Original Research Article

Effect of food matrix, cooking and preservation modalities on the outgrowth of *Bacilluscereus* spores in some starchy foods.

Abstract:

Aim:starchy products are very important in the fight against food insecurity in developing countries, where they are widely consumed. This study is aimed to assess the impact of cooking and storage methods on the spoilage of some cooked starchy products by spore-forming bacteria

Place and duration: Department of Microbiology of the University of Yaoundé I between June 2020 and September 2022

Methodology: In order to better understand the alteration of these foods byspore-forming bacteria, a survey was carried out among some households and restaurant owners in the city of Yaoundé to determine the cooking and preservation methods for cassava, maize and rice couscous. Then,cooking parameters and conservation modalities from the survey were simulated in laboratory in order to assess the deactivation kinetics of *Bacilluscereus.*

Results: It emerged from this experiment that cooking time varies according to the foodstuffs, the most important being maize. The packaging is mainly done in non-food plastic bags, and the thermos is used more often than the fridge and the pot. Cassava flour was more contaminated in aerobic spore (1.10^5 spores/g) than maize and rice (5.10^3 and 9.10^3 spores/g) after cooking of different starchy food enriched with *Bacillus cereus* spores, their inactivation was more important in rice followed by cassava and maize couscous as indicated by the Weibull-parameters which were respectively 1.1; 0.52 and 0.08.

It was also noted that increase of cooking time also reduced the time for spore to initiate outgrowth and this time was affected by the type of flour. These two parameters also affected the time 1 spore outgrowth to 5logufc/g.

Conclusion: Storage in the fridge is still the best way to store food, but it is used less often than the thermos flask. The use of electric thermostats set at temperatures around 60°C would increase the optimal shelf life and significantly reduce the risk of spoilage and foodborne illness.

Key word: Starchy food, *B. cereus* spores, spoilage

Comment [B1]: The methodology is not reproducible. Paraphrase.

1.INTRODUCTION

Food spoilage is a global problem that affects both developed and developing countries. The main cause of this loss is microbial spoilage which results in the loss of about 1/3 of food, especially in developing countries, thus accentuating the problem of food security and famine in these countries [1]. In Africa, and in Cameroon in particular, despite the culinary diversity and the many delicacies encountered, food insecurity and food-borne diseases remain major challenges[2]. Indeed, most of the foods consumed in tropical areas are starchy products that are mostly heat treated, including rice, maize and cassava. Couscous is a typically African food, a traditional dish with regular consumption, made from cereals (maize, wheat, millet) or tubers such as cassava or yam, and the names of this dish may vary from one region or country to another and differentiated with the name of the raw material used. For example, foufou corn or cassava foufou in Cameroon, attieke in Côte d'Ivoire or Too in Togo [3;4;5]. Despite the cooking of these foods, spore-forming bacteria such as *Bacillus* represent the dominant microbial flora, especially *Bacilluscereus* which have the ability to resist heat through the spores they produce and which subsequently germinate to produce toxins that cause illness or spoilage of these foods [6;7].*Bacilluscereus* is mainly involved in the spoilage of starchy products such as rice, pasta and couscous [8;9;10;11].The conditions of cooking, preservation and the raw material used to make couscous can vary from one country to another or from one region to another. While in North Africa, wheat is the main ingredient, in West and Central Africa it is more likely to be maize or cassava and yam [12]. It is a food that is mainly made for direct consumption and sometimes it is preserved for marketing or later consumption. The residual flora after cooking may therefore depend on the quality of raw material used or the cooking method, all of which will have an influence on the shelf life of the food and the risks associated with its consumption. The understanding of the mechanisms related to the alteration of couscous by spore-forming bacteria is in fact an opportunity toenrich the very weak data base explaining the outgrowth of spores according to the type of food matrix. The main objective of this work is therefore to evaluate the impact of the production and conservation methods of maize, cassava and rice couscous on the outgrowth of *Bacilluscereus* spores.

2. Materials and methods

2.1. Food Manufacturing Surveys

In order to get an idea of the production and conservation patterns of the different starchy products, a questionnaire was carried out to determine the cooking time during production, packaging and conservation methods. The survey was carried out randomly among people consisting of restaurant owners and housewives for a total number of 100.

2.2.Strain and culture media: *Bacilluscereus* ATC11966 was used in this work.Nutrient Broth (Oxoid, Basingstoke, UK) was used for the propagation of the strains. Regarding sporulation media, 1L of sporulating agar medium was prepared as indicated by [13], by dissolving, 5 g of peptone, 5g of sodium chloride, 1 g of meat extract, 2 g of yeast extract, 15 g of agar, 0.5 g of dissodic phosphate, 0,1g of calcium chloride, 0,04g of manganese sulphate and the pH adjusted to 7±0.2 before sterilization at 121°C for 15min. The medium was poured into sterile Petri dishes.

2.3.Production of spores: Strain previously stored at -80°C were propagated in nutrient broth (oxoid, Basingstoke,UK)at 37°C for 24h three time before been sowed in the sporulating medium. The incubation was done at 37°C for 7 days. Spores were harvested and washed as describe by [14]. Spores obtained were suspended in sterile distilled water and stored at -18°C for one month before use and considered as stock solution. These spores were more than 99,999% free of growing,sporulating cells and germinating spores as assessed through An “Ivymen System” optic microscope equipped with a phase-contrast device that was used to observe spores at a x100 objective.

