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Characterization of *Bacillus cereus* spores isolated from Algerian processed cheese

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The main purpose of this study is to characterize the isolated *Bacillus cereus* spores from processed cheese manufactured and marketed in Algeria. Sixty samples of four brands of processed cheese were analyzed. The panC gene sequencing identified four *B. cereus* spores belonging to Group III, according to the classification of Guinebretiere et al. (2010). In nutrient broth, values of D for *B. cereus* spores (LMBCF001, LMBCF002, LMBCF003, LMBCF004) vary between 4.76 min at 120°C and 93.75 min at 110°C and the values of ZT vary from 7.75 to 21.34°C. In processed cheese, D values obtained for the isolated spores vary between 7.12 min at 120°C and 21.53 min at 110°C. The minimum pH obtained from the four isolated *B. cereus* spores varies between 4.70 and 5.10. Furthermore the minimum aw varies between 0.940 and 0.951. The studied revealed that processed cheese is contaminated by *B. cereus* spores in spite of the pasteurization or ultra-high temperature (UHT) treatment. The contamination origin can be raw material such as milk powder or starch or cheddar. The study focused on the importance of bringing out the presence of this *Bacillus cereus* in such products.

Key words: *B. cereus,* bacterial growth, heat resistance, processed cheese.

INTRODUCTION

Processed cheese is the most consumed dairy product in Algeria. The amount consumed was more than 101 273 tons in 2015 (2.51 kg/year/inhabitant) (CNIS, 2015). The Algerian cheese market is dominated by five brands which hold about half of the market share. It could be commercialized under several kinds in block, semi liquid and solid forms with several flavors. The rest of the production is mainly concentrated in the Western region. The best brands use cheddar cheese as raw material, but many small manufacturers use mixtures of different raw materials.

Processed cheese consists of more recent technology

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than conventional cheese. This technology stabilizes milk nutrients for a longer time and preserves the “cheese aspect” to get the final product (Boutonnier, 2000). Walter Gerber and Fritz Steller invented how to process cheese in 1911 in Switzerland. Its production consists of blending, heating and texturing dairy products (cheese, butter and milk powder) and non-dairy products (agents, emulsifiers and salt). Processed cheese melts at 70 to 95°C for 4 to 15 min (Richonnet, 2016), depending on stirring capacities, final product texture and qualities preservation (Fox et al., 1996).

In Algeria, the process consists of mixture of several raw materials such as: cheddar cheese, milk powder, fat, milk proteins, modified starch and chemical conservatives (salts of cast iron, citrate and poly phosphates). The mixture is heated at 72 to 86°C or 92 to 94°C for 10 min for pasteurized, dissolved cheese, while for sterilized cheese UHT, the heat treatment applied is about 140°C for 2 to 4 s. The heat treatment applied is generally sufficient to destroy vegetative bacteria, but might not be sufficient to eliminate spores forming bacteria (Warburton et al., 1986).

