PHYSICOCHEMICAL CHARACTERIZATIONS OF ACACIA SIEBERANA VAR. SIEBERANA OF SUDANESE ORIGIN.

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Abstract

Twenty four authentic samples of Acacia sieberana var. sieberana were collected from South Kordofan (SK) and White Nile (WN) states. The samples were analyzed to determine average values, of moisture, ash, pH, specific rotation, refractive index, number average molecular weight, intrinsic viscosity, nitrogen content, protein content, total uronic acid and acid equivalent weight of the gum. For (SK)samples average values, for the above parameters, were found to be: 8.56 %, 1.61%, 4.34, +104°, 1.337, 2.13×106 Da, 8.56 ml/g, 0.37%, 2.45%, 9.2% and 1933 respectively, while those of WN samples were found to be: 9.12 %, 1.65%, 4.38, 103, 1.337, 2.29×106 Da, 9.39 ml/g, 0.37 %, 2.43%, 9.4 % and 1884 respectively. The cationic composition showed the following sequence: calcium > potassium> magnesium > sodium. Traces of: Fe, Ni, Co, Mn, Cu and Pb were detected. Percent average values of sugar composition show that galactose, arabinose, rhamanoe, glucuronic acid and 4-O-methylglcuronic acid for the gum samples from both locations are similar. For SK samples the values were found to be: 9.8, 57, 4.0, 2.7 and 6.5 respectively, while those of WN samples were found to be: 9.9, 56, 3.1, 2.4 and 6.6 respectively. The molecular weight distributions, of two composite samples, of the gum from the two locations indicate the presence of three main fractions. The weight average molecular weight of SK and WN, composite samples, is 1.8×106 Da and 1.6×106 Da respectively. The emulsifying capacity study of the gum shows that the gum is a grade one emulsifying agent.

Introduction:

Polysaccharide gums derived from varieties of Acacia species are used extensively in food and pharmaceutical industries (Millard and Balmert 1961; Tame-Said 1997). Although many Acacia species produce gums of potential quality, the varieties of marketable gums are limited. Considering the scarcity of gums during the last few years, it became important to explore new sources of gums and evaluate their potential application qualities. However, Sudan provides an ideal location for such exploratory studies, as it possesses a diversity of natural forests including species from non acacia resources (Sahni, 1968).

Acacia sieberana has been classified, taxonomically, as Gummiferae, Bentham’s Series 4 (Bentham, 1875). Limited studies on the properties of the gum were reviewed by Adriaens, 1939 and Anderson et al., 1973. Karamalla, 1999 reported some analytical data for three samples of sieberana species, namely A. sieberana var. sieberana, A. sieberana var. vermeseni and A. sieberana var. Villosa. The polysaccharide from Acacia sieberana var. sieberana gum is one of the available gum resources which are not exploited commercially.
The present work is an attempt to extend the scope of previous studies and to establish a frame of specifications for the gum from this species taking in consideration international guidelines for the specification for marketable gums from Acacia. It was also intended to investigate the effect of environmental variations on the gum quality. The work also was aimed to investigate some functional properties of the gum from this species.

Materials and methods:-

Materials:-
Gum samples were collected from (SK and WN) states in seasons 2006-2008 and authenticated by botanist of Ministry of Forestry-Sudan. The samples were cleaned from sand and bark impurities. Representative samples were powdered to a white-pale yellow powder using a pestle and mortar.

Methods:-
Standard methods of analysis to determine physicochemical properties were used (Chickamai et al., 1996). The gel permeation chromatography (GPC) method coupled to a multi-angle laser light scattering detector, a refractive index detector and a UV detector operated at 214 nm used in this study has been previously described (; Al-Assaf, et al., 2005).

The emulsification function is investigated by measuring the droplet size distribution of the emulsion at three different temperatures: as fresh sample and after storage for 3 and 7 days once at ambient temperature and once at 60 °C (using accelerated stability test). The samples were subjected to Mastersizer 2000, a laser diffraction particle size analyzer (Malvern Instruments). Distilled water was used as dispersant and a value of 1.450 was used for the refractive index for oil phase Octanoic/Decanoic acid triglyceride oil (ODO) (Katayama, et al, 2006).

