Sudan University of Science and Technology
College of Graduate Studies

Physicochemical Characterization of *Acacia tortilis* var. *spirocarpa* Gum from (Sudan)

توصيف الخواص الفيزيوكيمائية لصمغ السيال صنف السمر من (السودان)

A Thesis Submitted in Fulfillment of the Requirement for the Degree of Doctor of Philosophy in Chemistry

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April 2022

استهلال

بسم الله الرحمن الرحيم

(لَقَدْ رَضِيَ اللَّهُ عَنِ الْمُؤْمِنِينَ إِذْ يُبَايِعُونَكَ تَحْتَ الشَّجَرَةِ فَعَلِمَ مَا فِي قُلُوبِهِمْ فَأَنْزَلَ السَّكِينَةَ عَلَيْهِمْ وَأَثَابَهُمْ فَتْحًا قَرِيبًا)

صدق الله العظيم

سورة الفتح الاية 18

Dedication

To the souls of my parents
To my children.
And sisters.

Acknowledgement

Praise to Allah, the Most Gracious, the Most Merciful who helped me to achieve and complete this work.

My best regards to my supervisor **Dr. Elfatih Ahmed Hassan**, and **Professor Mohammed El-Mubark Osman**, for advising, supervising, guiding and encouraging me to fulfill this study.

I would like to acknowledge the Ministry of Higher Education, for financial support.

Thanks are extended to Professor Saphwan Al-Assaf, Hydrocolloid Research Center, Chester University: UK , Dr. Seifeldawla Awad Ibrahim , and Zahir Abdalsalam , Osama Taj Alser - Forest National Corporation (FNC), and the workers in sustainable Resource Management Project, for technical support.

Thanks are also due to everyone who contributed by different ways to complete this study and for their moral support.

Abstract

Authenticated twenty four samples of *A. tortilis* var. *spirocarpa* gum were collected from different trees from Khartoum and Gazira states -Sudan.

Physicochemical results show that , the mean values obtained for moisture content were 11.7and10.9%, ash content were 2.9 and 2.5%, pH values were 5.8 and 4.6 , specific optical rotation were +80.0 and +76.6 . Nitrogen content were 2.2 and 1.4%, protein content 13.8 and 8.4% , calorific value was 4.03Kcal mol^{-1}, absolute viscosity were 45cps and 23cps.

Percentage average values for rhamnose, arabinose and galactose values were found to be in range (4.5-2.9), (43.4- 40.3) and (17.1-13.9) respectively and glucouronic acid 8.17 and 8.43%, acid equivalent weight 2376.1 and 2301.5 . Cationic composition was studied using ICP technique showed that calcium has the highest value (8718-7727ppm) then potassium (3320-1087ppm), magnesium (1820-1330ppm) and sodium (98.9-42.3ppm).The rest of the elements Fe, Al, Mn, Co, Ni, Cr and the heavy metals Pb, Zn, Cu, As were found as traces. Molecular weight distribution was determined using Gel Permeation chromatography showed three main components arabino glactan protein (AGP), arabino galactan (AG), and glycoprotein (GP). Average molecular weight was estimated to be $(4.62\text{-}4.59)x10^5$ and the radius of gyration was 35-21nm.

Emulsification study showed that *A. tortilis* var. *spirocarpa* possessed high emulsifying power and stability as oil- in- water emulsion, the emulsion particles possessed smaller droplet size compared to those of other *Acacia* gums. The gum is classified as grade 1 emulsifier.

Rheological study of *A.tortilis* var. *spirocarpa* showed that the gum is Newtonian fluid at high shear rate and high concentration and the result of oscillatory test of the gum solution revealed a solid-like behaviour .

Results showed insignificant difference within samples collected from different location. On comparing between *A. tortilis* varieties*: spirocarpa* , *raddiana* and . *tortilis*, there are insignificant difference between them in the physicochemical and functionality properties, so they can be marketed as one species.

مستخلص

تم جمع أربع وعشرون عينة من صمغ السمر من أشجار محددة من ولايتين في السودان هما الخرطوم والجزيرة.

أظهرت النتائج الفيزيائية والكيميائية أن القيم المتوسطة التي تم الحصول عليها لمحتوى الرطوبة كانت 11.7 و 10.9٪ ، ومحتوى الرماد 2.9 و 2.5٪ ، وقيم الأس الهيدروجيني 5.8 و 4.6 ، والدوران البصري النوعي كان +80.0 و +76.6. كان محتوى النيتروجين 2.2 و 1.4٪ ، محتوى البروتين 13.8 و 8.4٪ ، القيمة الحرارية 4.03 كيلو كالوري مول $^{-1}$ ، اللزوجة المطلقة 45 سنتي ثانية و 23 سنتي في الثانية.

تم العثور على متوسط القيم المئوية لقيم الرامنوز والأرابينوز والجلاكتوز في المدى (4.5–2.9)، (43.4–40.3) و (17.1–13.9) على التوالي وحمض الجلوكورونيك 8.17 و 8.43٪ ، الوزن المكافئ للحمض 2376.1 و 2301.5. تمت دراسة التركيب الكاتيوني باستخدام تقنية ICP حيث أظهر أن الكالسيوم له أعلى قيمة (8718–7727 جزء في المليون) ثم البوتاسيوم (3320–1087 جزء في المليون) والمغنيسيوم (1820–1330 جزء في المليون) والصوديوم (98.9–42.3 جزء في المليون). تم العثور على باقي العناصر Fe، Al، Mn، Co، Ni، Cr والمعادن الثقيلة Pb، Zn، Cu، As على هيئة آثار. تم تحديد توزيع الوزن الجزيئي باستخدام كروماتوجرافيا التخلل الهلامي وأظهرت ثلاثة مكونات رئيسية لبروتين أرابينو جلاكتان (AGP) ، أرابينو جالاكتان (AG) ، وبروتين سكري (GP). قدر متوسط الوزن الجزيئي بـ (4.62–4.59) × 10^5 وكان نصف قطر الدوران 35–21 نانومتر.

أظهرت دراسة الاستحلاب لصمغ السمر تمتلك قوة استحلاب عالية وثباتاً مثل مستحلب الزيت في الماء، وتمتلك جزيئات المستحلب حجم قطيرات أصغر مقارنة بتلك الموجودة في صمغ الأكاسيا الأخرى. يصنف الصمغ كمستحلب من الدرجة الأولى.

دراسة ريولوجية صمغ السمر أظهرت أن الصمغ عبارة عن سائل نيوتوني بمعدل قص عالٍ وتركيز عالٍ ، وأظهرت نتيجة الاختبار التذبذب لمحلول الصمغ سلوكًا شبيها بالمادة الصلبة.

أظهرت النتائج اختلافًا طفيفًا بين العينات التي تم جمعها من مواقع مختلفة. عند المقارنة بين أصناف السمر والسيال والدتورتيليس، لا يوجد فرق كبير بينهما في الخصائص الفيزيائية والكيميائية والوظيفية ، لذلك يمكن تسويقها كنوع واحد.

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List of Abbreviations

FAO	Food and Agriculture Organization of the United Nation
WHO	World Health organization.
JECFA	The Joint Expert Committee of Food Additives of the FAO/WHO
GPC	Gel Permeation Chromatography.
ODO	Octanoic Decanoic acid triglyceride Oil.
UV	Ultra Violet.
LS	Light Scattering.
RI	Refractive Index.
AGP	Arabino Galactan Protein.
AG	Arabino Galactan.
GP	Glycoprotein.
VMD	Volume Median Diameter.
M_w	Weight average-Molecular weight
R_g	Radius of gyration
O/W	Oil-in-water Emulsion W/O Water –in –oil Emulsion
HPLC	High Performance Liqud Chromatography
AES	Atomic Emission Spectrometry
ICP	Inductive Coupled Plasma
PHRC	Philips Hydrocolloid Research Center
ESI	Emulsion Stability Index
HPSEC-MALLS	High Performance Size Exclusion Chromatography Multi Angle Laser Light Scattering
SANS	Small Angle Neutron Scattering
SAXS	Small Angle X-Ray Sattering.

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Chapter One

Introduction and Literature Review

Chapter One
Introduction and Literature Review

1.1. Introduction

1.1.1. Origin and Definition of Gums:

A great many plants exude viscous, gummy liquids, which when exposed to air and allowed to dry, form clear, glassy masses. The shapes of these masses vary from spherical, tear – drop balls typical of gum Arabic – produced for acacia trees to curved, ribbon – like strands of tragacanth from Astragolus, bushes. The color of these exudates vary widely from almost clear white(colorless) through shades of yellow, amber and orange to dark brown, some gums possess pink, red or green lines and some black , brownish gum are also found., depending on the species, climate, soil and absorbed impurities (Glickman, and Schachat,1959). The best grades of gum arabic are almost colorless with slight traces of yellow.

Water- soluble plant gums are usually odorless and in this respect differ markedly from the oil – soluble resinous exudates which have distinctive smell. The gums are usually tasteless, and bland, except for some species which have sweet taste. Gum contaminated with tannins usually has a harsh, bitter flavor twhich is a serious disadvantage in food application(Glickman, and Schachat,1959).

Gums are either hydrophobic or hydrophilic, high molecular weight molecules, usually with colloidal properties, when dissolve in an appropriate solvent or swelling agent produce gels, highly viscous suspensions, or solutions at low dry substance content.

Gums vary in hardness, which depend upon the amount of moisture present (12-16%). It cannot be used as a mean of classification as with minerals. Density is also

variable and depends upon the amount of air entrapped when the gum was formed. There are many plants gum known only four of them are of real commercial importance: arabic, ghatti, karaya and tragacanth.(Glickman,1969)

Acacia gums are a brittle, odorless and generally tasteless material that contains a number of neutral sugars, acids, calcium and other electrolytes. The main component of the gum is arabin, the calcium salt of the polysaccharide arabic acid. The gum is built upon a back bone of D-galactose unit with side chains of D-glucuronic acid with L- rhamnose or L- arabinose terminal units. The molecular weight of gum is large and estimates in the range of 200.000 to 600.000 Daltons. It is very soluble in water, but does not dissolve in alcohol (Glickman, *etal* 1959).

Acacia gums are composed of arabinogalactan-protein (AGP) type biopolymer. It contains a continuum of hyberbranched amphiphilic charged polysaccharide complexes differing by the amount of protein, type of sugars, sugar to amino acid ratios , degree of branching, conformation and physiochemical properties. It also contains minor components such as minerals, polyphenols and traces of lipids and enzymes (Sanchez, *etal* 2018). There are more than 1300 species of *Acacia* trees distributed over the world, 132 species in Africa and 32 species in Sudan.(Glickman, *etal* 1959) .

1.1.2. Procedures to Determine Structure of Gum:

Hirst and Jones (1949) described methods used to determine the structure of gum arabic and other acidic gums (Anderson, *etal* 1966). The steps are outlined below:

1- The homogeneity of the gum is determined.

2- The gum is converted to the free acid, and the equivalent weight of the acid is then ascertained.
3- A portion of the acid is boiled with 12% hydrochloric acid to liberate furfural and carbon dioxide which gives estimates of pentose and uronic acid contents.
4- Another portion of the acid is hydrolyzed with dilute acid, and liberated sugars are quantitatively identified.
5- The linkages are studies by three principal procedures, methylation analysis, periodate oxidation and auto-hydrolysis or room temperature hydrolysis to oligosaccharides.