Comment [B2]: Not clear. Is it 72 h or 24 h with subculture for 3 times? Use the right words.

2.4.Microbiological analysis of flours

The maize, rice and cassava flours were obtained respectively by grinding maize, rice and dried retted cassava chips, purchased in a market in Yaoundé, Cameroon. The search for spores in the latter was done according to the ISO7932 standard[15]. Indeed, 10g of flour of each sample was diluted in 90mL of distilled water, then decimal dilutions were made and introduced in a water bath at 80°C for 10min then cooled in an ice bath. The dilutions were then surface plated on nutrient agar and incubated at 37°C for 24 h. The spore concentration was obtained according to the formula

$$C = \frac{\sum N * Fd}{1,1 * V}$$

Comment [B3]: Formula is not clear. What is 1,1?

where ∑N is the colony sums from two consecutive dilutions; Fd is the dilution factor;V is the seeded volume in mL and C, is the microbial concentration expressed in cfu/mL. The concentration obtained was expressed in CFU/g.

2.5. Evaluation of spore deactivation and outgrowth kinetics in cassava, rice and maize couscous

The maize, rice and cassava flours were obtained respectively by grinding maize, rice and dried and reddened cassava chips, purchased in a market in Yaoundé, Cameroon

2.5.1. Production of couscous

Prior to flours used for couscous preparation after deliberate inoculum of spore, the flour was dried sterilized in autoclave for 121°C for 20min and microbial analysis in nutrient agar gave undetectable colonies.For the production of rice and cassava couscous 1.5 kg enriched with 2mL of spores contained $7,5*10^{8}$spores/mL in order to have 10^{6} spores/g of rice and cassava flour were cooked in a stainless steel pot containing 4.75 L of boiling water, into which the spore-enriched flour was introduced. The mixture was stirred with a pestle and additional boiling water was added as the mixture was stirred until the cooking was completed when the time of cooking was that selected from the population interview, after the maximum time determined by the survey. In the case of maize, the preparation was done in the same way, with the only difference that half of the flour (750g) is introduced into water (4L) and then brought to the boiling state and the other half of flour (750g) and water (4L) are also added and stirred until the end of the cooking with a wooden pestle.

2.5.2. Deactivation kinetics

During the cooking of the couscous, 20g of each test sample were taken with frequency describe in table1, packed and cooled in an ice bath for one minute, the search for residual spores was done according to the ISO7932 standard. The results obtained were fitted to the Weibull model in order to highlight the deactivation parameters and the distribution of resistance of the *B. cereus* spores.

Table 1: Experimental design for monitoring the evolution of spores deactivation during cooking

Food matrix	*Experimental time (min) For sample*				
Rice	0	10	25	40	
Cassava	0	10	25	35	
Maize	0	10	25	41	57

2.5.3. Spores outgrowth

The cooking time data obtained from the surveys were used to determine the minimum, average and maximum cooking time for each food. From there, the couscous enriched with spores (10^6 spores/g) were cooked at these different times and packed in plastic bags to form balls of 80mm ± 2. These couscous balls were then stored in food thermos flasks that were disposed at room temperature and other batch in a refrigerator. During storage a temperature profile in the thermos and in the refrigerator was evaluated. The microbial load evolution was assessed by taking randomly one ball from the thermos flask and from the refrigerator every 3 h over a period until physical observation of spoilage. 10g of each ball was diluted in 90mL of sterile distilled water, the resulting mixture was left to stand for 5min, then shaken every 3min for 15min. Then decimal dilutions were made and 0.1mL of each dilution was surface plated in Petri dishes containing nutrient agar and incubated at 37°C for 24hrs. The microbial load was obtained by applying the formula described above. The data thus obtained was fitted to the model of [16] using DMFIT software and the kinetic growth parameters (lag time, outgrowth rate and maximum load) were determined.

3.Results

3.1. Cooking, packaging and conservation patterns of couscous obtained from the survey

From the results of surveys reported in Table 2, it can be noted that the duration of cooking after boiling the water varies from 10 to 60 minutes depending on the product. Maize couscous takes longer than rice and cassava. Regarding maize cousous, the mode of the cooking times was 44.80 minutes, while 44.02min was the median and the mean cooking time was 41.7 minutes. In the case of Rice, the cooking time with the highest frequency was 26.80 minutes, while 30,30 min was the median and the mean cooking time was 25.41 minutes. The cooking time of cassava couscous with the highest frequency was 23.60 minutes, while 31.60 min was the median and the mean cooking time was 23minutes.

We also observe that plastic papers are the most used for couscous packaging (68.52%), followed by banana leaves (24.07) and nearly 7.41% of people do not package and leave in the pot. Most of the couscous is kept in a thermos flask (87.27%) for an average time of 19.44 hours. The couscous is kept in a refrigerator by 9.09% of the interviewed participants for an average of 28.40 hours and those who keep it in the pot (3.64%) do so for an average of 18 hours.

