Journal of Pharmaceutical and Applied Chemistry
An International Journal

http://dx.doi.org/10.18576/jpac/030303

Effect of Chemical Modification by Oxidation on The Physicochemical Properties of *Sweitenia mycrophylla* Gum. A Potential Excipient

O. Adeyanju[1,], E. A. Ewaoche[2] and J. Plavec[3]*

[1] Department of Chemistry, Faculty of Natural Sciences, University of Jos, Nigeria.
[2] Department of Pharmaceutical technology, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.
[3] National Institute of Chemistry, NMR Centre, Ljublijana, Slovenia.

Received: 19 Jul.2017, Revised: 12 Aug. 2017, Accepted: 17 Aug. 2017.
Published online: 1 Sep. 2017.

Abstract: Oxidized *S.mycrophylla* gum was prepared,characterized by FTIR, XRD, SEM, EDX and evaluated for its physicochemical characteristics. Oxidized gum showed improved physicochemical parameters, than the native gum with moisture content to be 3.5±40.10%. Ash 1.98±0.25%, protein 1.04 ± 0.05%, pH 4.95± 0.25, viscosity (CS), 56.32±6.79, refractive index 1.33±0.02, water holding capacity 94:63 ±8.45%, specific rotation - 15.2±0.09 degree Celsius,Emulsifying capacity 48.03±4.65cm^{-1} and swelling index 25.36±2.64%. Chemical modification via oxidation increased the physicochemical properties of the native gum. The experimental work, provide enough evidence to exploit this natural biopolymer in food and pharmaceutical formulations..

Keywords: Carboxyl group, *Sweitenia mycrophylla* gum, FTIR, XRD,SEM

1 Introduction

Polysaccharides from natural sources have some shortcomings like solubility, uncontrolled rate of hydration, thickening, drop in viscosity, storage and microbial contamination (Bharadia *et al*.,2004; Murali *et al*.,2000). modifications of polysaccharides produces new products with specific properties.Chemical modification such as oxidation, acetylation, carboxymethylation, hydroxypropylation and cross-linking are widely used. (Risica *et al*.,2005). Oxidation of gum can result in formation of water soluble derivative by using reactive groups, to substitute free hydroxyl groups along the macromolecule backbone (Samia *et al*.,2009). The oxidation of polysacharides increases their hydrophilicity and solution clearity and make them more soluble in aqueous system (Risica et al.,2009). Oxidation was selected as a chemical means of attaching pendant carboxyl groups(-COOH) due to its technical simplicity, low cost of chemical; reagents and wide range application to produce oxidized gum which can perform as a better binder in drug formulation.*Sweitenia mycrophylla* gum is non-starch polysaccharides obtained from the bark of *Sweitenia mycrophylla* tree (meliaceae), a large tree reaching a height of 30-40m and a girth of 3-4m, in favourable condition, it can reach 60m high and 9m girth. (Allemann, *et al*.,2003 and Elzalabam *et al*., 2011).The physicochemical, toxicological, structural characterization and application of purified *S. mycrophylla* gum as excipient had been investigated in previous studies. (Adeyanju, *et al*., 2015a; Adeyanju, *et al*., 2016a; Adeyanju, *et al*., 2015b). Evidently, there are no sufficient studies that confirms oxidation of this gum. Hence, this research aims at investigating the oxidation of the gum in order to improve its physicochemical characteristics for better efficacy in drug formulation . The results of this research is likely to highlight the effect of oxidation on some physicochemical properties of the gum.

2 Materials and Methods

Reagents: All reagents used in this study are of analytical grade

2.1 Collection and Preparation Of Samples

Gum was collected from the bark of *S. mycrophylla* tree in Owena Forestry, Ondo State, Nigeria.The plant was

* Corresponding author E-mail:adeyanju.olusola@yahoo.com

identified and authenticated at the herbarium of the forestry department, Federal University of Technology,Akure..Gum was tapped from the bark of the tree. The dried, cleaned gum sample was milled with a Kenwood blender (UK) and later sieved using a bin (mesh size-250microns) so as to obtain a fine and uniform sample, kept in labeled plastic container for subsequent analysis.

