Effect of Food Matrix, Cooking and Preservation Modalities on the Outgrowth of *Bacillus cereus* Spores in Some Starchy Foods

A. Nkoue Tong [a], M. R. Djoumene Kenne [a], R. Tchikoua [a], S. L. Sado Kamdem [a*] and François-Xavier Etoa [a]

[a] *Départment of Microbiology, Laboratory of Microbiology, University of Yaoundé I, P.O. Box812, Yaoundé, Cameroon.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors ANT, SLSK and FXE designed the study. Authors ANT and MRDK wrote de protocol, managed the analyses of the study and literature searches with author SLSK. Author SLSK performed the statistical analysis with author ANT. Authors ANT, SLSK and RT wrote the first draft of manuscript. All authors read and approved the final manuscript.

Original Research Article

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

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Methodology: In order to better understand the alteration of these foods by spore-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,

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*Corresponding author: E-mail sadosylvain@hotmail.com;

cooking parameters and conservation modalities from the survey were simulated in the laboratory in order to assess the deactivation kinetics of *Bacillus cereus.*

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 scaleparameters which were respectively 1.1; 0.52 and 0.08. It was also noted that increase in cooking time also reduced the time for spores to initiate outgrowth and this time was affected by the type of flour. These two parameters also affected the time of spore outgrowth to 5logufc/g.

Conclusion: Storage in the fridge is still the best way to store food, but the thermos flask is more often used. 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.

Keywords: Starchy food; B. cereus spores; spoilage.

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 Cameroon in particular, despite the culinary diversity and the many delicacies available, 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 [3-5]. Despite the cooking of these foods, spore-forming bacteria such as *Bacillus* represent the dominant microbial flora, especially *Bacillus cereus* 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]. *Bacillus cereus* is mainly involved in the spoilage of starchy products such as rice, pasta and couscous [8-11]. 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 to enrich 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 *Bacillus cereus* 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 among a hundred people consisting of restaurant owners and house wives.

2.2 Strain and Culture Media

Bacillus cereus 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 in 1L of distilled water, 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 each 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.

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

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

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 reddened cassava chips, purchased in a market in Yaoundé, Cameroon.

2.5.1 Production of couscous

The flour to be used for couscous preparation was deliberately inoculated with spores of *B. cereus*, 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 of flours was enriched with 2mL of spores containing $7,5*10^8$ 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 using the time selected from the result obtained from the population interviewed 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 boil 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 described in Table 1, 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, mean 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 placed 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 randomly picking one ball from the thermos flask and from the refrigerator every 3 hrs over a period until physical observation of spoilage. Ten grams 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 45.20 minutes, while 44.02min was the median and the mean cooking time was 42.10 minutes. In the case of Rice, the cooking time with the highest frequency was 27.20 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 24.00 minutes, while 32.00 min was the median and the mean cooking time was 23 minutes.

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.

3.2 Aerobic Spores 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 present in them. The results obtained are shown in Fig. 1. 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 Fig. 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 (Fig. 2). 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

Table 2. Production and conservation patterns of couscous

| Food Matrix | Cooking time (hours) | | | Storage and conservation modalities | | |
	Means	Median	Mode	Packaging modality	Storage modality	Means storage time (hours)
Maize	42.10	44.02	45.20	Plastic (68.52%)	Cooking pot (3.64%)	18.00
Rice	25.41	30.30	27.20	Banana leaf (24.07%)	Thermos flasks (87.27%)	19.44

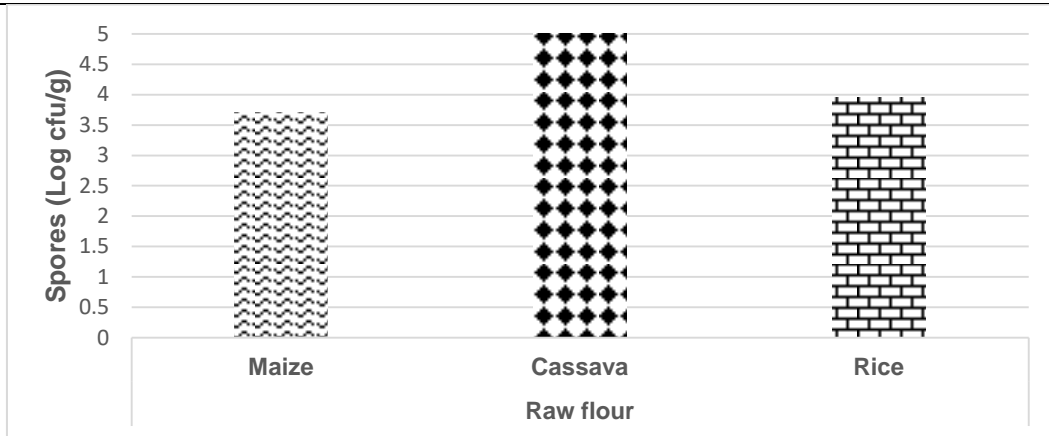| Cassava | 23.50 | 32.00 | 24.00 | Cooking pot (7.41%) | Fridge (9.09%) | 28.40 |