Table 2: Production and conservation patterns of couscous

	COOKING TIME (HOURS)			**STORAGE AND CONSERVATION MODALITIES**		
Food Matrix	Avarage	Median	Mode	Packaging modality	Storage modality	Average storage time (hours)
Maize	41.70	44.02	44.80	Plastic (68.52%)	Cooking pot (3.64%)	18.00
Rice	25.41	30.30	26.80	Banana leaf (24.07%)	Thermos flasks (87.27%)	19.44

Cassava	23.50	31.60	23.60	Cooking pot (7.41%)	Fridge (9.09%)	28.40

3.2. Aerobicspores contamination quality of raw flours used

The flours obtained from the market were analysed in order to get an idea of the amount of spores. The results obtained are shown in figure1. From this figure it can be seen that the spores contamination varies from 3 to 5 log cfu/g. Maize and rice flours have similar level of contamination. Cassava flour is the most contaminated with 5 log cfu/g

3.3. Thermal deactivation of spores in couscous from different raw material

During the cooking of the different couscous, a reduction of the spores was observed and the results are illustrated in figure 2. The kinetic parameters estimated after adaptation of Weibull model are also presented in table 3. It can be observed that the reduction of spores is more important in rice and maize than in cassava (Fig2). The highest deactivation amplitude parameter (1.103) is observed in rice. On the other hand, in maize, the deactivation amplitude is the lowest (0.077), and its deactivation speed tends to be constant with a value of n that approaches 1 (Fig 2, Table 3). In terms of individual cells, it can be observed that in rice couscous the average resistance time of the cells is very high (11.860 minutes) compared to what is observed in maize and cassava where the cells are less resistant to the cooking temperature.

Table 3: kinetics parameters of Weibull model

Food matrix	**Weibull parameters**		
	b	**n**	**Tc**
Maize	0.077	0.791	8.160
Rice	1.103	0.165	11.860
Cassava	0.524	0.350	3.040

Tc: Average resistant time of spore cells

3.4.Spores outgrowth in food

The decrease of cooking temperature to mesophile temperature is reached more quickly in the refrigerator than in the thermos (fig. 3a). The monitoring of the temperature evolution in the refrigerator shows that the temperature of the couscous decreases rapidly after 30 min and remains in the mesophilic zone for 120 min and then stabilises at 15°C (figure 3a). On the other hand, in the thermos, the couscous takes longer to cool down and goes into the mesophilic zone after 60 minutes and remains in this zone for about 300 min before with a stabilisationat 35°C (figure 3b). Indeed, the couscous stays more than 400 min in this temperature range in the thermos and less than 150 min in the refrigerator (figure 3).

3.4.1. kinetics parameters of spore outgrowth

The cooked couscous was stored in plastic bags and kept in the thermos at room temperature and in a refrigerator and the evolution of the microbial load was carried out and the results obtained made it possible to have kinetics such as presented by the figure4. It can be observed that outgrowth in fridge did not show visible lag phase and a slow growth rate, compared to thermos stored products. Indeed, maximum *Bacilluscereus* load where obtained 50h earlier in thermos than in fridge.

Table4 present the relation between the cooking time and the latency time of spore outgrowth in the different matrix. In the case of the thermos, it can be noted that the lag time is inversely proportional to the duration of the cooking of the food, in fact, the latency time of spores outgrowth was inversely proportional to the cooking timein different cooking food. The lowest latency is observed in corn couscous (9.90 10^{-9}h) and the highest is observed in rice couscous (5.30h) (Table 4).

Table4: Lag time for outgrowth of *Bacilluscereus* in cooked food and kept in thermos

Food matrix	Cooking Time(min)	Lag (hours)
Maize	57	9.90E-09
	41	7.50E-01
	25	3.50E+00
Rice	40	1.80E+00
	25	4.30E+00
	10	5.30E+00
Cassava	35	1.60E-08
	25	2.30E-08
	10	2.80E+00

It can be noted from table 5in general that the rate outgrowth of spores in the thermos is faster than in the refrigerator. Although there is no clear trend according to the duration of cooking, it can be noted that the highest outgrowth in the thermos (6.134 Logufc/h) is observed in the cassava couscous with had after 10 minutes of cooking. The lowest outgrowth rate (0.181 Logcfu/h) is observed in the corn couscous cooked after 57 minutes. On the other hand, in the refrigerator the highest outgrowth rate (0.178Logcfu/h) is obtained in maize with the lowest cooking time and the lowest rate (0.062Logcfu/h) is obtained in cassava with the lowest cooking time.

Table 5: Rate of outgrowth of *Bacilluscereus* spores in food

FOOD	Cooking Time(min)	Thermos	Fridge
Maize	57	0.181	0.015
	41	0.410	0.045
	25	0.323	0.178
Rice	40	1.091	0.130
	25	0.403	0.065
	10	0.969	0.064
Cassava	35	0.194	0.051
	25	0.276	0.122
	10	6.134	0.062

3.4.2. Amount of microorganisms associates with visual product spoilage

During the conservation of couscous, signs of alteration are generally observed in different ways depending on the type of microorganisms. In this work, the observation of a gelatinous paste in the case of *Bacilluscereus* generally materializes the degradation of starch products. After observation of the latter, the microbial loads were evaluated and the results obtained are reported in figure 5below. It can be seen from this figure that the microbial loads are higher in foods kept in the thermos compared to those kept in the refrigerator. The highest loads are observed in maize and the lowest in cassava.

In general, it can be noted that the time required to observe physical alteration and therefore to reach the maximum load during storage varies significantly according to the cooking time (*P=0.03*) and the storage mode (*P=0.01*). In fact, according to the results, it can be observed that in the refrigerator it takes longer than in the thermos and the products with the highest cooking time generally take longer to reach the spoilage load. Taking into account the fact that the appearance of signs of spoilage are most often noticeable when the microbial concentrations are above the relevant standards, the storage time needed to obtain the threshold value was evaluated and the results are recorded in the table below. It can be noted that cooking time (*P=0.04*) and type of storage (*P=0.04*) have a significant impact on the time needed to reach the unsustainable microbial load. In the thermos the highest storage time is 6.47h obtained from rice couscous cooked for 25min. In the case of the fridge the highest storage time is 59.02h observed with corn couscous cooked for 57min.

