ASSESMENT OF MICROBIOLOGICAL QUALITY OF YOGURT PRODUCED IN BAMENDA FROM DAY OF PRODUCTION TO END OF SHELFLIFE

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Abstract
The aim of this work was to analyse total bacteria, coliform, and fungi loads in Bamenda locally produced yogurt from the day of production to the expiry date. Nine yogurt samples were collected each in three batches, and from three different local producers. Three samples per batch were analysed the first day, three within the mid shelflife, and three the last day. Initial total living bacteria load varied from 5.44 ± 0.10 log(cfu/mL) to 6.50 ± 0.10 log(cfu/mL). For each producer, this load fluctuated with time. coliforms load was between 2.47 ± 0.11 log(cfu/mL) to 3.93 ± 0.10 log (cfu/mL). This load was more than the regulatory value. However, in some batches, some samples had coliforms load within the regulatory value. This load reduced during storage to fall less than 10^2 cfu/ml and even 0 in two batches. The initial fungal load was high (5.53 ± 0.10 to 6.37 ± 0.10 log(cfu/mL)) and remained high throughout the shelflife. The contamination was not systemic: Some yogurts locally produced in Bamenda should be recommended for consumption. However, it is not good for children. There is a need to continue training local producers for an improved microbial quality of locally made yogurt.

Key words: Yogurt, total viable count, coliforms, fungi, producer, storage, shelflife..

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INTRODUCTION

Yogurt is a fermented diary product produced by lactic fermentation of milk and is one of the most popularly fermented dairy products consumed throughout the world. In Africa and especially Cameroon, the presence of a traditional yogurt called Kossam (Darman *et al.*, 2013), and traditional dishes like Dakere (Essomba *et al.*, 2002), with Kossam as main part, is an evidence of acceptation of yogurt and milk product since millenaries (Rul, 2017). Since 1930s cow crossbreeds are being introduced in Cameroon (Tambi, 1991; Djoko *et al.*, 2003), leading to increase in milk production and transformation into yogurt (Tambi, 1991) with more and more peasants and corporation groups getting in yogurt production, which could represent a source of income and contribute to improve the welfare of dairy farmers.

Some dairy farmers of the Mezam Division in the North-West Region of Cameroon have been trained by researchers of the Food Technology and Post Harvest Laboratory (FTPHL) of the Institute of Agricultural Research for Development-IARD Bambui (Nchinda *et al.*, 2008). Technologies in milk collection, pasteurization, and yogurt making were extended to small-scale farmers and other milk processors within the ecological zone during that working period (Nchinda *et al.*, 2008; Nchinda & Mendi, 2008). However, the quality of yogurt being produced differs from one producer to another (Lamye *et al.*, 2017; Maïwore *et al.*, 2018).

Yogurt is produced by the controlled fermentation of milk by two species of bacteria; *Lactobacillus debruski* subsp. *bulgaricus* and *Streptococcus thermophilus* that have now been established as the yogurt

starters or yogurt cultures and should be found viable in yogurt. In order to respect this necessity, yogurt is not sterilised at the end of the process. Therefore, during yogurt making, the respect of good hygienic procedure (GHP) and good manufacturing procedure (GMP) is very important to produce safe yogurt which is not harmfull to the consumer and free from contaminant microrganisms such as Gram-negative psychrotrophs, coliforms, some lactic acid bacteria, and fungi (Marshall, 2001; Maïwore *et al.*, 2018). In addition, various bacteria of public health concern such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, pathogenic strains of *Escherichia coli* and enterotoxigenic strains of *Staphylococcus aureus* may also be found in milk and dairy products (Marshall, 2001). Therefore, a lot of interest have been placed on the microbiological examination of milk and dairy foods like yogurt.

Yogurt has been continually studied for its improvement and health benefits in particular. However continuous research needs to be done because it is a product whose outcome is dependent on many other factors including its initial microbial load, packaging and transporting parameters, its storage temperature and shelflife (Marshall, 2001). Alterations in these factors leads to significant effects on the product and its shelflife. Food is best consumed before its expiry date, and for yogurt this date also depends on the way retailers and consumers managed the product (Marshall, 2001; Ledenbach & Marshall, 2009). The need for norms in its production remains then indispensable. Traditionally, yogurt can be stored for up to four weeks. With more peasants and corperation groups getting in yogurt production in Cameroon and especially in Bamenda, it is important to carry out a research on the microbial evolution of yogurt with time to ascertain these locally made yogurt quality throughout its shelflife (Rul, 2017).