Some companies have effective means but many manufacturers do not have total control of hygiene. So there is an interest in assessing the health risk related to the consumption of processed cheese in Algeria. Thus, the cheese may be the cause of outbreak of food poisoning. In fact, dairy products are involved in 9.83 % cases of overall outbreak of food poisoning, with 19.56% of cases whose causal agent remains indeterminate according to Algerian Ministry of Trading (2015). The microbiological quality of processed cheese is not standardized in Algeria, but in a processed cheese quality control the standards used for this purpose are those applied to the soft cheese in the Algerian Official Journal No.35. Thus, the main researched micro-organisms are total Coliforms, fecal Coliforms, *Staphylococcus aureus*, *Clostridium* sulfite-reducers, *Salmonella* and *Listeria* (Ministerial order JO35, 1998). Otherwise, several microorganisms could be present in processed cheese as aerobic spores forming bacteria especially *Bacillus cereus*. In fact, *B. cereus* is an inevitable bacterium in dairy products (Lücking et al., 2013) and can survive processed cheese heating treatment; otherwise, it can be conveyed by different ingredients such as starch. In Algeria *B. cereus* is not researched as poisoning causal bacterium due to dairy products. So far, few or rare works have reported *B. cereus* and its toxins in processed cheese while some works have studied this bacterium on cheddar cheese, raw material used for manufacturing of processed cheese (Molva et al., 2009; Cherif-Antar et al., 2015).The *B. cereus* group presently consists of seven *Bacillus* species, that is, *Bacillus anthracis, B. cereus, Bacillus mycoides, Bacillus pseudomycoides, Bacillus thuringiensis, Bacillus weihenstephanensis,* and the most recently recognized member of the group, *Bacillus* cytotoxicus, which is thermotolerant. There are two types of *B. cereus* foodborne illnesses. The first type, which is caused by an emetic toxin, results in vomiting, whereas the second type, which is caused by enterotoxin(s), results in diarrhea. The emetic type is caused by a 45-kDa cyclic heat- and pH-stable peptide that is pre-formed in food “Cereulide”. The diarrheal type is caused by three component heat-labile enterotoxins, non-hemolytic enterotoxin (NHE), and hemolysin BL (HBL). Foods associated with the emetic type are commonly farinaceous while the diarrheal type is associated with meat, sauces, pudding, vegetables and milk products (Upasana and Labbé, 2016).

In this context, this study aims to research *B. cereus* in the commercialized processed cheese and its contamination level, its heat resistance, and the influence of some factors (aw, pH and lactic acid concentration) on *B. cereus* growth capacities.

MATERIALS AND METHODS

Samples of processed cheese

The processed cheese samples of four local trademarks (A, B, C and D) were bought directly from the local region of Mascara retail market (360 km west of Algiers) from February, 2011 to May, 2013. Samples were bought from refrigerated displays and had exceeded 50% of their shelf-life. The companies which make the marks A and D are situated in Oran (west of Algeria) whereas the companies making the marks B and C are located in Algiers. The companies manufacturing the marks C and D are considered as large-sized companies while those manufacturing the marks A and B are small-sized companies.

Determining processed cheese pH, aw and lactic acid

Processed cheese pH was measured with a pH-meter (Crisson microph 2001 model), introducing directly pH and temperature probes in a processed cheese sample at 20 to 25°C. Measures were recorded three times. The four processed cheese samples aw were measured with an aw-meter (FA-st/1, GBX France Scientific Instruments). D and L-lactic acids were analyzed adapting MEGAZYME D-LACTATE AND L-LACTATE kit recommendations and according to Noll (1984) and Gawehn (1988)’s methods.

Enumeration of *B. cereus* in processed cheese

Enumeration of *B. cereus* was carried according to the methods recommended through NF EN ISO 7932 norms (2005) in SAS (2010). It consists of the enumeration of presumptive *B. cereus* cells on whole selective Mossel agar (Tryptone (10.0 g) ; meat extract (1.0 g) ; D-mannitol (10.0 g) ; sodium chloride (10.0 g); phenol red (25.0 mg); sterile egg yolk emulsion (100.0 ml); bacteriological agar (13.5 g); and polymyxin B; pH 7.2±0.2) (Pasteur institute of Algeria). Twenty five grams of processed cheese were mixed with 225 ml of TSE (Biokar) and homogenized through a Stomacher (Lab-Blender 400). Decimal dilutions were prepared until 10-6. 0.1 ml of the each dilution was spread on whole

Mossel agar. The Petri dishes were inoculated for each dilution and then were incubated at 37°C for 24 to 48h. The presumptive colonies of *B. cereus* are big, pink (no fermentation of mannitol) and surrounded by a precipitation zone (lecithinase production). Then, the obtained presumptive *B. cereus* was streaked on blood agar and incubated at 37°C for 24h ± 2h. These colonies were supposed to belong to a *B. cereus* group.