Table 6: time to reach to a critical concentration of *Bacilluscereus* as influenced by type of food and cooking time

Food	**Cooking Time (min)**	**Time to reach to maximal load (hours)**		**Time to reach 5logufc/g (hours)**	
		Thermos	Fridge	Thermos	Fridge
Maize	57	30.42	239.02	4.89	59.02
Maize	41	14.59	67.84	1.85	10.06
Maize	25	12.73	11.14	3.45	0.10
Rice	40	4.06	24.06	2.74	7.90
Rice	25	11.60	50.40	6.47	13.48
Rice	10	4.35	47.07	5.93	9.57
Cassava	35	23.29	70.95	4.22	16.05
Cassava	25	13.47	22.27	2.23	5.05
Cassava	10	0.39	35.05	2.83	2.79

4.Discussion

The problem of post-harvest losses is a global reality, in developing countries like Cameroon, most starch products are generally dehydrated or processed into flour for better preservation. However, about 36.12% of processed products are lost at the distribution and consumption stage [17] . These losses are most often due to microorganisms, especially spore-forming bacteria that are able to resist the processing methods used. In this study, maize, cassava and rice flours purchased from local market had aerobic spore concentrations varing from 10^3 to 10^5 spores/g, with cassava flour which is the most contaminated of the three with a load of $1*10^5$ spores/g. Sporulating bacteria of the *Bacillus* genus are mainly found in starch-rich products and the proportion of starch contained in cassava is higher than that of the other two [18;19]. In addition, during the production of cassava flour, the retting stages accentuate the growth of *Bacillus*, and drying in the sun at varying temperatures and for varying lengths of time also favours the formation of bacterial spores [20]. Given these high microorganism concentrations in these foods, the risk of foodborne illness is also high. However, most of these flours, which are generally consumed as couscous after cooking. A survey carried out among some households in the city of Yaoundé in Cameroon shows a clear difference in the cooking and preservation methods of these three starchy products. It is noted that the majority of people cook maize couscous for 44 minutes, rice couscous for 26.8 minutes and cassava couscous for 23 minutes. This difference in cooking time could be due to the water absorption capacity of these flours, which varies according to the composition and structure of the starch in the flours [21;22].

According to the survey, couscous is mostly preserved in plastic bags, as opposed to banana leaves and cooking pots. Despite the Cameroonian government's ban on the use of plastic paper in the packaging of these dishes, it is still widely used, mainly because of the (low) cost and availability of packaging material. Plastic paper is cheaper than banana leaves, which are more common in rural areas. In urban areas, banana leaves are more expensive. The packaging is also done in a thermos as opposed to a refrigerator because the thermos keeps the food hotter which is more appreciated,the use of refrigerator for later consumption is also an alternative.

During the cooking of couscous, it is observed that the reduction of spores during cooking is proportional to the cooking time. Indeed, it is known that heat acts on microorganisms by denaturing their enzymes and nucleic acids. The longer the exposure time, the greater the damage caused, which explains why the reduction in the microbial load is proportional to time. According to the FDA, a heat treatment is considered effective when it eliminates at least 5logcfu/g [23]. In this work, we note that the greatest reduction observed during the cooking of rice is of the order of 2logufc/g. This small reduction could be explained by the thermal resistance of the spores. Indeed, the spores of *Bacilluscereus* are able to resist cooking temperatures for 1 hour[24], in their work, the authors have already shown that during cooking of food, heat treatments can reduce the fraction of spores in food. This resistance can also vary according to the composition of the material on which the treatment is carried out. Carbohydrate-rich foods such as starchy products can play a protective role in heat transfer [25;26] andspores resistance increases significantly with the lipid content of the medium [27;28]. This could explain why rice which contains less lipid than cassava and maize has the highest reduction in spores. However, the observed reduction of a very low amplitude, may cause by the resistance opposed to heat by hydrated starch, however small, may reflect a heterogeneity of the spore population, especially with the existence of a heat-sensitive fraction that is eliminated by heat[29;30].

Normal cooking times temperatures will reduce the residual flora to spores and it is their outgrowth that will cause food spoilage in general. In this work, it is observed that the outgrowth of spores varies according to the conditions under which the food has been stored. From the outgrowth kinetic parameters obtained, a lag phase is clearly observed in the case of foodstuffs stored in the thermos, whereas no lag phase is detected when these foodstuffs are stored in the fridge. This difference could be explained by the different thermal profile observed in the two cases, because in the fridge the temperature goes to the mesophilic zone only after 30 minutes, contrary to the thermos where it takes at least one hour, and with the psychotropic capacity of *Bacilluscereus*, this could explain why the alteration of the food starts quickly in fridge in comparison with the thermos. In the case of the latency observed in the thermos, it can be seen that this varies significantly according to the food and the duration of cooking. Indeed, the outgrowth of *Bacilluscereus* spores starts faster when the cooking time is high. Under these cooking conditions, it can be assumed that the cooking time for the residual spores in the various dishes allows for optimal activation of the spores, since the carbohydrate content of these dishes hinders the transfer of heat into the medium. It therefore takes longer for the spores in the medium to be optimally activated and therefore to germinate and multiply more quickly. In the case of outgrowth rates (development of cells from the spore), it can be noted from a statistical point of view that the cooking time and the type of food do not have a significant impact on the outgrowth of *Bacilluscereus* spores. On the other hand, the latter varies significantly according to the method of preservation. The highest outgrowth rates were observed during storage in the thermos. In the context of this work, it can be noted that *Bacilluscereus*, which is mesophilic, grows faster in conditions that are close to its optimal temperature [31;32]. In the thermos the temperature is maintained in the mesophilic zone for almost 400min while in the fridge it remains in this zone for only 150 min, which may explain the difference in speed observed in these conditions.