2.2 Purification Of Gum Samples

Dried crude gum (10g) was stirred in cold distilled water (250ml) for 2 hours at room temperature. The supernatant was obtained by centrifugation and made up to 500ml and ethanol solution was added (1:4 v/v) to precipitate all the carbohydrate. The precipitated material was washed again with ethanol, followed by distilled water and dried at room temperature milled with Kenwood blender (UK) and later sieved using a bin (Mesh size-250microns) kept in labeled plastic container for subsequent analysis.

2.3 Preparation Of Oxidised Gum

Standard method by Henry (2007) was used for the oxidation process. 10g gum was dispersed in 50cm3 distilled water. The pH of the slurry was adjusted to 9.0 using 3% Na0H. NaOCI was added slowly for a period of 90 minutes and constantly monitoring the pH at 9.0 and simultaneously cooling was done with crushed ice and NaOCI. The reaction proceeded for four hours after NaOCI addition was completed. The pH of the mixture was than adjusted to 7.0 with whatman No 1 filtered paper. The residue was then washed four times with distilled water and air dried at room temperature.

2.4 Determination Of Degree Of Oxidation And Carboxyl Percentage

The percent oxidation (%carboxyl) and degree of substitution (DS) was determined titrimetriclaly following the method of Wurzburg (1986b) with slight modification. One gram of the oxidized gum sample was suspended in 50ml of 75% ethanol solution. The slurry was then kept in a water bath at 500C for 30min with constant stirring. Thereafter the slurry was then cooled at room temperature and 40ml of 0.05m potassium hydroxide added. The slurry was allowed to stand for 24h at room temperature with occasional swirling. The excess alkali was then titrated with 0.5M hydrochloric acid using phenolphthalein as an indicator. The solution was allowed to stand for another 2h. any additional alkali that might leach from the sample was titrated. Blanks with raw gum was analyzed concurrently. The sample volume, the hydrochloric acid normality and the volume of hydrochloric acid required to titrate the blank and sample was recorded and calculated according to equation 1 below. This measurement was done in triplicate and the mean and standard deviation recorded.

$$\text{Carboxyl\%} = \frac{(\text{Vol.Blank}-\text{Vol.Sample}) \times \text{Mmolarity of HCL} + 0.043 \times 100}{\text{Sample weight}} \quad (1)$$

Degree of substitution which is the average number of sites per saccharide unit that posse a substituent group was calculated using equation 2;

$$DS = \frac{(162 \times Carboxyl\ \%)}{(4300 - [42 \times Carboxyl]\%)} -- (2)$$

2.5 Physicochemical Analysis Of The Native And Oxidized Gum

The moisture content was determined by drying to constant weight at 105°C (in a muffle furnace). (AOAC, 1984) Nitrogen conjent of the gum was determined by Kjeldah method (AOAC, 1984) using Gerhadkjeklotherm and vapodest system (Germany). Crude protein was calculated from the nitrogen content using the conversion factor of 6.25. pH, relative viscosity, water holding capacity, emulsifying capacity and swelling index were measured accordingly to the AOAC (1984). Specific rotation was determined on 0.2M ammonium hydroxide using an automatic digital polarimeter (model AA-10, optical activity Ltd, England) with sodium D line lamp.

2.6 Fourier Transform Infrared (FT-IR) And NMR Spectroscopy

The FT-IR spectrum of the sample was recorded in an FTIR spectrometer (Nicolet Magna-4R 560. MN USA), using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr.1H and ^{13}C NMR, ^{13}C DEPT and Solid State NMR of *Sweitenia mycrophylla* gum were recorded in an NMR (600 MHz) spectrometer (Agilent technologies, America). Chemical shifts were reported in ppm relative to an internal standard TMSP (Tetramethylsilanepropoinic acid). Peak integra were performed using Agilent software, America

2.7 X-Ray Powder Diffraction (XRD)

X-ray diffraction patterns of the gum was analyzed using a Siemens D5000 X-ray diffractometer (Siemens, Munish, Germany).

