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Viscosity and microbiology of the gum of *Sterculia setigera* Del.

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Abstract: Karaya gum or mbepp is produced by Sterculia setigera Del. in Africa. The value of the gum depends a lot on its viscosity and the absence of germs both of which are rarely studied. The objective of this study is to contribute to a better knowledge of the quality of karaya gum from Senegal. For this purpose, from gums originating from the region of Tambacounda, Senegal (i) the behavior of the viscosity of the gum as a function of the shear rate and (ii) the control of its microbiological quality were monitored. The viscosity of the two types of gum collected in the Bala, Daoudi and Malem Niani 1 sites (harvested during the hot dry period and one year old) and Malem Niani 2 (four months old and harvested during the dry-cool period) according to different shear rates has been studied. The results show that the viscosity of the *S. setigera* gum varies as a function of the interaction between the type of gum and the shear rate ($p < 0.0001$). The mbepp gum collected at Daoudi and Malem Niani is free from Salmonella and Escherichia coli contamination. On the other hand, total mesophilic flora (on PCA) is very important (> 3,106). These results helped to acquire new knowledge and to identify Senegalese karaya gum (mbepp) as a good quality gum.

Keywords. Karaya, Sterculia setigera, viscosity, *Salmonella*, *Escherichia coli*

1. INTRODUCTION

S. setigera is a species that exudes a gum (mbepp in Wolof language in Senegal or Karaya on the world market) very popular in the food, pharmaceutical, and cosmetic fields[1-2]. Today, it is found in a wide variety of products such as dietary products, desserts, medications, donuts, salty sauces, ready meals, ice cream and biscuits [3-9].

The physico-chemical properties of the vegetable gums vary according to the characteristics of the soil, the period of harvest, the site, the species[1,2,9]. According to Samba *et al.* [2], a comparison of viscosities of Senegalese karaya gum found that that of Bala (9913mPa) is higher (constant speed and temperature = 0.35 s^{-1} at 35 ° C) than those of Daoudi and Maleme Niani (on average 2872mPa). In India, karaya gum calibration takes into account several physicochemical variables including viscosity[10-11]. Colin-Henrion *et al.*[12] also find the solubilization of the pectins during cooking that the applied temperature (85 ° C) and the pH of the apple (3.5-4.0) contribute to the solubilization with a depolymerization of the parietal polysaccharides by an acid hydrolysis of the pectins. According to To [13], the viscosity of the serum of the GS (Granny Smith) apple variety purée is 10 times higher than that of GD (Golden Delicious).

The properties of the Senegalese karaya are little studied[1,2,14]. The further development of knowledge for a better appreciation of the physicochemical properties of this gum is essential. In addition, the gum is handled with bare hands or with dirty and inadequate equipment during harvesting and transport. Very hydrophilic, it is often the seat of development of microorganisms that can affect its commercial value. The use of gum sterculia in food and pharmaceutical preparations has shown that the product may contain pathogenic microbial agents (*Streptococci, Staphylococci, enterobacteria*) [1]. In addition, the drying techniques of this rubber are not yet fully controlled by operators who are unaware that a quality gum meets certain criteria to be acceptable in the international market. Senegalese society is a major consumer of mbepp gum, especially in areas where cereals are grown [2-15]. The microbiological control of the mbepp gum is in its necessary conditions.

The objective of this work is (i) to study the behavior of the viscosity of the gum as a function of the shear rate and (ii) to control its microbiological quality.

2. MATERIAL AND METHODS

The mbepp gums used are harvested in Senegal in the region of Tambacounda and dried at room temperature by producers Bala (14 ° 01 '63' 'North and 13 ° 10' 62 " West, altitude 65 m), Daoudi (14 ° 07 '42' 'North and 13 ° 58' 05 " West, altitude 33 m) and Malem Niani (13 ° 56 '00 "North and 14 ° 18' 00" West, altitude 36 m). The dry tropical climate of the region is of Sahelo-Sudanese type with two seasons (dry and rainy), the place between the isohyets 400 and 1,200mm. These sites are selected because they produce most of the Senegalese karaya gum[1-2.] They are then converted into powder after grinding the cleaned gum (IK MF10 grinder at 4000 rpm, Photo 1). The sieving of the gum is carried out in two stages: first with a sieve (1mm mesh), then with a second sieve finer mesh (0.5 mm) [2]. For microbiology, the preservation of the aged gum of one year was made in powder form in plastic jars with a cover hermetic. On the other hand, the gums aged 2 months were kept in the form of granules and it was at the time of their analysis that their grinding was carried out.

