Microbial quality and visual appearance of traditional baobab fruit nectar during storage

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In Senegal, traditional baobab (Adansonia digitata L.) fruit nectar (T-BFN) is the most popular drink from baobab fruit pulp and its consumption helps to fight poverty, malnutrition and generate income along the Senegalese value chain of non-timbered forest products. However, for a better competitiveness in the local market, traditional baobab fruit nectar must be microbiologically stable with an attractive visual aspect. The aim of this study is to evaluate the microbial quality during storage, and the impact of some treatments like homogenization on the visual appearance of traditional baobab fruit nectar processed in Senegal. The microbial shelf-life of pasteurized traditional baobab fruit nectar and the effect of homogenization at 0, 5, 8, 12, 13 and 14 MPa on the stability of visual appearance during storage have been studied. Pasteurized traditional baobab fruit nectar could be stored up to 190 days at 4°C without microbial spoilage. Homogenization at 14MPa stabilized the visual appearance of traditional baobab fruit nectar without sedimentation of the pulp or clarification for days at 4°C. In combination with pasteurization, homogenization may be addressed as an effective tool to prevent pulp sedimentation in traditional bottled baobab fruit nectar.

Key words: Baobab fruit nectar, microbial quality, visual appearance, storage, homogenization pressure, pulp sedimentation.

INTRODUCTION

In Senegal, traditional baobab (Adansonia digitata L.) fruit nectar (T-BFN) is the most popular drink from baobab fruit pulp. It is obtained by adding water (hot or fresh) to whole seeds, sugar, citric acid, milk or flavor generally according to consumer preference (Cissé et al., 2009; Diop Ndiaye et al., 2013; Maptouom et al., 2020; Cissé et al., 2021). It is often home made for self-consumption or processed at artisanal and sometimes at semi-industrial level. It is very popular and is found in all places of mass consumption, be it family and religious ceremonies, in hotels and restaurants, markets, supermarkets or in the street (Cissé et al., 2009).

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With an average registered production of 3 000 tons/year, baobab fruit is one of the most important non-timber forest fruits exploited in Senegal (DEFCCS, 2019). Baobab fruit pulp is rich in sugar (20-32%), calcium (254-655 mg/100g) and pectin (Haddad, 2000; Cissé et al., 2009; De Caluwé et al., 2010; Kaboré et al., 2011; Vincent et al., 2020; Adedokun et al., 2022). From the study of Cissé (2007), baobab fruit pulp has an interesting antioxidant capacity around 88 μmol trolox/g due to its richness in Vitamin C and phenolic compounds. It is also a good source of fibers with prebiotics-like activity with respectively 22.5 and 22.04% dry weight for soluble and insoluble fibers (Manfredini, 2002; Kaboré et al., 2011). Several studies have been reported on biological properties of baobab fruit pulp like anti-pyretic, analgesic, antibacterial, anti-inflammatory, antioxidant, antiviral, etc. (Baky et al., 2021).

Therefore, the consumption of baobab fruit pulp and derived products will be helpful to fight poverty, malnutrition and generate income along the Senegalese value chain of non-timbered forest products. Due to their high-water content and biochemical composition, fruit beverages are attractive medium for microbial growth, which could lead to various food borne diseases or changes in sensory quality (Khan et al., 2015; Kregiel, 2015; Fowoyo and Amadi, 2021). Microbial spoilage reduces the shelf-life of fruit beverages especially if the processing conditions are not controlled (Dudez et al., 2000; Kregiel, 2015). In Senegal, traditional fruit beverages, particularly T-BFN sold in the local market are often made at artisanal level by informal processors or at micro, small and medium scales. In most cases, Good Manufactured Practice (GMP) and Good Hygienic Practice (GHP) are not applied. In addition, few data are available on microbial shelf-life and storage conditions of T-BFN for informal and small and medium scale beverage processors who do not have enough money to control the microbial quality of products. Cissé et al. (2009) did accelerated aging test for 8 days at 37°C of T-BFN pasteurized at 80 °C/10 min and 90°C/5 min. They carried out stability test over several weeks to assess the stability of pasteurized T-BFN. James et al. (2022) studied the microbial contamination in processed baobab products in Kenya but their works focused on baobab candies and pulp. In this study of the microbial shelf-life of local fruit drinks in general and T-BFN in particular, there was an urgent need to improve their quality and ensure consumers’ safety.