Most households generally perceive food spoilage through perceptible physicochemical characteristics such as colour, smell, texture and taste. In this work, the perception of spoilage characteristics varies according to the storage modality from one food to another. The perception of odour, gas and the appearance of a gelatinous paste are more important with a higher *Bacilluscereus* load in the maize meal than in the cassava and rice. The residual spore load in maize couscous is higher and its composition, notably with a higher protein content than that of cassava and rice, could explain the major alteration observed [33;34]. The microbial loads obtained after the observation of microbial spoilage are all above 7logcfu/g and are very high in the thermos compared to the refrigerator. The optimum temperature for growth of *B. cereus* is between 28 and 35°C, but however, this bacterium can grow at a temperature range between 10 and 48°C [35;32]. In the thermos the temperature remains in the mesophilic zone for about 400min and in these temperature conditions *Bacilluscereus* has an important metabolic activity which explains the result obtained. Variable durations for the observation of spoilage are obtained according to the storage method and the duration of cooking of the food.

Beyond its spoilage character, *Bacilluscereus* is also known for its pathogenic character with the capacity to produce toxins which are at the origin of food poisoning. At the physical perception of the spoilage, the microbial loads are already largely superior to those recommended by the standards, therefore the food is at risk before the physical perception of spoilage. Most authors agree that *Bacilluscereus* produces important quantities of toxin when the threshold of the load of 10^5cfu/g is crossed [36;37;38]. In this work it was observed that the time needed to reach the risk load varies according to the cooking time and the cooking method. It is therefore understandable that alteration in the thermos takes less time and is therefore more risky. It would therefore be important to store these foods in temperature ranges that are above or below the growth temperatures of *Bacilluscereus* as shown in the work of [39] who recommends storage above 63°C and below 10°C to limit the growth of *Bacilluscereus*.The refrigeration storage is hence more recommendable than the thermos for it offers less time for *Bacillus cereus* growth.

5.Conclusion

Between rice, maize and cassava couscous, maize couscous has the longer cooking time according to Cameroonian practices (at 41.7 minutes). Despite the ban of non-food plastic packaging, is still widely used, and most of the food is kept in thermos flasks for a period of 18 to 21 hours. The reduction of spores, their growth and the optimum time for limiting the risk of spoilage and toxic infections vary essentially from one dish to another, and it is also affected by the length of cooking and the method of preservation. The maximum storage times in the thermos are 4.89h, 6.47h and 4.22h respectively for maize, rice and cassava. In the refrigerator, they are 59.02h, 13.48h and 16.05h for maize, rice and cassava respectively. The danger is therefore very high during storage in a thermos compared to storage in a fridge, showing the importance of raising people's awareness of the use of electric thermos set at temperatures above 63°C.

REFERENCES

1. FAO. Global foodlosses and foodwaste, Interpack in : Prévention du gaspillage et des pertes des produits de grande consommation : Le rôle clé de l'emballage. *Conseil National Français de l'Emballage.*(2011a). *3p. French*

2. Pouokam G. B., B. U. Saha Foudjo, Chi Samuel, PhilominaFankamYamgai, A. KamdaSilapeux, JoelTaguemkamSando, G. FankamAtonde and ChiaraFrazzoli.Contaminants in Foods of Animal Origin in Cameroon:(2017). A One Health vision for Risk Management “from Farm to Fork”. *doi: 10.3389/fpubh.2017.00197*.

3. Younoussa D , Momar T G , Mama S , Praxède G D , Amadou K , Jean-Paul B , Georges L. Importance nutritionnelle du manioc et perspectives pour l’alimentation de base au Sénégal (synthèse bibliographique*). Biotechnol. Agron. Soc. Environ. 2013 17(4), 634-643. French*

4. Krabi E R, Assamoi A A, Ehon A F. Production d’attieke (couscous à base de manioc fermente) dans la ville d’Abidjan. European Scientific Journal. 2015 *; (11)15: 1857 – 7881. French*

5. Thierry G, TCHUENGA S, Frédéric S. Le maïs : une céréale à multiples usages au Cameroun sous la menace des contraintes climatiques et de ravageurs. Afrique SCIENCE. 2017.13(6): 177 – 188.*French*

6. Lee H Y, Chai L C, Tang S Y, Jinap S., Ghazali FM, Nakaguchi Y, Nishibuchi M, Son R. Application of MPN-PCR in biosafety of *Bacilluscereus*s.l. for ready-to-eat cereals. Food Control. 2009. 20: 1068-1071.