2.1. Viscosity of the gum: In a device with total randomization, a two-factor test is set up:

- **Factor 1:** type of gum at four (4) levels: Bala, Daoudi, Malem Niani 1 and Malem Niani 2;

- **Factor 2:** shear rate at eight (8) levels: 0,35 s^{-1}, 0,7 s^{-1}, 1,4 s^{-1}, 2,8 s^{-1}, 5,6 s^{-1}, 11,2 s^{-1}, 22,4 s^{-1} et 44,8 s^{-1}.

Thirty - two (32) treatments are used and each treatment is repeated nine (9) times. The gums of Bala, Daoudi and Maleme 1 are harvested during the dry-hot period (March to June) and one (1) year old and the 4-month-old Malem 2 gum is harvested during the dry-cool period (November to February).).

The viscosity is measured with a VISCOTRON BRABENDER viscometer with coaxial cylinders, one of which is fixed and the other mobile. The device has an alcohol thermometer that is used to control the temperature of the water bath.

The viscosity (η) was calculated from the value read on the control block of the viscometer "S", using the following formulas:

Shear rate: $D = m. X\ s^{-1}$ (1)

Shear force: $\tau = B. S. Y$ Pa (2)

Viscosity $(\eta) = ([B \times S \times K] / m)$ mPas (3)

- m = rpm = revolution per minute (min^{-1})
- B = 3, scale given by the manufacturer
- S = digital reading
- X = shear factor = 1.4
- Y = force factor (precalibrated spring) = 0.0283
- K = calibration constant = 20.2.

B, X, Y and K are values given by the manufacturer of the viscometer used.

2.2. Microbiology of the gum

2.2.1. Background and crop conditions: The cultures are carried out on several media (buffered peptone water and peptone salt water, Plate Count Agar (PCA), DCL, Sabouraud medium) whose compositions are recorded in Table 1. A mother liquor solution is prepared with buffered peptone water. Then, bottles of 100 ml of buffered peptone water (pH for gum 6 to 7) are sterilized by autoclaving (121 ° C / 1 min) and after cooling, 1 g of gum powder is added per flask. After stirring, the flasks are incubated in an oven at 37 ° C for 24 hours for pre-enrichment.

A second salted peptone solution is prepared; three (3) flasks of 100ml and six (6) 9ml tubes are removed and the bottles are sterilized at 121 ° C / 15mn. One (1) g of gum powder is added to each 100 ml bottle of salted peptone water followed by mechanical stirring to obtain a concentrated solution of 10^{-2}. Dilutions up to 10^{4} are thus made. For inoculation, dilutions of 10^{-3} and 10^{-4} are used.

2.2.2. Identification of coliforms: Coliforms are enterobacteria that rapidly ferment lactose. "Fecal coliforms" and sometimes "presumptive Escherichia coli" are bacteria that produce gas from lactose at 44 ° C[16]. The choice of Sabouraud medium plus the antibiotic chloramphenicol, of the phenicols family, is explained by the fact that it limits the growth of germs other than fungi[14].

To determine the total coliforms, 1 ml of each dilution (10^{-3} and 10^{-4}) is taken and placed in petri dishes using a pipette; then 10 to 15 ml of the previously prepared 1/1000 deoxycholate citrate lactose (DCL) medium is added undercooled, after homogenization (uniform mixing with the inoculum by a slow horizontal circular movement). After drying, a second layer of DCL medium is added; after solidification, the incubation is carried out at 37 ° C. The same process is used for the determination of faecal coliforms. Incubation is done at 44 ° C for total coliforms. For each sample and dilution used, three repetitions are performed.

2.2.3. Mesophilic aerobic flora: For the determination of the mesophilic aerobic flora, 1 ml of the stock solution of each dilution (10^{-3} and 10^{-4}) is taken and then poured into petri dishes, then the Plate Count Agar (PCA) medium at pH 7 is added. . After homogenization and drying, a second layer of PCA is added followed by incubation at 30 ° C.

For the determination of yeast and mold, Sabouraud + Chloramphenicol (2.5 g) ready-to-use medium is used. Glucose and normal Sabouraud medium are prepared separately. The 2.5 g of Chloramphenicol (after filtration on 0.2 µm membrane) are sterilized under the laminar flow hood to the glucose solution cooled to 45 ° C after autoclave (bain-marie) then the whole is incorporated in the medium and then mixed before being poured into Petri dishes (3). Thus, a 100 µl sample (or 0.01 ml of dilutions (10^{-3} and 10^{-4})) is taken and spread in Petri dishes with glass spreaders and then incubated at 30 ° C. For each sample and dilution used, three repetitions are performed.

