Journal of Food Technology Research
2016 Vol. 3, No. 1, pp. 55-62
ISSN(e): 2312-3796
ISSN(p): 2312-6426
DOI: 10.18488/journal.58/2016.3.1/58.1.55.62

EFFECT OF CONTAMINATION BY FUNGI AND YEAST ON THE PHYSIOCHEMIC CHARACTERISTICS OF GUM ARABIC STORED IN SEMI-DESERT CLIMATE KHARTOUM CITY, SUDAN

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ABSTRACT

Structural properties of gum Arabic are one of the most important factors affecting the competitive prices in the domestic and overseas markets. Using laboratory standard methods, a number of methods were used to characterize the main alterations of the components of Acacia Senegal var. Senegal gum Arabic induced by inoculation with some species of fungi and yeast. Results revealed that, depending on the inoculated microbial species, the pH, viscosity, nitrogen and protein were decreased to various levels. The sugars content has noticeably been affected viz. galactose was entirely consumed by Saccharomyces cerevisiaeand Penicilliumnotatum while .rhamanose was drastically decreased by all microbial species under study. Likewise, the number average molecular weight was decreased by all species. Therefore, it may be concluded that the factors encouraging microbial growth must be given due consideration under gum Arabic storage conditions.

Keywords: Acacia senegal, gum Arabic, Microbial contamination, Physicochemical properties.

Received: 2 February 2016/ **Revised:** 9 April 2016/ **Accepted:** 20 April 2016/ **Published:** 2 May 2016

Contribution/ Originality

This study contributes to the existing literature by drawing the attention to the environmentally safe storage of gum Arabic by using new detection and analytical procedures that are logically acceptable by gum Arabic researchers, producers and retailers. Moreover, this study contributes significantly to the current body of knowledge pertaining to gum Arabic handling after harvest.

1. INTRODUCTION

Gum Arabic is the trade name for a natural forest product from the genus *Acacia*, it grows, most, prolifically in regions of tropical Africa, in particular the Republic of Sudan (Figure 1). During times of drought, the bark of the tree splits, exuding a sap that dries in small droplets or "tears" (Figure 2). Gum Arabic is manly obtained from *Acacia senegal*var.*Senegal* locally known as (*Hashab*). The genus *Acacia* was first recognized by Phillip Mellor. *Acacia Senegal* var.*senegal* trees, the main source of gum Arabic, spreads through what is known as the African Gum Belt (Glicksman, 1979). The main area of its occurrence is the central part of Sudan where the species is uniform and is found in pure stands giving Sudan the advantage of being the major producer and exporter of the best quality gum Arabic, supplying about 80% of the annual World requirements (Osman *et al.*, 1995).

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The structure of gum Arabic is described by Idris *et al.* (1998); Osman *et al.* (1995) have reported that the sugar and cationic composition of *Acacia senegal. var Senegal* samples originating from various sources vary considerably thus reflecting the difference in soil characteristics , various ages and different locations. Nitrogen and protein components play a very important role in the structure, physicochemical properties and functionality of gum Arabic, which was recently subjected to intensive investigation. There is a strong correlation between the proportion of protein in the gum and emulsifying stability (Dickinson *et al.*, 1991). Nitrogen content of *Acacia senegal.var Senegal* gum has been determined by Anderson (1977) and was found to be 0.29%. However,Osman (1993) reported that nitrogen content for the *Acacia. senegal. var Senegal* gum to be 0.31% and protein content was 2.4%. Gum Arabic is a natural complex product mixture of hydrophilic carbohydrate and hydrophobic protein components (FAO Rome, 1990). The hydrophobic protein component functions as an emulsifier which adsorbs onto its surface oil droplets while the hydrophilic carbohydrate component inhibits flocculation and coalescence of molecules (Anderson *et al.*, 1990). Osman (1993) reported an average moisture content of *A. Senegal gum arabic*of 13.0%. Recently, Younes (2009) reported the mean value of moisture content for *Acacia. senegal* gum as 11.01% and the range was 9.91% – 14.72%. Bokhary *et al.* (1983) found that natural gum Arabic carried appreciable numbers of microorganisms when cultured in different growth media, these included some fungi such as *Aspergillus*, *Curvularia*, *Alternaria* and *Helminthosporium* and bacteria, such as *Bacillus SPP.*, *Serratiamarcesens* and *Micrococcus varians*. No algae were detected.

