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EFFECT OF SOME ISOLATED BACTERIAL SPECIES ON THE PHYSICOCHEMICAL ASPECTS AND MAIN COMPONENTS OF GUM ARABIC (*ACACIA SENEGAL VAR. SENEGAL*)

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ABSTRACT

Physicochemical properties of gum Arabic are greatly affected by bacteria invasion before industrial processing. In this study, laboratory procedures as well as biochemical tests were used to assess the effect of some isolated bacterial species on the physicochemical aspects and main components of *Acacia senegal* var. *Senegal* gum Arabic. Degradation effects on the molecular weight of gum Arabic main components were remarkably affected in comparison to control. The arabinogalactan protein (AGP) was increased to six folds (from 3.80×10^6 to 22.69×10^6) by *Penicllium notatum*, and to three folds (11.03×10^6) by *Corynebacterium xerosis*, and to (7.08×10^6) by *Fusarium moniliforme*. Conversely, the AGP was decreased by *Aspergillus niger* from 3.80×10^6 to 1.36×10^6, whereas the bulk of the material corresponding to the protein-deficient arabinogalactan (AG) and galactoprotein (GP) component was decreased from 3.45×10^5 to 2.71×10^5. The results indicate that microorganisms have great impact on gum Arabic quality.

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INTRODUCTION

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 (Adam *et al.,* 2016a). Gum Arabic is a highly branched arabinogalactan polysaccharide, consisting mainly of calcium, potassium, sodium and magnesium salts with lower protein properties (Karamalla, 1999). It consists of three main fractions, AGP (10%), AG (90%) and glycoprotein (1%). The carbohydrate composition is galactose (40%), arabinose (30%), rhamnose (12%), glucuronic acid (15%) and has a branched structure with a β-1,3 linked galactan backbone (Street and Anderson, 1983). Siddig *et al*. (2005) reported that the AG fraction has a disk-like morphology with a diameter of 20 nm and a thickness below 2nm.

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Mahendran *et al*. (2008) stated that the AGP fraction was found to decrease on treatment with proteolytic enzyme although the third component (GP) was not affected. Using new techniques of gel permeation, flow field-flow fractionation and multi-angle laser light scattering, Phillips, Fenyo and Muller clarified the complex structure of acacia gum (Connolly *et al*., 1988). They stated that Acacia Senegal molecule has polymo lecular structure whereas gel permeation and size-exclusion chromatography has shown that Acacia senegal at least contains two fractions with different molecular weights and for both exudates, the highest molecular weight fraction contains the majority of the proteins but represents a minority percentage of gum. Fincher *et al*. (1983) and Connolly *et al*. (1988) reported that the 'wattle blossom' structure represents the highly branched compact structure of acacia gum from Acacia senegal. Arabinogalactans are attached to a protein skeleton forming the AGP fraction. The polysaccharide fraction is composed of a linear chain of β

(1,3)-linked galactose. In position (1,6), this chain is branched with side chains of galactose and arabinose. Rhamnose, glucuronic acid or methyl glucuronic acid units both found as chain terminations in the AG fraction (Street and Anderson, 1983). Gum Arabic is highly water soluble and is used as a food additive. Gum Acacia is used in pharmaceutical, cosmetic and food industries as an emulsifier and stabilizer. Dried supplements of gum Arabic increased fecal nitrogen excretion and decrease serum urea nitrogen concentration in patients with Chronic Renal Failure, and this was attributed to the increase of bacterial growth and activity in the gut (Bliss *et al.,* 1996). Gum Arabic was reported to be used for the treatment of inflammation of the intestinal mucosa (Gamal el-din *et al.,* 2003). Adam *et al.* (2016b) stated that the sugars content has noticeably been affected viz. galactose was entirely consumed by Saccharomyces cerevisiae and Penicillium notatum while. rhamnose was drastically decreased by all microbial species under study. Likewise, the number average molecular weight was decreased by all species. The objective of this study is to investigate the alteration on gum Arabic main components induced by selective microorganisms.

MATERIALS AND METHODS

Materials

Four samples of dried gum Arabic (*Acacia senegal*) were obtained from stores of Gum Arabic Company and from three different market locations at Khartoum, Sudan. All samples were harvested in the duration of 2005- 2008.