On the other hand, if microbial spoilage made fruit drinks unfit for human consumption, the visual aspect of the beverages is also a relevant parameter that contributed to the consumer’s purchase decision (Da Silva et al., 2019). In Senegal, local fruit- beverages like T-BFN packed in bottles (plastic or glass) exhibited a fast decantation during storage.

In fact, fruit juices and beverages exhibited a rapid sedimentation as a result of particles movements that aggregated under gravity to form sediment (Sallaram et al., 2014; Da Silva et al., 2019). This technological problem due to the natural composition of fruits beverages contributed to the low competitiveness of local fruit drinks in Senegalese market and has to be addressed in order to propose solutions especially for informal and small and medium-scale beverage processors. Since local fruit drinks are not attractive in bottle, local consumers prefer imported drinks that are better in appearance even if they are not always better in terms of nutritional quality. To improve the physical stability of fruits drinks, reducing the size of suspended particles and/or increasing viscosity are common methods (Da Silva et al., 2019).

Studies have been found in the literature concerning the prevention of decantation in different fruit beverages like cupuáçu juice (Da Silva et al., 2019) or apple juice (Gössinger et al., 2018); but no data are available on traditional baobab fruit nectar.

To be more competitive in the local market, traditional baobab fruit nectar must be microbiologically stable with an attractive visual aspect. Scientific data on microbial stability and visual aspect over time must be produced to improve our knowledge but also to allow informal and micro, small, medium scale beverage processors to have technological information to improve their products. Improving the visual appearance of traditional fruit drinks can increase the market access and reduce the level of imported drinks. Therefore, the aim of this study is to evaluate the microbial quality and the impact of homogenization on the visual appearance of traditional baobab fruit nectar processed in Senegal.

MATERIALS AND METHODS
Baobab fruits seeds were purchased at the wholesale fruit market (Sandica, Pikine), in Dakar, Senegal.

Preparation of traditional baobab fruit nectar for microbial analyses
Products were prepared according to preparation methods standardized by the Institute of Food Technology in Dakar (Diop Ndiaye et al., 2013) and in compliance with the Senegalese standard on Guava, Baobab, Ditax and Mango nectars, which is equivalent to the General Standard for Fruit Juices and Nectars (CODEX STAN 247-2005). Baobab fruit pulp was extracted by adding hot water to the baobab seeds (7 kg/l). After kneading, sieving and refining, the baobab pulp extract (3°Brix; pH 2.8) was mixed with sugar (130 g/l) and citric acid (2 g/l). After preparation, T-BFN was pasteurized at 95°C for 2 min with a three-stage electric pasteurizer operating at a maximum rate flow of 150L/h (Gillon-Pierre-et-fills, France). Pasteurized T-BFN was then hot filled in 250 ml aluminized bags that were kept five minutes before cooling with cold water to ensure the auto pasteurization of packaging. Pasteurized samples were divided into two batches; one batch stored at room temperature (25°C) and another in the refrigerator (4°C) for a maximum total period of 190 days. At the time of microbial control, three T-BFN packs of 250 ml each were taken at random from each storage batch.
Unpasteurized T-BFN was collected aseptically, just after preparation, in sterile bottles. It was transferred to the laboratory and analysed just once to see the initial contamination before pasteurization.

Microbial quality of T-BFN during storage

Microbiological analyses were carried out according to classical AFNOR (2002) standards methods. Since the products to be analysed are liquid, each test sample represented a stock suspension. Samples were transferred into sterile closed containers and gently homogenized with hand before performing a 10-fold serial dilution (from \(10^{-1}\) to \(10^{-6}\)) under aseptic conditions in tryptone broth sterile salt (BIOKAR). For each serial dilution, inoculation was done in two petri dishes and each beverage sample was analysed three times. The following germs were enumerated: Total aerobic bacteria at 30°C (TAB); yeast and moulds (YM); Thermo tolerant coliforms (TTC); Coagulase-positive pathogenic Staphylococci (CppS); Sulfite-reducing Clostridium (SrC); Bacillus cereus (Bc); Salmonella spp. (S) and Mesophilic Lactobacillus (ML).