The North East Wales Institute (NEWI, 1987) reported that gum Arabic did not contain *Escherichia coli*, *Staphylococcus*, *Salmonella* or *Clostridium* species, but contained species of *Aspergillus*, *Rhizopus*, *Penicillium*and other unidentified moulds. Yeasts and moulds and other microorganisms including thermophilic groups were noticed by Anderson and McDougall (1987); Idris *et al.* (1998) isolated from gum Arabic samples, a number of fungal genera including *Aspergillus*,*Penicillium*,*Cladsosporium*, *Gilocladium* and *Rhizopus*, together with some species of the bacterial genera of *Bacillus* and *Micrococcus*. No yeasts were detected.

This study investigates the effect of contamination by fungi and yeasts, during storage time, on the physicochemical properties of gum Arabic.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

This study was carried out in Khartoum City the capital of the Sudan where the raw gum was stored. The capital is located in the middle part of the country which is located between latitudes 15°26' and 15°45' N and longitudes 32°25' and 32°40' E, at an altitude of 405.6 m above sea level (Shakesby, 1991). According to Perry (1991) the main climatic conditions of Greater Khartoum are conditioned by its location on the southern fringes of the Sahara, the city experiences four climatic seasons, the winter season extends from mid-November to March, a minimum temperature ranging between 8°C and 10°C which falls to 5°C during night, and maximum temperatures varying from 23°C to 25°C, and a relative humidity which may sometime be as low as 20 per cent, the hot, dry summer season is well in place by the end of March, the maximum temperatures may exceed 45°C by the end of May, the rainy season covers the period from July to September, with August being the rainiest month. Generally, annual rainfall ranges between 110 and 200 mm. A short, hot (about 40°C) transitional season occurs between mid-September and the beginning of winter.

2.2. Source and Collection of Gum Arabic Samples

Adequate authentic samples of *Acaicasenegal var. senegal* gum Arabic harvested during years 2005, 2006, 2007 and 2008 were collected from Bahri, Omdrman and Khartoum. Approximately 1 kg raw gum Arabic was collected from each sampling unit. The samples were purified from bark and dust and then transported into labeled glass

containers to the department of chemical laboratory at Sudan University for Science and Technology, where they were kept and analyzed.

2.3. Physiochemical Analysis of Gum Samples

2.3.1. Sterilization of Gum Arabic Samples

The raw materials of gum Arabic samples were initially analyzed for physiochemical characteristics and then sterilized and analyzed again after sterilization to check out any alteration that may occur on the chemical contents.

2.4. Isolation of Yeasts and Fungi

The potato dextrose agar was used for isolation, counting and cultivation of yeasts and fungi.

2.5. Enumeration of Fungi and Yeast in Gum Arabic

Five species of fungi and only one species of yeast were grown on 30 g per liter in water solution gum Arabic medium. After 25 day incubation at room temperature, the fungi and yeast medium was filtered by filter paper No. 389 and then dried to a constant weight at 37°C for 12hr.

2.6. Determination of Ph Value

The pH value was determinated by using 3% aqueous solution (growth media at the end of the incubation period) at room temperature 25 °C using pH meter (CORNING/ Pinnacle / 555. pH /ion meder. Made in England).

2.7. Determination of Acidity

The titrable acidity is expressed as % uranic acid, determined by titration of a known amount of reconstituted with 0.1 N NaOH using phenolphthalein as indicator. The organic acids in media were formed during the fermentation as uric acid. The titratabe acidity of sample was estimated according to the method of AOAC (1984).

2.8. Optical Density

Culture turbidity was measured using a Bauch and Lomb Spectronic 20 spectrophotmeter (Made in England) at the wavelength of 540 nm.