   The rate of auto-hydrolysis gives information about the nature of the ring system since L- arabonose which occurs in the furanose form is usually hydrolyzed first.

1.1.3. Specification:

Gum arabic (*Acacia gum*) definitions is based on the American Food Chemical Codex, published in 1969 (FAO, 1969, WHO, 1969). The Joint Expert Committee for Food Additives (JECFA) of the FAO/WHO monograph on gum arabic in 1978 (JECFA, 1978) which has been reviewed in four years (1982, 1986, 1990, 1995). In 1990 (JECFA, 1990), significant changes were made to definitions, e.g. ranges for specific rotation (-26 to- 34) and nitrogen content (0.27 to 0.39%) were introduced. However, in 1995 JECFA, further recommended that specific rotation and nitrogen content are to be deleted from the definitions. Philips and William(1993) suggested that characterization of gum arabic is possible using four parameters, e.g. specific rotation, viscosity lysine and hydroxyproline composition. In 1996 (European Union, 1996) introduced the molecular weight limits. In 1997 *A. seyal* var *seyal* was accepted as closely related species (FAO, 1997). In 1998 Codex Alimentraius Meeting, the JECFA proposed specification for gum arabic, prepared

by JECFA for further consideration. In spite of objection to including *A.seyal* gum in the specification of the gum arabic. Another recommendation for the specification of gum arabic, where *A.seyal* as gum arabic, has been adopted but gums from other *Acacia* species are not included in these specifications.

In March 1999, the Codex Committee for Food Additives and Contaminants gave acceptance to the specification in category II (recommended for adoption after editorial changes, including technical revisions). Those editorial changes included:

1- The deletion of the synonyms gum Hashab, Kordofan and Talha.
2- The deletion of the sentences (gum from other *Acacia species* are non-included.
3- The deletion of the sentences referring to immunological differentiation and technological interchangeability.
4- This proposal was accepted and sent to Codex Alemintraius Committee at its 23 session, Rome July 1999. The approved specification for gum arabic establishes the definition as: Gum arabic is dried exudates obtained from the stems and branches of *Acacia senegal* (L.) and from *Acacia seyal* (Fam. *Leguminosae*).

1.1.4. Gum Arabic Composition:

- Hydrolysis of gum gave D-glactose, D-glucouronic acid, L-arabinose, L-rhamnose, and 6- O - (**β**-D-glucopyranosyluronic acid) –D - galactose.
- When ash-free arabic acid was heated in water, a degraded arabic acid, D-galactose, L-arabinose, L-rhamnose and 3- O – **β** – D - galactopyransyl, L-arabinose were obtained.
- Methylation and hydrolysis of gum arabic gave 1mole of 2,3,4,6-tetra-O-methyl-D-galactose + 5 moles of 2,3,4-tri-O-methyl-D-galactose + 3 moles of

2,4, di-O-methyl-D-galactose + 3 moles of 2,3,4-tri-O-methyl-D-glucuronic acid.

- Methylation hydrolysis of arabic acid gave the same reaction products as above accept for 2,3,4-tri-O-methyl-D-galactose, but 2,3,4-tri-O-methyl – L-rhamnose is produced + 2,3,5-tri-O-methyl – L-arabinose + 2,5-di-O-methyl – L-arabinose + 2,3-di-O-methyl – D-glucuronic acid.
- Degraded gum arabic was oxidized with sodium periodate and periodic acid to convert the aldobiouronic acid side chains to dialdelydes.
- The dialdelydes were reduced to dialcohols with sodium borohydride or with hydrogen and Raney nicked (without affect the main chain).(Glickman,*etal*.1959)

1.1.5. Structure of Gum Arabic:

Gums are a complex polysaccharide neutral or slightly acidic salt. The major minerals are calcium, magnesium , sodium and potassium.

The structure of gum was determined by subjected it to a series of smith degradation using periodates. They obtained seven distinct degradation products. Further analysis of each product was used methylation and gel permeasion chromatography (GPC) showing that uronic acid and rhamnose residues where eleiminated first, they are located as periphery of the molecule. The core was found to consist of a **β** – (1,3) galactopyranose chain with branches linked through 1,6- position (Anderson, *etal* 1966). The gum composed of six carbohydrate moieties glactose, arabinopyranose, arabinofuranose, rhamnose, glucuronic acid and 4- o-methyl glucuronic acid.

- The main structural feature is a central chain of - galactopyranose unit with 1-3 bonds and side chains of 1,6- linked galactopyranose units terminating the

glucuronic acid residues. Both contain a small amount of nitrogenous material that cannot be removed by purification.

- Three random models were proposed to represent the core, each being consistuent with experimental data and structural fragments:
- Model of structure based on arabinogalactan protein (Street, *etal* 1983) proposed a structure which is branches composed of 116 galactose unit linked by (1,3) with six branches attached to the chain by (1 ,6) links Fig. 1.1 . The average molecular mass is about 19.0000 . Then the model of the whole molecule is obtained. Churms, *etal* (1983) carried out smith degradation studies, different values for the molecular size and composition were reported. They proposed that the galactan core consist of 13- **β**- 1,3 -D-galactopyranosyl residues having two branches which would give single repeating subunits of molecular mass 8000 with in the whole molecule.
- Anderson, *etal*. (1966) had showed that proteinous component was located almost with in the highest molecular mass fraction and the remaining lower molecular mass components were polysaccharide. Anderson *etal.* (1987) carried out a series of smith degradation on the whole gum. They found that the protein was present in all of the degradation products, although the sugar-protein ratio was far higher at the core of the molecule (11:1) than at the periphery (40:1) .
- Akiyama, *etal*. (1984) showed that gum interacted with yariv reagent indicating the molecule was a kind of arabinogalactan protein complex (AGP). They suggested that the binding side was the hydroxyproline rich domains. The studies showed the presence of hydroxyproline oligo arabino side and serine carbohydrate linkages. Vandevelde, *etal.(*1985) obtained a a molecular mass distribution, using GPC monitoring that eluent by UV absorption at 214 nm. The resulting chromatograms showed three or four

main peaks, indicating high molecular mass fraction (30%) which suggested to be (AGP) complex. Cannolly, *etal* (1988) treated gum with protolytic enzyme and monitored the molecular mass distribution before and after treatment. The overall molecular mass was found to decrease. Durvallent, *etal* (1989) showed the weight average molecular mass change from 720000 to 180000 after protnease treatment, where number average molecular mass remained constant 190000-180000 . The results led to suggestion the gum had a wattle blossom structure with a number of poly saccharide units linked to a common polypeptide chain as propose by Ficher, *etal* (1983) for AGPs generally Fig.1.2., Fig.1.3. Randall, *etal* (1989) fractionated the gum using hydrophobic affinity chromatography and isolated four fractions. charbohydrate composition of each have different relative size and protein content and was refered to as an arabino galactan (AG). The minor fraction was rich in protein and was refered to as (AGP) and glycoprotein (GP).

- Qi, *etal* (1991) reported that the carbohydrate component when subjected to alkaline hydrolysis and following subsequent analysis they concluded that the carbohydrate was attached to the poly chain in small units of 30 sugar residue through galactose hydroxyl proline linkage. This was supportd by electron microscopy studies , they suggested that the structure resemble a twisted hairy rope in contra to wattle blossom model Fig.1.4.

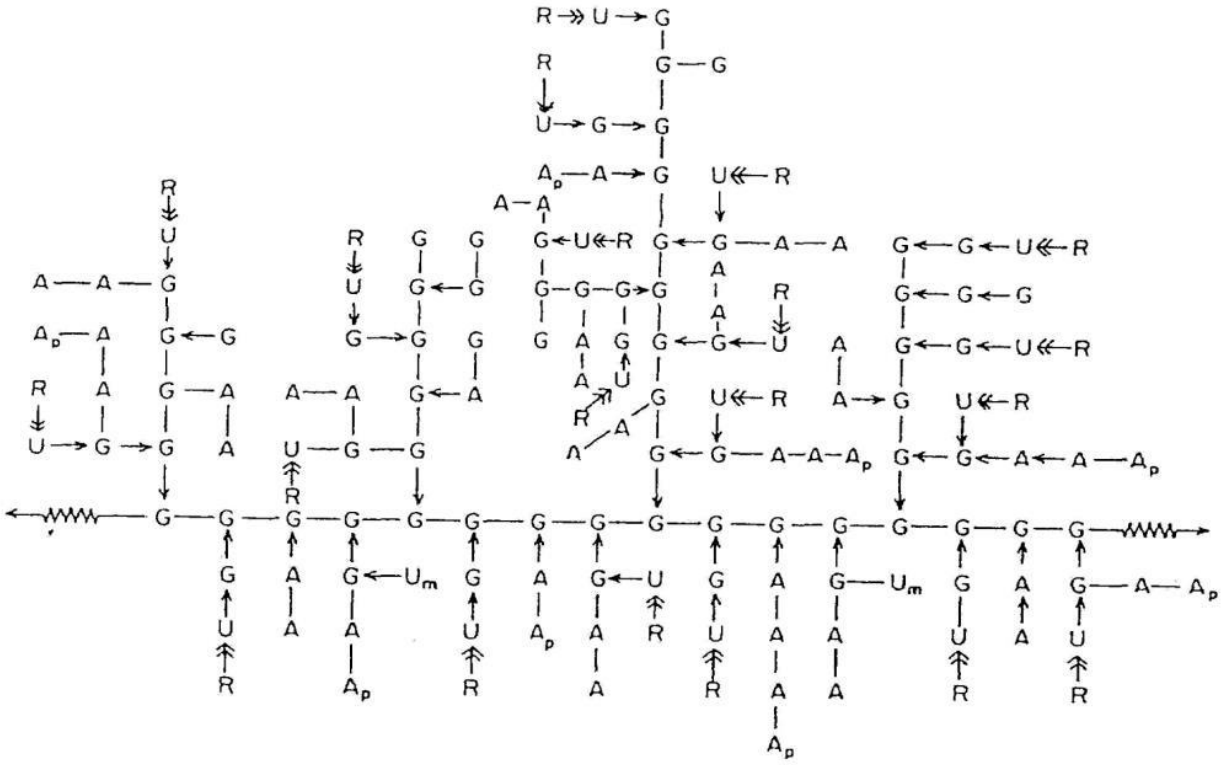


Fig. 1.1. The Proposed Structure of the Polysaccharide from *Acacia senegal* Gum (Street and Anderson, 1983)

