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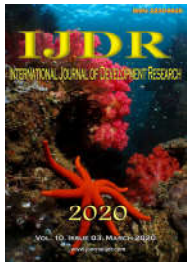

RESEARCH ARTICLE OPEN ACCESS

EFFECT OF SOME MICROBIAL SPECIES ON GUM ARABIC PHYSICOCHEMICAL CHARACTERISTICS

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ABSTRACT

Characterization of gum Arabic is of paramount importance especially when it is intended to be used as a natural additive in a wide spectrum of pharmaceutical and food industries. In this study, laboratory procedures as well as biochemical tests were used to investigate the effect of some bacteria species on the characteristics of *Acacia Senegal* var. *Senegal* gum Arabic stored for different periods. The results revealed that dried gum Arabic, kept under long-term storage, is more resistant to biodegradation than the freshly harvested one. In general, the pH, viscosity, nitrogen and protein were decreased to various levels in accordance with the bacterial species used. The various sugars level has also been affected; galactose and arabinose were totally consumed by microorganisms, whereas rhamanose level was partially decreased by all microbial species used in this study except for C. *Xerosis* where it was increased from 12% to 36%. The relatively high utilization of gum Arabic components by the microorganisms investigated indicates the probability of gum Arabic deterioration by the growth bacteria.

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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 the 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). It is mainly 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, spreading through what is known as the African Gum Belt (Gliksman, 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). Gum Arabic chemical composition with respect to sugar and cations is variable according to the tree age and location where the tree is grown (Idris *et al*., 1998; Osman *et al.,* 1995).

Nitrogen and protein component 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, 1991). Nitrogen content of *Acacia senegal. varsenegal*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. varsenegal* 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, 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). The moisture content of *Acacia senegal* gum is in the range from 11% to 16.1%. (Randall *et al*., 1989; Osman, 1993; Younes, 2009). The gum from *Acacia senegal*is a water soluble polysaccharide of the hydrocolloid group and comprised mostly of arabinogalactan and protein moiety, in addition to some mineral elements (Murwan, 2008). Solutions of Gum Arabic are viscous; the viscosity increases slowly up to 25% concentration, above which viscosity increases much more rapidly in proportion to gum content. Increased temperature reduces viscosity considerably. Gum Arabic has low viscosity despite its high molecular weight (Glicksman, 1969). Studies of flow of gum solution play an important role in identification and characterization of their molecular structure. Kaufman and Falcetta (1977) showed that viscosity could be presented in many terms such as relative viscosity, specific viscosity, reduced viscosity and intrinsic viscosity.

Aqueous solutions of gum are slightly acidic with a pH of 4.2 to 4.6, increases in pH result in a gradual increase in viscosity until pH 6 then increased until pH 12 where it remained constant (Thomas, 1928). Karamallah *et al*., (1998) reported the pH mean value of 4.66 for the 755 authentic *A. senegal* gum samples, collected in season 1994/1995. The same authors, in the same study , reported the mean value of 4.54 for commercial samples collected between 1992 and 1996 . Crude gum Arabic is slightly acidic because of the presence of few free carboxyl groups of its constituent acidic residues, D-glucuronic acid and its 4-O-methy1 derivatives, the pH value has been determined by Younes (2009), and he reported a value of 4.78 for *Acacia. senegal.* Deterioration of gum Arabic by bacterial action has been previously approved and well documented in the literature review. Physicochemical properties of gum Arabic are greatly affected by bacteria invasion before industrial processing (Adam *et al*. 2018). Accordingly, this study was to investigate the biodegradation of dry Gum Arabic quality that stored for different times.

MATERIALS AND METHODS

Description of study area: This study was carried out in Khartoum City, the capital of the Sudan where the raw gum was stored pending export. The capital is in the middle part of the country which is located between latitudes 15º26’ and 15º45’ N and longitudes 32º25’ and 32º40’ 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 8ºC and 10ºC which falls to 5ºC during night, and maximum temperatures varying from 23ºC to 25ºC, 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 45ºC 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 40ºC) transitional season occurs between mid-September and the beginning of winter.

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 marketplaces. Approximately 1 kg of 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 chemistry laboratory at Sudan University for Science and Technology, where they were kept pending analysis.