Microbiological work was conducted in the microbiology laboratory of the Department of Soil and Environment Sciences, Faculty of Agriculture, University of Khartoum, Sudan

All types of bacteria (*bacillus spp.* were *B. cereus*, *B.polymyxa, B. licheniformis*, *Corynebacterium xerosis*, *Staphylococcus epidermis*, *Sterptococcus bovis*) and fungi (*Penicllium notatum, Rhizopus nigricans, Aspergillus niger, Fusarium moniliforme*) were isolated from gum Arabic, *Lactobacillus* sp. was isolated from camel milk.

The main medium used in this study was gum Arabic as carbon and nitrogen source, and then sodium hydroxide (1N) was added to adjust pH.

Methods

Three grams of Gum Arabic were sterilized by autoclaving for 10 min at 121ºC and 15lb/in, then dissolved in sterile distilled water; pH was adjusted by adding few drops of sodium hydroxide (1N). The bacteria and fungi isolates were grown in aseptic bottles of 200 ml capacity containing 100 ml of gum Arabic medium.Then the inoculated medium was incubated at 28 ºC for bacteria and at room temperature for fungi for 25 days, and then dried to constant weight. Chemical analyses were run in Phillips Hydrocolloids Research Centre (PHRC), Glyndwr University, Wrexham, LL11 2AW, UK; Molecular weight parameters were monitored by GPC-MALL using detection systems Light Scattering (LS), Refractive Index (RI). Protein profile (UV) was detected at (214nm). Each of molecular weight of whole gum, molecular weight of AGP peak, % AGP, Rg (root mean square radius of gyration) were investigated to study biofractionation of gum Arabic.

RESULTS AND DISCUSSION

Fractionation of *Acacia senegal. var. Senegal*

Figure (1) shows standard gum Arabic fractionation while figure (2) represents control gum fractionation. Gum Arabic has three different fractions, and the elution profile of *Acacia senegal* (figures 1 and figure 2) are the GPC profiles of the standard sample obtained from Phillips Hydrocolloids Research Centre (PHRC, Glyndwr University, UK) and control sample (as monitored by LS, RI, and UV detectors) was for detecting the amount of protein in each fraction**.** The LS response showed two distinctive peaks for gum Arabic, the first peak indicates the high molecular weight of AGP. The second AG peak is broader with a lower response and it accounts for the rest of the gum. The RI indicates the concentration of the two main fractions of AGP and AG, the concentration of the AGP is lower than AG for gum, therefore, its peak was smaller. The UV response shows three different peaks, the first one for AGP, AG and GP, which has a protein core and carbohydrates attached to it. The control figure, was similar to the standard obtained from Phillips Hydrocolloids Research Centre (PHRC) except for the third peak; this peak has low protein when compared with the standard.

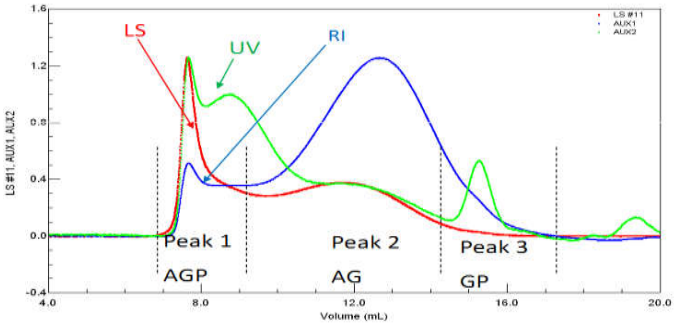

Fig. 1. GPC chromatogram showing the elution profiles monitored by light scattering (LS) (red), refractive index (RI) (blue) and UV light at 214 nm (green) detectors for *Acacia enegal* sample (standard sample