2.2.4. Determination of Salmonella: One (1) milliliter of the pre-enriched suspension (i.e., 1 g in 100 ml of the buffered peptone water and incubated at 37 ° C / 24 h) is taken in 9 ml of broth rappaport to obtain a dilution of 10 ml. $^{-3}$. One (1) milliliter of this last dilution (10^{-3}) is taken to obtain a 10-4 dilution and incubated at 37 ° C / 24 hours. For each sample (Daoudi, Malem Niani) and each dilution used three repetitions are performed.

3. RESULTS

3.1. Viscosity of mbepp gum: The dynamic viscosity of the Sterculia gum varies according to the interaction between the type of gum and the shear rate ($p < 0.0001$). For a shear rate of $0.35 s^{-1}$, the viscosity is higher with Bala's gum (9912 ± 216mPas) followed by that of Malem Niani 2 (9002.5 ± 216mPas). The lowest viscosities are obtained with Daoudi and Malem Niani gum 1 (2832.75 ± 216mPas). Viscosity maintained the same trend as before with speeds of $0.7 s^{-1}$, $1.4 s^{-1}$ and $2.8 s^{-1}$ (**Figure 1**).

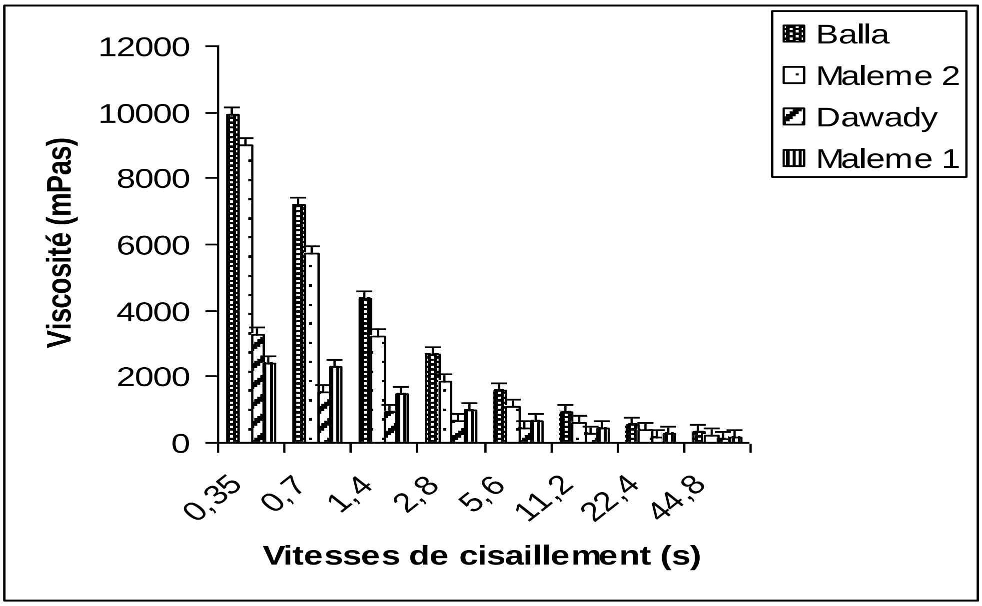

Figure 1: Interaction between shear rate and gum origin (p = 0.0000).

The viscosities of the Bala and Malem Niani gums 2 to $5.6s^{-1}$, are on average 1334.15 ± 216mPas. The viscosities of the Daoudi and Malem Niani 1 gums also do not vary, with an average of 534.95 ± 216mPas. The viscosity of Balla gum is higher at $5.6s^{-1}$ (1601 ± 216mPas) than that of Daoudi (418.2 ± 216mPas). On the other hand, for the speeds of $11.2s^{-1}$ and $44.8s^{-1}$, the viscosities do not vary according to the type of rubber **(Figure 1).**

The viscosities of the Malem Niani 1 ($0.35s^{-1}$ and $0.7s^{-1}$), Malem Niani 2 ($2.8s^{-1}$) and Bala ($5.6s^{-1}$) gums do not vary significantly, average of 2042.62 ± 216mPas. The viscosities of the Daoudi ($0.35s^{-1}$), Malem Niani 2 ($1.4s^{-1}$) and Bala ($2.8s^{-1}$) gums also did not vary significantly, averaging 3058.4 ± 216mPas (**table 1).**