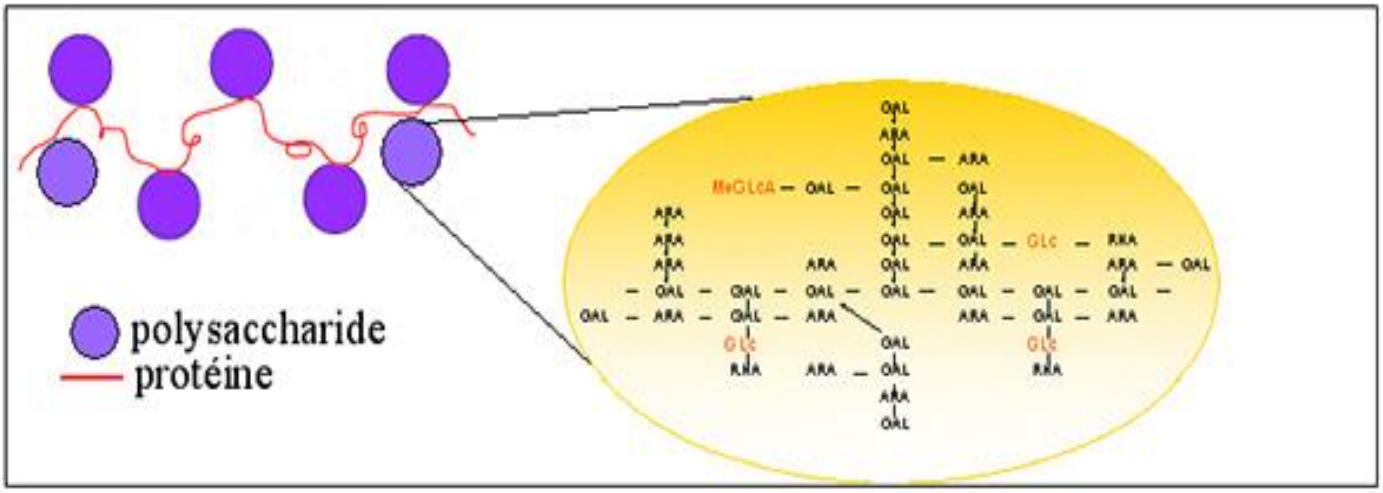


Fig. 1.2. Schematic Representation of Wattle-blossom Model (Finche1983)

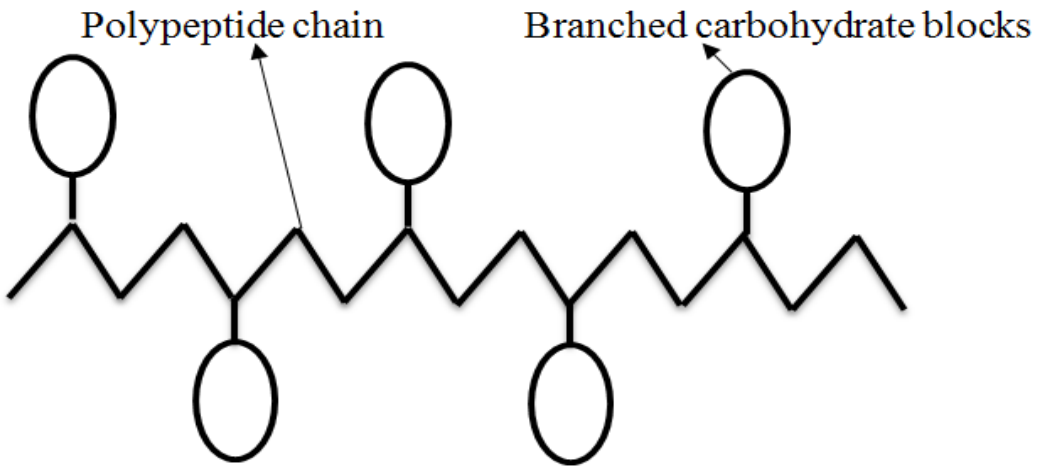


Fig.1.3. The Wattle blossom model of the Arabinogalactan–protein (Fincher, 1983).

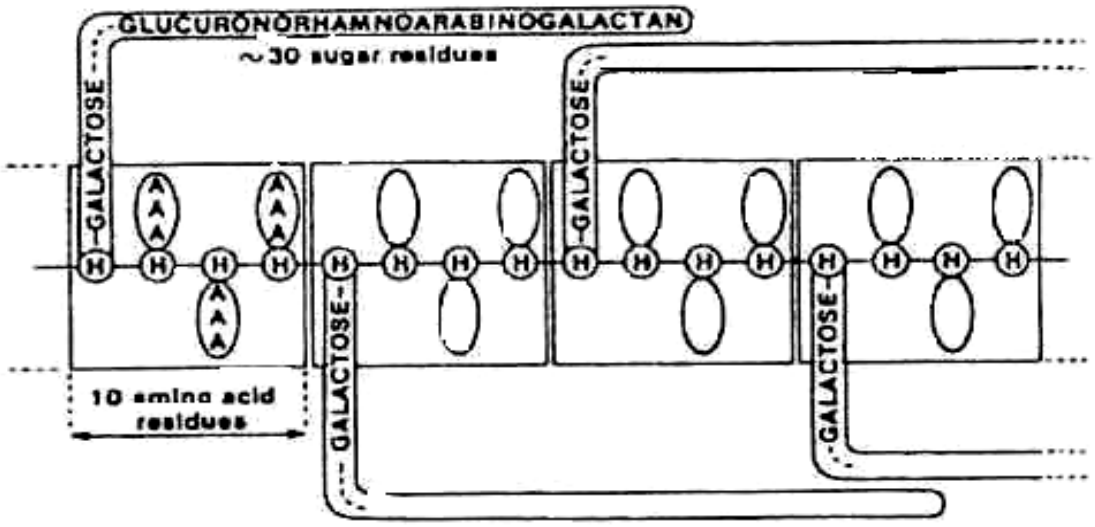


Fig 1.4. Twisted Hairy Rope model for the high molecular mass fractions of *Acacia senegal* Gum (Qi *etal*. 1991)

1.1.6. Components and Mesoscopic Structures of Gum Arabic :

Gum species were first separated through their molar mass using size exclusion chromatography into araninogalactan protein, arabinogalactan,

glycoprotein (Randall, *etal* 1988,Vandevelde, *etal* 1985), it was also separated into three main fractions using hydrophobic interaction chromatography (HIC) (Randall, *etal* 1989, Osman, *etal* 1993,. Renard, *etal* 2006) example of Renard, the first fraction represented most of the gum arabinogalactan-peptide (AGp)with a low protein content (1.1%) and high molecular weight $2.9x10^5$ $gmol^{-1}$, the second fraction was arabino galactanprotein complex (AGP) contained 9% protein and molecular weight of $1.9x10^6 gmol^{-1}$ protein content and the third fraction refered to glycoprotein (GP) with molecular weight $2.5x10^5 gmol^{-1}$ and highest protein content 24.6%.

The first model for the arabinogalactan fraction from *A*. gum proposed by Sanchez *etal* (2008) using combination of high- performance size exclusion chromatography – Multi Angle Laser Light Scattering (HPSEC-MALLS), Small Angle Neutron Scattering (SANS) coupled to Abinitio calculation, and various microscopic techniques from small angle neutron scattering experiments in change screening conditions, arabinoglactan appeared to be a dispersion of two-dimensional structures with a radius of gyration of ~ 6.3nm and an inner dense branched structure. Inverse Fourter Transmission of arabinogalactan scattering from factor revealed a disk-like morphology with a diameter of ~ 20nm and a thickness below 2nm – Abinitio calculation on the pair distance distribution function produced a porous oblate ellipsoid particle with a central intricated "network". Both Transmission Electron Microscopy and Atomic Force Microscopy confirm the thin disk model and structural dimensions.

Mohendran *etal*. (2008) investigated the structural characteristics of the gum from *A.senegal*. They found that the arabinoglactan protein complex has a molecular mass of (1-2) x 10^6 Da and consists of a polypeptide chain possibly containing ~ 250 amino acids with short arabinose side chains and much larger blocks of the

carbohydrate of molecular mass ~ 4.0 x 10^4 Da attached. The carbohydrate is highly branched. They suggested that these carbohydrate blocks may have a thin oblate ellipsoid.

Carbohydrate structure, which has been reported for the AG component (Sanchez *etal*. 2008). They found that the molecule adopted a very compact conformation with R_g of 36 nm. They concluded that the polysaccharide moieties were linked through both O- serine and O- hydroxypyroline residues.

Dror *etal* 2006 used Small-Angle-X-Ray and Neutron Scattering combined with Cryotransmission Electron Microscopy (SAXS), investigate its mesoscopic structure in aqueous solutions.Scattering measurements revealed an intricate shape composed of many spheroidal aggregates assigned to the poly saccharide with small amount of large coils. A dual chromatoghraphic separation together with Small Angle X-Ray (SAXS) and Neutron Scattering Structural (SANS) characterization was carried out by (Atgiè, *etal* 2019), the different species present in gum arabic cannot be easily classified in distinct families .They are builds from various combination of two building blocks. One block corresponds to polysaccharide., described as three dimensional multi-scale porous cholloids. The other block corresponds to protein chain Fig.1.5. A large array poly saccharide/protein conjugates was identified, which differ in size , hydrophobicity and amino acid. Small angle neutron scattering reveals that large scale structures are spreads in gum Arabic solutions possess multi-scale structures that mainly impacted by concentration and ionic repulsion.

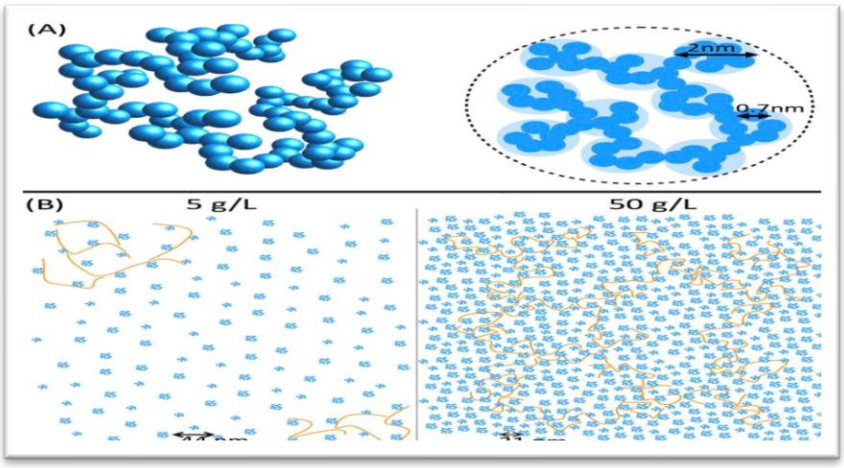


Fig. 1.5. Different *species* present in gum arabic(Atigè, 2019)

1.1.7. Application of Gums :

Exudate gums are used in an overwhelming number of applications, mainly situated in the food area. However, there are also considerable non-food applications. Gum Arabic is being widely used for industrial purposes such as a stabilizer, a thickener, an emulsifier and an encapsulating in the food industry and to a lesser extent in textiles, ceramics, lithography, cosmetic and pharmaceutical industry (Dauqan,2013). Gums imparts certain properties to food, that cannot be obtained from other materialsTable 1.1,Table 1.2. It is nontoxic, odorless, colorless, tasteless and completely water soluble, and does not affect the flavor, odor or color of other food ingredients. (Glickman,1969).

Gum Arabic readily dissolves in cold and hot water in concentrations up to 50%. Because of the compact, branched structure and therefore small hydrodynamic volume, gum Arabic solutions are characterized by a low viscosity, allowing the use of high gum concentrations in various applications. Solutions exhibit Newtonian behavior at concentrations up to 40% and become pseudoplastic at higher concentrations.