2.8 Microstructure Studies By Scanning Electron Microscopy (SEM)

Morphological features of the gum were studied with a JSM – 5600LV scanning electron microscope of JOEL (Tokyo, Japan).

3 Results And Discussion

3.1 Result

Table 1. Physicochemical characteristics of the native and Oxidised *S. mycrophylla* gum

Parameter	Raw Gum	Oxidized Gum
Moisture content (%)	7.68±0.15	3.54±0.10
Ash content (%)	2.70±0.30	1.98±0.25
Protein content (%)	2.08±0.30	1.04±0.05
pH	5.50±0.10	4.90±0.25
Relative viscosity	22.10±0.50	56.32±6.79
Refractive Index	1.34±0.01	1.33±0.02
Water holding capacity (%)	64.90±4.25	94.63±8.4
Specific rotation (^{0})	-25.46±0.40	-15.20±0.09
Swelling index (%)	17.50±1.10	25.36±2.64
Emulsifying capacity (cm^{-1})	17.29±0.20	48.03±4.65

Data are mean±sem of triplicate results

PLATE 1.*S.mycrophylla* crude exudate Gum

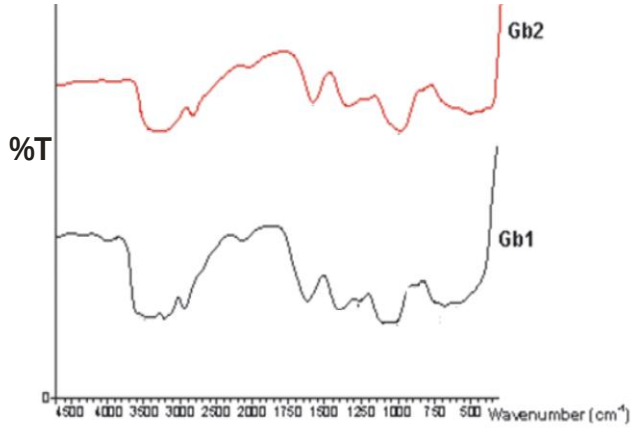

Fig 1. FTIR of Native(Gb1) and Oxidized(Gb2) *sweitenia mycrophylla* gum

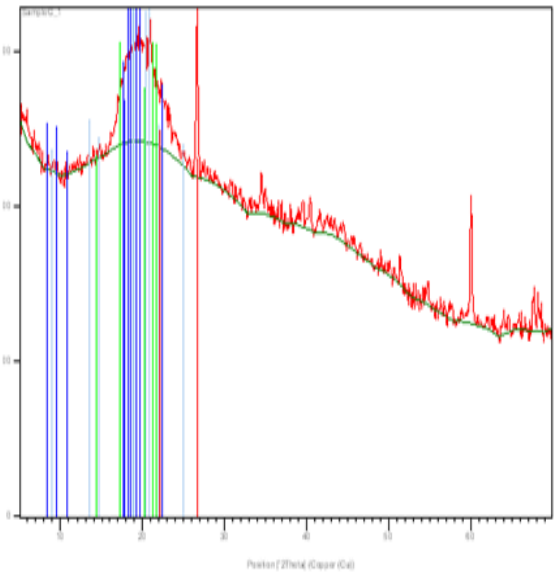

Fig 2 .XRD of native *S. mycrophylla* gum

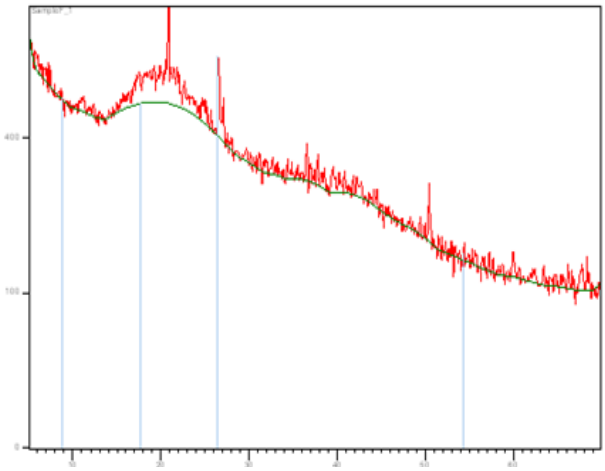

Fig 3. XRD of oxidized *S. mycrophylla* gum

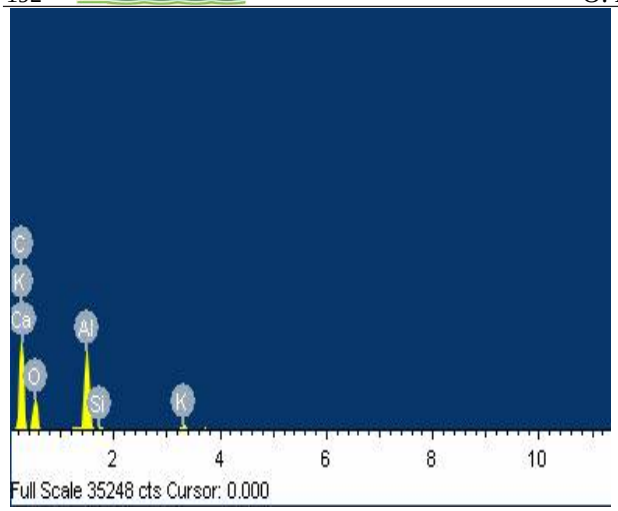

Fig 4 . EDX of native *S. mycrophylla* gum

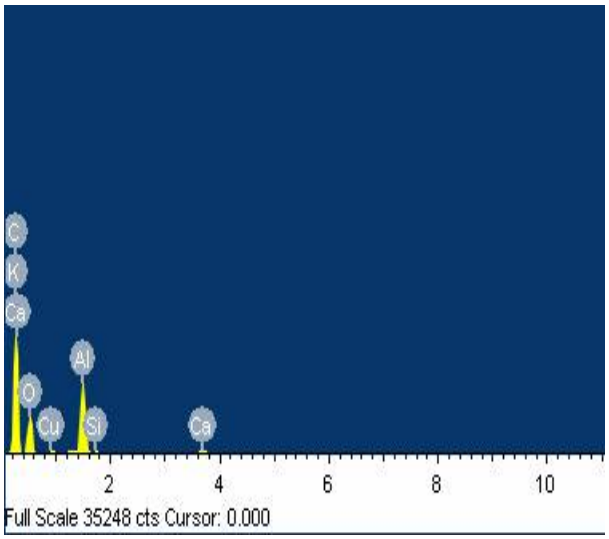

Fig 5.EDX of Oxidized *S. mycrophylla* gum

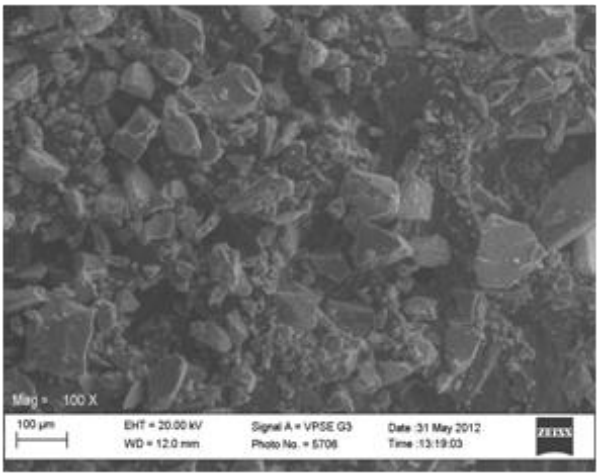

Plate 2a .SEM of Oxidized S. *mycrophylla* gum

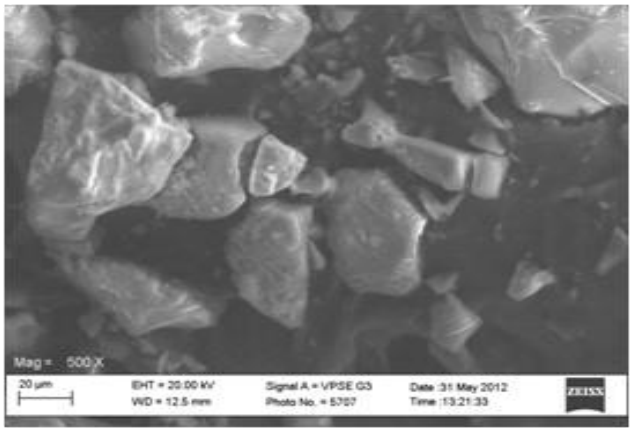

Plate 2b. SEM of Native *S. mycrophylla* crude exudates gum

3.2 Discussion