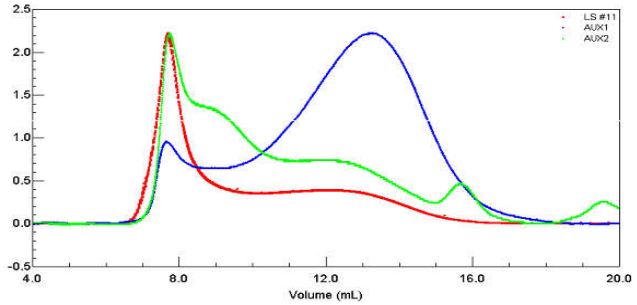

Fig. 2. GPC chromatogram showing the elution profiles monitored by light scattering (LS) (red), refractive index (RI) (blue) and UV light at 214 nm (green) detectors for control sample (Table 1sample 12)

Bio-fractionation and molecular weight distribution of gum *Acacia senegal. var. Senegal:* Figures 3, 4 and 5 show similar elution profiles for molecular weight distribution of gum Arabic *Acacia senegal. var. Senegal* due to biofractionation by of the genus *Bacillus viz. B. licheniformis, B. cereus* and *B.polymyxa* (Table 1) sample 8, 16 and 17) which showed less hydrolysis effect. The LS response of light reflects the mass and concentration and showed two distinctive

peaks. The first peak has a lower response since it corresponds to the higher molecular weight material AGP complex content and the M_w of AGP decrease from $3.80x10^6$ to ~ $2x10^6$ for all *Bacillus sp* (Table 1). The second peak showed a high and a big size response (AG + GP). It increased to $3.55x10^5$ and $4.28x10^5$ for *B. cereus*, and *B.polymyxa*, respectively, however,. with respect to *B. licheniformis* it decreased to $3.28x10^5$.

The RI, concentration detector response has also showed two peaks, which were different when compared to LS response. The first peak has almost disappeared, with elution volumes of ~ 7.5 ml. The RI concentration detector response also showed two other peaks, which are different from LS response, and they represent about ~16% of the total mass and the second peak is broader with higher response corresponding to the

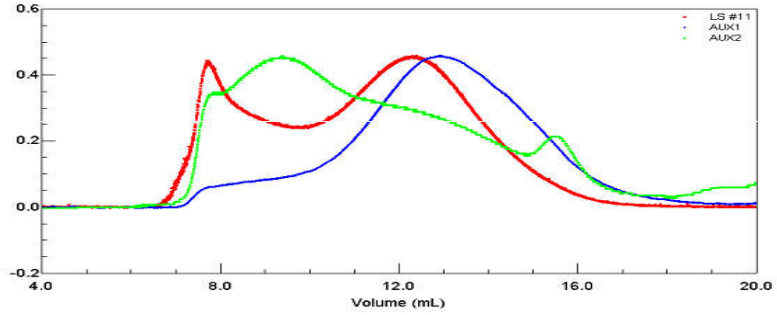

Fig. 3. GPC elution profiles showing the effect of *B. Licheniformis* (Table 1 sample 8) on the molecular distribution of gum Arabic

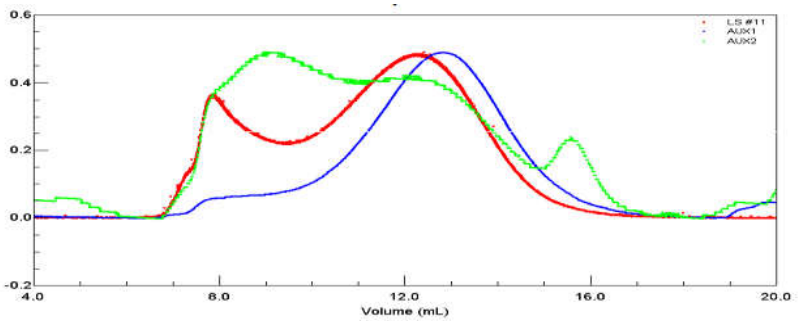

Fig. 4. GPC elution profiles showing the effect of *B. cereus* (Table 1 sample 16) on the molecular distribution of gum Arabic