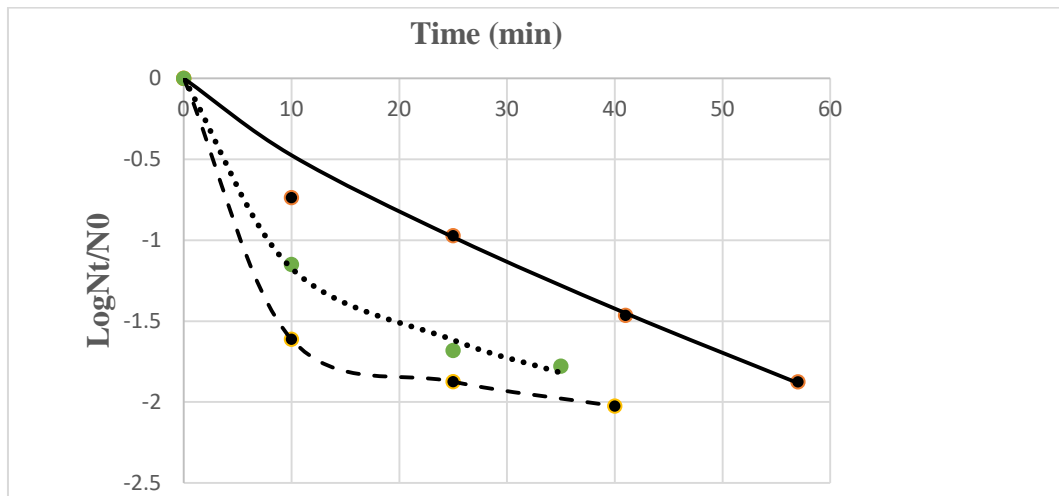**Fig. 1. Amount of aerobic spores in raw flours obtained from market**

Fig. 2. Reduction kinetic of *Bacillus cereus* spores as function of cooked food matrix (thick line: Maize; broken line: Rice; dotted line: Cassava)
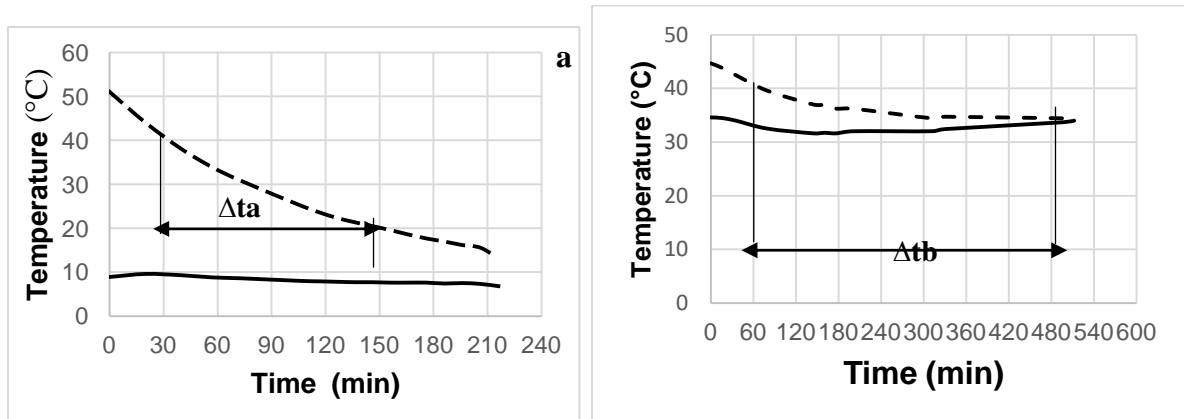

Fig. 3. Temperature profile in fridge (solid line: fridge; broken line food) (a) and thermos flask (solid line: thermos flask; broken line food) (b) during conservation.
Δt: length of time in the mesophilic temperature

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 (Fig. 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 stabilisation at 35°C (Fig. 3b). Indeed, the couscous stays more than 400 min in this temperature range in the thermos and less than 150 min in the refrigerator (Fig. 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 in Fig. 4. 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 *Bacillus cereus* 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 time in 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).

It can be noted from Table 5 in 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 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 4. Lag time for outgrowth of *Bacillus cereus* in cooked food 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

Table 5. Rate of outgrowth of *Bacillus cereus* 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 associated with visual product spoilage

During the conservation of couscous, signs of alteration are generally observed in different ways depending on the type of microorganisms.

