International Journal of Multidisciplinary and Current Research

Research Article

Production and microbiological evaluation of three types of "Dèguè", a local fermented drink made from milk in Benin

TCHEKESSI C. K. Célestin¹*, BOKOSSA Auréole¹, AGBANGLA Clément², AZOKPOTA Paulin³, DAUBE Georges⁴, SCIPPO Marie-Louise⁴, KORSAK Nicolas⁴, ANGELOV Angel⁵ and BOKOSSA YAOU Innocent¹

¹Laboratory of Microbiology and Food Technology (LA.MI.TA), Department of Vegetable Biology, Faculty of Sciences and Techniques (FAST), University of Abomey-Calavi (UAC); 04BP 1107 Cotonou, Benin; Tel. ++ 229 95 96 29 42;

³Laboratory of Molecular Biology and Formulation of Food, Department of Nutrition and Food Sciences, Faculty of Agronomic Sciences (FSA), University of Abomey-Calavi (UAC); 01BP 526 Cotonou, Benin; Tel. ++ 229 97 22 12 49;

⁴Department of Science of Foodstuffs (DDA), Faculty of Veterinary Medicine, University of Liege (ULg); Boulevard Colonster, 20-Bldg. B43 bis, B-4000 Liege 1, Belgium;

⁵Department of Biotechnology, University of Food Technologies; 26 Maritza Blvd, 4000 Plovdiv, Republic of Bulgaria; Tel. ++ 35932603608.

¹*Corresponding author: TCHEKESSI C. K. Célestin; 04BP 1107 Cotonou, Republic of Benin; Tel. ++ 229 97 81 00 40.

Accepted 20 July 2014, Available online 01 Aug 2014, Vol.2 (July/Aug 2014 issue)

Abstract

This study consists to finalize some technologies for the production of a fermented drink called dèguè. This drink is widely consumed in Benin and other countries in sub-Saharan Africa. Following three different technologies, we had produced three (03) types of dèguè respectively with maize flour, sorghum and millet. These types have been analyzed and their microbiological characteristics were evaluated. The microbiological analysis results obtained from the experiments have shown that lactic acid bacteria, yeasts and molds were the dominant microflora of dèguè and varied respectively 7.22log10CFU/g to 7.55log10CFU/g for lactic acid bacteria and 7.78log10CFU/g to 8.44log10CFU/g for yeasts and molds. Moreover, the statistical analysis of these results showed that there was no significant difference at 5% (p < 0.05) between the three types of dèguè. The values of lactic acid bacteria obtained were consistent with the standard ($\geq 10^7$ /g) attached to the yoghurt. No type contained neither total coliforms nor thermotolerant coliforms. The products (dèguès) were also free of pathogenic microorganisms such as Escherichia coli, Staphylococcus aureus and Salmonella.

Keywords: cereals, milk, dèguè, fermented drink, production.

1. Introduction

Raw milk is a highly nutritious product. It is a suspension (micellar casein in equilibrium, somatic and microbial cells), an emulsion of fat globules and lactose with a solution of hundreds of other soluble molecules of which serum proteins high nutritional value, minerals, growth factors, vitamins, hormones [1]. However, the wealth of nutriments and water made him a highly perishable product.

To keep the milk, man constantly sought to preserve it by transforming it. Among the transformation process available, we notice fermentation.

Fermentation is one of the oldest technologies used for food preservation [2]. It is a technology upon which millions of people in the Third-World for the preservation of their food to make them available to the average consumer. For several decades, fermented foods are very important in the human diet; they are mostly made based cereals, tubers and/or milk [3], [4], [5]. In Benin, most fermented products are prepared with cereals. These cereals are available throughout the country. They are primarily used in the food and consist mostly of maize, millet and sorghum.

Moreover, the fermented products of these cereals often serve refreshing beverages commonly consumed in Benin in particular in urban areas where they appreciated especially during periods of extreme heat. Production plays an important role in the Beninese domestic economy [4]. Among these fermented cereal foods placed "dèguè".

As for the fermentation of milk, it consists in the degradation of lactose into lactic acid and lactic acid bacteria multiplication. As products of this fermentation we have the fermented milks.

According to French law, fermented milk is a dairy produce made exclusively from raw materials of dairy origin (milk and milk components), having undergone pasteurization and fermentation by specific

²Laboratory of Genetics and Biotechnology, Faculty of Sciences and Techniques (FAST), University of Abomey-Calavi (UAC); BP526-Commune of Abomey-Calavi, Benin; Tel. ++ 229 66 44 50 07;

microorganisms and characterized by a lactic acid content minimal (0.6%) [6]. Thus, the yogurt is fermented milk obtained by the development of specific thermophilic lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus bulgaricus* to be planted simultaneously and be alive in the product in an amount of at least ten (10) millions bacteria per gram of product [7]. In Senegal, the fermented milk is very popular due to its acid but pleasant taste, but also through the expansion of supply by the addition of other foods, such as cereals [8]. This milk added to dumplings of cereals flour is called in Benin "dèguè".