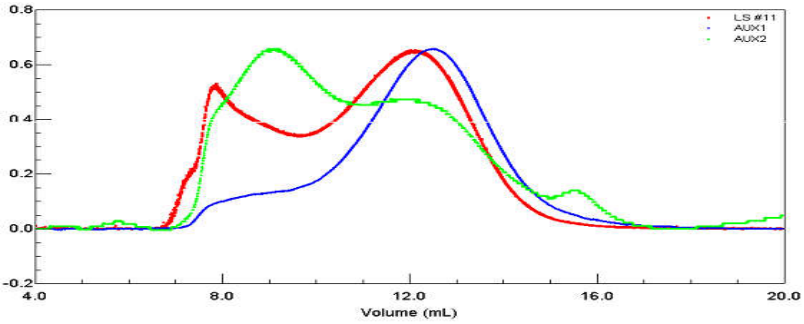

Fig. 5. GPC elution profiles showing the effect of *B.polymyxa* (Table 1sample 17) on the molecular distribution of gum Arabic

Table 1. Summary of M_w Parameters

Microbe code NO.	Wnx10^5 (whole gum)	Rg/nm	M_w AGPx10^6	% mass (AGP)	Rg -AGP	M_w(AG+GP)x10^5	% mass
S1*	4.49	41	4.86	3.12	60	3.05	96.88
S2	2.84	23	1.36	1.20	31	2.71	98.80
S3	10.04	68	22.69	3.05	76	3.17	96.97
S4	4.82	56	7.08	2.70	78	2.99	97.30
S7	3.62	29	11.03	0.65	56	2.87	99.35
S8	3.73	23	2.63	1.95	42	3.28	98.05
S12	7.43	64	3.80	11.30	73	3.54	88.70
S15	3.92	22	2.36	1.10	46	3.69	98.90
S16	4.07	22	2.31	2.65	65	3.55	87.35
S17	4.95	24	2.42	3.38	46	4.28	96.62
S20	7.89	43	4.67	7.70	65	4.66	92.30
S21	5.40	28	4.02	4.10	49	3.92	95.90

S1*: *Rhizopus nigricans*
S2: *Aspergillus niger*
S3: *Penicillium notatum*
S4: *Fusarium moniliforme*
S7: *C.xerosis*
S8: *B. licheniformis*
*S12 control sample
S15: *Staphl. epidermis*
S16 : *B. cereus*
S17: *B.polymyxa*
S20: *Sterpto. bovis*
S21: *Lactobacillus*

major peak which appeared at the elution volume of ~10.5 ml to 14 ml (~ 84 % of the total mass). For *Acacia senegal* gum the RI response is opposite in direction to that in LS as shown in figure (3) This is possibly because of its low concentration and that the AGP was only ~2 or 3 % of total of gum Arabic, and its peaks was smaller than AG and GP which constitute ~97%. The UV response showed three different peaks. The first one (peak 1) is for the AGP which has a protein core with a carbohydrate attached to it, but it has comparatively decreased.

marked resistance to enzymes; this may be due to the fact that this fraction has different amino acids composition from that linked to the AGP fraction, and that aspartic acid, leusine and glycine being the main components. In contrast, the peak of the lower molecular weight (peak 2) has been apparently increased. The UV elution profiles showed that the treatment with protease enzyme has gradually induced a decrease in the intensity of peak 1 and an abrupt increase in the intensities of peaks 2 (Osman *et al.,* 1993).

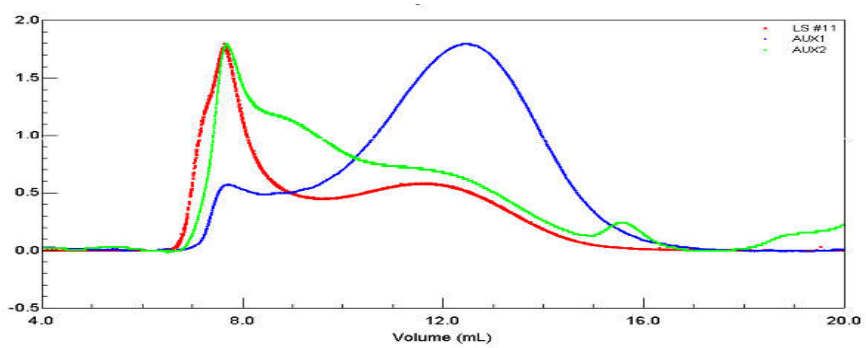

Fig. 6. GPC elution profiles showing the effect of *Strepto. bovis* (sample 20) on the molecular distribution of gum Arabic