Preparation of *B. cereus* spores suspension

Pre-cultures of *B. cereus* strains were realized in Brain Heart Infusion (BHI). 300 µl was spread on Fortified Nutrient Agar (FNA) supplemented with 50 mg/l of MnSO4,H2O and 60 mg/l of CaCl2. Cultures were incubated at 37°C for 5 to 7 days. Sporulation was checked daily by microscopic examination, and spores were harvested when at least 90% of the cells had produced spores. Then, agar medium surface was scraped with rake in a sterile water at 4°C. Spores were washed and centrifuged at 6500r/min for 5 min. Recovered supernatant spores are then centrifuged at 6500r/min for 30 min. The washed suspension was diluted in distilled water and centrifuged twice following the same procedure. The final washed spores were heated at 70°C for 15 min and then cooled in ice for 5 min. Finally, the spores stock was kept in 30% of glycerol. The final concentration could be at 1010 spores/ mL.

Molecular identification of isolates *B. cereus*

The isolated *B. cereus sensu lato* was identified by sequencing of the 16S rDNA gene. Genomic DNA was extracted by using Qiagen kit, PCR. The PCR was carried out by mixing 300ng of DNA and 0.2 mM of NTP (Eurogentec, Seraing, Belgium), 2.5 mM of MgCl2, 0.25 µM of every primer, 0.75 U of AmpliTaq polymerase (Perkin-Elmer, Courtaboeuf, France) and 9 µl of AmpliTaq 10X buffer with no MgCl2 (Eurogentec) for a final volume of 90µL. The PCR reaction was performed by using standard primers: Forward primer 27f (5'-GAGTTTGATCMTGGCTCAG-3') and reverse primer 192r (5'-GNTACCTTGTTACGACTT-3') (Weisburg et al., 1991). PCR cycle was realized in a thermo-cycler (BIO-RAD): a 5-minute start cycle at 94°C, followed by 30 15-second cycles at 94°C, a 30-second cycle at 55°C, a 30-second cycle at 72°C and a final 7-minute extension at 72°C. The obtained amplicons were sequenced in AGCT Biotech Company in Heidelberg, Germany. Finally, the obtained sequences were blasted with the NCBI database (http://www.ncbi.nlm.nih.gov/ BLAST /). The affiliation of *B. cereus* strains was determined according to Guinebretiere et al. (2008)'s procedure. PCR products were purified using "High Pure PCR Product" kit (Roche Diagnostics, Mannheim, Germany) and amplicon sequences were stringed together using Sym'Previus online software (https://www.tools.symprevius.org/bcereus/).

Determination of the minimum growth values for pH and aw

The minimal value of pH and aw allowing growth of the four strains of *B. cereus* were carried out in nutrient medium broth (Fluka, France). The studied pH range (4.51 to 9.35) was adjusted by adding HCl (0.1N) or NaOH (0.1N), and then sterilized by filtration. Otherwise, the aw was adjusted with NaCl according to Morales et al. (2006). The studied aw ranged between 0.940 to 0.982, and n was autoclaved. Finally, 5 ml of medium was inoculated with 10µl of spores suspension and then incubated at 37°C for 24 h. Growth ability is determined by visual observation.

***B. cereus* spore heat resistance**

The heat resistance studied was made by the method of capillaries after preparation of spore suspension. The spores stock was diluted at 1/100. 100 µl of spore dilution was then introduced in the sterile capillaries. The capillaries were sealed and then placed into a glycerol-thermostated water bath at different temperatures (105, 110, 115, 120 and 125°C). After heat treatment, capillaries were removed at regular time intervals and instantly cooled in ice water. Tube ends were scratched with a glass saw, blazed up and cracked. Tube content was expelled with 1 ml Tryptone-Salt dilution towards a 9 ml-tube with the same solution. Decimal dilutions were then realized. Finally, 1ml of every dilution was sown on nutrient agar at 37°C for 24 to 72 h.