7. Dolores R, Cristina M. Rosell and Antonio M. Risk of *Bacillus cereus* in Relation to Rice and Derivatives. Foods. 2021: 10: 302. Availablehttps://doi.org/10.3390/foods10020302

8. Cadel SS, DeBuyser M.L, Vignaud M.L, Dao TT., Messio S, Pairaud S., Hennekinne JA, Pihier N. and Brisabois **A** Toxi-infections alimentaires collectives à *Bacillus cereus* : bilan de la caractérisation des souches de 2006 à 2010 Bulletin épidémiologique, santé animale et alimentation /*Spécial Risques alimentaires microbiologiques.* 2012**;** *50: 57-61.*

9. Di biase M Study on spore-forming *Bacillus* species involved in break spoilage, contamination risk evaluation and bio-preservation tool. UniversitàDegliStudi Di Bari, Aldo Moro. 2013..

10. Carter L, Chase HR, Gieseker CM, Hasbrouck NR, Stine CB, Khan A, Ewing-Peeples LJ, Tall BD &Gopinath GR. Analysis of enterotoxigenic*Bacilluscereus* strains from dried foods using whole genome sequencing, multi-locus sequence analysis and toxin gene prevalence and distribution using endpoint PCR analysis. International Journal of Food Microbiology.2018; 284: 31-39.

11. Yu S, Yu P, Wang J, Li C, Guo H, Liu C, Kong L, Yu L, Wu S, Lei T, Chen M, Zeng H, Pang R, Zhang Y, Wei X, Zhang J, Wu Q. & Ding Y. A study on prevalence and characterization of Bacillus cereus in ready-to-eat foods in China. Frontiers in Microbiology. 2020; 10: 3043.

12. Mohammed Ziane. Caractérisation, identification et étude de la thermorésistance de souches de *Bacilluscereus* isolées de semoule de couscous. "*THESE*" :UniversiteAboubekrBelkaidTlemcen. 2015.*French*

13. Etoa F. X., A. Nkoue Tong, J. J. EssiaNgang and S. L. SadoKamdem.Effect of selected conditions on spore populations outgrowth dynamics and time to single spore outgrowth distribution in *Bacillussubtilis* and *Bacilluscereus*spores population. Microbiology Research Journal International.2017**;** 18(2): 1-16.

14. Christian A, Rudi F. Effect of sporulation medium and its divalent cation content on the heat and high-pressure resistance of *Clostridiumbotulinum* type E spores. Food Microbiology. 2014; 44:156-167

15. ISO 7932 NF EN. Microbiologie des aliments – Méthode horizontale pour le dénombrement des Bacillus cereus présomptifs – Technique par comptage des colonies à 30°C. ISO/IEC 17025 :2005. General requirements for the competence of testing and calibration laboratories.2005.http://www.iso.org/iso/catalogue_detail.htm? csnumber=39883, (Date accessed: 15 June 2016)

16. Baranyi J, Roberts T. Mathematics of predictive food microbiology. International. Journal of Food. Microbiology.1995; 26: 199-218

17. FAO. Pertes et gaspillages alimentaires dans le monde –Ampleur, causes et prévention. Rome. 2012.

18. Ehling-Schulz M, Vukov N, Schulz A, Shaheen R, Andersson M, Martlbauer E, et al. Identification and partial characterization of the nonribosomal peptide synthetase gene responsible for cereulide production in emetic Bacillus cereus. Applied. Environment And Microbiology. 2005 ;71; 105–113. doi: 10.1128/AEM.71.1.105-113. 2005

19. Ehling-Schulz M, Frenzel E, Gohar M. Food-bacteria interplay: Pathometabolism of emetic Bacillus cereus. Frontiers in Microbiology. 2015 ; 6: 704.

20. Ezo'oMengo Fabrice. Impact de la flore sporulée bactérienne des cossettes de manioc rouies sêchées sur la durée de rouissage du manioc. *Mémoire de Master.* 2010 **;***56 Pages.French*

21. Singh J, Owen J Mc, Harinder S. Physico-chimical and morphological characteristics of New Zealand Taewa (maori potato) starches. Carbohydrate Polymer. 2006

22. ParisaFallahi, KasiviswanathanMuthukumarappan& Kurt A. Rosentrater Functional and Structural Properties of Corn, Potato, and Cassava Starches as Affected by a Single-Screw Extruder, *International Journal of Food* Properties.2016;19(4): 768-788, DOI: 10.1080/10942912.2015.1042112

23. FDA (Food and Drug Administration) – *Bacteriological Analytical Manual – Chapter 14 :Bacillus cereus.*

24. Byrne B; Dunne G; Bolton D J (2006). Thermal inactivation of *Bacillus cereus* and *Clostridiumperfringens* vegetative cells and spores in pork luncheon roll. Food Microbiology

25. Sarang, S., Sastry, S.K. and Knipe, L. (2008) Electrical conductivity of fruits and meats during ohmic heating. J Food Eng 87, 351–356

26. Guillermo C ID , Santiago C and Pilar M. Physiology of the Inactivation of Vegetative Bacteria by Thermal Treatments: Mode of Action, Influence of Environmental Factors and Inactivation Kinetics. Foods. 2017;(6):107; doi:10.3390/foods6120107

27. Chmal-Fudali E and Papiewska A. The possibility of thermal inactivation of Alicyclobacillusacidoterrestris spores in fruit and vegetable juices. Biotechnology FoodSciences.2011; 75: 87-96