In origin, dèguè is a product made from milk and millet flour. It is a food widely consumed in Benin and beyond to West Africa.

This alimentary product is sold in local markets in Benin, along the lanes, public places, but also in schools and university campuses [9]. Production of dèguè generates jobs of thousands of people especially women, whose education and skills in food processing are often limited. These kinds of foods (aliments) are defined by the FAO (2007) [10] as food and beverages ready for consumption prepared and/or sold by street vendors or fixed, especially in streets and other similar public places. They are produced without any rules of good practices of hygiene of production and marketing. Thus, production environments are not safe, low adequate infrastructure and low grade. Other problems also exist in producing, for example, the non-control manufacturing parameters such as temperature, fermentation processes, etc. This lack of control parameters can cause food-borne infections.

Faced with all these problems related to the consumption of food streets and given the socio-economic importance of dèguè, it is timely important to finalize technologies for the production of different type of dèguè of good health and nutritional qualities.

Material

Maize (*Zea mays L.*), millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) are the three cereals used; they are bought at the local market of the town of Abomey-Calavi.

The yogurt "nature" composed of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* has been bought at the Togoudo pharmacy in the city of Abomey-Calavi. Milk powder of brand LACSTAR of Irish origin bought at the market in Dantokpa at Cotonou served animal material. The water from the National Water Company of Benin (SONEB) was used in the production of dèguè.

Methods

Production of dèguè

Three types of dèguè were manufactured respectively based flours of maize, sorghum and millet, but before this

it is necessary to get the pellets of these cereals and fermented milk.

Manufacture of the pellets

The cereal grains weighed, cleaned (sorted out, sieved) and washed three times with tap water were drained and then dried for forty-eight (48) hours under sunlight. Afterwards, the dried clean grains were weighed again and ground in a mill drives brand N°A PREMIER GRINPING with a frequency of 1760tours/min, its power is 25.50kwa to obtain the different flours. 1kg of flour was divided into three equal parts. We took two thirds (2/3) of the aforesaid flour that are mixed with 700mL of water; then stir well and a paste is obtained. The pellets have been made by hand rolling by mixing gradually the third (1/3) of the flour with dough and turning regularly for 5 to 10 minutes. The pellets formed are finally steamed for 15 to 20 minutes. After cooling, they are ready for use.

Production of fermented milk

The fermented milk was obtained from the reconstituted milk powder. Thus, we took 1kg of milk powder that we mix with seven (07) liters of warm water at 35°C; the mixture is then pasteurized at 80°C for 05 minutes then cooled to 45°C. The reconstituted milk was inoculated with nature yoghurt. Finally, the fermented mixture is left at 45°C for 8 hours. For 1kg of milk are added two pots of yoghurt 125g each.

Assessment of microbiological quality

Microbiological analysis consisted in counting the total mesophilic flora [11] on PCA (Oxoid CM 0325), yeasts and molds [12] on Sabouraud Dextrose Agar (Oxoid CM 0041) at chorampphénicol (0.05g/l), lactic acid bacteria [13] on MRS-Agar (Oxoid CM 0361), total coliforms [14] and thermotolerant coliforms [15] on VRBA-Oxoid CM 0107 (Violet Red Bile Agar), *Staphylococcus aureus* [16] on Baird Parker Agar (Oxoid CM 0275 BP) egg yolk and potassium tellurite and *Salmonella* [17] on SS Agar. The counting was done by counting the colonies [18]. Microbiological analyzes were performed in triplicate on each sample dèguè.

Statistical analyzes of data

The Excel software was used to perform the calculations. Analysis results were processed using SPSS 16.0 software that permitted to make analysis of variance (ANOVA) and Tukey's test for comparison of means. The significance level of 5% is selected (p < 0.05).

Results and discussion

Manufacture of the pellets

The results of the production technology of different types of pellets are shown in Figure 1.