Determination of gum Arabic sugar content: The galactose, arabinose and rhamnose content gum Arabic were determined by high performance liquid chromatography (HPLC)(Thomas and Svoronos, 2003). Prior to analysis, the samples were hydrolysed into their constituent sugars.The hydrolysates were filtered (0.45, what man) and analysed using an HPLC system (Model No.410 Refractometer, Model No.717 Autosample, Model No.600 Controller. Waters Operating Corporation, Mass .01757,USA) Linked to a S5 amino column (250 mm X 4.6 mm; Waters. USA). The sample was injected (lOudm3) in to the column and eluted using a mobile phase of 75:25 V/V acetonitrile: water (filtered and degassed before use). Analysis was performed at ambient room temperature (30C°) and the flow rate maintained at 1.0 cm3 min'1. The retention times of the monosaccharides were monitored using a differential refractometer (R 410, waters). The retention times obtained were compared to those determined using D- galactose, L-arabinose and L- rhmnose (Sigma Chemical CO., Ltd.) as standards. The area percent of each peak was calculated by millinum program, which connected with RI. 410 detector.
The sugar percent was calculated as:

$$\text{Component sugar (i) \%} = \frac{\text{Area percent of component x 100}}{\text{Total area percent}}$$

RESULTS AND DISCUSSIONS

Effect of sterilization of gum Arabic components: No significant changes were observed on the gum Arabic components due to sterilization except nitrogen and protein percentages (Table 1). This may attribute to the high temperature which denaturizes protein and destroy its structure

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

*=Number averge molecular weight

Effects of bacteria species on physicochemical parameters of gum Arabic

Acidity and pH: The initial acidity of media was adjusted to pH 7.3 (acidity was 0.01) to match bacteria growth (control sample). Acidity was decreased to different levels according to bacterial species inoculated. The lowest pH value of the investigated samples was 6.401 (acidity was 4.0) which resulted from *B.lichenisfarium* activity whereas the highest pH value was 6.98 resulted from the activity of *Lactobacillus* (Table 2). Different pH values have been observed between the upper (6.9) and the lower (6.401) limits for the other *bacillus* species activity. In general, Corynebacterium species exhibited pH values ranged between 6.359 and 6.777 whereas the unique micrococcus species (*M. marians)* revealed pH value of 6.777. Streptococcus species revealed pH values of 6.244, 6.525 and 6.800 for *Strep. bovis, Sterp. faccalis and Sterp. faccalis,* respectively. Decrease of pH in the observed gum Arabic media might be due to bacterial activities.

Table 2. The effects of bacteria species on some physicochemical parameters of gum Arabic

Property/ content Determined	Control Sample	Bacteria species														
		Bacillus						Conyebacterium			Microc-occus	Streptococcus			Staphylococcus	
		B. cereus	*B. lactoba-cillus*	*B.licheni-sfarium*	*B. thuring-reris*	*B. polym-yxa*	*B. macer-ans*	*C. bovis*	*C. xeros-is*	*C. murim*	*M. marian-s*	*Strep. bovis*	*Sterp. facca-lis*	*Strep. facei-um*	*Staph. Epide-rmis*	*Staph. aureus*
pH	7.3	6.53	6.98	6.40	6.69	6.55	6.42	6.36	6.46	6.78	6.78	6.24	6.53	6.80	6.49	6.69
Acidity	0.01	2.5	1.5	4.0	1.9	2.3	3.0	3.2	2.5	1.9	1.9	3.6	2.6	1.9	2.2	2.3
Optical density	0.0	0.08	0.15	0.08	0.17	0.01	0.04	0.21	0.12	0.11	0.11	0.06	0.03	0.02	0.08	0.05
Viscosity (%)	10	7.0	7.0	7.0	7.0	8.0	7.0	8.0	7.0	12.0	12.0	8.0	7.0	7.6	8.0	6.0
Nitrogen (%)	0.28	0.008	0.007	0.008	0.007	0.008	0.010	0.007	0.013	0.014	0.014	0.008	0.007	0.007	0.010	0.010
Protein (%)	1.85	0.055	0.046	0.055	0.046	0.055	0.064	0.046	0.085	0.092	0.092	0.055	0.046	0.046	0.065	0.065
Galactose (%)	38	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Arabinose (%)	27	0.22	0.5	0.0	0.0	0.45	0.66	0.0	0.0	0.1	0.84	0.46	0.84	0.0	2.1	0.0
Rhamanose (%)	12	0.66	0.5	0.0	0.0	0.1	0.66	1.2	36.6	3.3	0.03	0.36	0.13	0.0	1.5	0.0
Number average molecular weight (Mn)	2.46 $x10^5$	2.92 x 10^4	2.44 $x10^5$	1.83 x 10^4	1.22 x 10^4	2.67 x 10^4	2.91 x 10^3	4.50 x 10^4	1.7 $x10^4$	2.13 $x10^4$	5.11 $x10^4$	2.71 $x10^4$	1.74 x 10^4	9.78 x 10^3	4.36 x 10^4	1.49 x 10^4
Reduction of Mn%	-	88.2	1	88.5	96.6	89.2	98.8	81.8	93.1	91.4	79.3	89	93	96.1	82.3	96