28. Samapundo S, Heyndrickx M, Xhaferi R, De Baenst I. and Devlieghere F**.** The combined effect of pasteurization intensity, water activity, pH and incubationtemperature on the survival and outgrowth of spores of *Bacillus cereus* and *BacillusPumilus*in artificial media and food products. *International Journal of Food Microbiology***.** 2014*;181: 10-18.*

29. Pandey R, TerBeek A, Vischer NOE, Smelt JPPM, Brul S, Manders EMM.Live cell imaging ofgermination and outgrowth of individual *Bacillus subtilis* spores; the effect of heat stress quantitativelyanalyzed with SporeTracker. *PLOS ONE.* 2013; *8(3): 58-72*

30. Alicja K, Xiao Y, Boekhorst J, WellsBennik M, Nierop M, Abee T. Analysis of germination capacity and germinant receptor (sub) clusters of genome sequenced *Bacilluscereus* environmental isolates and model strains. Applied Environment and Microbiology.2017; 83:1- 16.

31. Valero M, Hernandez-Herrero LA, Giner MJ. survival isolation and characterisation of a psychrotrophic*Bacilluscereus* strain from a mayonnaise-based ready to eat vegetable salad. Food Microbiology .2007 ; 24: 671-677.

32. RymNouriaBenamara.Identification et caractérisation de spores de *Bacillus cereus* isolées de fromages fondus fabriqués en Algérie.2017**.**

*33. Kombou M N, Joseph A.*Composition protéique et minérale de quelques plats traditionnels cuisinés en milieu urbain (Yaoundé). *Science and TechnoldgyReview*, 1984 **;***1 : 31-44.*

34. Anse-*ciqual. 2020 table de composition des aliments : French*

35. Guinebretière S, Auger N, Galleron M, Contzen B, De Sarrau M-L, Granum D, Lereclus P, De Vos, Nguyen-The C, Sorokin A. *Bacilluscytotoxicus* sp.nov.is a novel thermotolerant species of the *Bacillus cereus* Group occasionally. International Journal System Evolution Microbiology. 2013 ;63: 31-40

36. European Food Safety Authority (EFSA). Risks for public health related to the presence of Bacillus cereus and other *Bacillusspp.* including Bacillus thuringiensis in foodstuffs. EFSA J. 2016, 14, 4524

37. Bursova S, Necidova L, Harustiakova D. Growth and toxin production of Bacillus cereus strains in reconstituted initial infant milk formula. Food Control.2018; 93: 334-343.

38. Rouzeau-Szynalski K, Stollewerk K, Messelhausser U, Ehling-Schulz M. Why be serious about emetic *Bacilluscereus*: Cereulide production and industrial challenges. Food Microbiology. 2020; 85.

39. Glasset B, Herbin S, Guillier L, Cadel-Six S, Vignaud ML, Grout J, Pairaud S, Michel V, Hennekinne JA, Ramarao N, Brisabois A. *Bacilluscereus*-induced food-borne outbreaks in France, 2007 to 2014: epidemiology and genetic characterisation. Eurosurveillance.2016;21(48), 30413. http://dx.doi.org/10.2807/1560-7917. ES.2016.21.48.30413. PMid:27934583.

Figure captation

Figure 1: Amount of aerobic spores in raw flours obtained from local market

Figure 2: Reduction kinetic of *Bacilluscereus* spores as function of cooked food matrix (thick line: Maize; broken line: Rice; dotted line: Cassava)

Figure 3: Temperature profile in fridge (solid line: fridge; broken line food) (a) and thermos flask (solid line: thermos flask; broken line food) (b) during conservation.

Figure 4: Outgrowth kinetic of *Bacilluscereus* spores in fridge (broken line) (a) and thermos flask (solid line) (b) during conservation