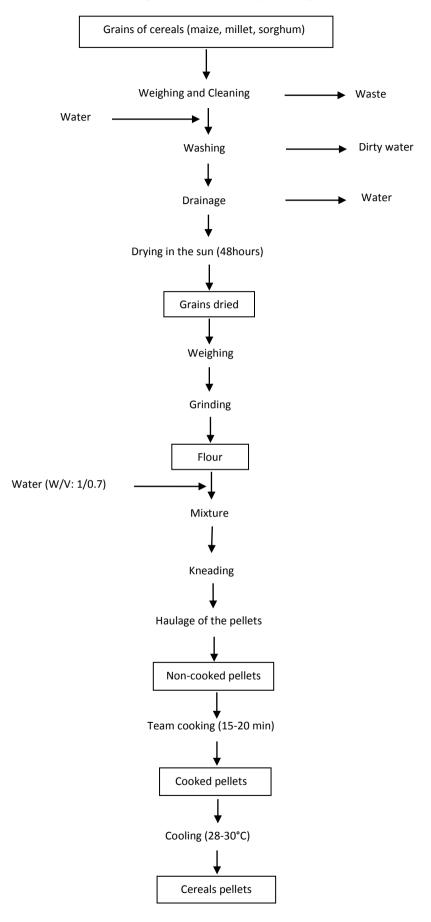


Figure 1: Technological diagram of production of cereals pellets

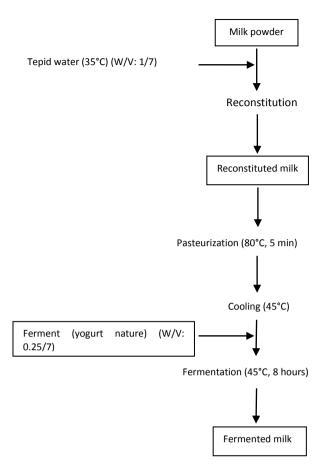


Figure 2: Technological diagram of production of fermented milk

The most important unit operation of the technological diagram of the production of the pellets is the haulage (Figure 1). It is essentially manual and tedious. Rolling called over know-how with respect to the adjustment of the size of the pellets, by adding water or by the intensity of mixing. This allows having pellets too soft, medium and acceptable sizes. This is a stage that determines the quality of dèguè. The finalized technology provides three types of pellets. It differs from the one traditionally known based millet flour described by Agbanzoume in 2005 [19] by the use of other flour.

Production of fermented milk

The results of production of fermented milk are shown in Figure 2.

Production of the three types of dèguè

The results of the technological manufacturing of the three types of dèguè are shown in Figure 3.

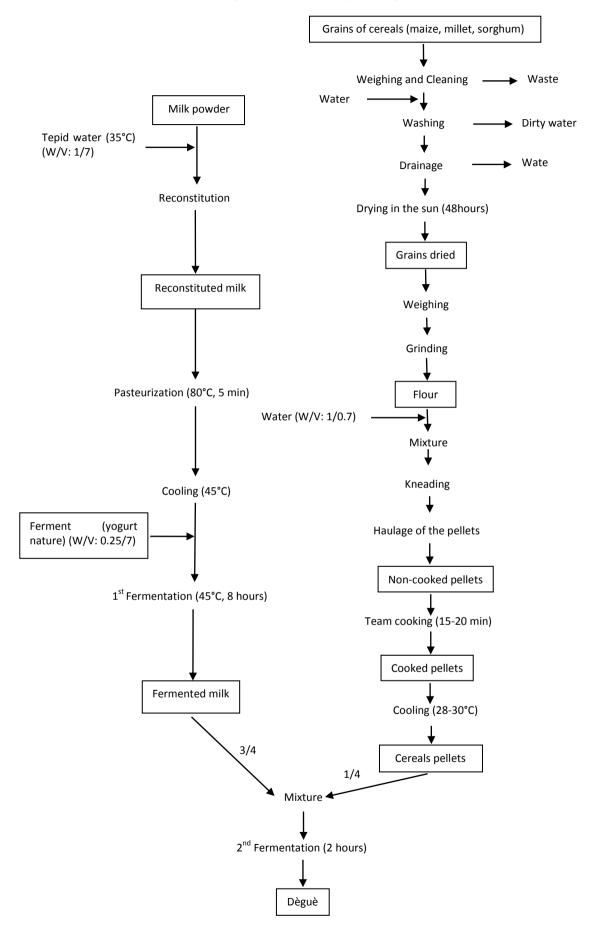
According to Figure 3, mixing and fermentation are the major operations of the manufacture of these types of dèguè. After experiments, the formula 25% of cereals pellets and 75% of fermented milk is adopted. All dèguè

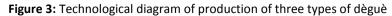
were prepared according to this formula which ensures the organoleptic characteristics necessary and acceptable in our finished products. The second fermentation which lasts 2hours provides thorough mixing and fermentation microorganism's revivification. Adding cereal products stimulates the growth of lactic acid bacteria during the second fermentation. This phase promotes the production of lactic acid to allow yeasts growth. This experiment was also made by Tchekessi *et al.* in 2013 [4].