The aim of this study is to assess the microbiological quality of yoghurt produced in Bamenda and how it evolves to the end of shelflife set by the producer. This, by following the dynamism of the total bacteria count, coliforms counts as well as total fungal counts.

METHODS

Sample purchase and sampling

Plain yogurt samples were collected from three producers (Mb, Qu, Sb) from three subdivisions of the Mezam Division in the North West region of Cameroon.

From each producer, three different batches of the yogurt were sampled (A, B, C). In each of these three batches, nine 1L yogurt bottles were purchased for analyses. Three samples of each of the batches were cultured on the first day of its shelflife, three at middle of shelflife and three at the end of shelflife. These samples were labelled and transported in a cooler under aseptic conditions to Bambili and were kept in a refrigerator at 4°C containing yogurts for sale.

Preparation and sterilization of materials

0.1% peptone (BR 52, 100 tablets, Oxoid; England) water solution was used as solvent for dilution (Shen & Zhang, 2017). Nutrient agar (NA) (Lifesave Biotech, San-Diego, USA) was used for total plate counts; Sabouraud dextrose agar (SDA) (Lifesave Biotech, San-Diego, USA) for the culture of fungi; and MacConkey agar (MCA) (Lifesave Biotech, San-Diego, USA) to evaluate coliforms present in the yogurt samples. Each culture media was prepared according to the manufacturer's procedure (Jay, 2000; Nisha, 2013; Ray & Bhunia, 2014; Nisha, 2015; Tankeshwar, 2016), and then all material was sterilized. Petri dishes in a dry oven at about 140°C for 24 h; working area cleaned with bleach, and swabbed with 70° alcohol (Jay, 2000; Ledenbach & Marshall, 2009; Ray & Bhunia, 2014; Lamye *et al.*, 2017); and micropipette tips, prepared media, peptone solution autoclaved at 121°C for 15 min (Tankeshwar, 2016). After autoclaving, the media were removed and placed in a water bath at 45°C.

Culture and enumeration of total plate count, coliforms and fungi

Then pour plate method was used for culturing.

For total bacterial plate count, 1 mL of a 10^{-5} and one of a 10^{-4} dilutions were measured using micropipette and put into the different Petri dishes. 15 mL of NA media was poured into the corresponding plates, gradually swirled to mix and allowed to solidify. Then the plates were inverted and incubated at 37°C for 24 h (Nisha, 2013).

For coliforms evaluation, 10^{-1} and 10^{-2} dilutions were plated the same way using MCA. Then the plates were inverted and incubated at 37°C for 24 h (Nisha, 2015).

For fungi count, 10^{-3} and 10^{-4} were equally plated the same way using SDA. Thereafter the plates were not inverted as others and incubated at room temperature for 5 days (Tankeshwar, 2016). From the mid shelflife, 10^{-4} and 10^{-5} were used for fungi evalauation.

All the plates were in duplicates, however there were three control plates.

After incubation, the colonies were enumerated on an electric lit Gallenkamp colony counter by counting the discrete colony-forming units (CFUs) per plate and the results recorded.

Data analysis

The number of colonies, dilution, batches, samples, media, days, were recorded in an excel sheet. Then the colony forming unit (CFU) were calculated as $cfu/mL = cfu/plate \times dilution\ factor$. Correction (greater colonies per plate) were made as a Log_{10} transformation (Rul, 2017). Afterwards, ANOVA was made to compare means that were separated by Duncan at a confidence level of 5% using StatGraphic 11.0 software (Statgraphics, 2014). Figures were drawn with Excel and data were presented as mean±SE.

RESULTS AND DISCUSSION

General microbial load

The total viable bacteria count varied with producers, and samples from the producer Sb had the highest total bacteria count (6.50±0.10 log (cfu/mL)), while those from producer Mb had the lowest (5.44±0.10 log (cfu/mL)) (F=31.68, p=0.0000). Same observations were made for coliforms load and samples from producer Sb had the highest coliforms load (3.93±0.10 log (cfu/mL)), while those from producers Mb and Qu had the lowest (F=54.13, p=0.0000). For fungi, samples from the producer Qu had the lowest load (5.53±0.10 log (cfu/mL)), and there was no statistical significant difference between the loads from Mb and those from Sb (F= 18.28, p=0.0000) (Table 1).