Effect of pH on *B. cereus* heat resistance

Effect of pH on *B. cereus* heat resistance was studied in nutrient broth according to the same procedure as previously described for only LMBCF002 strain. The choice of this strain was based on the preliminary results of heat resistance: it seems to be the most representative and the pH change was made for only this strain. The pH of the medium was adjusted to different values (7, 6.5, 6, 5.5, 5 and 4.5) with HCl (1N). It was sterilized through a 0.22 µm filtration membrane. After inoculation of the pH adjusted nutrient broth with LMBCF002 strain, decimal dilutions were performed. Then, 1 ml of each dilution was sown on nutrient agar at 37° C for 24 to 72 h. The decimal reduction time (D) and sensitivity to treatment (z pH) of LMBCF002 *B. cereus* spores were estimated using GraphPad PRISM 6 (GraphPad Software, San Diego, CA, USA).

Determination of *B. cereus* heat resistance in processed cheese

The heat resistance of *B. cereus* in processed cheese was conducted as follows: 45 g of processed cheese was inoculated with spore suspension at 1% and then homogenized in a Stomacher (Lab-Blender 400). Each spore was inoculated in the cheese trademarks, which were isolated. Mixtures were filled into ampule of 1 mL type Wheaton (Sigma Aldrich, France) with sterilized syringe and needle (Spinal Needle quincke Type point 18GA3.00IN, 1.2x75mm). The ampule was sealed and then treated at different temperatures (110 and 120°C), at a rate of ten experimental points between 15 and 90 min. After the heat treatment, ampules were removed at time intervals and instantly cooled in iced water. The first series of ampules was removed, plated on nutrient agar and incubated for 24-48 hours at 37°C. In addition, two other Wheaton ampules were incubated at 37°C for 5 days. The surviving bacteria not affected by the incubation conditions have developed in the food matrix. The presence of surviving bacteria in the ampule is highlighted by seeding in agar nutrient medium. The time of decimal reduction (D) of *B. cereus* spores in processed cheese was estimated by GraphPad PRISM 6 (GraphPad Software, San Diego, CA, USA).

Determining heat resistance parameters

The time of decimal reduction (D) and sensitiveness to treatment (z) of *B. cereus* spores were estimated by GraphPad PRISM 6 (GraphPad Software, San Diego, CA, USA). D values for *B. cereus* were calculated using the average slope for a given treatment. The

Table 1. Characterization of four Algerian processed cheese.

Variable	A n=3	B n=3	C n=3	D n=3
pH	5.750±0.005	5.84±0.01	6.16±0.02	5.74±0.01
Water activity (a_w)	0.978±0.005	0.980±0.004	0.975±0.004	0.965±0.002
Lactic acid (g/100g)	0.151±0.002	0.294±0.005	0.095±0.006	0.211±0.004

Table 2. Enumeration of *B. cereus* in four Algerian processed cheese.

Variable		A n=15	B n=15	C n=15	D n=15
B. cereus (*CFU*/g)	Min	$9x10^2$	$1.5x10^4$	$1.9x10^4$	10^3
	Moy	$1.2x10^3$	$1.9x10^4$	$2.7x10^4$	$1.6x10^3$
	Max	1.4×10^3	2.5×10^4	3.4×10^4	1.9×10^3

value of the inverse slope obtained by plotting log10 D value versus temperature represents the Z value.

RESULTS AND DISCUSSION

Physicochemical and biochemical pH, aw and lactic acid dose of processed cheese

pH, aw and lactic acid concentration of processed cheese

Table 1 illustrates the results of physicochemical and biochemical analysis of four processed cheese samples. The results showed that pH varies from 5.74±0.01 to 6.16±0.02. The results are comparable to pH values (5.2 to 6.7) reported by Boutonnier (2000) and Roustel (2014). Even though pH values reported in this study were slightly superior to the product criterion of 5.6 recommended by the Canadian Food Inspection Agency (2014), they belong to 5.4 to 6 interval recommended by *Codex Alimentarius* (SAS, 2010). Concerning the aw results of the processed cheese, they varied from 0.965±0.002 to 0.980± 0.01. They are like those recorded (>0.970) by Rüegg et al. (1977). However, they are superior to values (0.840 to 0.940) indicated by the Canadian Food Inspection Agency (2014).