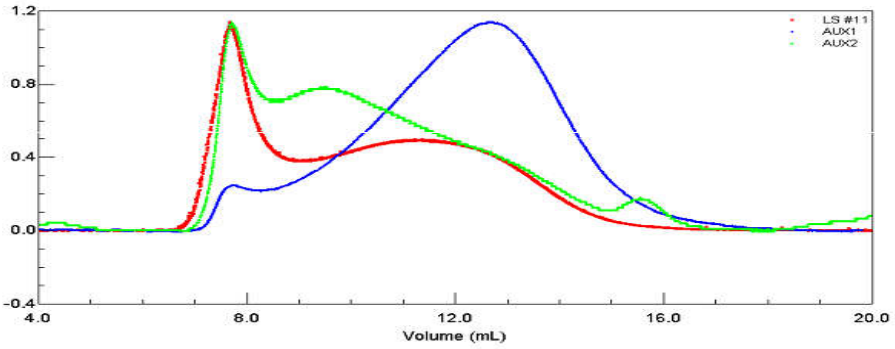

Fig. 7. GPC elution profiles showing the effect of *Lactobacillus* (Sample 21) on the molecular distribution of gum Arabic

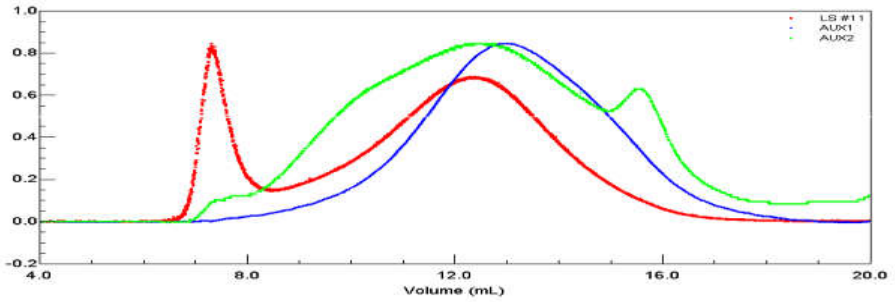

Fig. 8. GPC elution profiles showing the effect of *C.xerosis* (Sample 7) on the molecular distribution of gum Arabic

The second peak (peak 2) appears after the AGP and corresponds to the AG but has comparatively increased. The third peak (peak 3) which corresponds to GP also was lowered. Peak 1, of the higher molar mass, decreased in concentration after digestion by *Bacillus* enzymes. Thus indicating that glycosidic bonds have been broken by microbial enzymes, and some of the sugar was linked with fraction 2. The results demonstrated that the high molecular mass AGP component is hydrolyzed by the microbial enzyme and its molecular mass was reduced from 7.43×10^5 to 3.73×10^5, indicating that large carbohydrate blocks are linked to a polypeptide chain in accordance with the "wattle blossom" type structure (Connolly *et al*., 1988, Randall *et al*., 1989, and Osman *et al*., 1993). Loss in weight (about 40%), as noticed in peak 2, showed a

On the other hand, slight changes have been observed in the elution chromatogram measured by RI. It was evident that there was an increase in amount of the AG and GP compounds, demonstrating the hydrolysis of AGP by *Bacillus* enzymes producing both AG and GP. The UV is another indicator of enzymatic degradation. The high molecular mass in peak 1, indicating that glycosidic bonds have been broken. Figures 6 and 7 and were seemed to be similar, while figure 8 was different in the GPC chromatogram, demonstrating the effect of *Bacillus* species as well as fermentation by *Strepto. bovis,Lactobacillus* and *C.xerosis* (Table 1 samples 20, 21 and 7) on molecular weight distribution. The LS for *Lactobacillus* and *C.xerosis* showed a decrease in molecular weight of 5.40×10^5 and 3.62×10^5; respectively. But there was an increase

by *Strepto. bovis that amounted to* $7.89x10^6$. However, Figure 8 that represents *C.xerosis* has a major hydrolysis effect on the protein of AGP which shifted to aggregate on the AG and GP. The LS and RI response reflected the mass and concentration with two distinctive peaks. The first one has a high response since it corresponded to the high molecular weight material of AGP complex content. The second peak has a lower response and similar to that found in the control sample. The UV response showed three peaks, but the first one (AGP) was degraded to lesser extent compared to *C.xerosis* with *Strept. bovis* and *Lactobacillus* activities.