In the same column, value followed by the same letter are not significantly different (α=0.05).

Microbial variation per batch and time for each producer

Three batches from two producers had bacterial load less than 10^6 cfu/mL. In addition, few variations in total bacteria count was observed with time within each batch. These variations were not the same in the batches, even from the same producer (Figure 1).

Table 1: Overall batches and time microbial qualityof yogurt per producer

Producer code	Log (cfu/mL)		
	Bacteria	Coliforms	Fungi
Mb	5.44±10[a]	2.47±0.11[a]	6.37±0.10 [b]
Qu	6.11±0.10 [b]	2.87±0.09 [b]	5.53±0.10[a]
Sb	6.50±0.10[c]	3.93±0.10 [c]	6.11±0.12 [b]
F (p)	31.68 (0.0000)	54.13 (0.0000)	18.28 (0.0000)

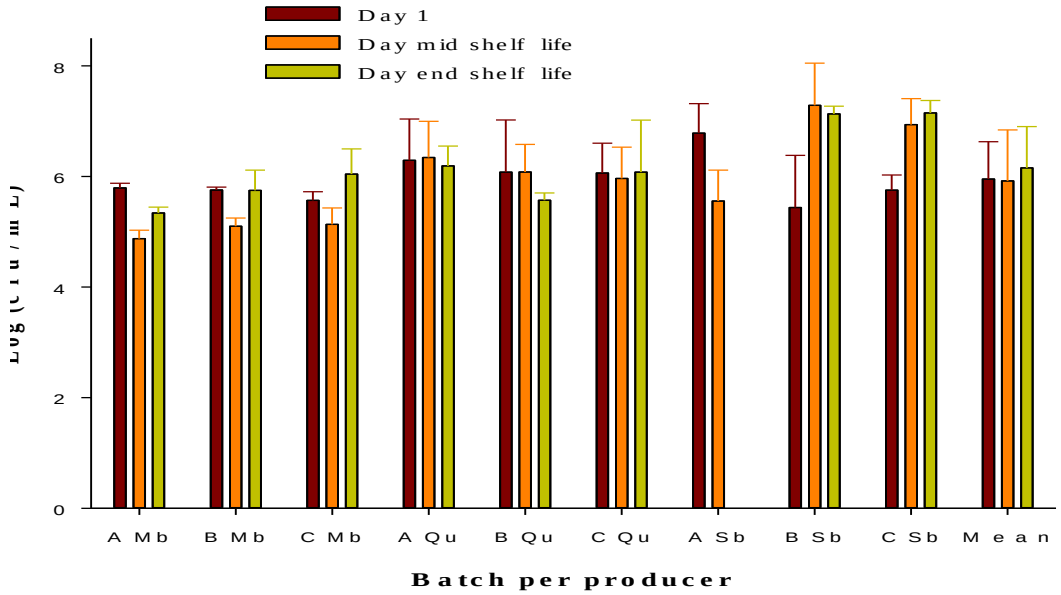

Figure 1: Total bacterial plate count of yogurt variation with producer, batch, and time.
AMb: batch A of the producer Mb; BMb: batch B of the producer Mb; CMb: batch C of the producer Mb; AQu: batch A of the producer Qu; BQu: batch B of the producer Qu; CQu: batch C of the producer Qu; ASb: batch A of the producer Sb; BSb: batch B of the producer Sb; CSb: batch C of the producer Sb.