Microbiological characteristics

The results of microbiological analysis of different types of dèguè are presented in Table 1.

Statistical analysis of microbiological results show that all values of p-value greater than 0.05 are obtained (p> 0.05). This study reveals that there is no significant difference at the 5% level between the three types of dèguè products (Table 1). Total mesophilic aerobic germs, yeasts and molds of each type are higher than the respective values 10^5 /g and 10^2 /g set by AFNOR for yoghurt. A difference can be explained by the incorporation of cereal pellets in fermented milk. As for the results of lactic acid bacteria, the values obtained are consistent with standards ($\geq 10^7$ /g) set for yoghurt and





Types of Dèguè	Studied parameters						
	Total mesophilic flora (Log10CFU/g)	Yeasts and molds (Log10CFU/g)	Lactic acid bacteria (Log10CFU/g)	Total coliforms (Log10CFU/g)	Thermotolerant coliforms (Log10CFU/g)	Staphylococcu s aureus (Log10CFU/g)	Salmonella (Log10CFU/g)
Dèguè Maïs	7.85±0.97 ^a	8.44±0.86 ^ª	7.22±0.06 ^a	<1ª	<1ª	<1ª	Abs/25g
Dèguè Mil	7.31±0.19 ^a	7.78±0.99 ^a	7.49±0.02 ^a	<1ª	<1ª	<1 ^a	Abs/25g
Dèguè Sorgho	7.82±0.51 ^ª	7.91±0.38 ^ª	7.55±0.14 ^ª	<1ª	<1 ^ª	<1 ^a	Abs/25g
Norms of yoghurt (germs)	10 ⁵ /g	≤10 ² /g	≥10 ⁷ /g	10/g	1/g	Abs/g	Abs/25g

Table 1: Microbiological characteristics of three types of dèguè

In each of the columns, means of triplicate samples were recorded with different superscripts differ significantly (P < 0.05).

± Standard deviation; Abs: Absent.

 $(\geq 10^6/g)$ set for probiotic fermented milks by AFNOR NF V 04-600, 2001 cited by Beal and Sodini in 2003 [20]. Total and thermotolerant coliforms, Staphylococcus aureus and Salmonella did not grow after seeding. This shows that the production of three types of degue comply the standards of viewpoint hygienic quality and merchantability. Analysis of the results indicates overall that the dominant flora of dèguè is constituted of lactic acid bacteria and yeasts and molds. Therefore, the introduction of cereals flour dumplings would further enriched in milk fermented by lactic acid bacteria and yeasts and molds. These results are consistent with those obtained by Gymnase in 2011 [21] which states that cereals contain prebiotics which stimulate the growth of microorganisms in the case of bacteria. Indeed, lactic acid bacteria from their carbohydrate metabolism caused by acidification of the food become favorable to growth of yeasts and molds. Which acidification is the hygienic point of view a major asset because it prevents the growth of most pathogens (Tchekessi et al., 2013) [4]. Thus, the preservative effect of lactic acid bacteria in the manufacture and storage of fermented foods is mainly acidic conditions they create, by converting carbohydrates into organic acids. These results are consistent with those of Mavhungu in 2006 [22].

Conclusion

This study allows us to finalize a technology for the production of three types of dèguè. The dominant flora of each type is lactic acid bacteria and yeasts and molds. The products contain no coliforms, E. coli, dèguè Staphylococcus aureus and salmonella. These manufactured products comply with the microbiological standards. Dèguè maize, dèguè sorghum and dèguè millet developed have great nutritional importance. They can be classified as probiotic foods because containing significant living cells capable to improve the intestinal flora of the consumer by giving it more immunity.

Acknowledgements

The authors sincerely thank the Ministry of Higher Education and Scientific Research (MESRS) of Benin for

having made available the financial resources that enabled the realization of this study.

References

[1]. S. Lortal, J. F. Boudier, (2011), Valorisation de la matière première lait, évolution passé et perspectives, Innovations Agronomiques (13): 1-12.

[2]. K. H. Steinkrause, (1995), Handbook of indigenous fermented foods, Marcel Dekker, New York-USA, 76p.

[3]. I. Bokossa Yaou, C. K. C. Tchekessi, J. Banon, C. Agbangla, K. Adeoti, P. Dossou-Yovo, (2013), Etude socio-économique de production d'une pâte traditionnelle fermentée "gowé" fabriquée à base de maïs au Bénin, J. Rech. Sci. Univ. Lomé (Togo), Série A, 15(3) : 347-358.