weight of AG and GP components was slightly changed from $3.54x10^5$ to $3.69x10^5$ g/mol. The digestive enzymes excreted by *Staph epidermis* into of gum Arabic medium, affected protein components. The peak for high molecular weight was degraded resulting in two peaks, thus indicating that all gum was converted into one single peak with low molecular weight similar to that for AG. This result indicates that the AGP was degraded to AG and GP. The elution profile obtained by UV presented in Figure 9 is completely different when compared with the RI profile that showed three molecular masses for gum Arabic sample when compared with control sample.

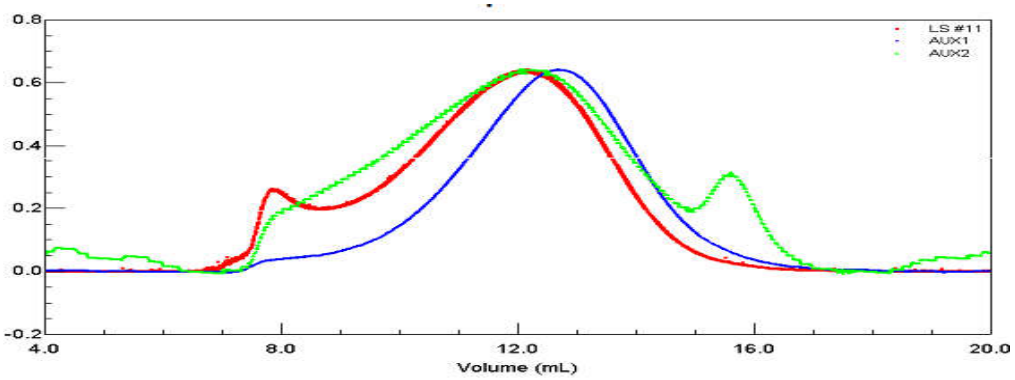

Fig. 9. GPC elution profiles showing the effect of *Staph epidermis* (Sample 15) on the molecular distribution of gum Arabic

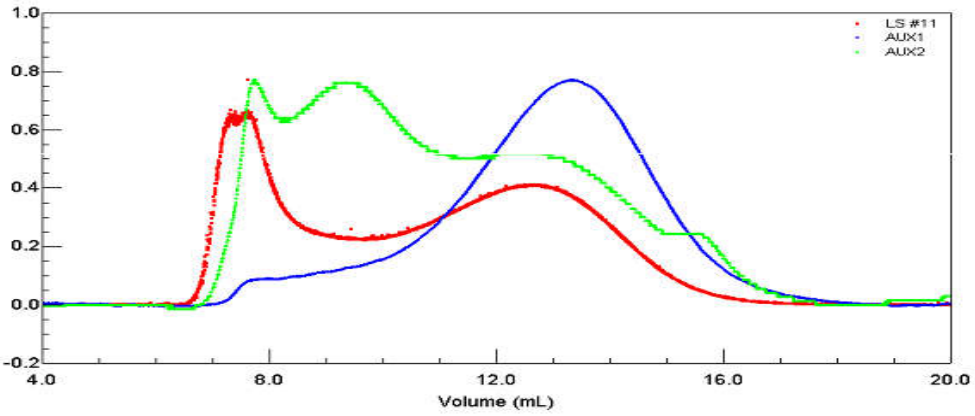

Fig. 10. GPC elution profiles showing the effect of *Rhizopus nigricans* (sample 1) on the molecular distribution of gum Arabic