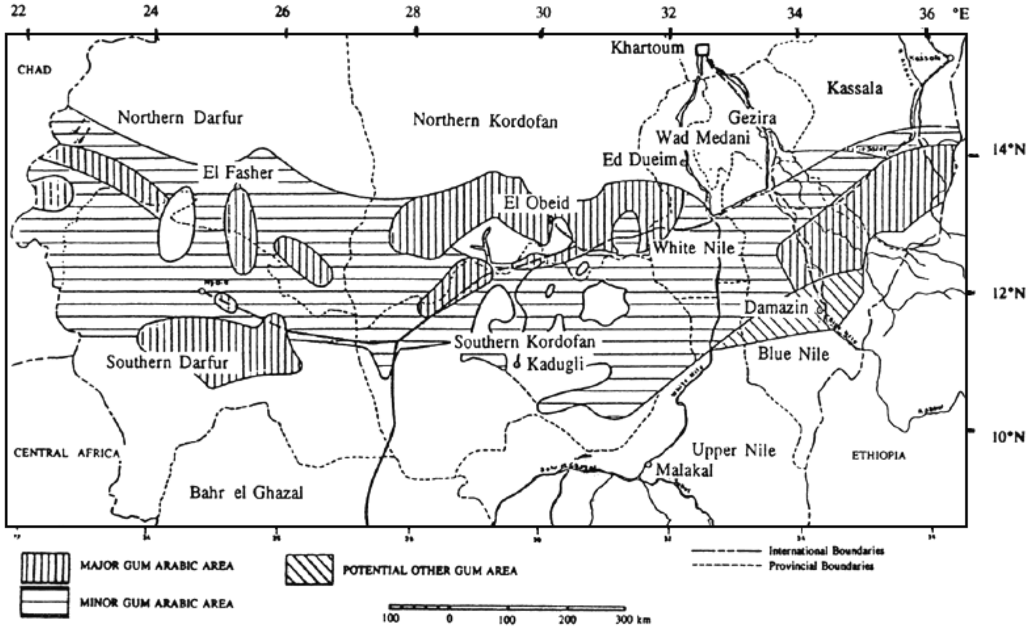

Figure 1. Map of the gum Arabic belt in Sudan (after Kananji, 1993)

The presence of natural enzymes such as pentase, arabinase and galactase in gum Arabic media may contribute to biodegradation hydrolysis of gum Arabic media which eventually decreases the pH value and changed its color into deep dark yellow. Decrease of pH values of gum Arabic samples after biodegradation demonstrated the liberation of carboxylic groups into the media as a result of microbial action. Variation of acidity levels in the investigated gum Arabic samples may be attributed to the diversity of microorganisms' activity, which decomposed the investigated media. There was a converse relationship between titratable acidity and pH measured. However, high acid levels in gum Arabic are often associated with low pH values.

Optical density: According to Table (2), optical density of the investigated gum Arabic medium samples under study was determined by spectrophotometer. Optical density readings were varied according to bacterial species inoculated. The highest optical density was 0.21, which observed in sample treated with *Corynebacterium bovis*, while the lowest optical density (0.01) was observed in sample treated with *B. polymyxa.* Changes of optical density readings in gum Arabic medium could be an indication of its suitability for the enumeration of bacteria species growth, to some extent, *B. thuringreris* grew more than other species under investigation. However, *Corynebacterium bovis* shows better growth rate than of *Micrococcus, Streptococcus* and *Staphylococcus* species.

Viscosity: Highest viscosity is (12.0) was observed in each of *Corynebacteriummurim* and *Micrococcus marianss*pecies while different values for the rest. The increasing of viscosity by *Micrococcusmarians* and *C. murim* may be attributed to the production of biopolymers (biomass). However, it seems that there is a converse relationship between growth rate and viscosity measured. The growth of the other different species of *Corynebacterium, Micrococcus, Streptococcus and Staphylococcus* decreased the viscosity by different degrees that ranged between 7.0 and 8.0 spc. These results are shown in (Table 2).