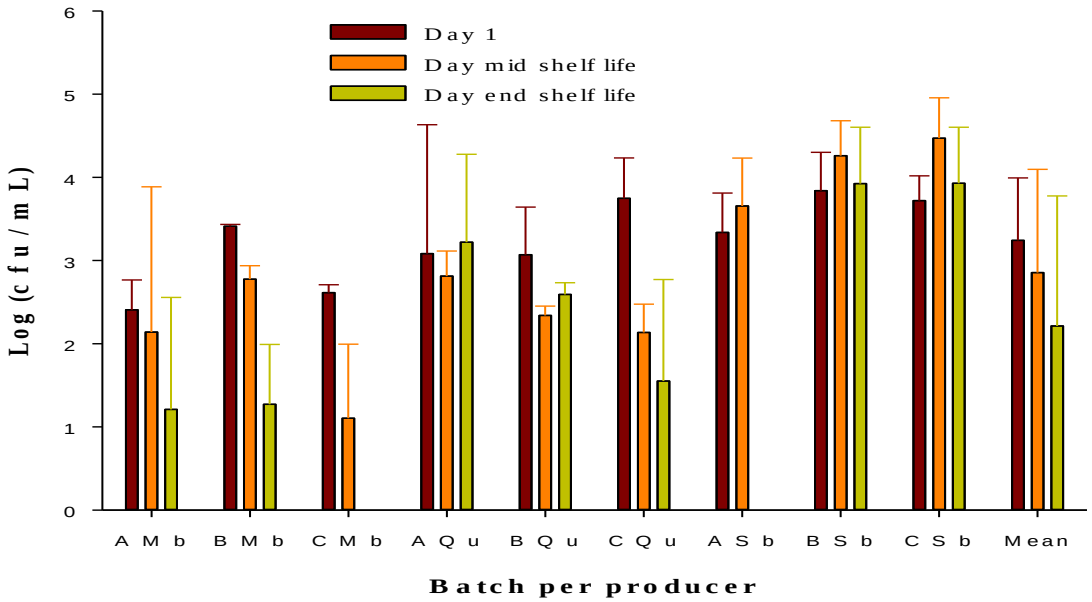

Figure 2: coliforms load of yogurt variation with producer, batch, and time.
AMb: batch A of the producer Mb; BMb: batch B of the producer Mb; CMb: batch C of the producer Mb; AQu: batch A of the producer Qu; BQu: batch B of the producer Qu; CQu: batch C of the producer Qu; ASb: batch A of the producer Sb; BSb: batch B of the producer Sb; CSb: batch C of the producer Sb.

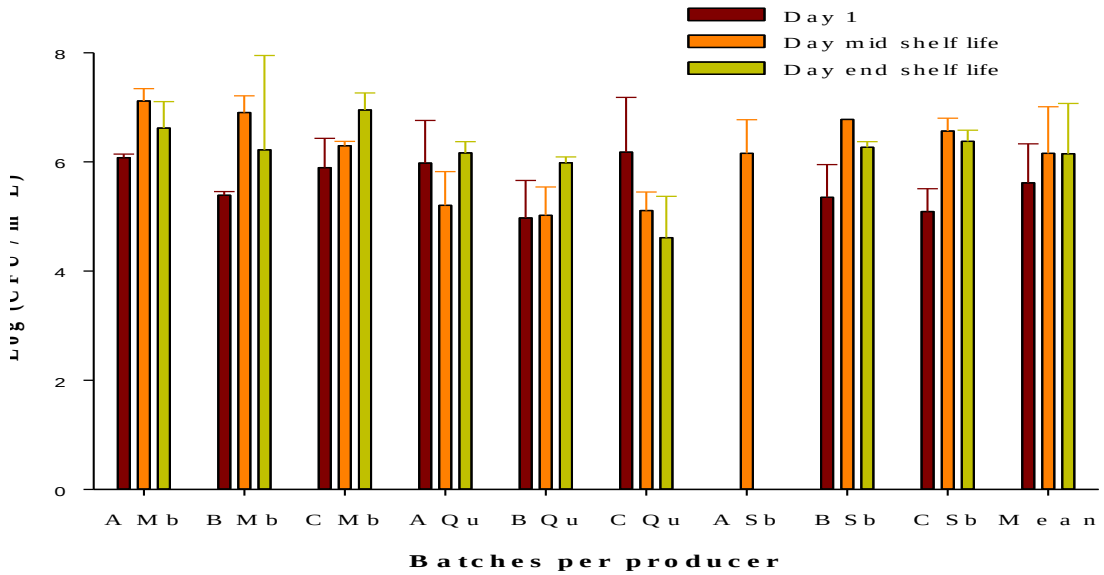

Figure 3: Fungi load of yogurt variation with producer, batch, and time.