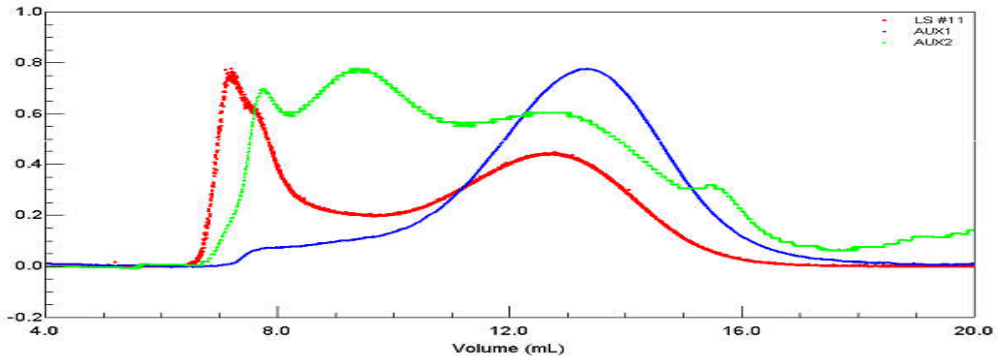

Fig. 11. GPC elution profiles showing the effect of *Fusarium moniliforme* (sample 4) on the molecular distribution of gum Arabic

The second peak appeared immediately after AGP peak and corresponds to AG, but *C.xerosis* showed irregular shape. Finally, the third peak elutes was corresponding to GP; it was not detected on LS since it has low molecular weight. However, it appeared clearly in *C.xerosis treatment*. Figure 9 showed a decrease in molecular weight (M_w) from $7x10^5$ to $3.43x10^5$ by*Staph epidermis* sample. The AGP M_w value decreased from $3,8x10^6$ to $2.36x10^6$ whereas the molecular

Bio-fractionation and molecular weight distribution of gum *Acacia senegal* by fungal species

The structural characteristics of the gum exudates of *Acacia senegal* have been investigated before and after being attacked by fungal enzymes. Elution profiles (LS, RI and molar masses (M_w) distributions of the fungi *Rhizopus nigricans,* and *Fusarium moniliforme* for treated crude gum Arabic are shown in Figures 10, 11 and 12 (Table 1samples 1,2, and 3).

Two peaks were observed in the medium of *Rhizopus nigricans* as has previously been observed in control gum Arabic sample that presented in Fig (2). However, there is an insignificant change in each LS and RI responses due to the higher fraction in molecular masses by the species as shown in Figure 10. It decreased the molecular weight from $7.43x10^5$ to $4.49x10^5$. The molecular weight in peak 1 is $4.86x10^6$ for AGP. The slight increase in molecular weight may be due to biological processing products linked with AGP part such as sugars. In contrast, the peak of lower molecular weight (peak 2) exhibited a mild decrease to $3.05x10^5$. The result of UV elution profiles indicated that the treatment of gum Arabic with each of *Rhizopus nigricans* and *Fusarium moniliforme* showed gradual increase of the intensity of peak 1 while the increase of the intensities of peaks 2 and peak 3 has abruptly occurred (Figures 10 and 11).

GPC has a M_w of $22.69x10^6$ which means that means all the AG and the GP were hydrolyzed to AGP thus accompanied with reductions in molecular weight for all gum to $4.82x10^5$. In general, the M_w of gum was reduced to $3.16x10^5$ after biodegradation. The whole modification of the species on Acacia *senegal* gum medium composition, after fungal treatment, suggests that the selective enzymes may have hydrolyze the peptide and glaycid bonds between carbohydrate and protein. It may be noticed that peak 2 in all figures showed marked resistance to hydrolytic degradation by microbial enzymes. This may be due to the occurrence of the difficult to hydrolyze amino acids composition linked to the original compound. The results demonstrate that the large carbohydrate blocks are linked to a polypeptide chain in accordance with the "wattle blossom" type structure (Connolly *et al*., 1988; Randall *et al.,* 1989, Osman *et al*., 1993).