[4]. C. K. C. Tchekessi, I. Bokossa Yaou, J. Banon, C. Agbangla, K. Adeoti, P. Dossou-Yovo, E. Assogba, (2013), Caractérisations physico-chimiques et microbiologiques d'une pâte traditionnelle "*gowé*" fabriquée à base de maïs au Bénin, J. Rech. Sci. Univ. Lomé (Togo), Série A, 15(2) : 377-387.

[5]. J. Banon, (2012), Evolution de la flore microbienne au cours de la fermentation d'un produit alimentaire fermenté du Bénin: cas de Ablo, Mémoire de Master, FAST/ UAC, Bénin, 57p.

[6]. A. Saint-Eve, (2006), Compréhension de la libération et de la perception des composés d'arôme en condition de consommation : cas du yaourt brassé aromatisé, Thèse de Doctorat, Institut National Agronomique Paris-Grignon, 273p.

[7]. M. Apfelbaum, M. Romon, M. Dubus, (2004), Diététique et nutrition, 6^{ème} édition, III Masson, Collection Abrèges, Paris, 535p.

[8]. B. Musabyemariya, (2011), Conditions de production et biodiversité bactérienne des produits laitiers fermentés artisanaux au Sénégal, Thèse de doctorat, Biologie Appliquée et Modélisation des Systèmes Biologiques, Université Polytechnique de Bobo-Dioulasso, 178p.

[9]. C. K. C. Tchekessi, J. Banon, I. Seni, J. Gandeho, C. Agbangla, P. Azokpota, A. Angelov, I. Bokossa Yaou, (2014), Socioeconomic study of a Fermented Drink "Dèguè" made with Milk and Cereals in Benin, Int. J. of Multidisciplinary and Current Research, vol.2, 626-632.

[10]. FAO, (2007), Les bonnes pratiques d'hygiène dans la préparation et la vente des aliments de rue en Afrique, FAO, 00153 Rome, Italie, 188p.

[11]. ISO 4833, (2003), Microbiologie des aliments, Méthode horizontale pour le dénombrement des micro-organismes, Technique de comptage des colonies à 30°C sur PCA, 19p.

[12]. NF ISO 21527-2, (2008), Microbiologie des aliments, Méthode horizontale pour le dénombrement des levures et

moisissures, Partie 2 : technique par comptage des colonies dans les produits à activité d'eau inférieure ou égale à 0,95, 9p.

[13]. NF ISO 15214 (V 08-030), (1998), Microbiologie des aliments, Méthode horizontale pour le dénombrement des bactéries lactiques mésophiles, Technique par comptage des colonies à 30°C sur la gélose MRS, 7p.

[14]. NF ISO 4832 (V 08-015), (2006), Microbiologie des aliments. Méthode horizontale pour le dénombrement des coliformes, Méthode par comptage des colonies obtenues à 37°C, 6p.

[15]. NF ISO 4832 (V08-060), (2009), Microbiologie des aliments, Dénombrement des coliformes thermotolérants par comptage des colonies obtenues à 44°C, Biokar diagnostics Violet Red Bile Agar (VRBA), 4p.

[16]. NF EN ISO 6888-1/A1, (2004), Microbiologie des aliments, Méthode horizontale pour le dénombrement des staphylocoques à coagulase positive (*Staphylococcus aureus* et autres espèces), Partie 1 : Technique utilisant le milieu gélosé de Baird-Parker, Amendement 1 : Inclusion des données de fidélité, 18p. [17]. ISO 6579, (2002), Microbiology of food and animal feeding stuffs, Horizontal method for the detection of *Salmonella spp*, Fourth edition, 34p.

[18]. J. Guiraud, P. Galzy, (1980), L'analyse microbiologique dans les industries alimentaires, Edition l'Usine Nouvelle, Paris-France, 236p.

[19]. K. S. Agbanzoume, (2005), Contribution à la mise au point d'une technologie de production de Dèguè, Mémoire de Maîtrise, FAST/UAC, Bénin, 61p.

[20]. C. Beal, I. Sodini, (2003), Fabrication des yaourts et des laits fermentés, Techniques de l'ingénieur, F6 315-7, 31p.

[21]. A. P. Gymnase, (2011), Approche microbiologique des yogourts et probiotiques, Epalinges, Erik Hansen, 3m², Travail de Maturité, 48p.

[22]. N. J. Mavhungu, (2006), Isolation and Caracterization of Lactic Acid Bacteria from «Ting» in Northern Province of South Africa, Degree MSc Microbiology, University of Pretoria, South Africa, 84p.