Emulsion preparation:-
Distilled water was added to about 12 g of the gum sample (based on dry weight) in glass bottle to become about 40 g in total with a concentration of 30 % (w/w) gum solution. The sample was agitated on a tube roller mixer overnight until the sample completely dissolved and hydrated. Exact calculated grams for each samples (~27 - 28 g) of the prepared gum solution was filtered using 100 µm mesh then mixed with 0.52 ml of 10 % (W/V) sodium benzoate solution as a preservative, and 0.48 ml of 10 % (W/V) citric acid solution to adjust the pH to 4, distilled water was added until the total weight become 32.0 g. then, 8.0 g ODO oil was added to the gum solution to give a total of 40 g and final concentration of 20%. The mixed solution was homogenized for 3 minutes using a polytron (PT-2100) homogenizer at 26000 rpm. Impeller (PTDA21 9 mm tip diameter) was used as dispersing tool. To achieve small particle size < 1 micron, the pre-emulsified mixture was homogenized using a high-pressure Nanomizer (NM2-L100, Yoshida a kikai Co. Ltd.). In order to achieve effective disaggregation of the gum it was passed twice at 50 M Pa. The final emulsion was divided into two aliquots and kept in closed glass universals. One of the aliquots was kept at 60 oC in the Vacuum Oven (Gallenkamp. OVA031.XX1.5), and the other was kept at ambient temperature (Sakata et al, 2006).

Results and discussion:-

Tables (1 and 2) show the results of the physicochemical characterization of the samples from SK and WN states. Average values for % moisture, % ash, %nitrogen, % protein, % total uronic acid pH, specific rotation, number average molecular weight, intrinsic viscosity, and equivalent weight are almost identical for all samples, however very slight variations are observed.

Low intrinsic viscosity indicates a highly branched globular structure (Barrow, 1979). Percentage of nitrogen and protein for SK and WN samples are three times that of Acacia seyal (Hassan et al., 2005) and are in the same order for those of Acacia senegal (Osman et al., 1995). Eequivalent weights for SK and WN samples are slightly different and are higher than the values reported for Acacia seyal (Hassan et al., 2005). The high equivalent weight for Acacia sieberana var. sieberana may be attributed to its high molecular weight and relatively low uronic acid content.

Tables (3 and 4) show the major cationic compositions of Acacia sieberana var. sieberana gum samples from SK and WN respectively. The results indicate that the major elements are in the order Ca > K > Mg > Na for SK samples and in the order Ca > K > Na > Mg for WN samples. These differences may be attributed to differences in soil element composition.

Tables (5 and 6) show the sugar compositions of Acacia sieberana var. sieberana gum for SK and WN samples respectively. The results indicate two facts: arabinose: galactose ratio is >1 and rahmnose Content is low. These two facts are typical features of gums from the Gummeeferae (Anderson and McDougall, 1987).
Table 1: Physicochemical data of *A. sieberana* var. *sieberana* collected from South Kordfan state.