Standard enumeration methods by counting bacterial colonies on agar culture media were used. For this purpose, specific culture media were used to highlight the species of bacteria sought.

Microbial quality of resultant nectar

1) For TAB enumeration, 1 ml of the decimal dilutions was inoculated in plate count agar medium (PCA, HIMEDIA) under aerobic conditions and incubated at 30°C for 72 h ± 3 h according to ISO 4833-1 (2013).
2) YM were enumerated after inoculation of 1 ml of samples in yeast extract, glucose and chloramphenicol agar (YGC, LD) under aerobic conditions and incubated at 25°C for 3 ± 5 days (NF V08-059, 2002).
3) For ML enumeration, inoculation (1 ml sample) was done in depth on Man Rogosa and Sharpe agar medium (MRS, CONDA), poured into two petri dishes. After the mixture solidified, a second layer of the same medium was used to cover the medium. Plates were incubated at 30°C for 24 to 48 h (NF ISO 15214, 1998).

Hygienic status during nectar production

1) For the enumeration of TTC, 1 ml sample was inoculated in depth on bile, crystal violet, neutral red lactose (VRBL, BIO-RAD) agar medium into two petri dishes (double layer technique) and incubated at 44°C for 24 h (NF V 08-060, 2009).
2) For the detection of CppS, 1 mL sample was used for enrichment on hyper salted lactose broth (Chapman broth). After that, isolation was carried out on Baird Parker’s (LD) selective glucose medium. Petri dishes were incubated under aerobic conditions at 37°C for 24 to 48 h. To perform the coagulase test, a portion of each selected colony was removed and inoculated in sterile tube containing heart-brain broth. After incubation at 37°C for 24 h ± 2 h, 0.1 ml of each culture was aseptically added to 0.3 ml of rabbit plasma in sterile hemolysis tubes and incubated at 37°C for 4-6 h. The reaction is considered positive when the coagulum occupies at least half the volume initially occupied by the liquid (ISO 6888-1, 1999).
3) SrC was detected by inoculating 1 mL sample in two tubes in which 20 ml of tryptose sulphite cycloserine agar (TSC, SCHARLEAU) was added. Incubation was performed at 37°C under anaerobic conditions for 20 ± 2 h.
4) For the enumeration of Bc, 0.1 ml of sample was inoculated on solid selective Bacillus cereus medium (SCHARLEAU) and incubated at 30°C under anaerobic conditions for 18 to 24 h and then 48h (NF EN ISO 7932, 2005).
5) The detection of Salmonella requires four successive phases. A pre-enrichment stock suspension (25 ml of T-BFN in 225 ml of buffered peptone water) was homogenized using a Stomacher and incubated at 37°C for 16 to 20 h (De Smedt et al., 1986). After that, 0.1 and 2 ml of the pre-enrichment culture were transferred into two different tubes containing respectively 10 ml of Rappaport-Vassiliadis broth and 20 ml of selenite-cystine medium. The two media were then incubated respectively at 42 and 37°C for 18-24h. Isolation was done on the selective solid medium by the streak method. Incubation was done at 37°C for 24 to 48h. Colonies presumed to be Salmonella were isolated and purified on nutrient agar (ISO 6579-1, 2017). The results were expressed in \(\log_{10}\) CFU/ml as the number of colony-forming units per millilitre of traditional baobab fruit nectar.