Moreover, for the food water activity, the lower microorganisms are heat resistant and therefore the heat treatment is ineffective. Generally, the minimum aw (causing bacterial sporulation is less than that for vegetative growth; it is estimated to *B. cereus* 0.950 (Lozach, 2001). This explains the presence and growth of *B. cereus* germ in the studied samples. Otherwise, the lactic acid concentration in processed cheese varied from 0.095 to 0.211 g/100g as summarized in Table 1. They were lower than the recommended values of cheese paste (<0.3g/100g) and traditional cheese (<1.2g/100g) reported by Arthur and Prashanti (2013).

The lowest lactic acid concentration was explained by the non- addition of lactic acid in processed cheese. The lactic acid could be produced by lactic acid bacteria from lactose. Lactic acid fermentation is generally a fast process. For certain types of cheese such as Cheddar, it must be completed before it is pressed (Henning et al., 2006). That clarifies an important presence of bacteria in studied products, because lactic acid in cheese might have antibacterial effects. This research field is quite new as mentioned by Puah et al. (2013).

Initial contamination of processed cheese

Results concerning searching and numbering of *B. cereus* on complete Mossel agar are shown in Table 2. The results showed that the four studied (A, B, C and D) trademarks processed cheese were contaminated by *B. cereus sensu lato* with maximal concentration equals to 1.4x103 to 3.4x104 CFU/g. These values are approximatively near to risky concentration assigned at 105spores/g of product according to Salustiano et al. (2009). This concentration could be increased depending on consumers' behaviors and high contamination can cause a harm to public health.

Identifying and determining molecular origin of *B. cereus*

The total four isolates selected show characteristic colonies on Mossel agar obtained from different processing cheese samples. After sequencing 16S rDNA gene, molecular identification confirmed that presumed *B. cereus* belonged to *B. cereus* group and the identified

Table 3. Minimal pH and aw (NaCl) for four B. cereus strains isolated from four Algerian processed cheese.

Strains	pH min	a_w min
LMB_CF001	4.72	0.940
LMB_CF002	4.93	0.940
LMB_CF003	5.10	0.951
LMB_CF004	5.10	0.951

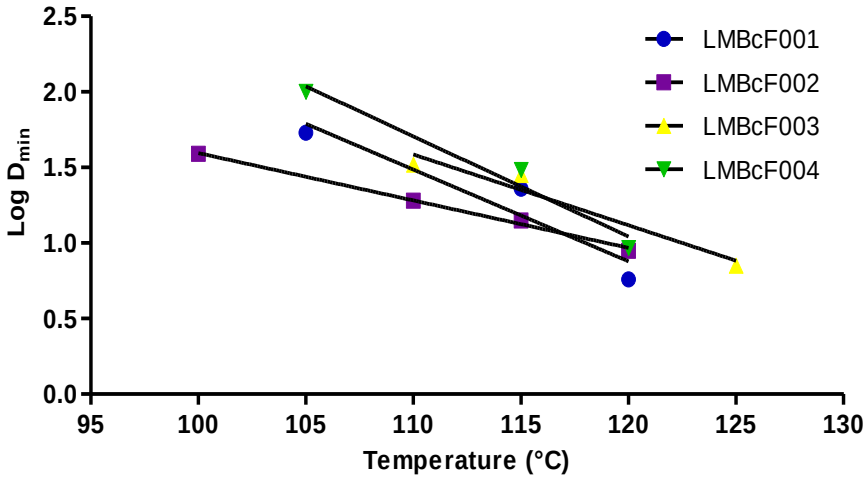

Figure 1. Survival curve for four B. cereus spores isolated from Algerian processed cheese in synthetic medium.

trains were codified as follows: LMBcF001, LMBcF002, LMBcF003 and LMBcF004.