The pH of the solutions is normally around 4.5-5.5, but maximal viscosity is found at pH 6.0. Gum Arabic has excellent emulsifying properties. The hydrophobic polypeptide backbone strongly adsorbs at the oil-water interface, while the attached carbohydrate units stabilize the emulsion by steric and electrostatic repulsion. Fractionation studies show that, although emulsifying properties generally improve with increasing molecular weight and protein content, the best results are obtained with mixtures of different fractions. Seemingly, the heterogeneous nature of the gum makes it an excellent emulsifier (Dauqan, *etal* 2013)..

Table 1.1 International specifications of quality parameters of gum Arabic

Property				**Range**
Moisture content				(%) 13-15
Ash content				(%) 2-4
Internal energy				(%) 30-39
Volatile matter				(%) 51-65
Optical rotation (degrees)				-26- -34
Nitrogen content				(%) 0.26-0.39
Cationic composition (ppm)				
Copper	Iron	Manganese	Zinc	
52-66	730-2490	69-117	45-11	

Table 1.2. Characteristics of gum from *A sengal* and *A. seyal*

Property	***Acacia senegal***	***Acacia seyal***
4-O-meth1 glucuronic acid	1.5	5.5
% nitrogen	0.36	0.15
Specific rotation/degrees	-30	51
Average molecular mass (M_w)	380,000	850,000
% carbohydrate		
galactose	44	38
arabinose	27	46
rhamnose	13	4
glucuronic acid	14.5	6.5

1.1.7.1. Food Applications:

Gum Arabic is mainly used in the confectionery industry, where it is incorporated in a wide range of products Table 1. 3 . It has a long tradition of use in wine gums, where it produces a clarity that is higher than can be obtained with other hydrocolloids. Furthermore, it prevents sucrose crystallization, provides a controlled flavor release and slows down melting in the mouth, making the wine gum long lasting. It also provides the appropriate texture to these candies, which are easily deformed in the mouth but do not adhere to the teeth . In lower-calorie candy, gum arabic is used to compensate for the loss of texture, mouthfeel and body, resulting from the replacement of sugars by artificial sweeteners. It is also used in chewing gum as a coating agent and as a pigment stabilizer. In aerated confectionery products, such as marshmallows, nougats and meringues, gum Arabic acts as a whipping and stabilizing agent. It is also used in toffees and caramels as an emulsifier, to maintain a uniform distribution of the fat across the product. In jelly products, it is used to provide a fibrous, fruit-like texture . Gum Arabic is widely used as an emulsifier in the manufacture of soft drinks. Due to its stability in acid conditions and its high solubility, gum Arabic is well suited for use in citrus and cola flavor oil emulsions. High levels of gum are used to ensure a complete coverage of the interface and to prevent flocculation and coalescence of oil droplets. Normally, a weighting agent is added to increase the oil-phase density, inhibiting destabilization due to creaming . Gum Arabic can also form a stable cloud in the drink, imitating the effect of added fruit pulps and juices. Gum Arabic is used increasingly as a source of soluble fiber in low-calorie and dietetic beverages. In powdered beverage mixes, gum Arabic is added to produce the same opacity, appearance, mouth feel and palatability as natural fruit juices . In microencapsulation, liquid, solid or gaseous substances are

coated with a protective layer to prevent chemical deterioration and the loss of volatile compounds. It is a useful technique to convert liquid food flavors to flowable powders that can be used in dry food products. Gum Arabic is an effective encapsulation agent because of its high water solubility, low viscosity and emulsification properties and is used in soups and dessert mixes. Gum Arabic is also used to prevent gelation in canned gravy based pet foods, as it inhibits the extraction of proteins from the meat into the gravy .(Dauqan, *etal* 2013).

Table 1.3. Function of Gum in Food Production (Glickman,1969):

Function	Application
Adhesive	Bakery glaze
Binding agent	Sausages
Bulking agent	Dietetic food
Crystallization inhibition	Ice cream, sugar syrups
Clarifying agent	Beer wine
Cloud agent	Confectionery
Emulsifier	Salad dressings
Encapsulation agent	Powdered fixed flavors
Film former	Sausage castings protective coatings
Flocculating agent	Wine
Foam stabilizer	Whipped toppings
Ceiling agent	Puddings, deserts aspices
Mold released agent	Gum drops, jelly caudies
Pending protective colloid	Flavor mayonnaise
Stabilizer	Chocolate milk
Suspending agent	Processing meats
Swelling agent	Cheese frozen foods
Syneressis inhibition	Jams pie fillings sauce
Whipping agent	Topping icings

1.1.7.2. Non-Food Applications

In cosmetics, gum Arabic functions as a stabilizer in lotions and protective creams, face mask, hair cream, face powder compact.

where it increases viscosity, imparts spreading properties and provides a protective coating and a smooth feel. It is used as an adhesive agent in blusher and as a foam stabilizer in liquid soaps, prepare products envelopes, lable stamps, glue, transparent cement. . Gum Arabic is also used in the preparation of etching and plating solutions in the lithography industry (sensitizer for lithographic platers elements in light-sensitive composition).. It is used as a dispersant in paints and insecticidal/acaricidal emulsions, respectively keeping the pigments and active components uniformly distributed throughout the product. In the textile industry, it is used as a thickening agent in printing pastes for the coloration of knitted cellulose fabrics, used as sizing and finishing agents. Other applications are ink and pigment manufacture, ceramics and polishes . In Pharmaceuticals (Glickman, *etal* 1969) , as suspending agent, a mucilage used to aid in suspension of insoluble drugs, a syrup in flavor vehicles and suspending agent in calamine suspension, code liver oienment, magnesia suspension. As emulsifying agent in prepare cotton seed oil and as antiseptic preparation in ophthalmic infections and binder agent in tablets. In medicine in treatment of low blood pressure in saline injections and in treatment of nephritic edema as colloid injection.

,

1.1.8. ***Acacia tortilis* (*Vachellia tortilis*)**:

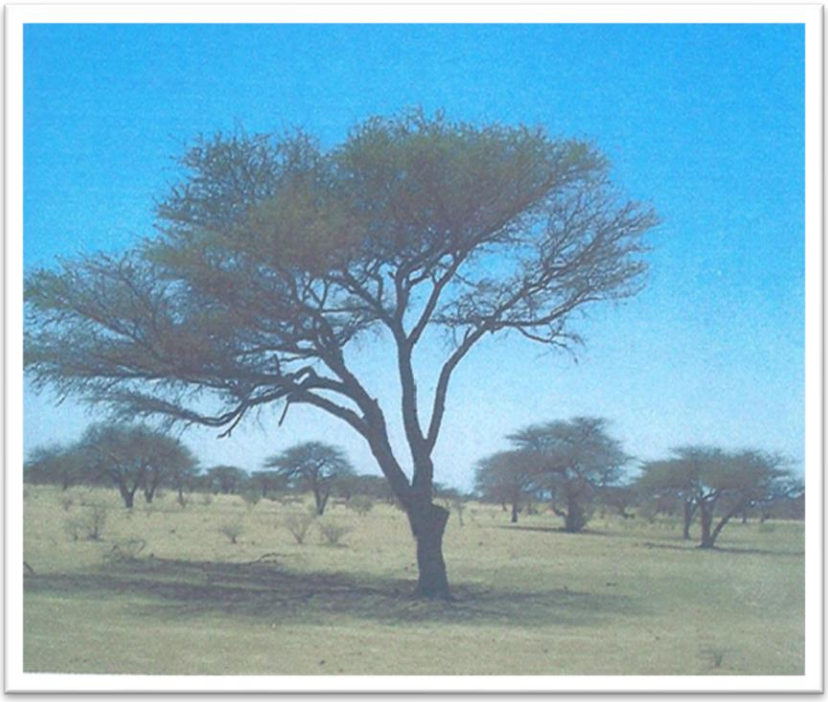
Acacia until recentily 1300 species world wide were classified as *Acacias*, about 960 are native to Australia with the remainder in tropical to warm temperature regions of Europe, Africa , Southern Asia and Americas.

Acacia tortilis often called the umbrella thorn for its distinctive spreading crown. It spreads in seasonally dry areas of Africa and Middle East.

A. tortilis (forsk) Hayne (subfamily *Mimosoidea* family *Leguminosae*) is one of about 135 African A. species. Unlike the Australian acacias, African *Acacias* are armed with thorns and produce highly palatable pods.(Brenan,1983).

A. tortilis include four recognized subspecies, *spirocarpa,*Fig.(1.6) *and raddiana* Fig.1.7., *tortilis* Fig 1.8 , *hetracantha* (Brenan, 1983). *A. tortilis* varies from multi-stemmed shrubs (var. *tortilis*) to trees up to 20m tall with rounded (*var. raddiana*) or flat-tapped (var. *hetracantha* and var. *spirocarpa*) crowns. The presence of very long thorns and two thorn types, long-straight and shorter hooked distinguish *A. tortilis* from other *Acacias* .

Fig.1.6. *A.tortilis* var. *spirocarpa*



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- **Fig. 1.7. *A.tortilis* var. *raddiana***

-

Fig.1.8. *A.tortilis* var.*tortilis*

-

1.1.8.1. Characteristics of *Acacia tortilis:*

It is a tree which grows up to 21m. (69ft.) in height. The tree carries leaves that grown to approximately 2.5cm (1 in) in length with between 4 to 1 pair of pinnae each with up to 15 pairs of leaflets. Flowers are small and white, highly aromatic, and occur in tight clusters. Seeds are produced in pods which are flat and coiled into a spring like structure.

The plant is known to tolerate high alkalinity, drought, high temperatures, sandy and stony soils, strongly stopped rooting surfaces, and sand lasting.Also, plants older than 2 years have been observed to be somewhat frost resistant. *A. tortilis* is widely occurring and variable species within which four subspecies are recognized.

There is some overlap in subspecies *tortilis* and subspecies *raddiana*. The subspecies are separated by the presence or absence of pubescence on the pods their wide and differences in the pubescence of the branchlet.

Four varieties in different ecological zone:

- var. *tortilis* Sahel, Middle East
- var. *raddiana* Sudan, Middle East, Sahel
- var *spirocarpa* Sudan, Eastern Africa, Sahel
- var. *hetracantha* Southern Africa

1.1.8.2. Keys to Subspecies. and Varieties:

1. Pods glabrous or neary so, edlandular:

1.Yong branchlets, petioles and leaf-rhachides glabrous or subglabrous, crown irregularly rounded, distribution north of the Equator (*ssp.raddiana* var. *raddiana*).

2.Yong branchlets, petioles and leaf-rhachids, shortly pubescent, crown flattened, distribution in Southern Africa (*ssp.hetracantha*).

3. Pods appressed-puberulous to densely pubescent orotomelleous, sometimes spreading hair, pods 3-5mm wide, shortly pubscent, eglandular shrubs or trees, 2-6m high (*ssp. tortilis*).

4. Pods pubescent or tomentellous with short hairs or with spreading longer hairs, numerous dark, reddish glands among the hairs visible with a hard lens, 6-13mm wide.

Pods pubescent or tomentellous with short hairs or mostly up to 0.75mm long, scattered longer hairs sometimes also present (*ssp. spirocarpa* var. *spirocarpa*).