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In this work, the observation of a gelatinous paste in the case of *Bacillus cereus* generally materializes the degradation of starch products. After observation of the latter, the microbial loads were evaluated and the results obtained are reported in Fig. 5. 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.
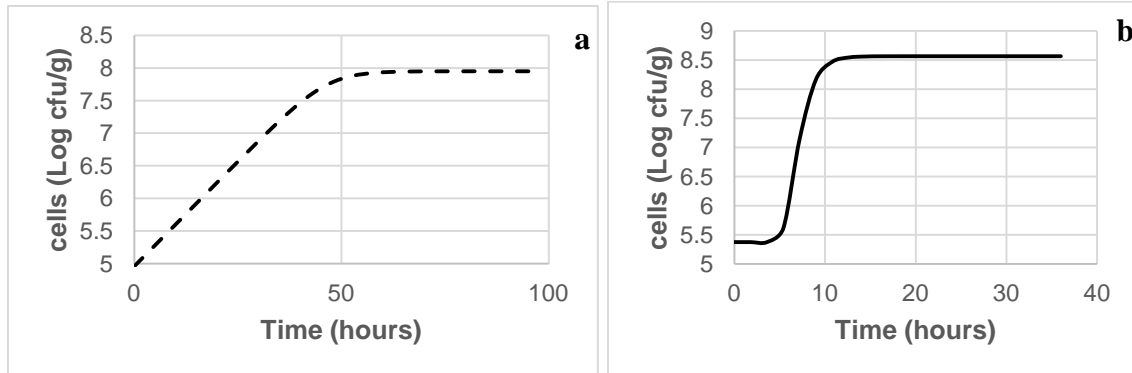

Fig. 4. Outgrowth kinetic of *Bacillus cereus* spores in fridge (broken line) (a) and thermos flask (solid line) (b) during conservation
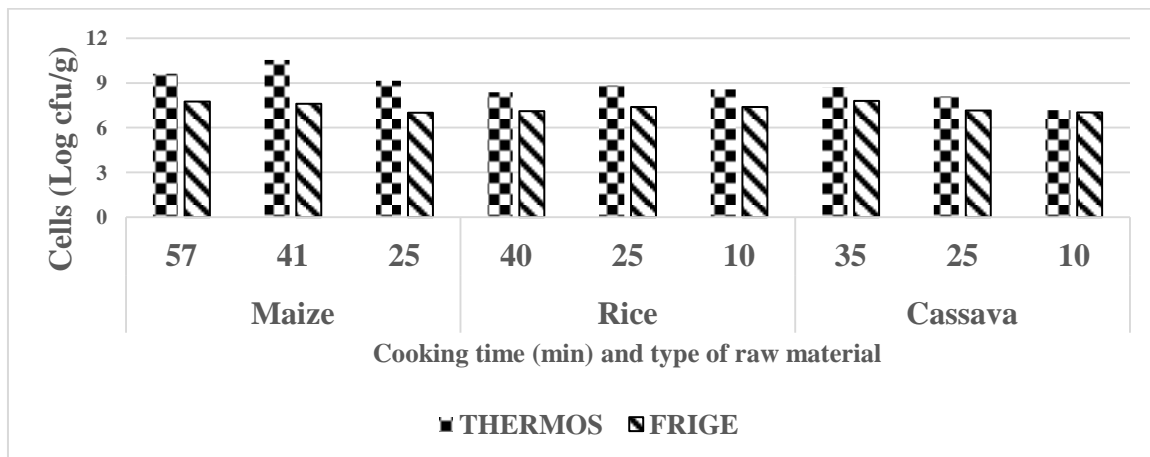

Fig. 5. *Bacillus cereus* cells at load food spoilage

Table 6. Time to reach to a critical concentration of *Bacillus cereus* 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

In general, the time required to observe physical alteration and 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 of microbiology, the storage time needed to obtain the threshold value was evaluated and the results are recorded in the table. 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.

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 varies 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 maize and rice [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 27.20 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. Banana leaves are natural packaging that is highly valued by the population. They retain heat well, but are also very perishable [23]. There is less water condensation in the food with banana leaves, which allows for a longer shelf life of the food compared to plastic water where water condensation is important [24]. 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 5 log cfu/g [25]. 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 *Bacillus cereus* are able to resist cooking temperatures for 1 hour [26], 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 [27,28] and spores resistance increases significantly with the lipid

content of the medium [29,30]. 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 [31,32].

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 *Bacillus cereus*, 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 *Bacillus cereus* 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 *Bacillus cereus* 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 *Bacillus cereus*, which is mesophilic, grows faster in conditions that are close to its optimal temperature [33,34]. 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 *Bacillus cereus* 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 [35,36]. 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 [37,34]. In the thermos the temperature remains in the mesophilic zone for about 400min and in these temperature conditions *Bacillus cereus* 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, *Bacillus cereus* 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 allowed by the standards, therefore the food is at risk before the physical perception of spoilage. Most authors agree that *Bacillus cereus* produces important quantities of toxin when the threshold of the load of 10^5cfu/g is crossed [38-40]. 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 *Bacillus cereus* as shown in the work of [41] who recommends storage above 63°C and below 10°C to limit the growth of *Bacillus cereus*. 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 42.10 minutes). Despite the fact that non-food plastic packaging is banned, 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 5.29hrs, 6.47hrs and 4.22hrs respectively for maize, rice and cassava. In the refrigerator, they are 59.02hrs, 13.48hrs and 16.05hrs 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 on the use of electric thermos set at temperatures above 63°C.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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