Table 1 shows the physicochemical parameters of the native and oxidized S. *mycrophylla* gum. The swelling capacity in water'expressed in percent was 25.36% (Table 1). The result shows that the oxidized gum has a high swelling index compared to the native gum with swelling index of 17.5% (Dickson *et al*., 1991). The relatively high swelling index at pH= 4.9 implies that the gum may be used as a matrix former in controlled drug-release (Emeje *et al*., 2008). The pH measurement shows that the gum solution was slightly acidic. The pH value of 5.5 (Table 1) is in good agreement with reported pH values for gum *arabic* and *anacardium occidentale* (Cashew gum) by several authors (Abu *et al*., 2007).The acidity of the oxidized plant gum is not unexpected since they are known to contain salts (Ca, Mg, K, Na and Fe) of acidic polysaccharides, the activity of which is due to uronic acids in their structure (Abu *et al*, 2007). The pH of an excipient is an important parameter in determining its suitability in formulations since the stability and physiological activity of most preparations depend on the pH.(Goycoolea *et al*.., 1997).Moisture content of the oxidized gum is 3.54% (Table 1) and compares favourably with the minimum standards (<15%) for good quality gum according, to European specification (E-14) (WHO, 1996).The total ash value of the native gum was found to be 2.70% (w/w) (Table 1) and was reduced to 1.98% as a result of oxidation.This falls within the acceptable level of less than 4% for gum arabic