Pods more or less densely clothed with long whitish spreading hairs 0.75-3mm long, shorter hairs and glands also present (*ssp.spirocarpa* var.*crinita*).

1.1.8.3. Varieties of Subspecies.:

1- *ssp. raddiana* var. *raddiana.*
2- *ssp. raddiana* var. *pubescence.*
3-*ssp.raddiana* var . *savi.*
4-*ssp.spirocarpa* var. *spirocarpa.*
5-*ssp.spirocarpa* var. *crinita.*
6-*ssp.tortilis* var. *tortilis.*
7-*ssp.tortilis* var. *lenticellosa.*
8-*ssp.tortilis* var. *pubscens.*
9-*ssp.hetracantha*

1.1.9. Ecology:

A. tortilis occurs throughout dry Africa, from Senegal to Somalia, and down into South Africa Fig 1.9. In Asia in Southern Arabia in Iran found in Sahara and extended to desert.

A. tortilis occurs from sand dunes and rocky scarps to alluvial valley bottoms, avoiding seasonally water logged sites. A very drought resistant species, grow in areas with annual rainfall as low as 40mm and as much as 1200mm with dry seasons of 1-12 months. The temperature vary from 0 to 50 degree Celsius. The

tree favors alkaline soils. *A. tortilis* form a deep tap root in sandy soils reaching 35m deep.

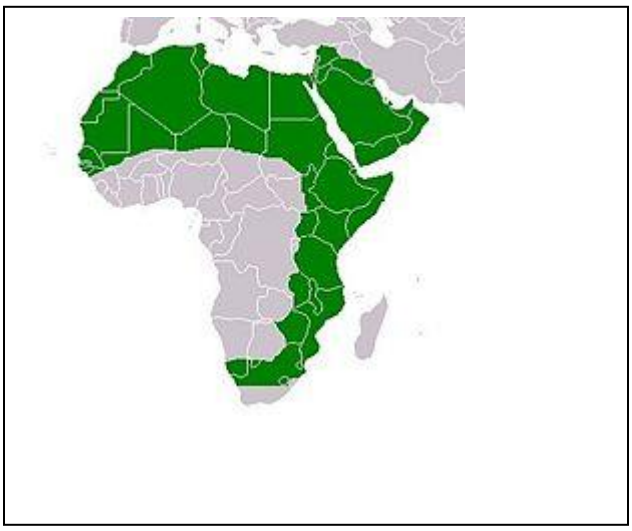

Fig. 1.9. Distribution of *Acacia tortilis* Trees

1.1.10. Uses:

- Forage: In semi- arid areas, *A. tortilis* provides a stable browse especially for camels and goats.
- Pods: Are collected for sale in markets as animal and human food. Also fed to locating animals to increase milk yields.
- Pods and Leaves: Have a good level of digestible protein and rich in minerals (LeHouerous,1989).
- Seeds**:** Are high in crude protein and phosphorus.
- Silvi pasture: Provide shade for animals.
- Sand Dune Stabilization and Sheitebelts: In Somalia and United Arab Emirates.
- Wood: Very good charcoal and fuel wood.

- Flowers provide a source of good quality honey in some regions.
- Fruits are eaten.
- The bark yield tannin and the inner bark cordage.
- Thorny branches are used for enclosures and livestock pens.
- Roots are used for construction of nomad huts.
- Leaves, bark, seeds and a red gum are used in many local medicines.
- Two active compounds for treating asthma have been isolated from the bark.(Hagos, *etal* 1987)
- Gum from the tree is edible and can used as gum arabic.
- Parts of the tree including roots slots and pods used by native as decoration, weapons, tools and medicine.
- Also it is important for rehabilitation of degraded arid land. It tolerates drought wind, salinity and wide range of soil types and has benefit of fixing nitrogen in the soil vial its interaction with symbiotic root bacteria and erosion control.(Wikipedia).

1.1.11. *Acacia tortilis* Subspecies *spirocarpa:*

1.1.11.1. Classification:

- Subspecies *spirocarpa* var. *spirocarpa* (Hochst ex. A. Rich) (Brenan,1983), is restricted to Eastern Africa, occurring in Sudan, Ethiopia, Somalia, Uganda, Kenya, Tanzania, Malawi, Mozambique, Zimbabwe and Botswana.
- Subspecies *spirocarpa* var. *crinita* (chiov. The var. *crinita*) has been found in Somalia, Kenya and Tanzania (Long hair pods to var. *spirocarpa*).

1.1.11.2. *Acacia tortilis* subspecies *spirocarpa* var. *spirocarpa:*

1.1.11.2.1. Descriptions (Elamin, 1990) :

Tree 2-21m high with flattened crown young, branches densely pubescent. Umbrella -like and flat topped. Leaves are compand and leaflet (6-22 pairs) are very small (1-4mm long x 0.6-1mm broad). Spines paired, two types, long, straight and white, cor short brownish and hooked, they range from 1.2 to 8 cm in length. Flowers are white, cream or yellow and highly aromatic. Fruits characteristic twisted browny pod . Pods with spreading or curved hairs, with numerous red –black glands conspicuous when seen with lens. (Elamin, 1990). Dark smooth or rough and fissured grey to black brown.

1.1.11.2.2. Distribution:

Is widely spread in crown usually flat and spreading in a grass and Savana of Central, Northen and South East Sudan along rivers, seasonal valleys and khors or gravely soils. Also it extends to semi-desert scrubland and dry grass (Elamin , 1990).

1.1.11.2.3. Taxonomy:

Domain	=	*Eukaryota*
Kindom	=	*Plantae*
Phylum	=	*Angiospermae*
Class	=	*Docotyledonae*
Oder	=	*Fabales*
Family	=	*Fabaceae*
Subfamily	=	*Mimosodideae*
Genus	=	*Acacia*
Species	=	*Acacia tortilis*

A. tortilis (Forssk) hayane *spirocarpa* (Hochst-ex A. Rich) Brenan(1983).
(Dharani, 2006).

Binomial Name	=	*Acacia tortilis*
Local name	=	*Samor, Sumor*
English name	=	*Karamoja , Thorny tree*

Fig. 1.10. One Stem *A.torrilis* var. *spirocarpa*

1.1.11.2.4. Botanical Classification: **(Elamin, 1990):**

Family = *Leguminoesae*

Subfamily = *Mimosoideae*

Genus = *Acacia tortilis*

SSP = *spirocarpa*

Latin name = *Acacia tortilis* (forssk*) Hayne*

English name = *Thorny tree*

Arabic name = *Samur*

1.2. Physicochemical Properties of *Acacia* Gums :

The physicochemical properties of gums, established as quality parameters and assist to differentiate between different *Acacia* gums include moisture, total ash, pH value, Specific optical rotation, molecular weight distribution, viscosity, nitrogen content protein , uronic acid cationic composition and sugar content.

1.2.1. Moisture Content:

Moisture content facilitates the solubility of hydrophilic carbohydrate and hydrophobic proteins in gums.(Dauqan, *etal* 2013).

The mass of analyte can be determined indirectly by measuring a change in mass due to its loss or the mass of coumpound formed as the result of a reaction involvining .The percent. Loss on drying was determined according to the JECFA method which is a measure of the moisture loss when the sample was heated to 5 hours at 105^{o}C (Al-Assaf, *etal* 2005).

A moisture analyzer consist of a precision laboratory balance and drying chamber that is attached to the laboratory balance. The drying chamber provides stable temperature on moisturing process. Such design of moisture analyzer makes the drying process different from traditional method. The functioning of a moisture analyzer is basically. Precise determination of mass of weighed sample before drying process, and during this process with no need to take the sample out of drying chamber . Automatic finish of a drying process, drying till dry mass or drying till elapse of set time interval.

Calculating of drying process result by algorithm for selected drying profile forwarding data from drying processes to a printer or computer if there is a need to prepare drying process documentaries.

1.2.2. Ash Content

:

Total ash content is used to determine the critical levels of foreign matter, acid insoluble matter, salts of calcium, potassium and magnesium (Dauqan ,*etal* 2013). Ash content indicates the presence of inorganic elements exist in salt form (Anderson and Dea,1968) . Siddig (2005), shows that the type of soil clay or sand affected the ash content significantly.

1.2.3. pH value:

Measurement of acidity or alkalinity of water soluble substances , pH stands for potentiality of Hydrogen (wikipeadia).

1.2.4. Specific Optical Rotation:

Optical rotation is used to determine the nature of gum sugars and to identify the source of production. Many substances possess the inherent property to rotate the plane of incident polarized light, this property is called optical activity. It is also used to employed the purity of substance. The optical rotation is the angle through which the plane of polarization is rotated when polarized light passes through a layer of solution. Substances are described as a dextrorotatory(+) or levorotatory(-) according to whether the plane of polarization is rotated clockwise or counterclockwise, respectively(Elhag, 2018).

Optical rotation was measured using digital polarimeter equipped with 250mm tube filled with the test solution at room temperature. Specific rotation was calculated by the following relation:

$$\text{Specific optical rotation } [\alpha]_D^L = a\ \frac{100}{CL}$$

Where: α is the observed angle of rotation of the solution in circular degrees, C is the grams of substance per 100 ml of solution, and L is the path of the solution in decimeters (Moffit and Young, 1956).

1.2.5. Total Nitrogen and Protein:

The Kjeldahl method was developed for determining the nitrogen content in organic and inorganic substances. The technique apparatus have been modified over the years, but the basic principle introduced by Johan Kjeldahl are still the same (AOAC, 1990).

A micro Kjeldahl method was used to determine the total nitrogen in *A. tortilis* var. *spirocarpa* gum samples, the nitrogenous compound was digested with concentrated sulfuric acid in the presence of selenium and copper sulfate – potassium sulfate catalyst to yield ammonium sulfate. An excess of sodium hydroxide is added and ammonia is distilled in steam, absorbed in boric acid and titrated with hydrochloric acid using methylated as indicator. The reaction involved in these steps can be shown as follows:

- Sample + $H_2SO_{4\,(conc.)}$ + Catalyst + Heat = $(NH_4)_2SO_4$
- $(NH_4)_2SO_4 + 2NaOH = 2NH_3 + Na_2SO_4 + 2H_2O$
- $NH_3 + H_3BO_3 = NH_4^+ + H_2BO_3^-$
- $H_2BO_3^- + HCl = H_2BO_3 + Cl^-$

1.2.6. Sugar Composition:

HPLC is widely considered to be technique mainly for biotechnological, biochemical research, and for the pharmaceutical industry, is a well widely used in a lot of fields, such as cosmetic, energy environmental

and food industries . The purpose of analyzing the gum samples by HPLC was to determine the relative concentrations of each sugar residue present in the sample, namely rhamnose (Rha), arabinose (Ara), galactose (Gal) and glucuronic acid (GlcA).

1.2.7. Acid Equivalent Weight and Total Uronic Acid:

Uronic acids constitute a major component of natural polysaccharides. Several methods can be used to determine it in its compounds, they are:

1- Colorimetric methods.
2- Decarboxylation methods.
3- Volumetric methods.

Potentiometric titeration was used for the determination of acid equivalent weight, using the procedure adopting by Anderson *etal* 1983 ,Vandevelde,1985, Randall *etal* 1989, Osman, *etal* 1995, Hassan, *etal* 2005. Uronic acid percentage was calculated from the same source.