2.9. Determination of Viscosity by Brookfield Viscometer

Viscosity was measured using HAAKE visotester 6 plus. About 3% solutions and the viscosity of gum solution was read directly in centipoises/second (cps/s) 3 times and averaged out.

2.10. Determination of Total Nitrogen and Protein

The Kjeldahl method was used to determine the total nitrogen in gum Arabic samples according to AOAC (1984).Protein content was calculated using nitrogen conversion factor resulting from amino acid analysis (Anaderson *et al*., 1986).

2.11. Determination of Number Average Molecular Weight by Osmometry

Gum solutions (3% w/v) were prepared by dissolving the gum in sufficient amount of distilled water, the solution were treated with different microorganisms species for 25 days and filtered through Whitman 41 filter paper. Then different concentrations were made for each original solution and their corresponding osmotic pressures were measured using Osmomat^{R} 050 (Colloid – Osmometr). The temperature measurement was recorded,

the plot of (π/ C) vs. (C) gave a straight line for each solution of all the gum samples and average number molecular weight was calculated from the intercept to the plot as described in the following equation

The Van't Hoff equation: $\pi / C = RT / M_n$

3. RESULTS AND DISCUSSION

3.1. Sterilization of Gum Arabic Samples

No significant changes were observed on the gum Arabic components due to sterilization except nitrogen and protein percentages (Table 1).

3.2. The Effect of Fungi and Yeast Species on Some Physicochemical Parameters of Gum Arabic

3.2.1. Acidity, Ph and Mycelial Dry Weight

Table (2) shows the effect of the growth of fungi and yeasts on the physiochemical properties of gum Arabic. According to the this study, it could be demonstrated that gum Arabic acts as a good medium for fungi and yeast enumeration. The pH value was decreased from 6.8 in control sample to various lower levels according studied species. Accordingly, acidity has increased from 0.002 in control sample to higher various levels, especially in case of *Aspergillusniger*. Absence of significant mycelial growth in control samples and its occurrence in the investigated samples is another indicator of the suitability of gum Arabic as a potential growth medium for fungi and yeasts, as was previously reported by Cochrane (1958). According to Table 2, the relatively high acidity (pH of 4.3) that found in the medium of *Aspergillusniger* , after biodegradation , indicates the rapid growth of this species compared to the others. On the other hand, *Penicilliumnotatum and Fusarimmoniliforme*showed low increase in acidity (pH 6.6 and pH 6.7 for each, respectively) thus indicating the unsuitability of gum Arabic as a medium for such species. In general, all species showed a relative increase of acidity after biodegradation of gum Arabic medium. It was found that the presence of gum Arabic, as a carbon source, for enumeration of fungi and yeast, has no positive or significant effect on the sporulation of *Fuserimmoniliforme*while *Aspergillusniger*was clearly affected as was previously reported by Griffin and Gareen (1968).

3.3. Optical Density

3.3.1. Viscosity

After incubation for 25 days the viscosity of the *Acacia senegal* gum was reduced as result of microbial degradation. However, the initial viscosity of gum Arabic was 10 cps. The decreasing rate of viscosity as shown in table 2 varied according to the species involved. The initial viscosity of gum Arabic medium was reduced by all fungi and yeast species. The viscosity reduction ranged between 4 and 7 cps. The highest value of viscosity was obtained for *S. cerevisiae*. It has been noticed that all of treated gum Arabic samples have low values of viscosity levels when compared with control sample. Mhinzi (2003) reported that the viscosity of *Acacia senegal var. senegal* and *Acacia seyal var. fistula* from Tanzania was ranged between 1.7 – 4.6 and 3.4 – 5.7, respectively.