On the other hand, after sequencing *pan*C gene according to Guinebretière et al. (2010), these four *B cereus* strains belong to group III with 97% homology. It consists in a mesophilic group matching with *Bacillus thuringiensis* III, *B. cereus* III or *Bacillus anthracis* species. This strain group is generally cytotoxic and the emetic strains are notably included in this group (Guinebretiere et al., 2008). The fact that isolates belong to group III does not necessary mean the strains harbor the emetic toxin gene. They produced the toxin in food product. The *B. cereus* strains of this group are not involved in the diarrheic syndrome but are involved in emetic syndrome (Guinebretiere et al., 2008; Kumari and Sarkar, 2014). *B. cereus* concentrations are toxicogenic above 105spores/g of the product (Salustiano et al., 2009).

Determining pH and aw minimum values

Few works have studied the impact of pH and aw on *B. cereus* growth. The minimal growth pH for studied strains varied from 4.72 to 5.10 (Table 3). They are higher than the values (4.63 and 4.65) reported for *B. cereus* group III by Carlin et al. (2013). Otherwise, minimal aw values obtained varied from 0.940 to 0.951. They are slightly superior to values (0.941 to 0.944) recorded by Carlin et al. (2013). This may due to the differences of used methods. *B. cereus* is a pathogen, Gram-positive, strictly aerobic or facultative anaerobic bacteria with a 25°C to 37°C optimal growth temperature (Abee et al., 2011) and a 4.9 to 9.3 pH growth acidity (Bermúdez-Aguirre et al., 2012).

Studying *B. cereus* spores heat resistance

Studying B. cereus spores and determining heat resistance parameters

Heat resistance results are shown in Figure 1. The results showed that when temperature of treatment increases, time of decimal reduction (D-value) decreases. At 115°C, the D115°C varied from 23.15 to 32.63min.

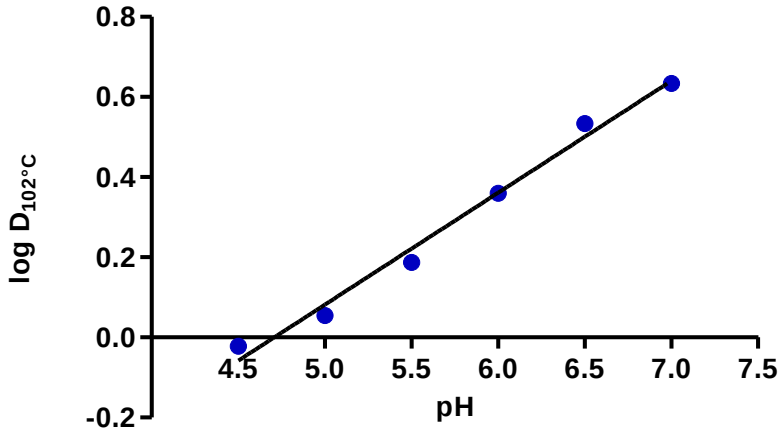

Figure 2. Influence of pH value on LMB_CF002 *B. cereus* heat resistance.

Otherwise, D110°C values ranged from 33.33min to 93.75 min higher than D110°C values documented by Gonzalez et al. (1999) which ranged from 0.080 to 0.36 min. The lowest D-values were observed at 120°C (4.76). D120°C values vary from 4.76 to 9.3 min and are higher than those mentioned by Russell (1999), indicating D120°C value 2,37min.

In this study, *B. cereus* LMBCF002 appeared as the most resistant strain, whereas *B. cereus* LMBcF003 was the most sensitive. D decimal value strain differs according to the phylogenetic group of bacterial species (ANSES, 2011).