| Sample code | Moisture % | Ash % | pH | Specific rotation | Refractive index | Equivalent weight | Intrinsic viscosity | Nitrogen % | Protein % | Uronic acid anhydride % | Sample code |
|-------------|------------|-------|----|-------------------|------------------|-------------------|-------------------|------------|-----------|------------------------|-------------|
| SK1         | 8.19       | 1.35  | 4.25| 4.25              | 1.33             | 8.19              | 1.35              | 4.25       | 1.35      | 2.52                   | SK1         |
| SK2         | 8.99       | 1.33  | 4.23| 4.23              | 1.33             | 8.99              | 1.33              | 4.23       | 1.33      | 2.31                   | SK2         |
| SK3         | 8.92       | 1.42  | 4.25| 4.25              | 1.33             | 8.92              | 1.42              | 4.25       | 1.42      | 2.34                   | SK3         |
| SK4         | 7.31       | 1.38  | 4.25| 4.25              | 1.33             | 7.31              | 1.38              | 4.25       | 1.38      | 2.34                   | SK4         |
| SK5         | 8.19       | 1.35  | 4.25| 4.25              | 1.33             | 8.19              | 1.35              | 4.25       | 1.35      | 2.34                   | SK5         |
| SK6         | 8.99       | 1.33  | 4.23| 4.23              | 1.33             | 8.99              | 1.33              | 4.23       | 1.33      | 2.34                   | SK6         |
| SK7         | 8.92       | 1.42  | 4.25| 4.25              | 1.33             | 8.92              | 1.42              | 4.25       | 1.42      | 2.34                   | SK7         |
| SK8         | 7.65       | 1.38  | 4.25| 4.25              | 1.33             | 7.65              | 1.38              | 4.25       | 1.38      | 2.34                   | SK8         |
| SK9         | 8.19       | 1.35  | 4.25| 4.25              | 1.33             | 8.19              | 1.35              | 4.25       | 1.35      | 2.34                   | SK9         |
| SK10        | 8.99       | 1.33  | 4.23| 4.23              | 1.33             | 8.99              | 1.33              | 4.23       | 1.33      | 2.34                   | SK10        |
| SK11        | 8.92       | 1.42  | 4.25| 4.25              | 1.33             | 8.92              | 1.42              | 4.25       | 1.42      | 2.34                   | SK11        |
| SK12        | 7.31       | 1.38  | 4.25| 4.25              | 1.33             | 7.31              | 1.38              | 4.25       | 1.38      | 2.34                   | SK12        |
| Mean        | 8.56       | 1.61  | 4.37| 4.37              | 1.33             | 8.56              | 1.61              | 4.37       | 1.61      | 2.34                   |              |
| SD          | 0.65       | 0.23  | 0.11| 0.11              | 0.0006           | 0.65              | 0.23              | 0.11       | 0.11      | 0.0006                 |              |
| CV %        | 7.55       | 14.4  | 2.58| 2.58              | 0.05             | 7.55              | 14.4              | 2.58       | 2.58      | 0.05                   |              |
| Sample code | Equivalent weight | Moisture % | Ash % | Protein % | Uronic acid anhydride % | Nitrogen % | Intrinsic viscosity | Mn (× 10^6 Da) | Refractive index | Specific rotation | pH | Ash % | Moisture % | Mean | SD | CV |
|-------------|-------------------|------------|-------|-----------|------------------------|------------|--------------------|----------------|----------------|-----------------|----|-------|------------|------|---|----|
| WN1         | 1797              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.7               | 9.9            | 1.337          | 101             | 4.35 | 4.48  | 8.19       | 9.6  | 0.6 | 6.7 |
| WN2         | 1834              | 9.4        | 1.2   | 9.5       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
| WN3         | 1827              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
| WN4         | 2001              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
| WN5         | 1914              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
| WN6         | 1834              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
| WN7         | 1827              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
| WN8         | 2001              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
| WN9         | 1914              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
| WN10        | 1834              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
| WN11        | 1827              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
| WN12        | 2001              | 9.6        | 1.2   | 9.2       | 4.38                   | 0.36       | 11.2               | 9.6            | 1.337          | 102             | 4.35 | 4.48  | 8.99       | 9.6  | 0.6 | 6.7 |
Table 3: The major cationic content in ppm, of Acacia sieberana var. sieberana gum (SK).

| Sample code | aN | K | aC | gM | eF | iN | oC | nM | Cu | bP |
|-------------|----|---|----|----|----|----|----|----|----|----|
| SK1         | 624| 1600 | 13663 | 1176 | 28 | 100 | 0  | 7  | 1  | 3  |
| SK2         | 580| 1250 | 12601 | 375  | 10 | 106 | 4  | 6  | 2  | 4  |
| SK3         | 341| 1430 | 17301 | 434  | 17 | 89  | 0  | 9  | 0  | 2  |
| SK4         | 342| 2080 | 14842 | 773  | 18 | 78  | 6  | 4  | 2  | 0  |
| SK5         | 423| 1800 | 12997 | 821  | 15 | 76  | 1  | 5  | 3  | 4  |
| SK6         | 284| 2443 | 22082 | 780  | 21 | 86  | 0  | 2  | 2  | 3  |
| SK7         | 521| 852  | 16430 | 648  | 19 | 85  | 0  | 3  | 0  |    |
| SK8         | 262| 1210 | 20150 | 268  | 23 | 94  | 4  | 9  | 0  | 2  |
| SK9         | 282| 1480 | 15410 | 440  | 19 | 68  | 1  | 5  | 2  | 0  |
| SK10        | 425| 945  | 12105 | 283  | 14 | 64  | 3  | 4  | 1  | 4  |
| SK11        | 228| 1355 | 10285 | 329  | 17 | 73  | 0  | 7  | 3  | 5  |
| SK12        | 342| 1284 | 15200 | 420  | 16 | 82  | 5  | 5  | 0  | 4  |
| Mean        | 388| 1477 | 15256 | 562  | 18.1 | 83.4 | 2  | 5.5 | 1.6 | 2.6 |
| SD          | 129| 454  | 3380  | 278  | 4.6 | 12.6 | 2.3 | 2.2 | 1.2 | 1.8 |
| CV%         | 33.2| 30.7 | 22.2  | 49.4 | 25.3 | 15.1 | 112.8 | 39.9 | 73.5 | 69.0 |