3.4. Nitrogen and Protein Content

Nitrogen and protein contents in control sample were 0.28% and 1.85%, respectively (Table 2). The highest levels of nitrogen and protein contents (0.112% and 0.74%, respectively) were obtained for samples treated with *Aspergillusniger*, which shows a relatively little growth in liquid medium compared to the others. Consequently, its efficiency in degrading nitrogen and protein was less than the other fungal species. This result may be due to deficiency of specific protein-hydrolysis enzymes, or probably due to low content of nitrogen itself in the gum Arabic medium used. The other four fungal species viz. *Penicilliumcitreonigrum*, *Rhizopusnigricans*, *Penicilliumnotatum*, and *Fusariummoniliforme* were all able to grow on gum Arabic medium, since the nitrogen and protein contents after

fermentation were similar in all species studied. Mhinzi (2003) reported that the nitrogen% of *Acacia senegal var. Senegal gum* and *Acacia seyal var. fistula gum* from Tanzania ranged between 0.32 – 0.37 and 0.25 – 1.42, respectively.

3.5. Sugars Content

The relatively high level (38%) of Galactose in gum Arabic control sample was completely consumed (0.0%) by both *Saccharomyces cerevisiae* and *Penicilliumnotatum* species, whereas in gum Arabic samples treated with *Penicilliumcitreonigrum* the level was found to be 7.4% after fermentation (Table 2). This may be attributed to the efficient growth of yeast *Saccharomyces. cerevisiae* and *Penicillium. notatum*. On the other hand, the relatively high content of galactose that was detected in the samples treated with *Penicilliumcitreonigrum* indicates the absence of the relevant degrading enzymes. Arabinose and rhamanose levels in control sample (27% and 12%, respectively) were also consumed to various degrees, depending on the species used in the treatment.

3.6. Number Average Molecular Weight after Biodegradation by Fungi Species

The number average molecular weight (M_n) of *Acacia senegal gum* samples under study was determined by osmometery measurement and is presented in table (2).

The number average molecular weight of *Acacia Senegal gum* samples ranged between $2.85x10^4$ and $1.51x10^4$. The *F.moniliforme* showed the highest value while *R. nigrians*was the lowest. The decrease in the number average molecular weights indicates a change of the molecular weight structure of the original components.

4. CONCLUSION AND RECOMMENDATIONS

Inoculation of gum Arabic with some species of fungi and yeast has deteriorated the main components that characterize its structure and the physicochemical properties as well, and therefore, affecting its functionality when it is intended to be used in pharmaceutical and food industries. It is recommended that the factors encouraging microbial growth must be given due consideration under gum Arabic storage conditions.

Funding: This study received no specific financial support.

Competing Interests: The authors declare that they have no competing interests.

Contributors/Acknowledgement: The authors would like to thank Mr.Altag Mustafa and Mr.MostafaAltoum of the Microbiology Laboratory, Dept. of Soil and Environment Sciences, Faculty of Agriculture, University of Khartoum, for their technical help and support throughout this study.

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Table-1. Some physiochemical properties of gum Arabic samples before and after sterilization

Tests	Sample Under Study	
	Before Sterilization	After Sterilization
Moisture (%)	12	12
pH	4.53	4.53
Acidity (ml)	0. 58	0.57
Nitrogen (%)	0.42	0.28
Protein (%)	2.77	1.85
Viscosity (spc)	10	10
M_n*	2.46x 10^5	2.46x 10^5
Sugars total (%)	79	77

*=Numberavergemolecularweight

Table-2. The effect of fungi and yeast species on some physicochemical parameters of gum Arabic

Property/content Determined	Control Sample	Fungi and yeast species					
		Aspergillusniger	Penicilliumcitreonigrum	Saccharomyces cerevisiae	Rhizopus. nigriansc	Penicilliumnotatum	Fusariummonforme
pH	6.8	4.340	5.312	6.174	6.435	6.631	6.7
Acidity	0.002	5.0	4.8	3.0	2.6	2.0	1.8
Mycelial dry weight (%)	0.0	0.050	0.51	0.15	0.12	0.55	0.65
Optical density	0.0	0.050	0.070	0.086	0.111	0.035	0.036
Viscosity (cps)	10	5.0	4.0	7.0	4.0	5.0	6.0
Nitrogen (%)	0.28	0.112	0.028	0.014	0.014	0.014	0.014
Protein (%)	1.85	0.74	0.185	0.092	0.092	0.092	0.092
Galactose (%)	38	3.9	7.4	0.0	0.26	0.0	2.4
Arabinose (%)	27	11.0	2.3	1.3	0.11	0.86	0.6
Rhamanose (%)	12	0.6	0.05	0.8	1.0	10.0	0.4
Number average molecular weight (Mn)	$2.46x10^5$	$1.64x10^4$	$2.21x10^4$	$1.7x10^4$	$1.51x10^4$	$1.88x10^4$	$2.85x10^4$