1.2.8. Cationic Composition of *A. tortilis* var. *spirocarpa:*

The cationic compositions of ash content are used to determine the specific levels of heavy metals in gums.(Lelon, *etal* 2010).

Inductively coupled plasma (ICP), the most common used technique for the determination of trace concentrations of elements in samples are based on atomic emission spectrometry (AES) are used to dissociate sample molecules into free atoms, thermal source such as flames, furnaces and electrical discharge .More recently, other type of electrical discharges, namely plasma have been used as atomization/excitation source for AES plasma. Plasma sources offer several advantages compared with flame and electro-thermal methods. The advantages are

that is a multi-element technique and it has wide range. Plasma electron temperatures can range between ~6000 K and ~10000 K (~6ev - ~100 ev) compared to the surface of the sun. ICP discharges are of relatively high electron density, on the order of 10^{15} cm^{-3}. As a result, ICP discharges have wide applications where a high density plasma (HDP) is needed.

Current plasma source (DCP) provides a much easier method of handling liquid and gaseous samples. Spectra for dozzen of element can be recorded at the same time which is important when the sample is very small. Plasma sources also permit determination of non-metals such as chlorine, bromine, iodine and sulfur.

ICP-AES applications include the determination of small quantities of metallic compounds in wine, arsenic in food, trace elements in soil and trace elements bound to proteins. Food and food products can be prepared for ICP analysis by either wet oxidation or dry ashing and samples with a wide range of elemental concentrations can usually be handled without the need to dilute or concentrate, since the ICP has a fairly large dynamic range .(Zurera, *etal*.1993).

1.2.9. Viscosity:

A measure of the resistance of flow due to internal friction when one layer of fluid is caused to move in relationship to another layer. The poise represents absolute viscosity, the tangential force per unit area of either of two horizontal planes at unite distance apart, the space between, being filled with substance.

1.2.9.1. Relative Viscosity (η_{rel}):

The ratio of the viscosities of the polymer solution (of started concentration and of the pure solvent at the same temperature. Also Known as solution solvent viscosity.

1.2.9.2. Specific Viscosity(η_{sp}):

The relative viscosity of a polymer solution of known concenteration minus 1, usually determined at low concentration of the polymer, for example 0.5 gram per 100 milliliters of solution or less.

1.2.9.3. Reduced Viscosity (η_{red}):

The specific viscosity(η_{sp}) divided by the concentration, expressed in g/ml.Also Known as viscosity number.Reduced viscosity is expressed in the following equation

$$\eta_{red} = \eta_{rel} - 1/C$$

Where C = concentration , η_{rel} = relative viscosity

1.2.9.4. Inherent Viscosity (η_{inh}):

The quotient of the natural logarithm of relative viscosity and the concentration. Inherent viscosity(η_{inh}) is expressed in the following equation:

$$\eta_{inh} = \ln \eta_{rel} / C$$

Where C : concentration , η_{rel} = relative viscosity

1.2.9.5. Intrinsic viscosity(η) :

The ratio of a solution s specific viscosity to the concentration of the solute, extrapolated to zero concentration. Intrinsic viscosity reflects the capability of a polymer in solution to enhance the viscosity of the solution. The viscosity behavior of macromolecular substances in solution is one of the most frequently used approaches for characterization. The intrinsic viscosity munber is defined as the limiting value of the specific viscosity / concentration ratio at zero concentration.

The intrinsic viscosity is determined by measuring the relative viscosity at several different concentrations and the extrapolation the reduced viscosity to zero concentration.

The variation of the viscosity number with the concentration depends on the type of molecule as well as the solvent. The intrinsic of linear substance is related to the molecular weight or degree of polymerization with linear macromolecules viscosity number measurement can provide a method for rapid determination of molecular weight has been established.intrinsic viscosity is calculated by determining the reduced viscosity**(** η_{sp}/c **)** and extrapolating to infinite dilution.

Huggin ,s equation **(** η **)** = $\lim_{C\to 0} \eta_{red}$

Craemar,s equation **(** η **)** = $\lim_{C\to 0} \eta_{inh}$

Where C = concentration of polymer in grams per 100 milliliters of solution

Also Known as limiting viscosity number.

1.2.9.6. Apparent Viscosity:

The value obtained by appling the instrumental equations used in obtaining the viscosity of a Newtonian fluid to viscomerer measurements of a Non-Newtonian fluid.

1.2.9.7. Absolute Viscosity:

The tangential force per unit area of two planes at unit distanc apart when the space between them if filled with a fluid and one plane moves with unit velocity in its own plane relative to the other , also known as coeffient of viscosity.

Absolute (Brookfield) viscosity usually refers to a viscosity measurement performed with Brookfield Viscometer (HAKE VISCOMETER 6 PLUS).

1.2.9.7.1. Operation:

The viscometer motor rotates the spindle at a defined speed (measured in rpm) on shear rate and the viscometer measures the resistance to rotation and reports a viscosity value. Various spindle designs can be employed, depending on the nature of the sample and the requirements.

1.2. 10. Calorific Value:

Gums are a profitable natural wellspring of dissolvable dietary fibers that reaches 85% of its weight. Soluble dietry fibers help diminish the total cholesterol and the low density lipoprotein cholesterol, which impact coronal heart desease. Gum arabic is a prebiotic fiber, raise the exents of lactic acid bactria and bifidobacteria in healthy subjects. It was fermented gradually , created by short-chain fatty acids and its fecal absorbability was around 95%. The addition of GA dietary fiber to dairy items enhanced their nutirtional quality, since it makes it conceivable to redue the fatty substance by utilizing dietary fibers as a substitute of fat without loss of value (Mariod, 2018).

1.3. Molecular Weight :

1.3.1. Gel Permeation Chromatography

Gel permeation chromatography (GPC) is an analytical technique that separates dissolved macromolecules by size based on their elution from columns filled with a porous gel. When GPC is coupled with light scattering, viscometer and refractometer detectors (known as triple detection), it can measure absolute

molecular weight, molecular size , intrinsic viscosity and generate information on macromolecular structure, conformation, aggregation and branching.

By using GPC to measure molecular weight and these other properties, it is possible to characterize molecules such as synthetic polysaccharides polymers, as well as natural polymers . GPC Fig. 1.11, coupled on line to absolute molecular weight determining device (such as a light scattering photometer) and concentration sensitive detector (as RI and UV) is currently the best available technique for the quick and absolute determination of polymer molecular weights and their distribution. The LS detector utilizes the principle that the intensity of light scattered elastically by a molecule (Raleigh Scattering) is directly proportional to the molecular weight (mass detector). By using the RI detector connected directly after the LS it was be able to measure the molecular weight of each fraction as it elutes from the GPC column.

In addition to these two detectors, also an ultraviolet (UV) detector at 214 nm can be used which specifically shows the amount of protein in the gum fraction.

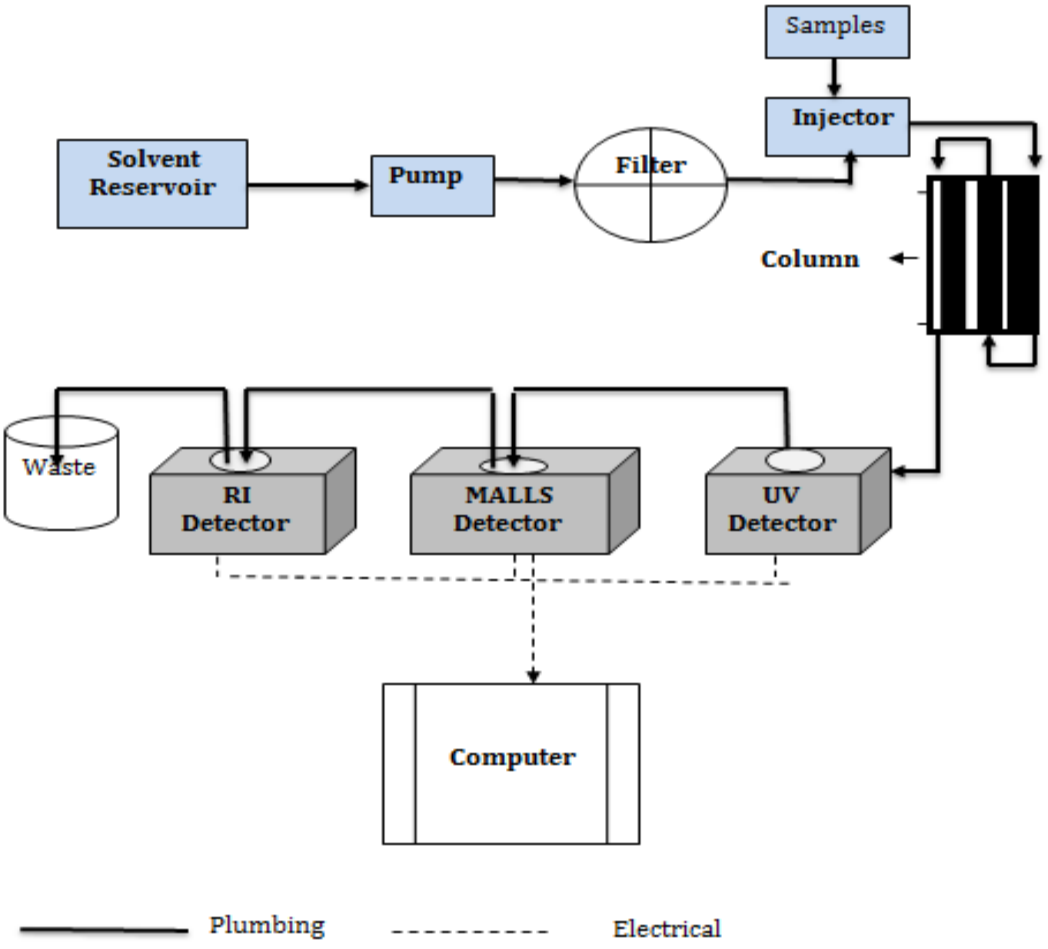

Fig. 1.11. A typical GPC-MALLS flow diagram.

Solvent enters the degasser and is pumped through in-line filter before the sample is injected and pass through columns and detectors.(Hamouda,2017).

1.3.2. Advantages (Wikipedia):

As separation technique, GPC has many advantages. First of all, it has a well-defined separation time due to the fact that there is a final elution volume for all unretained analytes. Additionally, GPC can provide narrow bands although this aspect of GPC is more difficult for polymer samples that have broad ranges of molecular weights present finally, since the analytes do not interact chemically or physically with the column, there is a lower chance for analyte loss to occur.

Determine molecular weight of polymers and mass distributions quick, easy , disadvantage : limit number of peaks.

1.3.3. Theory of Separation:

A column is made up of swollen gel particles and the solvent used to swell the gel in a suitable tubular container. An equation is given below:

$$V_t = V_o + V_i + V_m$$

Where V_t = the total value of the column (which can be measured).

V_o = the volume of liquid outside the gel matrix (known also void or dead value).

V_i = the volume of liquid inside the matrix.

V_m = the volume of the gel matrix.

1.3.3.1. How it Works:

GPC separates based on the size of the analytes Fig . 1.12. This differs from other separation techniques which depend upon chemical or physical interactions to separate analytes. Separation occurs via the use of porous beads packed in a column.