SD = standard deviation; CV = coefficient of variation

Table 4: The major cationic content in ppm, of Acacia sieberana var. sieberana gum (WN).

| Sample code | aN | K | aC | gM | eF | iN | oC | nM | uC | bP |
|-------------|----|---|----|----|----|----|----|----|----|----|
| WN1         | 1500 | 5012 | 15712 | 420 | 28 | 190 | 5  | 16 | 7  | 3  |
| WN2         | 850  | 4109 | 16840 | 280 | 27 | 210 | 3  | 12 | 2  | 9  |
| WN3         | 1025 | 3025 | 13500 | 580 | 8  | 200 | 3  | 10 | 6  | 6  |
| WN4         | 1400 | 2758 | 12280 | 846 | 45 | 225 | 2  | 12 | 4  | 4  |
| WN5         | 425  | 4400 | 15000 | 850 | 38 | 275 | 5  | 8  | 5  | 0  |
| WN6         | 1075 | 2860 | 13100 | 825 | 15 | 290 | 3  | 6  | 5  | 7  |
| WN7         | 736  | 2005 | 10284 | 480 | 9  | 80  | 2  | 7  | 2  | 6  |
| WN8         | 628  | 2443 | 8484  | 158 | 11 | 82  | 1  | 6  | 7  | 4  |
| WN9         | 961  | 1214 | 17411 | 176 | 12 | 79  | 1  | 9  | 2  | 0  |
| WN10        | 1198 | 1042 | 20152 | 325 | 18 | 96  | 0  | 4  | 6  | 3  |
| WN11        | 1661 | 8513 | 16433 | 344 | 15 | 109 | 1  | 3  | 5  | 4  |
| WN12        | 1676 | 2722 | 20084 | 326 | 16 | 115 | 2  | 2  | 4  | 3  |
| Mean        | 1095 | 3342 | 14940 | 467.5| 20 | 163 | 2.3 | 7.9 | 4.6 | 4.1 |
| SD          | 405  | 2016 | 3594  | 253 | 12 | 78  | 1.6 | 4.1 | 1.8 | 2.6 |
| CV%         | 37.0 | 60.3 | 24.1  | 54.1 | 58.7 | 48.0 | 66.7 | 52.1 | 40.0 | 64.8 |

SD = standard deviation; CV = coefficient of variation
Table 5: The sugar composition of Acacia sieberana var. sieberana gum (SK).

| Sample code | Galactose% | Arabinose% | Rhamanose% | Glucuronic acid % | 4-O-methylglucuronic acid % |
|-------------|------------|------------|------------|-------------------|-----------------------------|
| SK_1        | 10         | 58         | 4.6        | 2.6               | 6.6                         |
| SK_2        | 11         | 56         | 5.3        | 0.0               | 9.4                         |
| SK_3        | 9          | 60         | 3.8        | 2.8               | 5.0                         |
| SK_4        | 9          | 59         | 4.1        | 3.0               | 5.6                         |
| SK_5        | 10         | 54         | 3.9        | 5.2               | 4.5                         |
| SK_6        | 8          | 58         | 4.6        | 0.0               | 9.7                         |
| SK_7        | 9          | 57         | 0.0        | 3.4               | 7.1                         |
| SK_8        | 8          | 59         | 2.5        | 0.0               | 9.3                         |
| SK_9        | 9          | 62         | 6.0        | 1.9               | 5.4                         |
| SK_10       | 11         | 57         | 5.2        | 3.6               | 5.6                         |
| SK_11       | 12         | 54         | 4.8        | 5.6               | 3.6                         |
| Mean        | 9.8        | 57         | 4.0        | 2.7               | 6.5                         |
| SD          | 1.5        | 2.8        | 1.6        | 1.96              | 2.0                         |
| CV%         | 15.2       | 4.9        | 40.4       | 71.9              | 31.1                        |

SD = standard deviation; CV = coefficient of variation

Table 6: The sugar composition of Acacia sieberana var. sieberana gum (WN).