Nitrogen and protein content: Table (2) showed a significant decreasing in both nitrogen and protein content in the investigated gum Arabic samples after biodegradation by microorganisms, by different degrees from microbial species. It is believed that this decreasing can be taken as an indicator to demonstrate that the extracellular enzymes were produced for hydrolysis of protein. This hydrolysis may lead to changes in the medium components structure and eventually alters its molecular weight. The highest values of the protein and nitrogen contents obtained were 0.092% and 0.014% for *C. murim*and*M. marians,* respectively. Four variant species *B. cerus*, *B.lichenisfarium, B. polymyxa,* and *Strep. bovis*havegiventhe same nitrogen value (0.0084 %), and protein value (0.05250%) contents and corresponding losses in weight were found to be 97.1%. However, same values (0.0098 %) nitrogen and (0.06125%) protein and losing weight (96.5%) were found to be in each *Staph. epidermis and Stroph. aureus.* Nitrogen and protein contents were found to be 0.007% and 0. 0.046% for both of *Lactobacillus, C. bovisSterp.faccalis, Strep.faceium*.

Sugar contents: Table (2) shows change in the sugar contents of gum Arabic samples after biodegratation. Sugar contents were measured using HPLC technique after hydrolyised by the different microbial species for 25 days incubation time. Galactose contents were found to be 0.0% except *Strophaureus* which it was 0.1. Decomposition of galactose is probably attributable to enzyme action that attached some units of its side chains. Most of bacteria, including *Bacillus* and *Corynebacterium* and *Micrococcus* species utilize arabinose sugar for aerobic growth. Moreover, the sugar content for arabinose, and rhamnose were 0% for species as *B.lichenisfarium, B. thuringreris, Strep. Faceium* and *Strophylococcus. aureus.* Rahmanose was found to be 0.0 % for various bacterial species including *B.lichenisfarium, B. thuringreris*, *Stroph. Aureus* and *Strep. faceium.* Gum Arabic sugars were completely broken-down by the enzymes that excreted by *B.lichenisfarium B. thuringreris* and *Stroph. aureus* and the three sugar contents were found to be 0.0% as a result of consumption by bacterial activities. This may be attributed to the fact that these sugars may have been used for physiological activities. The study of the sugar content, aspects of the molecular structure and various acids support that the idea that urorinic acids are located on the periphery of the molecular structure of gum Arabic. *C. xerosis* synthesized a new polysaccharide from rhamanose sugar cleaved occurred by rhamanoidase enzyme. This may be lead to increasing rhamanose content; it became 36% (tetra-polymer), either by elimination of oxygen molecule from galactose or addition of CH to araboinose to synthesis this sugars.

Figure 2. Nodules of gum Arabic

Number average molecular weight (Mn): Treatment of gum Arabic with *Lactobacillus* resulted in a marked increase the number average molecular weight (M_n) (2.44x10^5) comparing with that before biodegradation. *B.macerans* degraded gum Arabic two folds (2.91x10^3) lower than the original gum sample (Table 2). Growth of all bacteria species in gum Arabic medium exhibited a clear reduction in M_n. This may indicate the formations of spherical molecules, which were removed from the original structure and molecular of weight may lead to a new structure formation. The reduction in number average weight (M_n) related to the lowering of the molecular weight (M_w) specially the molecular weight of proteinaceous component (short peptide chains). On the other hand, it is possible that the reduction in (M_n) could be attributed to the elimination of hydrogen bonds characterizes by polysaccharides polymers.

Conclusion and recommendation

According to the current study, the relatively high utilization of gum Arabic components by the microorganisms investigated indicates the probability of gum Arabic deterioration by the growth bacteria. Some of the investigated species have biodegraded gum Arabic components more than the others showing high activity and rapid growth in gum Arabic media. On the other hand, some of which has revealed low activity and slow growth in gum Arabic media. However, as general, all species show a relative increase of activity and growth when inoculated in gum Arabic as a growth medium. It is recommended to store raw gum Arabic in dry conditions to avoid attacking by fungi and yeasts. It is recommended that gum Arabic should be used as a cheap local media to laboratory enumerate many different kinds of bacteria species.

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