AMb: batch A of the producer Mb; BMb: batch B of the producer Mb; CMb: batch C of the producer Mb; AQu: batch A of the producer Qu; BQu: batch B of the producer Qu; CQu: batch C of the producer Qu; ASb: batch A of the producer Sb; BSb: batch B of the producer Sb; CSb: batch C of the producer Sb.

The initial coliforms loads were more than 10^2 cfu/mL. In the batches from producer Mb and in a batch from producer Qu, these loads reduced with time. Reduction that brought the coliforms loads to less than 10^2, and even less than 10 cfu/mL. For the other batches, it reduced before increasing (Figure 2). For two producers (Mb and Qu) at least a sample in a batch had the initial coliforms load less than 10^2 cfu/mL (Figure 2). On the last day, a batch from producer Mb, and another one from producer Sb had a null coliforms load that is illustrated by the absence of coliforms the last day of the shelflife (Figure 2).

The initial fungi loads were at least 4.97±0.69 cfu/mL (BQu's log10 value). Moreover, in exception of the third batch from the producer Qu, these loads varied in a non statistically significant way but remained more than 10^4 (Figure 3).

CONCLUSION

The initial microbial load (5.44±0.10 to ±0.10 log(cfu/mL)) was less than the expected 10^7 cfu/g as recommended by the Codex Alimentarius entry for fermented milk (CODEX-STAN-243-2003, 2011) and the 10^8/g in non-frozen yogurt but more than the 4.70 (log cfu/g) accepted in most of the states in USA (Marshall, 2001). However, the loads we found were less than those obtained in yogurt samples collected from producers in Bamenda from 2012 to 2013 (Lamye *et al.*, 2017). Therefore, our yogurt may not have completed fermentation (Utpal *et al.*, 2015), and may need adjustment in quantity of stater culture used, temperature, or time of incubation. This total plate count was not different from the 6.1 x 10^5 cfu/mL found in Onitsha Metropolis, Anambra State, South-Eastern Nigeria (Ifeanyi *et al.*, 2013), and the $0.57x10^4$ and $1.72x10^6$ cfu/mL found in fermented milk from Maroua (Maïwore *et al.*, 2018). The difference with the result obtained by Lamye and collaborators (2017) can be attributed to the fact that they worked with samples from producers different from those we worked with. The similitude with samples from Maroua and Nigeria can attest that local producers may be producing in almost the same manner.

The coliforms load was high as compare to the regulatory 10 cfu/g (Rul, 2017), and within the 0.00±0.00 to 5.76 ±0.76 x 10^4cfu/mL found in

fermented milk from Maroua (Maïwore *et al.*, 2018), not different from those found in homemaid yogurt samples in Bamenda in 2012 and 2013 (Lamye *et al.*, 2017). However, no initial coliforms load was high as the 4.4 x 10^5 cfu/mL found in yogurt samples in Nigeria (Ifeanyi *et al.*, 2013). The initial coliforms load variation was similar to the variation found in Maroua (Maïwore *et al.*, 2018) and in Nigeria (Ifeanyi *et al.*, 2013). Since coliforms bacteria belong to the larger group of gram negative asporogenous facultatively anaerobic glucose-fermenting bacteria of the family Enterobacteriaceae, and that all of which are killed by pasteurization (Rul, 2017), the presence of coliforms in all the batches is an evidence of nonrespect of good manufacturing or of good hygienic practices. Therefore, we suspected a poor pasteurisation procedure, starter contamination, or postpasteurisation contamination. The postpasteurisation procedure can be at the level of adding sugar or packaging. All what is added after pasteurisation should be kept in an asceptic way. Fortunately, the fact that some samples in some batches had coliforms load less than the regulatory one, and that there was a wide intra-batch variation, this contamination was not systemic: some good samples are produced in Bamenda. However, there is a need to improve in others. The reduction of coliforms load during storage has also been also noticed in yogurt samples from Nigeria (Ifeanyi *et al.*, 2013), and can be because yogurt pH is not friendly to coliforms. Therefore, it may be preferable to conserve locally produced yogurt for about 2/3 the shelflife before consumption. However, it will not be adviceable to give it to infants.