Effect of homogenization pressures on the visual aspect of T-BFN during storage

T-BFN was prepared and pasteurized as previously. After hot water extraction (7 l/kg), kneading, sieving, refining and formulation (130g/l sugar cane and 2 g/l citric acid), T-BFN was pasteurized at 95°C for 2 min with a three-stage electric pasteurizer operating at a maximum rate flow of 150 l/h (Gilion-Pierre-ett-fils, France). The pasteurized traditional baobab fruit nectar was homogenized in a continuous process by connecting the outlet of the pasteurizer to the inlet of the homogenizer to avoid contamination after thermal treatment. The following homogenization pressures were applied to the pasteurized T-BFN: 0, 5, 8, 12, 13 and 14 MPa using an ALM2 Pierre Guérin homogenizer (France). After homogenization, T-BFN was packed into 250 ml cleaned plastic bottles and stored at 4°C. The impact of the homogenization pressures was evaluated directly by visual observation of the presence of the serum phase and pulp sedimentation. Photographs were taken using a Lumix camera.

Statistical analysis

All statistical analyses were performed using SPSS 20.0 (IBM stats software). The Student-Newman-Keuls (SNK) test was used to determine the difference at a=0.05.

RESULTS AND DISCUSSION

Microbial quality of traditional baobab fruit nectar during storage

Table 1 presents the microbial composition expressed in \(\log_{10}\) CFU/ml of pasteurized traditional baobab fruit nectar during storage at 4°C and 25°C between 0 (zero time) and 190 days. In the absence of microbiological criteria in the Senegalese standards applicable to local fruit juices, the results were compared to the microbiological criteria of foreign references such as the guidelines for interpretation (F0-54, 2018) and the Federation of Commerce and Distribution Criteria (FCD, 2019) as well as with data from the literature on similar products.

Indeed, in the Senegalese standard for fruit juices and nectars, it is only mentioned that baobab nectar must be free of microorganisms capable of growing under normal storage conditions and must not contain any substance originating from microorganisms in quantities that could
present a health risk without specifying microbiological criteria (NS 03-92, 2009; NS 03-96, 2009). However, the standard recommended that baobab nectar should be prepared in accordance with the Recommended International Code of Hygienic Practice for Canned Fruits and Vegetables and the general principles of food hygiene recommended by the Codex Alimentarius Commission (CAC/GL 21, 1997; CODEX STAN 247, 2005).

Depending on the type of ingredient, the level of bacterial population in fruits and vegetables ranged from 3 to 7 log_{10} CFU/g (Pla et al., 2005; Abadias et al., 2008; Korir et al., 2016; Krahulcová et al., 2021). Raw foods containing a number of spoilage microorganisms aerobic plate count at 21.1°C less than 4 log_{10} CFU/ml are rated as good and are safe for consumption (Buyukkural et al., 2015; Khadka et al., 2017). For non-bottled drinks, the number of foodborne pathogens such as E. coli, Staphylococcus aureus and Clostridium perfringens must be less than 2 log_{10} CFU/ml while Salmonella spp. must be not be detected in 25ml (CFS, 2014). In ready-to-eat food in general, Clostridium perfringens, Staphylococcus aureus and other coagulase-positive staphylococci and Bacillus cereus must be less than 1, 1.30 and 4 log_{10} CFU/ml respectively (CFS, 2014).

Only total aerobic bacteria (TAB) were detected while yeast and moulds (YM), thermo tolerant coliforms (TiC), Coagulase-positive Pathogenic Staphylococci (CppS), Sulfite-reducing Clostridium (SrC), Bacillus cereus (Bc), Salmonella (S) and Mesophilic Lactobacillus (ML) were not found both in unpasteurized and pasteurized samples.