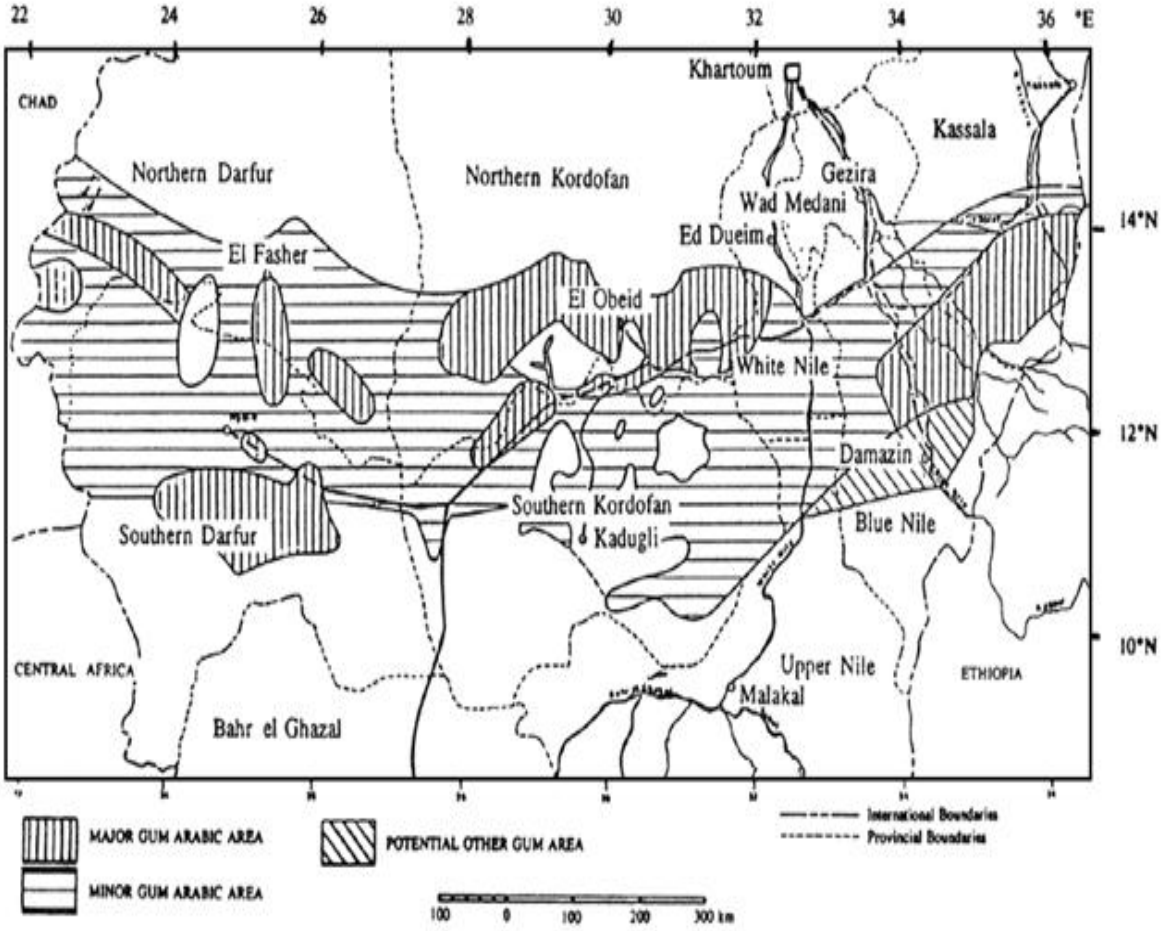

Figure-1. Map of the gum Arabic belt in Sudan (after (Kananji, 1993))

Figure-2. nodules of gum Arabic