reported by WHO (1998) for food and pharmaceuticals. The very low values of ash show that the gum has a good quality of mineral content with low level of contamination. This was confirmed in the EDX analysis showing the presence of Ca, K, Si, Al O and Cu in good concentration (Fig. 4 and 5). Ohwoavworhua and Adelakun (2005).

The relative viscosity of the gum was found to be 56.32 (Table 1) compared to the native gum Arabic 24.81. According to Wang *et al.*, (2003), molecular association in fluids greatly influences their rheological behaviours. Increase in viscosity with concentration may be probably due to increasing number of high molecular weight polymeric chair of the gum per unit volume (-COOH-) and increased interaction between these chains in aqueous solution or dispersion. However, higher relative viscosity of the gum solution suggests the presence of high molecular weight in the gum chemical constitution which was introduced during oxidation.

The value for protein content obtained.2.08% (Table 1) fairly agree with that of *acacia* gum (0.5- 2.7%) (WHO, 1996). The moderate protein content in the gum sample is noteworthy. This is because protein content is known to have effects on the emulsifying behavior of gum with the best emulsion capacity and stability found in gums with higher nitrogen content (Randall *et al*, 1988; Dickinson, 1991). The specific rotation of the aqueous gum was found to be optically active (-25.46º) (Table 1). This shows that the sugar present is laevorotatory.