| Sample code | Galactose% | Arabinose% | Rhamanose% | Glucuronic acid % | 4-O-methylglucuronic acid % |
|-------------|------------|------------|------------|-------------------|-----------------------------|
| WN_1        | 12         | 57         | 5.1        | 4.5               | 5.3                         |
| WN_2        | 9          | 63         | 3.4        | 1.8               | 8.0                         |
| WN_3        | 10         | 61         | 6.0        | 2.3               | 4.9                         |
| WN_4        | 11         | 58         | 4.2        | 4.4               | 5.2                         |
| WN_5        | 9          | 60         | 0.0        | 0.0               | 5.6                         |
| WN_6        | 11         | 43         | 2.7        | 0.0               | 8.8                         |
| WN_7        | 9          | 57         | 0.0        | 2.5               | 6.7                         |
| WN_8        | 10         | 48         | 4.5        | 3.0               | 6.6                         |
| WN_9        | 8          | 56         | 3.4        | 3.2               | 7.2                         |
| WN_10       | 9          | 57         | 0.0        | 2.8               | 6.8                         |
| WN_11       | 10         | 54         | 3.8        | 1.6               | 7.9                         |
| WN_12       | 11         | 56         | 4.2        | 3.1               | 6.7                         |
| Mean        | 9.9        | 56         | 3.1        | 2.4               | 6.6                         |
| SD          | 1.2        | 5.5        | 2.1        | 1.4               | 1.2                         |
| CV%         | 11.7       | 9.9        | 66.1       | 58.9              | 18.4                        |

SD = standard deviation; CV = coefficient of variation

Figures 1 and 2 show the GPC elution profiles for SK and WN respectively. The profiles reveal the presence of three main fractions differing very much in their molecular weight, emphasizing the polydispersity of the gum. These fractions resemble the arabinogalactan-proteins (AGP), arabinogalactan (AG) and glycoprotein (GP) described for Acacia senegal (Randall et al., 1988). However, the distribution patterns of these fractions are slightly different indicating different detailed structure of the three components.
Fig. 1: GPC elution profiles of *A. sieberana* var. *sieberana* gum (SK).

Fig. 2: GPC chromatogram showing the elution profiles monitored by light scattering (red), refractive index (blue) and UV (214 nm, green) detectors for *A. sieberana* var. *sieberana* gum (WN).

Table 7 also shows the weight average molecular weight for the gums from *A. sengal*, *A. seyal* and *A. sieberana* var. *siebarana*. The values for the first fraction for all gums are slightly different (eluted at almost the same volume). For the second fractions, for all gums the values are quite different indicate different retention time and hence different molecular weights. *A. sieberana* var. *siebarana*, from both locations, has the highest value of molecular weight followed by *A. seyal* and *A. Senegal* (Fig. 3).
Table 7: Molecular weight parameters and root mean square radius of gyration ($R_g$) of A. senegal, A. seyal and A. sieberana var. sieberana (two locations) determined by GPC-MALLS.

| Sample code | $M_w$ processed as one peak | $R_g$ (nm) | $M_w/M_n$ | $M_w$ processed as two peaks | $R_g$ (nm) | $M_w/M_n$ |
|-------------|-----------------------------|------------|-----------|-----------------------------|------------|-----------|
| A. senegal Control | $1.1 \times 10^6$ | 32.1 | $3.7 \pm 0.3$ | $4.5 \times 10^6$ | 56.1 | $1.8 \pm 0.0$ |
| A. seyal Control | $1.7 \times 10^6$ | 26.6 | $2.4 \pm 0.2$ | $4.7 \times 10^6$ | 37.6 | $1.4 \pm 0.1$ |
| A. sieberana (SK) | $1.8 \times 10^6$ | 22.3 | $1.2 \pm 0.1$ | $5.1 \times 10^5$ | 41.1 | $1.7 \pm 0.1$ |
| A. sieberana (WN) | $1.6 \times 10^6$ | 19.1 | $1.7 \pm 0.1$ | $4.8 \times 10^6$ | 38.3 | $1.1 \pm 0.0$ |

Note: The high molecular weight peak was processed separately and the remainder of the gum processed as the second peak; $M_w$ = weight average molecular weight; $M_n$ = number average molecular weight.

Fig. 3: Comparison of the elution profiles monitored by refractive index detector for the gums: A. senegal (black), A. seyal (red), A. sieberana var. sieberana (SK) (violet) and A. sieberana var. sieberana (WN) (green).

Figures 4 and 5 show Particle size distributions of A. sieberana var. sieberana (SK) and (WN) emulsions, all particle sizes are almost less than (~1 micron) and the changes in the distributions with time and temperature for the gum from both locations are appreciably small, indicating good emulsification properties.
Summary and conclusions:-
The physicochemical study of Acacia sieberana var. sieberana gum shows the followings characterizations: positive optical rotation, low viscosity and low rhaminose content. The GPC fractionation of this polysaccharide gum shows the presence of three fractions and the protein is distributed among all these fractions with considerable ratios. From a functional application view, the gum is considered a grade one emulsifier.

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