The smaller analytes can enter the pores more easily and therefore spend more time in these pores, increasing their retention time. These smaller modules spend more time in the column and therefore will elute last. Conversely, larger analytes spend little if any time in the pores and are eluted quickly. Al columns have a range of modules weights that can be separated.

If an analytes is either too large or too small, it will be either not retained or eluted with the free volume outside of the particles (V_o), while analytes that are

completely retained are eluted with volume of solved held in the pores (V_i). The total volume can be considered by the following equation, where V_g is the volume of the polymer gel and V_t is the total volume.

$$V_t = V_g + V_i + V_o$$

V_g = the volume of the polymer gel

As can be inferred, there is a limited range of molecular weights that can be separated by each column and therefore, the size of the pores for the packing should be chosen according to the range of molecular weight analytes to be separated.

For polymer separations the pore sizes should be on the order of the polymers being analyzed. If the sample has a broad molecular weight range, it may be necessary to use several GPC columns in tandem with one another fully resolve the sample.

The volume of liquid at which the polymer elutes from the column (V_e) is related to the physical parameters of the column by the relationship:

$$V_e = V_o + V_d\, V_i$$

Where V_e the elution volume = V_R the retention time

V_o the void volume of the column

V_i the internal volume of the column

K_d the distribution coefficient (based on relative concentration between phases).

$$\text{Then } V_d = V_e - V_o/V_i$$

$$V_t = V_o + V_i$$

$$\text{Then } K_d = V_e - V_o/V_t - V_o$$

The physical significance of K_d can be explained accordingly:

$K_{d=}$ o when the solute is completely excluded from pores $0< K_d < 1$ when there is a partial exclusion, the solute particles are distributed between V_o and V_t .

$K_d = 1$ the solute is free into or out of the pores. Average solute concentration at V_o and V_t are equal $K_d > 1$ enthalpic interaction between solute and packing.

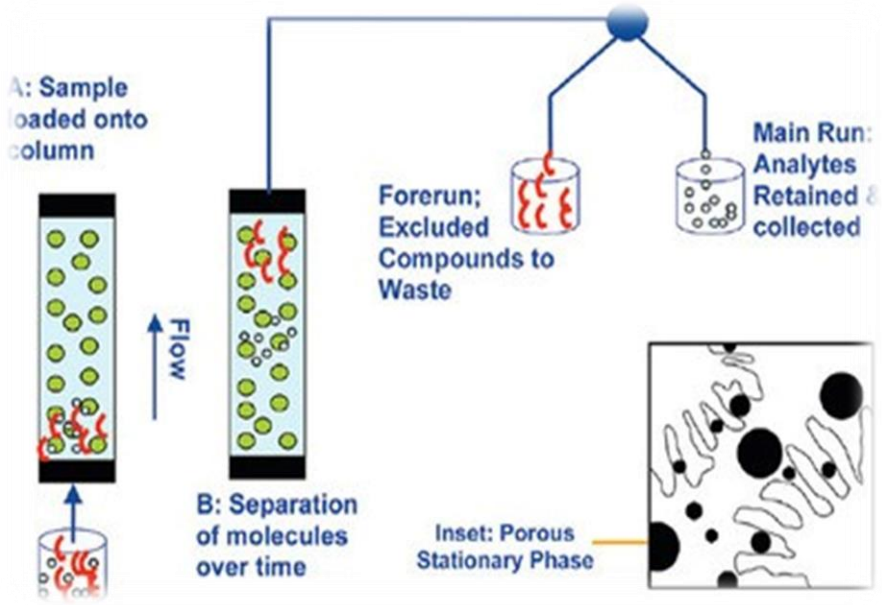

Fig 1.12. Schematic Diagram of GPC Separation

1.4. Emulsification

One of the most functional roles of food hydrocolloids is in the preparation of emulsions and in the control of emulsion shelf-life. Product applications include carbonated soft drinks (Tan, 2004), ice-cream (Goff, 1997), sauce and dressings (Sikora, *etal* 2008). Most hydrocolloids can act as stabilizers (stabilizing agents) of oil – in – water emulsions, but only a few can act as emulsifiers (emulsifying agents).

The latter functionality requires substantial surface activity at the oil-water interface, and hence the ability to facilitate the formation and stabilization of fine droplets during and after emulsification (Dickinson, 2003, 2004).

The most widely used polysaccharide emulsifiers in food applications are gum arabic (*Acacia senegal*), modified starches, modified celluloses, some kinds of pectin, and some galactomannans (Dickinson, 2003, Garti & Reichman, 1993). The surface activity of these hydrocolloids has its molecular origin in either (i) the non-polar character of chemical groups attached to the hydrophilic polysaccharide backbone (in hydrophobically modified starch / cellulose or (ii) the presence of protein component linked covalently or physically to the polysaccharide (some gums, pectins, etc.).(Dickinson, 2003)

Protein ingredients derived from milk and eggs are the most commonly used food emulsifying agents, but these are not hydrocolloids (Dickinson, 1992). Due to its unique hydrophilic character, gelatin is really the only protein that can properly categorize as a hydrocolloid. Gelatin does have some emulsifying ability, but its more characteristic roles as a colloid stabilizer and gelling agent.

Food biopolymers find extensive application in foods because of their capability to modify texture and interfacial activity . An important function of many hydrocolloid ingredients in oil-in- water emulsion is as a structuring, thickening, gelling agent in the aqueous medium or thickening agent which they find use in soaps and toppings white as gelling agents they are used in products like jam, jelly, marmalade, restructured foods and low sugar calorie gels.

Gums are used as stabilizers, thickeners, emulsifiers and encapsulating agents in confectionery, bakery, dairy products, beverage(Verbeken, *etal* 2003) and to a lesses extent in textile, ceramic, lithography, cosmetic and pharmaceutical industries

(Sanchez, *etal* 2008).The arabinogalactan protein (AG) is the hydrophobic region of the gum molecule. The great fliexibility of AGP structure allows molecules to be easily deformed at oil/water interface (Sanchez, *etal* 2002). While hydrophilic carbohydrate component inhibits flocculation and coalescence of molecules through electrostatic and steric repulsion in food additives(Lelon, *etal* 2010).

Functionalities of molecular structure in applications, in the food sector depend on the action of gum arabic as a protective colloid, stabilizer, or adhesive when it contact with water, it s ability to thicken and provide a dietary food fiber with low colorific value. It can impact its desirable qualities through its influence over the viscosity, body and texture of the food. It is also a good emulsifier and a foam stabilizer in beer and when used in spray – dried flavors, since it is capable of film forming and giving an impenetrable film around the flavor particle. Confectionary application depend on its ability to interact and bind water, thicken as a gel and so prevent sugar crystallization. Here also its emulsification quality is important to enable fat to distributed throughout the product and not more to the surface and make the food appear greasy. In this application as in beverages, it needs to be able to coat the fat or oil droplet using its high molecular protein structure.

As a stabilizer in dairy products it needs to prevent water crystallization and the formation of ice crystals and is thus depended on its water absorbing properties. In the baking industry it is used because of its adhesive property, in glazes and toppings. In pharmaceuticals sectors, it is used as a suspending agent, in syrups, emulsions, antiseptic preparations, medicines wound dressings, cosmetics and adhesive. In paints, inks lithography and textiles. (Al-assaf, *etal* 2005).

1.4. 1. Backgrounds

Randall, *etal*1998 rep32orted that the AGP complex is the main component responsible for gum Arabic ability to stabilize emulsion by association of the AGP amphiphilic protein component with protein component with the surface of oil droplets, while the hydrophilic carbohydrate fraction is oriented towards the aqueous phase, preventing aggregation of the droplets by electrostatic repulsion.

Emulsion stability is significantly affected by the type of oil used. Cotton seed oil gave the most stable emulsion while groundnut oil resulted in lower stable emulsion. increase of the length of the stirring time is significantly increase stability of the emulsion also stability affected by gum grades, other factors of concentration and temperature did not significantly influence emulsion stability (Sabah Elkhair, *etal* 2008). Previous studies have shown the stability of oil in water emulsions (O/W) depends on both the type and concentration of ingredients contained into emulsion as well as processing and storage conditions (Mc Clements,1999), this is agreed with the study of (Sabah Elkhair,2008) emulsion stability affects by the type of oil , stirring time and also gum grade but the study is,disagreed in factors like concentration and temperature that does not influence emulsition stability. Minerals decrease emulsion stability due to an electrostatic screening effect, pH also affects stability , when it is 2.5 stablility decreases and when it is (4.5-5.5) stability increases. (Dickinson, *etal* 1991).

Removal of the protein by treatment with proteolytic enzyme reduce the emulsification properties (Randall, *etal*1988).

The increase of AGP percentage in emulsion is helped to achieve more uniform homogeneity of oil droplet population (Castellani, *etal* 2010). Increasing of AGP content from 11 to 28% result in the formation of emulsion with relatively smaller

droplet sizes and better stability.Further increase in the AGP content to 41% result in the formation of emulsions with larger droplets.In spite of the larger droplet sizes, these emulsions are extremely stable.The AGP content plays avital role in emulsion stability and droplet size (Han,2019). Many factors affect the stabilization such minerals could decrease the emulsion stability presumably due to an electrostatic screening effect, pH also affected the emulsion, less stable at pH2.5 than high pH level 4,5-5.5 (Dickinson, *etal*1991).

1.4. 2. Emulsion and Emulsifiers:

An emulsion is defined as a dispersion of droplets of one liquid into another, the two being immiscible (Dickinson, 1992). Emulsions are thermodynamically unstable system rapidly or slowly separated into two immiscible phases over a period of time. (Borwankar, 1992) due to the high free energy of the interface between the two phases ,and are stabilized by improving of their kinetic stability.

Viscosifying agent (gum) is common additives that improve emulsion stability in foods. These compounds have little surface activity but stabilize emulsions by increasing the viscosity of the continuous phase such that collsions between droplets of the dispersed phase are less frequent and phase separation takes longer to occur.

Emulsions can break by variety of processes include flocculation, coalescence, creaming, sedimentation, and Ostwald ripping (Walstra, 1997). Equilibrium is reached when the area of contact between the two phases is at minimum. Emulsifiers show the rate of phase separation, also, they have an amphiphilic molecular structure and concentrate at the interface between two phases. An attractive method of evaluating emulsifiers would directly measure the area of contact between the two phases. An effective emulsifiers is therefore one that:

i) Rapidly reduces the interfacial tension at the freshly formed oil – water interface.
ii) Binds strongly to the interface once adsorbed.
iii) Protects the newly formed droplets against flocculation or coalescence. (Dickinson, 2009).

Emulsifying agents are added to emulsions to stabilize the two phases. It acts on interface and increase the kinetic stability of an emulsion, so the size of droplets does not change significantly with time, thus stabilizing the emulsion.Emulsifiers are typically have a polar or hydrophilic (i.e. water soluble) parted a nonpolar (i.e. hydrophobic or lipophilic) part. Thus they tend to have more or less solubility either in water or in oil.