The emulsifying capacity of the native and oxidized gum was found to be 17.29cm^{-1} and 48.03cm^{-1} thick respectively, (Table 1). A higher turbidity is an indication of a better emulsion capacity. In addition to protein content of gum, the typical molecular structure and high molecular weight are responsible for good emulsifying properties (Yadav *et al.*, 2007). A similar correlation between molecular weight and emulsion stability of gum Arabic was reported by Dickinson *et al.*, (1991). Refractive index of the gum sample was found to be 1.34 (Table 1). This may prove to be a qualifying index for this gum. Awad El Karium, (1994) reported that refractive index for Acacia senegal gum was 1.338 and seyal gum was 1.337.

Water holding capacity of the gum was found to be 84.90%. the water holding capacity of the gum is the

ability to hold water and does not only depend on the functional group of carbohydrate that are hydrophilic but also on the protein present in the gum, since they also contain functional groups that are able to bind with water molecule. Thus addition of other substance can be accommodated and this may improve the texture of the overall product. (Karamalla *et al.*, 1998).

In the FTIR analysis, the polysaccharide unit of glucose with hydroxyl group (-OH) as the major functional groups appear in the region (3600-3200cm-1) and disappeared when the gum was oxidized. There was introduction of carboxyl group and the spectrum now processed peak around (1800cm-1-1500cm-1). (Fig. 1) The FTIR of unmodified and oxidized gum show similar bands such as those at 2924-2893 (symmetric and a symmetric CH), 1415cm-1 (-OH bending 1200-800cm-1) and (C=0 and C-C stretching) differences could be seem in the bands at 1620cm-1 and 1430cm-1 attributed to asymmetric and symmetric stretching of carboxylate groups. The existence of bands attributed to –C00- in unmodified gum and the increase in the band intensity related to carboxylate group of the oxidized gum (1620 and 1430cm-1) confirm the oxidation of the polysaccharide.(Dodi *et al*., 2001; Agrawal, 1992; Jiang, et al., 2000; Kato *et al*., 2003).

The SEM analysis of the native gum shows irregular particle size (plate 2a) while the SEM of the oxidized gum (plate 2b) was found to be fibrilar, indicating loss in particle morphology that was observed in the native form of the gum. The x-ray diffraction analysis of the native gum (fig 2) shows numerous halves with weak peaks conforming the amorphous nature of the gum while the XRD of the oxidized gum shows more regular pattern with few sharp peaks confirming the level of crystallinity of the oxidized gum (fig 3). The EDX of the native gum sample (fig 4) shows that the gum sample contain various elements such as carbon, potassium, calcium, oxygen, copper, aluminium, silicon, calcium and manganese. The high ratio of carbon to oxygen indicate the presence of a sugar polymer. Though, virtually all the elements present in the native gum are also present in the oxidized gum (fig 5) but the concentration of these elements increased for the oxidized gum. This may be as a result of the purification of the native gum sample.

4 Conclusion

Modification (oxidation) of *S.mycropylla* gum has an effect on some of the physiochemical properties of the gum such as moisture content, insoluble matter, pH, viscosity and swelling index. This may increase the gum efficacy in application.

Acknowledgement

The authors are grateful to National Institute of Chemistry NMR centre Ljublijana, Slovenia for their technical support.

References

[1] Abu Baker, A. A., (2007). Analytical and structural studies on some plant gum exudates. MSc, Faculty of Agriculture, University of Khartoum.

[2] Adeyanju, O. Lajide, L. Ajayi, O.O., Amoo, I. and Plavec, J. (2015a). Physicochemical and structural characterization of *Sweitenia mycrophylla* gum.Academic Journal of Biosciences (SAJB). 3(3): 231-238.

[3] Adeyanju, O., Lajide, L., Ajayi, O. O., Amoo, I.A. and Plavec, J. (2015b). 1D and 2D NMR characterization of Sweitenia mycrophylla gum. International Journal of current Research in Chemistry and Pharmaceutical Sciences. 2(1): 4-13.