Fungal load was high throughout the shelflife. In a batch, it was even so high that isolated colonies were not observed. No sample with no fungi was found as in some fermented milk products made in Maroua (Maïwore *et al.*, 2018). This fungal load was similar to those observed in Nigeria (Ifeanyi *et al.*, 2013). However, the variation with time was not as pronounced as found in Onitsha Metropolis, Anambra State, South-Eastern Nigeria (Ifeanyi *et al.*, 2013). Of the 61 yeast species found on milk and dairy products, 21 were presents on yogurt whereas nine of about 89 species of mould were also present on yogurt, with much present on raw milk (Garner *et al*., 2017). Intoxication due to fungi in yogurt have neen reported (Lee *et al*., 2014). Since there was also a high initial coliforms load, the high fungi load in our locally made yogurts can be due to raw milk contamination, contamination of former yogurt used as ferment, or wing that can cary spores into the process chamber. This can induce production of mycotoxin, off-odour and flavour (Garner *et al*., 2017). There is a need to control animal feeds, raw milk collection (Garner *et al*., 2017), wing getting in the room where yogurt is being produced and use biopreservation using antifungal bioprotective cultures or fermentates (Garner *et al*., 2017). If not possible, then use safe antifungi like natamycin (Snyder *et al.*, 2016).

Yogurt from local producers needs improvement in microbiological quality. This can be done by adjusting the pasteurization process and improving in GHM and GMP

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REFERENCES

[1] Darman, D. R., Lendzemo, V., Essia-Ngang, J. J. & Etoa, F.-X., 2013. Processing of "Kossam" an African sour fermented milk beverage from northern Cameroon. *Annals of Food Science and Technology*, **14**(2), pp. 261-269.

[2] Essomba, J.-M., Dury, S., Edjenguèlè, M. & Bricas, N., 2002. *La consommation des produits laitiers à Ngaoundéré au Cameroun: l'émergence des MPE (micro et petites entreprises).* In [éd.] Christine Raimond, Eric Garine et Olivier Langlois. Ressources vivrières et choix alimentaires dans le bassin du lac Tchad. Université de Paris X-Nanterre, Paris, Institut de Recherche pour le Développement, pp. 457-475.

[3] Rul, F., 2017. Yogurt: microbiology, organoleptic properties and probiotic potential. In: R. C. Ray & D. Montet, éds. *Fermented food—Part II: Technological interventions.* Food Biology Series, 9781138637849. ff HAL-01579303f éd. s.l.:CRC Press, pp. 418-450.

[4] Tambi, E. N., 1991. *Dairy production in Cameroon: Growth, development, problems and solutions.* In

Branckaert, R.D.S ed., La biotechnologie au service de al production et de al santé animales. [Online] Available at: http://www.fao.org/3/U1200T/u1200T0g.htm#dairy%20production%20in%20cameroon:%20growth,%20development,%20problems%20and%20solutions [Retrieved on the 24 August 2019].

[5] Djoko, T. D.; Mbah, D. A.; Mbanya, J. N.; Kamga, P.; Awah, N. R.; Bopelet, M. 2003. Crossbreeding cattle for milk production in the tropics: effects of genetic and environmental factors on the performance of improved genotypes on the Cameroon western high plateau. *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux*, **56**(1-2), pp. 63-72.

[6] Nchinda, V. P.; Mendi, S. D.; Imele, H.; Kamga, P.; Ngassam, S. B.; Chi, C. M., 2008. Cost and returns of smallholder yoghurt production enterprises in the Western highlands of Cameroon. *Journal of Applied Biosciences*, **9**(1), pp. 372 - 377.

[7] Nchinda, V. P. & Mendi, S. D., 2008. Factors influencing the adoption of yoghurt technology in the Western Highlands Agro-ecological zone of Cameroon. *Livestocks Research for Rural Developmennt*, **20**(7), p. 102. Retrieved August 24, 2019, from http://www.lrrd.org/lrrd20/7/nchi20102.htm.

[8] Lamye, G. M., Lunga, P. K., Pamo, T. E. & Kuiate, J.-R., 2017. Seasonal microbial conditions of locally made yoghurt (Shalom) marketed in some regions of Cameroon. *International Journal of Food Science*, **2017**(4), pp. 1-16. Article ID 5839278, https://doi.org/10.1155/2017/5839278. DOI: 10.1155/2017/5839278.

