria; beer B1 possesses a bactericidal activity on the gram positive as well as on gram negative bacteria. We can therefore say that there is a concordance between the popular practices and results obtained. B1 could therefore contribute to the fight against food contaminations and allow use of soft methods for food preservation. We must note that, this beer will not be directly used as preservative; it should be fractioned in order to extract the active principle responsible for this bactericidal activity. After many studies, this active component could be used as food preservative.

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