These results showed the good hygiene level of unpasteurized and pasteurized traditional baobab nectar as TAB was 1.41 log_{10} CFU/ml in unpasteurized T-BFN and 1.24 log_{10} CFU/ml in pasteurized T-BFN. However, the results are different from those obtained by Cissé (2009) in unpasteurized baobab nectar (ratio fruit water 1/3) in which total aerobic bacteria (2.29 - 5.75 log_{10} CFU/ml), mesophilic lactobacillus (2.20 - 4.11 log_{10} CFU/ml) and moulds (1.30 - 3.53 log_{10} CFU/ml) were detected. Yeasts were detected at a level < 1 log_{10} CFU/ml, while in pasteurized baobab nectar (70°C/10 min), TAB and moulds were less than 2 log_{10} CFU/ml (Cisse et al., 2009). The results were also lower than those reported in papaya juice (6.3 log_{10} CFU/ml) by Khan et al. (2015). Krahulcová et al. (2021) reported also total aerobic bacteria from 2.9 to 7.3 log_{10} CFU/ml in different smoothie samples collected in six food service establishments in Slovakia. James et al. (2022) reported an amount of TAB at 3.08 and 4.3 log_{10} CFU/ml, respectively in baobab fruit pulp from formal and informal baobab processors sector in Kenya.

According to FCD (2019) criteria guidelines, YM and lactic flora in unpasteurized fresh fruit juices are respectively 4 and 4.69 log_{10} CFU/ml at the factory outlet in their initial industrial packaging. In pasteurized juices, the number of TAB varied from 4 to 6 log_{10} CFU/ml according to the requirements of F-054 Rev05 (2018). As TAB, mesophilic ML and coliforms are considered as process hygiene criteria, the present results pointed out the respective GMP and GHP during the process. As

### Table 1. Microbial composition of traditional baobab fruit nectar during storage at 4 and 25°C.

<table>
<thead>
<tr>
<th>Microbiological parameters (Log_{10}CFU/ml)</th>
<th>Unpasteurized</th>
<th>Traditional baobab fruit nectar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
<td>4°C 62D</td>
</tr>
<tr>
<td>Aerobic mesophilic counts at 30°C</td>
<td>1.41</td>
<td>1.24^{cX} (0.017)</td>
</tr>
<tr>
<td>Yeast and molds</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Thermo tolerant coliforms</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Coagulate-positive Pathogenic Staphylococci</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sulfite-reducing Clostridium</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Bacillus cereus (LOG_{10}CFU/0.1mL)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mesophilic Lactobacillus</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Salmonella (absence in 25 g)</td>
<td>ND</td>
<td>NA</td>
</tr>
</tbody>
</table>

Means values ± standard deviation of three samples. Different letters in miniscule, denote significant differences between the storage times for the same storage temperature. Different letters in majuscule denote significant differences between storage temperatures for the same storage time.

Source: Authors
the acceptable limit of bacteria load in drinks for human consumption should not exceed $7 \log_{10}$ CFU/ml (ICMCF, 2005), traditional baobab fruit nectar processed in our study conditions is safe for consumption. However, the microbial safety of T-BFN has to be evaluated in order to assess the microbial shelf-life.

The microbiological problems encountered in beverages and nectars as well as in fruit juices are due to the presence and growth of microorganisms of alteration that lead to the degradation of the hygienic and marketable qualities of the product, and microorganisms responsible for food-borne illnesses (Philip, 2005). During storage at 25°C, only TAB and YM were detected among the microorganisms sought. The TAB increased significantly $4.19 \log_{10}$ times after 34 days of storage compared to initial load (Table 1). However, this result was under acceptable limit of bacteria load in drinks for human consumption ($7 \log_{10}$ CFU/ml). The presence of YM implied unfitness for consumption. Therefore, in our conditions of study, T-BFN has a limited microbial shelf-life that was less than 34 days when it is stored at ambient temperature. Pasteurisation only killed vegetative microbes and pasteurized products should be stored below 20°C.

During storage at 4°C, only TAB was detected among the microorganism sought with significant variation after 34, 62 and 190 days compared to initial load. Nevertheless, after 190 days of storage at 4°C, the TAB level was still under than permissible limits in fruits juices. Therefore, in our conditions T-BFN can be stored up to 190 days at 4°C without microbial spoilage. These results are satisfactory and indicated good refrigeration (at 4°C) that kept the food in a state close to its initial state. The lower the temperature is, the slower the multiplication of microorganisms.