The heat resistance depends on its physiological state, temperature and living environment, food composition (lipid…etc.) and characteristics (pH, aw, etc..). Else, the starch in some processed cheese types may explain increase of the heat resistance in heating medium (Oteiza et al., 2003). Nevertheless the study results do not match with those recorded by Mvou Lekogo (2010)'s works.

The sensitivity of heat treatment (ZT) values varies from 7.75 to 21.34°C. The z-values depend on bacteria strains. Diverse factors (surrounding environment during treatment, treatment parameters, etc.) can greatly influence microorganisms' heat resistance (Levy, 2010). ZT values for LMBCF002 match with ZT values reported in the literature for *B. cereus* spores. On the other hand, ZT values for LMBCF001, LMBCF003 and LMBCF004 strains are higher. The highest ZT value ever (21.34°C) was recorded with LMBCF003 *B. cereus*.

B. cereus Z value varies from 8 to 12.5°C, according to ANSES (2011); from 8 to 8.6°C according to Byrne et al., (2006) and from 6.7 to 10.1°C, according to Caudrillier (2008). Mayoraz (2006) reported a 15°C Z value, while characteristic group III ZT values are 8.4°C, according to Luu-Thi et al. (2014).

Studying pH effect on B. cereus heat resistance

It is documented that pH is used as a hurdle to limit microbial development. Food acidification is frequently used as a microbial development limiter. After studying pH influence (pH = 7, 6.5, 6, 5.5, 5 and 4.5) on LMBCF002 strain resistance to heat at 102°C, the results obtained (Figure 2) clearly assess that amount of *B. cereus* decreases as pH does: important when pH = 7 and reverse when pH = 4.5. By the same way, treatment length varies as pH does: decreasing from 15 minutes when pH = 7 to less than 5 minute when pH = 5 at 102°C. The same decrease as heat treatment increases with sample. The resistance decrease is related to proton concentration. Such decrease might not be abrupt but progressive (Weiss, 1921). At 102°C D value (D102°C) for the strain studied LMBCF002 decreased from 4.30 to 0.95min; for pH=7 and pH=4.5 respectively. The result suits those quoted by Lopez et al. (1997) and Couvert (1999). The obtained ZpH=3.58 value was close to ZpH reported by Gaillard et al. (1998). They record a 4.08 ZpH value for a pH range of 4.5 to 6.6 with a constant temperature.

Determination of *B. cereus* heat resistance in processed cheese

All studied *B. cereus* strains have the same surviving kinetic in processed cheese as well as in treatment medium with a temperature from 110 to 120°C (Figure 3). Bacteria heat resistance in food matrix differs in treatments processed in heating medium. The heat resistance of bacteria in food is different to that obtained in the laboratory environment since the decimal reduction time is higher in the food matrix for all samples studied