[4] Adeyanju, O., Lajide, L., Edah, A.O. Adesemuyi, M.F. and Plavec, J. (2016a). Effect of Acetylation on physicochemical characteristics of Sweitenia mycrophyllagum.A Potential excipient.Journal of Pharmaceutical and Applied Chemistry. 2(1): 13-17.

[5] Agrawal, P. K. (1992). Determination of the chemical structure of complex polysaccharides by heteronuclear NMR spectroscopy.Phytochemistry.31; 3307.

[6] Allemann, J. and Hammes, P.S. (2003).Chemical composition of South Africa Plectranthusesculentustubers.South African Journal of Science. 99: 127-129.

[7] AOAC (1984) Association of official analystChemists, official methods of analyst, 13lh ed. Horwitz, W. (EN) Benjamin Franklin Station.Asngton D.C 16:96-99.

[8] Bharadia T. R., Kanwar, M., Lai, R. and Gupta, A.(2000). Natural gums and modified natural gums as sustained release carriers. Drug Development and Industrial Pharmacy 26(10):1025-1038.

[9] Dickinson, E., (1991). The role of hydrocolloids instability particulate dispersions and stabilizer for the industry for the food Industry. Vol. 4 Philips, Wedlock D.J Williams P.A (Eds.) IRL Press, Oxford, pp:249-263.

[10] Dodi, G., Hritcu, D. and Popa, M.I. (2011).Carboxymethylation of Guar gum; synthesis and characterization.Cellulose chemistry and Technology. 45(3-4, 171-176.

[11] Elzalabani SM, E. l., Askary H. I., Mousa O. M., and Issa, (2011).“Genetic and botanical profiling of (L.)Jacq.AndSwietaniamacrophyllaKing”.Egyptian J. of biomedical Sciences, 35:190-228.

[12] Goycoolea F.M. Calderon de la BarcaAM.Balderrama JR Valenzuela JR (1997) Immunological and functional properties of exudates gum from north western Mexicanmesquite (prosop'ssppjn comparison with gum arable. Int. J BiolMarcomol 21:29-36.

[13] Henry, M. O. (2007). Effect of chemical modification on starch of some legume flours.Pakistan Journal of Nutrition(67): 167-171.

[14] Kato, Y., Matsuko, R. and Isogai, A. (2003).Chemical oxidation of sago starch.Carbohydrate Polymer. 51:69-72.

[15] Mirau, P. A. (2004). A practical guide to understanding the NMR of Polymers: Wiley: Hoboken, N. J. 81-102.

[16] Ohwoavworhua F. O. and Adelakun TA (2005) Some physical characteristics of microcrystalline cellulose obtained from raw cotton of cochlospermumplanchonii. Trop J. Pharm. Res 4:1-7.

[17] Randall. R.C; Philips GO; Williams P.A (1988) The role of the pretentious component on the emulsifying properties of gum arabic: Food Hydrocolloids, 2, 131-140.

[18] Risica D., Barbetta, L., Vischetti, C. and Dentini, M (2009).Chemical modification of Gum in Kenya.Polymer. 51:1972-1974.

[19] Risica, D., Dentini, M. and Crescenzi, V. (2005).Isolation and characterization of Guar Gum.Polymer.46: 1247.

[20] Samia, E.A, Babiker, E.M. Kara malla, A. (2009) Analytical studies on Gum exudates of anogeissusleiocarpus Pakistan Nutritions(16): 782-786.

[21] WHO (1996). Trace Elements in Human Nutrition, WHO Geneva, 26: 478.

[22] Yadav.M.P; Fishman, M.L; Chau.H.K Johnston D.B Hicks K.B. (20.07) Molecular characteristics of corn fiber gum and their influence on its emulsifying properties.Cereal chem. 84 (2) 175-180.