Table 1: Composition of the culture media

Composition	Weight
Plate Count Agar medium (PCA): enumeration of total aerobic mesophiles (total mesophilic flora) for 1 liter	
Peptone	5g
Yeast extract	2,5g
Peptone	10g
Glucose	1g
Demineralized Water	1l
Sabourand environment: enumeration of yeasts and molds for 1 liter	
Glucose	20g
Chloramphenicol	0,5g
Agar	15g
DCL (Deoxycholate Citrate Lactose) medium at 1/1000: enumeration of faecal coliforms, for 1 liter	
Peptone/tryptone	10g
Lactose	10g
Agar	15g
Na Citrate	1g
Iron Citrate III	1g
Na deoxycholate	1g
K_2HPO_4 (Na)	2g
NaCl	5g
Neutral red	30mg

3.2. Microbiology of Sterculia gum (mbepp): The 2-month-old mbepp gum harvested in Daoudi and Malem Niani during the hot period (April) does not contain Salmonella or Escherichia coli. On the other hand, the total mesophilic flora (on PCA) is very important :> 3 106 **(Table 2).**

For one-year-old Bala, Daoudi and Malem Niani gums collected at the same time, an absence of faecal and total coliforms is noted, as is an absence of fungal growth. The presence of Salmonella is noted on the samples (one year old) of Bala and Malem Niani **(Table 3).**

Table 2: Microbiological gum of *S. setigera* aged 2 months

Medium	**Dilution**	**UFC/g Daoudi**	**UFC/g Malem Niani**
PCA (mesophilic)	10^3	(incompatible) > 3 10^6	(incompatible) > 3 10^6
	10^4	5 10^7	8,5 10^6
Sabouraud (yeasts and molds)	10^3	2 10^4	< 10^4
DCL (coliformes)	10^3	Transplanted on Uri and Rambach Agar	
Uri Select (E. coli)	Colony seeding (from DLC)	absence	absence

Table 3: Microbiological of 12 month old Sterculia gum

Medium	**Dilution**	**UFC/g Dawady**	**UFC/g Malem Niani**	**UFC/g Balla**
PCA (mesophilic)	10^3	-	-	-
	10^4	5 10^6	2 10^5	2 10^6
Sabouraud (yeasts and molds)	10^3	absence	absence	absence
DCL (coliforms)	10^3	Transplanted on Uri and Rambach Agar		
Uri Select (E. coli)	Colony seeding (coming from the DLC)	absence	absence	absence
Rambach		absence	présence	présence

4. DISCUSSION

The viscosity of the *S. setigera* gum varies depending on the interaction between the type of gum and the shear rate. The viscosity of the Bala gum (April) (0.35 s^{-1}) is higher (9912mPa) than that of Malem Niani 2 (February), 9002.5mPa for the same shear rate. This confirms the results of Samba et al.[2]. On the other hand, the gums of Malem Niani 1 (April) and Daoudi (April), for the same shear rate, give a lower viscosity (2832.75mPa). The viscosities of Bala gum and Malem Niani 2 are comparable at a speed of 5.6 s^{-1}. However, at a rate of 11.2 sec^{-1} to 22.4 sec^{-1}, the viscosity is not influenced by either the age of the gum or the site of harvest. With Xanthan gum, thresholds of 700, 2500 and 7000mPa are determined with a Rheomat 30 viscometer for gum concentrations of 3, 5 and 10 g / l respectively[17]. By comparison, To13 found a value of 136mPas for a Granny Smith variety puree and 13.3mPas for a Golden Delicious variety puree

It is also possible to obtain identical viscosities for mbepp gums harvested at different periods or sites. Thus, the gums of Malem Niani 1 (for the speeds $0.35s^{-1}$ and $0.7s^{-1}$), Malem Niani 2 ($2.8s^{-1}$) and Bala ($5.6s^{-1}$) give the same viscosity, for an average of 2042.6mPa. The viscosity of the Daoudi gum ($0.35s^{-1}$) is the same as that of Bala ($2.8s^{-1}$) and Malem Niani 2 ($1.4s^{-1}$), for an average of 3058.4mPa. According to Brahim, the viscosity decreases as the temperature increases for the same shear rate, in accordance with the rheological predictions. According to the same author, the effect of temperature on the viscosity is even lower than the shear rate is large. A study of Saidou19 on Triumfetta cordifolia and Bridelia thermifolia shows that the viscosity of the extracts decreases for bark drying temperatures above 70 ° C. This drop in viscosity of the extracts is more the result of a conformational change of the polysaccharides than of their degradation. The study of the rheological behavior of these polysaccharides shows a rheofluidifying, viscoelastic, little thixotropic behavior and with flow threshold constraints between 0.2 and 5Pa and between 0.5 and 1Pa for T. cordifolia and B. thermifolia respectively. Concentrations between 0.52 and 0.82 g / l.