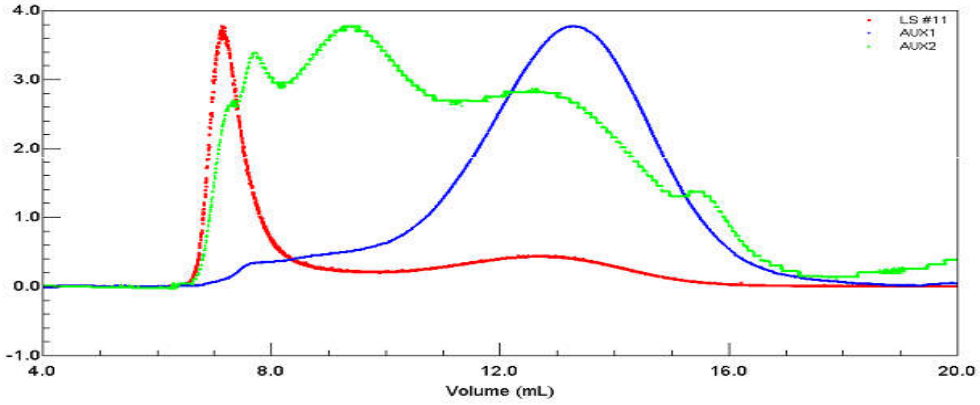

Fig. 12. GPC elution profiles showing the effect of *Penicllium natatum* (sample 3) on the molecular distribution of gum Arabic

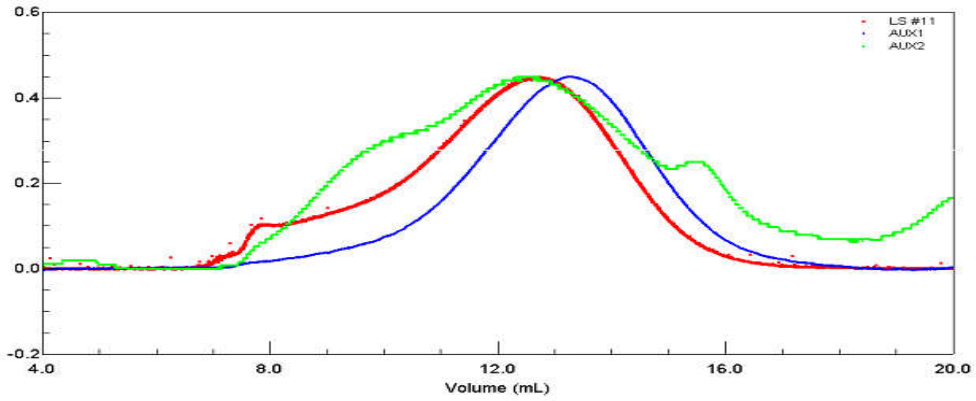

Fig. 13. GPC elution profiles showing the effect of *Aspergillaus Niger* (Sample 2) on the molecular distribution of gum Arabic

Due to *Penicllium notatum* activity, the increase of molecular weight to $10.04x10^5$ may be attributed to either newly syntheized compounds or to some compounds transferred from AG and GP to form AGP with M_w of $7.04x10^6$ due to dipolymarization of protein structure. Figure (13) shows that the structural characteristics of the gum exudates of *Acacia senegal* which were investigated after being fractured by *Aspergillaus Niger* enzymes. The results demonstrated that the high molecular mass AGP component was slightly hydrolyzed by the protein enzymes and its molecular mass decreased from 3.80×10^6 to 1.36×10^6, indicating that large carbohydrate blocks that linked to the polypeptide chain have partially been decreased. *Aspergillus niger* has differently and strongly affected the medium compared to other fungal species of *Rhizopus nigricans,* and *Fusaruim moniliforme* since the first peak has disappeared due to the activity of protein enzymes.

GPC molecular weight distribution encouraged degradation of AGP, AG and GP but with lowering concentration. However, AGP has almost disappeared in some microbial treatments. In some cases the AGP molecular weight was increased due to synthesis or regeneration of new molecules, (carbohydrates and protein) by *C. xerosis* and *B. cereus* and the fungal speices *Penicillium notatum*, *Fusarium monilifome* and *Aspergillus niger* introducing di or tetra polymers for sugars and proteins.

Conclusions and recommendations

The gum Arabic quality is greatly deteriorated by microorganisms. The AGP was increased to six folds (from 3.80×10^6 to 22.69×10^6) by *Penicillium notatum*, and to three folds (to11.03×10^6) by *Corynebacterium xerosis*, and to (7.08×10^6) by *Fusairum moniliforme*. In contrast, the AGP

was decreased by *Aspergillus niger* from 3.80×10^6 to 1.36×10^6, whereas the bulk of the material corresponding to the protein-deficient AG and GP component was decreased from 3.45×10^5 to 2.71×10^5. It is recommended that the factors encouraging microbial growth must be given due consideration under gum Arabic storage conditions.

Acknowledgement

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