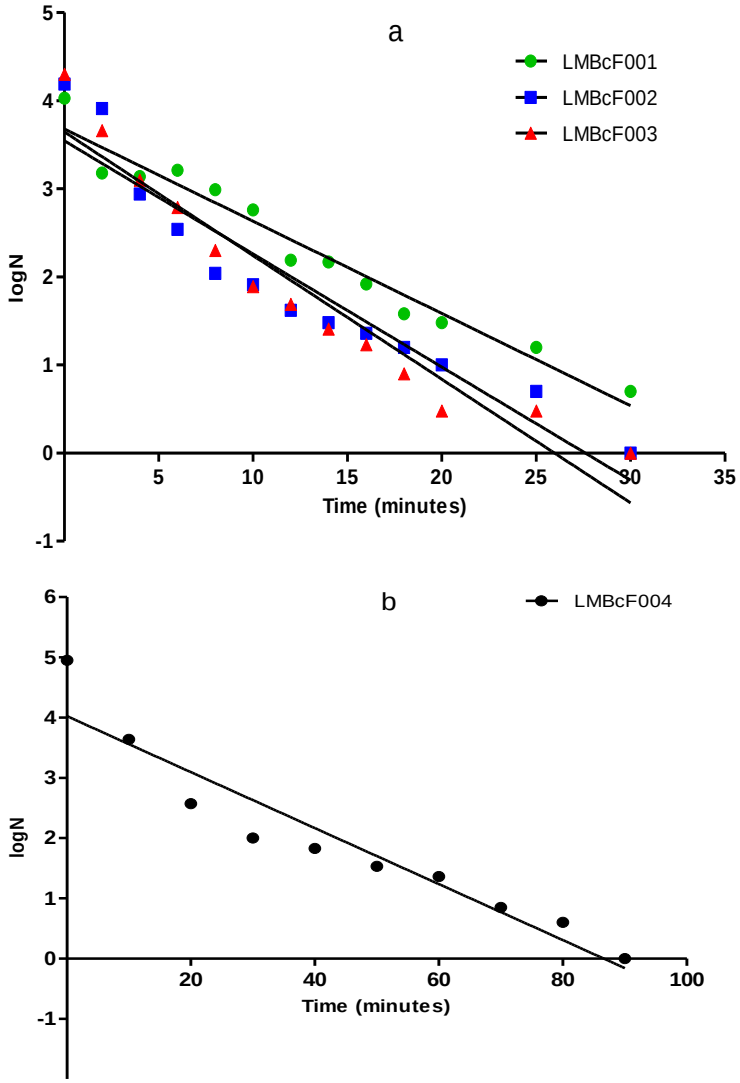

Figure 3. Survival curve for four B. cereus spores isolated from Algerian processed cheese in processed cheese (LMBCF001, LMBCF002 and LMBCF003 B. cereus at 120°C (a); LMBCF004 B. cereus at 110°C (b)).

compared to laboratory conditions. The D110°C and D120°C values appear superior to that obtained in nutrient broth : D120°C value was in the range of 7.12 to 9.55 minutes vs. 4.76 to 9.3 min in nutrient broth. The study notes that there is no total destruction. Bacteria persistence might be explained according to matrix type: in solid matrix, local microenvironments where aw is weaker. Bacteria located in these microenvironments are more heat-resistant compared to global population (Coroller et al., 2006). Otherwise, food products are more complex than nutrient broth and potentially include components that might protect spores (Leguerinel et al., 2005; Samapundo et al., 2014). It is therefore most likely that the macromolecular compounds in foods that is, fat, proteins, and starch may influence the effect of a heat treatment on the survival or outgrowth of spores in food products (Samapundo et al., 2014).

Conclusion

In Algeria, processed cheeses are the most popular cheese compared to other cheese types considered as luxury products. Nevertheless, processed cheese can be hygienically corrupted during preservation due to microbial contaminations. Among microbial contaminations, *B. cereus* is globally part of dairy products alteration flora. The *B. cereus* issue does not figure

among cheese microbiological specifications (Ministerial order JO35, 1998). Microbiological analyses assess presence of *B. cereus sensu lato* in all studied processed cheese samples. *B. cereus* isolated strains belong to group III according to Guinebretiere et al. (2010) classification.

Studies concerning resistance to heat assess that selected spores are resistant to heat treatment during processed cheese production. After validating results in food matrix, we know that these spores can grow in processed cheese. *B. cereus* spores have indeed demonstrated a considerable growth potential in processed cheese. Results might partly explain why unidentified agents responsible of food poisoning are so important in 2015 Algerian statistics. Percentage of unidentified agents represents 19.56% and could be caused by germs as *B. cereus*. Regulations should be amended to take in consideration a contaminant like *B. cereus*. *B. cereus* incidence on cheese can be due to its spore adhesion properties on milk industry surfaces (Marchand et al., 2012; Lücking et al., 2013).

Conflict of interests

The authors have not declared any conflict of interests.

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