[9] Maïworé, J.; Baane, M. P.; Tatsadjieu, Ngoune; Fadila, J. A.; Yaouba, M. Yero; Montet, D., 2018. Qualité microbiologique et physico-chimique des laits fermentés consommés à Maroua (Cameroun). *International Journam of Biological and Chemical [*

[10] Marshall, R. T., 2001. Chapter 4: Frozen desserts. In: E. H. Marth & J. L. Steele, éds. *Applied dairy microbiology.* Second éd. s.l.:Marcel Dekker, Inc, pp. 93-125.

[11]Ledenbach, L. H. & Marshall, R. T., 2009. Microbiological spoilage of dairy products. In: W. H. Sperber & M. P. Doyle, éds. *Compendium of the microbiological spoilage of foods and beverages.* New York: Springer Science+Business Media, LLC 2009, pp. 41-68.

[12] Shen, C. & Zhang, Y., 2017. *Food microbiology laboratory for the food science student: A practical approach.* Cham: Springer International Publishing AG. p. 104. DOI 10.1007/978-3-319-58371-6.

[13] Jay, J. M., 2000. *Modern food microbiology.* Sixth éd. Gaithersburg(Maryland): Aspen Publication p. 625. ISBN: 0-8342-1671-X.

[14]Ray, B. & Bhunia, A., 2014. *Fundamental food microbiology.* Fifth éd. s.l.:CRC Press, Taylor & Francis Group, LLC. p. 626.

[15] Nisha, R., 2015. *Sabouraud Dextrose Agar (SDA): Principle, composition, uses and colony morphology.* [Online] Available at: https://microbeonline.com/sabouraud-dextrose-agar-sda-principle-composition-uses-colony-morphology/ [Retrieved on the 15 February 2016].

[16] Tankeshwar, A., 2016. *Nutrient Agar: composition, preparation and uses.* [Online] Available at: https://microbeonline.com/nutrient-agar-composition-preparation-uses/ [Retrieved on the 20 March 2016].

[17] Nisha, R., 2013. *MacConkey Agar (MAC): Composition, preparation, uses and colony characteristics.* [Online] Available at: https://microbeonline.com/macconkey-agar-mac-composition-preparation-uses-and-colony-characteristics/ [Retrieved on the 15 February 2016].

[18] Statgraphics, 2014. *Statgraphics Centurion user manual.* 1 éd. s.l.: Statpoint Technologies, Inc. p. 305. https://www.hearne.software/getattachment/75ca04f6-56bc-41e6-8bf5-a131b7597e01/User-Manual.aspx.

[19]CODEX-STAN-243-2003. Codex standard for fermented milks. [auteur du livre] FAO/WHO. [éd.] FAO and WHO. *Milk and milk products.* Second. Rome : Food and Agriculture Organization of the United Nations, 2011, pp. 6-16. http:// www.codexalimentarius.org.

[20]Utpal, K. P., Khan, M. R., Akhterujjaman & Jahangir, A., 2015. Performance analysis of industrially and traditionally used cultures in yogurt production considering microbial risk. *American Scientific Research Journal for Engineering, Technology, and Sciences*, **12**(1), pp. 92-99.

[21] Ifeanyi, V. O., Ihesiaba, E. O., Muomaife, O. M. & Ikenga, C., 2013. Assessment of microbiological quality of yogurt sold by street vendors in Onitsha Metropolis, Anambra state, Nigeria. *British Microbiology Research Journal*, **3**(2), pp. 198-205.

[22]Garnier L., Valence F., & Mounier J., 20017. Diversity and control of spoilage fungi in dairy products: An update. *Microorganisms*,5(42), pp.1-33.

[23] Lee S.L., Billmyre R.B. Li A., Carson S., Sykes S.M., Huh E.Y., Mieczkowski P., Ko D.C., Cuomo C.A., & Heitman J., 2014. Analysis of a Food-Borne Fungal Pathogen Outbreak: Virulence and Genome of a *Mucor circinelloides* Isolate from Yogurt. *mBio* **5**(4), pp. 1-12. e01390-14. doi:10.1128/mBio.01390-14.

[24] Snyder A.B., Churey J.J., Worobo R.W., 2016. Characterization and control of *Mucor circinelloides* spoilage in yogurt. *International Journal of Food Microbiology*, 228, pp. 14-21.