**Effect of homogenization pressures on the visual aspect of T-BFN during storage at 4°C**

Figure 1 presents the impact of homogenization pressures at 0, 5, 8, 12, 13 and 14 MPa on the visual aspect of pasteurized traditional baobab fruit nectar after 1D (day) and 54 D (days) of storage at 4°C. Homogenization is a unit operation commonly used in the processing of fruit and vegetable juices in order to improve their physical stability by reducing the size of suspended particles (Zamora and Guamis, 2015; Patrignani and Lanciotti, 2016; Da Silva et al., 2019).

High-pressure homogenization (HPH) application ranged from 3 to 500 MPa continuously in fluid products (Castagnini et al., 2014; Betoret et al., 2015). In our study, pressures below 20 MPa were applied since our homogenizer operated at 5, 8, 12, 13 and 14MPa. The impact of homogenisation pressures was assessed directly by visual observation of the presence of the serum phase after 1 and 54 days of storage at 4°C. From Figure 1, the non-homogenized traditional baobab fruit nectar showed a serum phase and a sediment phase that are more or less of the same height after 1 and 54 days of storage at 4°C. In homogenized T-BFN, the visual height of the serum phase decreased with the progressive increase of pressure (5, 8, 12 and 13 MPa). At 14 MPa no difference was found after 1 and 54 days of storage at 4°C but in comparison with the control sample, no serum phase occurred after 1 and 54 days of storage. The present results indicated a positive effect of homogenization on the visual appearance of traditional baobab fruit nectar which could be stored up to 54 days at 4°C without appearance of pulp sedimentation. Thus, homogenisation at 14 MPa is sufficient to stabilise the visual appearance of T-BFN. According to Siebert (1999), turbidity and sediment formation in fruit juice is due to the presence of starch, pectin, polyphenols and proteins especially in the absence of microbial growth.

Yu et al. (2018) obtained similar result in taro pulp where HPH treatment (0-60 MPa) improved the stability of the taro pulp suspension. On the other hand, different results were observed for pineapple pulp where homogenized samples showed sedimentation of the pulp after ten days of storage at 25°C (Silva et al., 2010). This can be explained by the accumulation of particle size aggregated, which are different in size for each fruit. Indeed, according to Lopez-Sanchez et al. (2011), each plant cell wall behaved differently when treated with HPH. Homogenisation reduced the particle size and improved the stability of the suspension in fruit drinks. During this operation the pulp is passed through a narrow orifice and then broken down into small particles, resulting in high stability, no sedimentation and a smooth texture. The results offered interesting prospects for small-scale beverage manufacturers as homogenizers working at a pressure below 20MPa are easier to have and more suitable for their production level than high-pressure homogenizers.

However, high-pressure homogenisation and ultrahomogenisation offered the dual advantage of microbiologically stabilization beverages while also allowing for physical stability of the beverage. The HPH could be used as a valuable tool to reduce particle sedimentation and serum separation (Kubo et al., 2013; Salehi, 2020). The reduction in particle size during homogenization can be related to the greater stability of the homogenized products. Therefore, homogenization may be addressed as an effective instrument for preventing pulp sedimentation in traditional baobab fruit nectar.

**Conclusion**

Pasteurized traditional baobab fruit nectar could be stored up to 190 days (6 months) at 4°C without microbial spoilage. When the storage was carried out at room
temperature (25°C) its microbial shelf life was reduced less than 34 days. The present results have also demonstrated that the application of homogenization, particularly at 14 MPa could be efficient to maintain during 54 days at 4°C the visual appearance of traditional baobab fruit nectar without appearance of pulp sedimentation and serum phase. These results would be

useful for informal and micro, small, medium scale beverage processors, who will have technical information to produce safe and attractive traditional baobab fruit nectar. However, in order to generate more knowledge on traditional baobab fruit nectar, further studies should be carried out on the evolution of nutritional and sensory quality during storage at 4°C of pasteurized and

Figure 1. Effect of homogenization at 0, 5, 8, 12, 13 and 14 MPa on the visual appearance of traditional baobab fruit nectar after 1 (1D) and 54 days of storage at 4°C.
Source: Authors
homogenized (14Mpa) traditional baobab fruit nectar.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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