It is possible that the difference in viscosity found in the context of this study between the different gums is related to their acidity: Bala (pH = 6.75, April) the weakest followed by Malem Niani [2] (February) and Daoudi (April, pH between 6.23 and 6.20). However, the acidity of the gum Malem Niani [1] is stronger (5.53). It is possible that the gum Malem Niani 1 has more difficulty in releasing its acidity with conservation. The difference in viscosity between the different gums can also be explained by the presence of impurity (debris, sand, other) because the gum analyzed was not of the quality Hand Pecket Selected (HPC) 15. In the case of tomato concentrate, an increase in solubilization of pectins coincides with the decrease in serum viscosity20. According to Ouerdane and Mahfoud [21], the rate of elongation of the viscoelastic fluid increases with the inertia and concentration of the solution.

The viscosity of a gum solution varies according to several factors namely the particle size, the gum concentration, the pH, the temperature, the calcium ion, the shear rate, the age of the gum [22]. According to Verbeken *et al*.[9], in dry form, the karaya gum stored in powder form loses its viscosity more rapidly than when it is stored in the form of granules. According to Hsu [23], with tomato juice the comparison of two treatments Hot Break (92 ° C, 2 min) and Cold Break (60 ° C, 2 min) shows that the viscosity of the juice decreases with the Cold Break and increases with the Hot Break . The difference between the viscosities obtained with the two treatments is attributed to the enzymatic activity and the solubilization of the pectins that it entails.

The results of this study also show that viscosity decreases with increasing shear rate. These results are similar to those of Simon [24]. According to the latter, when the velocity gradient (expressed in s-1) increases, the apparent viscosity (expressed in steps) decreases. Under a growing shear effect, the galactomannan chains will disentangle and orient themselves in parallel. This reorganization of the molecules is responsible for the decrease in viscosity.

Actions in the direction of a better control of the techniques of exploitation, drying and storage are therefore essential. From our results, the freshly harvested mbepp gum shows neither Salmonella nor E. coli. It therefore conforms to the pathogen level for international trade as a raw material. On the other hand, the mesophilic total flora (on PCA) was very important and superior to the norm of the Pharmacopoeia: 100 times or 1000 times more (the maximum microbial level was of the order of 10 000 germs / g), whereas many industrial companies require a lower rate1-14. Karaya gum is 100% natural and vegetarian, free from pesticides and GMOs. It does not contain gluten and is rich in fiber. It is called E416 according to the EFSA additives standard (the European Food Safety Authority). According to EFSA and JECFA (Joint FAO / WHO Expert Committee on Food Additives), Karaya gum does not pose any safety or health concerns and no ADI is required (Admissible Daily Intake)) for the Karaya3 gum.

Daoudi gum, whose storage conditions are not adequate, presents a fungal pollution, hence the interest of reinforcing the capacities of producers in terms of equipment for collecting, transporting and preserving gum in order to avoid pollution5. The contamination found with Bala gum and Malem Niani aged one year and kept at room temperature in powder form in sealed plastic jars is a problem. From where in the future the interest to study the best conditions of conservation (powder or granule) of the Senegalese karaya gum. Even if it is accepted that the freshly harvested gum is free from contamination1 this is not excluded from the possibility of contamination if the packaging conditions do not meet the standards required by the world market to have a quality gum. Solutions and jellies of karaya gum require condoms as they are prone to bacterial attack. They are easily preserved with a mixture at a maximum of 0.17% methyl and 0.03% propyl o-hydroxybenzoate as well as with glycerine and propylene glycol. Benzoic acid, as well as 0.1% sodium benzoate, effectively preserves karaya gum solutions [9].

5. CONCLUSION

The results of this study show that for Senegalese karaya gum, it is possible for different harvest periods, sites and shear rates to obtain equal and significant viscosities. Malem Niani [1] (0.35s-1 and 0.7s-1), Malem Niani 2 (2.8s^{-1}) and Balla (5.6s^{-1}) give the same viscosity. (2042,6mPa). The viscosity of the Daoudi gum (0.35s-1) is the same as that of Balla (2.8s^{-1}) and Malem Niani 2 (1.4s-1), on average 3058.4mPa. These results also show the importance of the market value that Senegalese karaya gum could have, especially since it does not contain Salmonella or E. coli. It therefore conforms to the pathogen level for international trade as a raw material. However, for sustainability of this market value, there is a need to strengthen the capacity of producers in terms of harvesting, transportation and conservation processes. It is also important to put at their disposal all the small equipment or tool necessary for a better conservation of the physicochemical properties of the mbepp gum.

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