Figure5: *Bacilluscereus* cells at load food spoilage

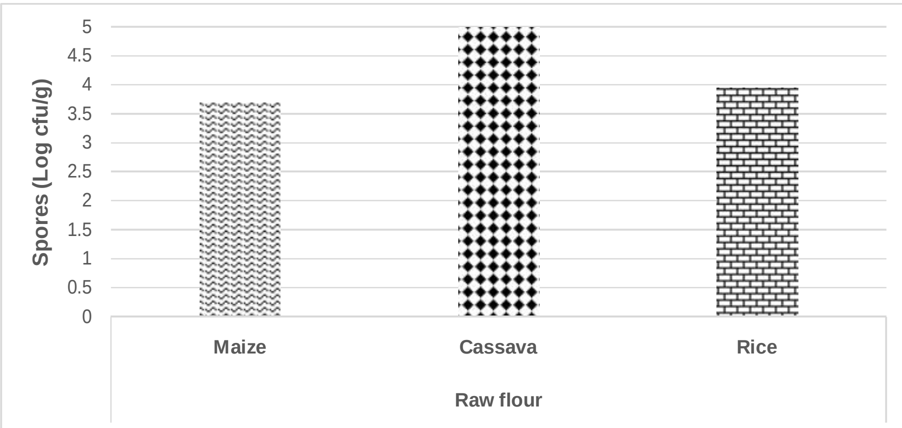


Figure 1: Amount of aerobic spores in raw flours obtained from market

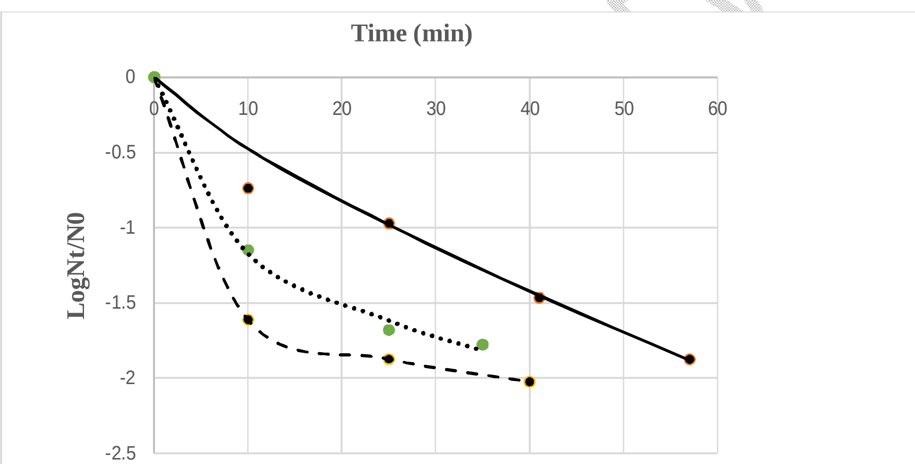
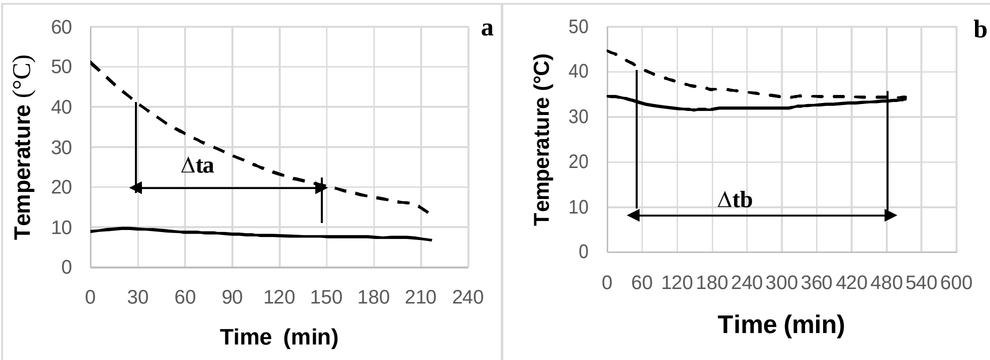


Figure 2: Reduction kinetic of *Bacilluscereus* spores as function of cooked food matrix (thick line: Maize; broken line: Rice; dotted line: Cassava)



Δt: length of time in the mesophilic temperature

Figure 3: Temperature profile in fridge (solid line: fridge; broken line food) (a) and thermos flask (solid line: thermos flask; broken line food) (b) during conservation.

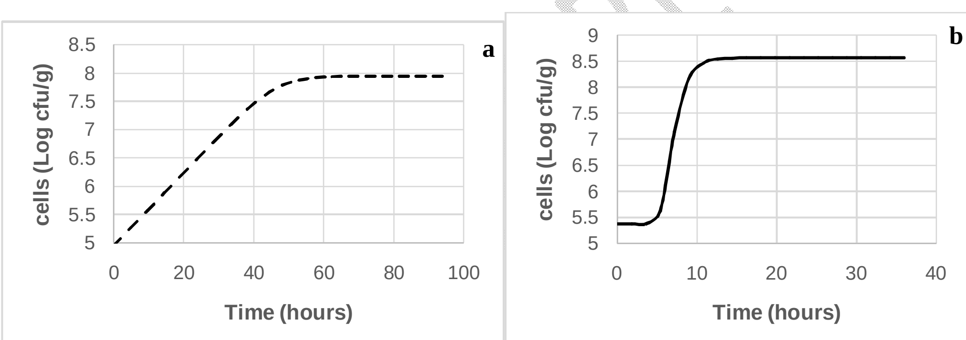


Figure 4: Outgrowth kinetic of *Bacilluscereus* spores in fridge (broken line) (a) and thermos flask (solid line) (b) during conservation

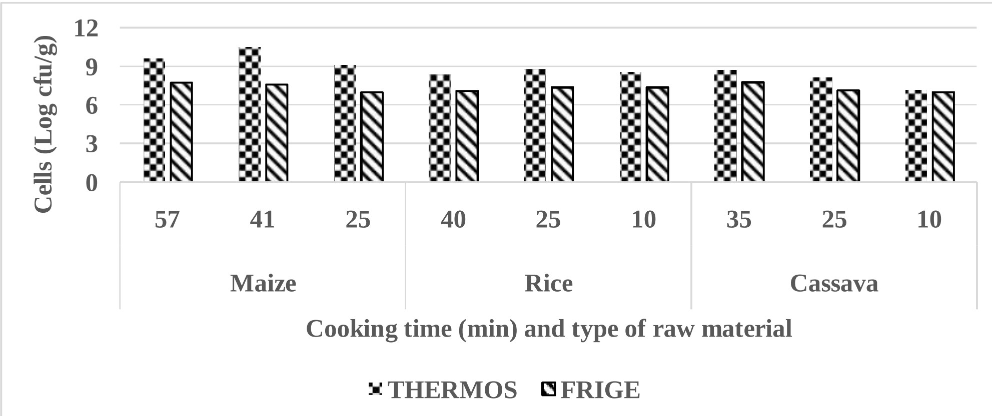


Figure5: *Bacilluscereus* cells at load food spoilage