1.4.3. Emulsion Preparation:

1- Emulsification – gelation is a method frequently used to prepare protein micro-particles. This method involves a process to stabilize emulsions of oil droplets in aqueous protein solution (O/W) or aqueous protein droplets in oil phase, followed by droplets in oil phase (W/O), followed by gellification of the protein by heating, chemical or enzymatic crosslinking.
2- A course premix is created by rapid mixing of the ingredients. This is sufficient to break up the dispersed phase into large droplets, and allow adsorption of the emulsifiers prior to final emulsification (Wilde, *etal* 2009).

1.4.4. Homogeneity:

There are two main methods/principles commonly used to homogenize emulsions in the food industry:

1- Mechanical (e.g. rotor – stator) and high pressure. Mechanical methods induce high shear fields to break up droplets.(Walstra,1983).
2- High pressure homogenizers are now very common, and simply force the premix through a narrow orifice or valve at high pressure (typically 10-100 MPa). Forcing the emulsion through a valve at high pressure creates turbulence and very high shear forces, thus breaking up the droplets.
3- Cross flow membrane emulsification (XME): A premix is forced through a porous substrate, into the continuous phase.(Charcosset and Fessi, 2005) . The droplet size produced is dependent on the interfacial tension and the pore size.

There are various methods to measure indices:

i) Emulsifying capacity (EC).
ii) Emulsifying stability (ES).
iii) Emulsifying activity (EA).

1.4.5. Emulsifying Stability (ES):

Is a measure in terms of the amount of oil or cream separating from an emulsion during a certain period of time at a stated temperature and gravitational field (13000 g).

1.4.6. Theories of Emulsification:

Several processes separate the oil in water emulsion, these processes may depend on temperature gravitational field strength and the concentration of oil in the emulsion.

1- **Repulsion Theory**: It proposes that the emulsifying creates a film over one phase that forms globules, which repel each other. This repulsive force causes, them to remain suspended in the dispersion medium.

2- **Surface Tension Theory**: Emulsification takes place by the reduction of interfacial tension between two phases.
3- **Viscosity Modification**: Certain emulgents such as *acacia* increase the viscosity of the medium, which helps create and maintain the suspension of globules of the dispersed phase.

Several breakdown processes may occur for emulsions Fig.1.13. , they are:

1- **Flocculation**: When droplets are attracted to each other and spontaneously form a flac.
2- **Creaming**: When droplets rise or sink according to buoyancy.
3- **Coalescence**: When droplets merge with each other to form larger droplets.
4- **Ostwald Ripening**: When smaller droplets decrease in size until they disappear and the free molecules redeposit on larger droplets.

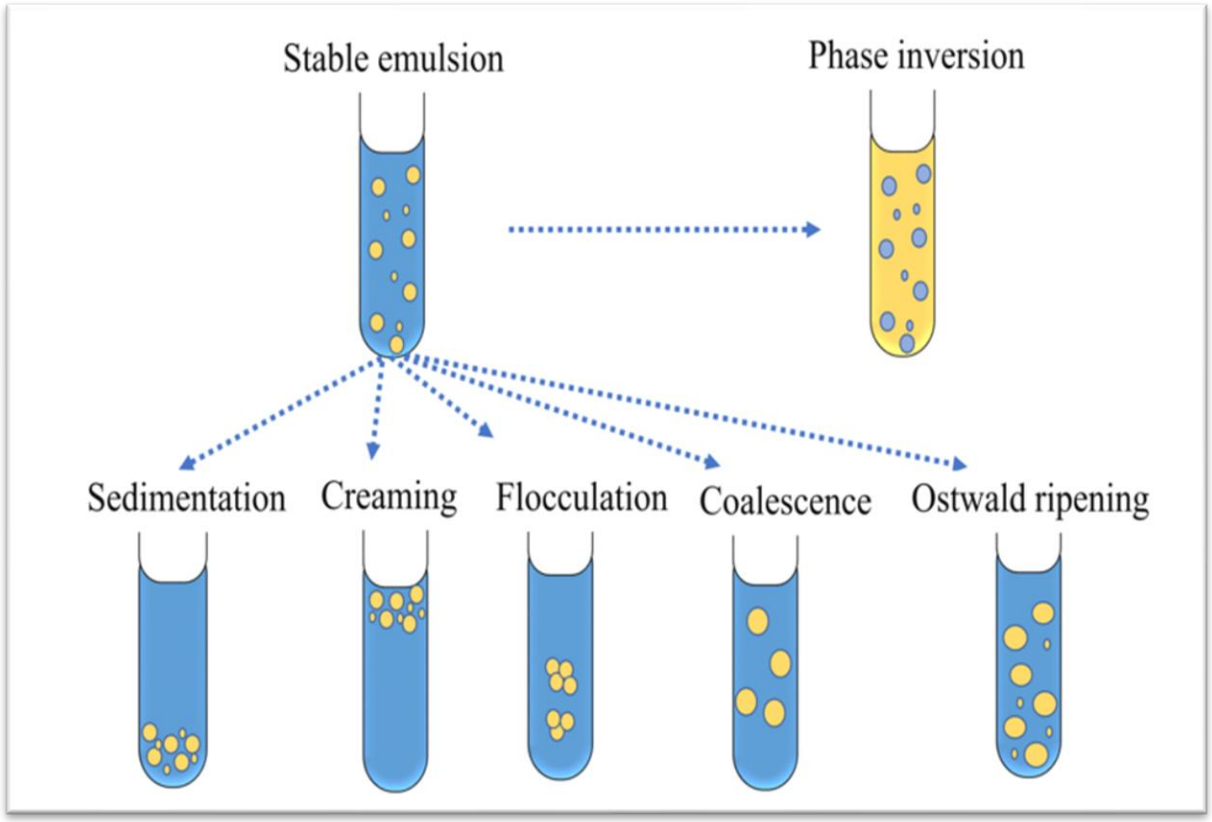

Fig. 1.13. Schematic Representation of Emulsion Breakdown

1.4.7. Mechanical Methods of Emulsification

An emulsion is prepared by mixing oil phase and gum aquesous solution and other additives, the mixed solution is homogenized energetically for 3 minutes by using a bench-top high-speed mixer (a Polytron PT-2100 homogenizer Fig.1.14. at high speed 26000 rpm. The high-speed rotation of Polytron homogenizer generates a shear stress in the gap between stator part and rotor shaft which causes the large droplets to be broken into smaller ones for making emulsion, then an average droplet size of < 1 μ and narrow particle size distribution is achieved by subjecting the pre-emulsion to high-pressure valve homogenizer (Nanomizer NM2-L100-D07, Collision type S generator (diameter 84 μ), Yoshida Kikai Co. Ltd. See Fig.1.15) at 50 MPa in two passes.

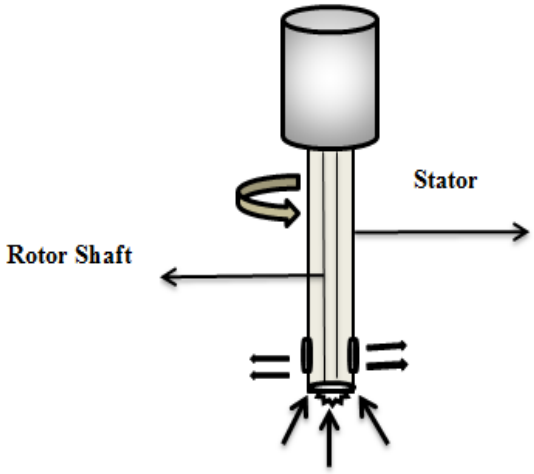

Fig.1.14. Structure and homogenization mechanism of Polytron.

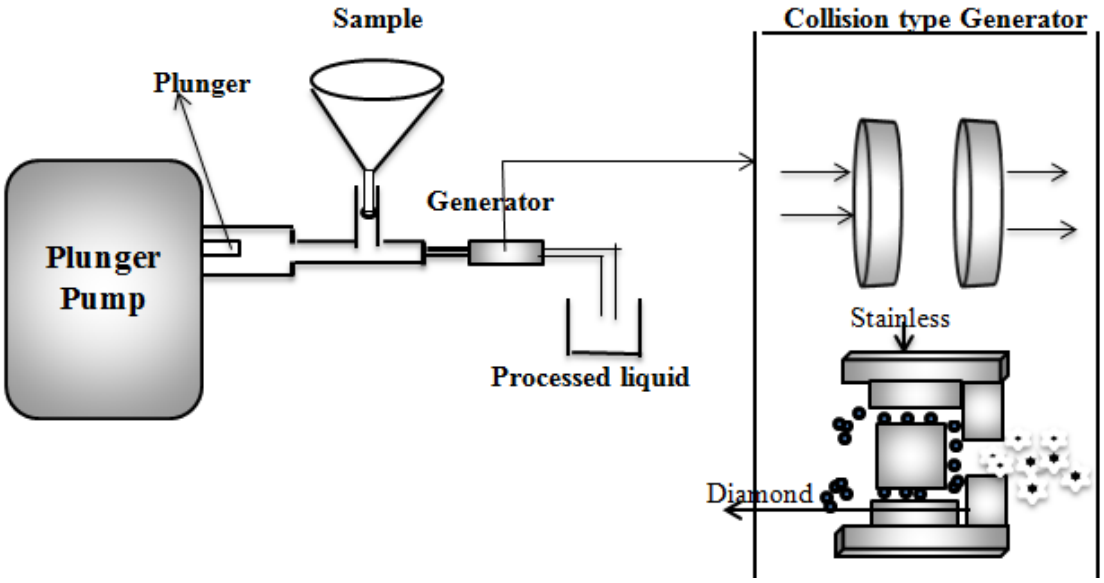

Fig. 1.15. Structure and homogenization mechanism of Nano-Mizer (high pressure homogenizer) gives a combination of intense shear, cavitation and turbulent flow conditions which cause the larger droplets to be broken down into smaller ones.

The most direct method to measure emulsion particle size and distribution, which is theoretically least subject to errors, is electron microscopy . However, this technique cannot be used routinely to determine size distribution. The rapid methods of particle sizing are based on light scattering. Laser diffraction is the most widely used technique for particle size analysis, where four types of interaction occur between light and particle; diffraction in outline of particle (Fraunhofer diffraction, which is evaluation of the wave fields diffracted from plane aperture), reflection on both inside and the outside surfaces of particle, refraction on the interface between particle and dispersion medium, and absorption of light in the particle inside. According to these interactions, the intensity shape of the scattered light is changed due to the particle diameter. Particle size analysis by laser diffraction is measured by detecting this change. The basic scheme of particle size analysis system is shown in Fig.1.16. Once an emulsion has been formed it is often necessary to characterize the emulsion, specifically in terms of it is average size and it is size distribution. The importance of particle size in determining emulsion stability is evident from Stockes law, Equation:

$$V = 2r^2(d_2 - d_1)\, g9\mu$$

Where: V is velocity of separation (or rate of creaming) (cm/sec) , r is droplet radiu(cm), d_2 is density of continuous phase, d_1 is density of disperse phase (g/cm^3), g is acceleration of gravity and μ is viscosity of the continuous phase (g/cm.sec).

According to stokes law, the particle size changing will have big effect on the emulsion stability because the velocity of creaming or sedimentation is related to the square of the radius of the particle size.

The scattered pattern is determined by the relation between the wavelength of light and particle diameter. If the range of particle diameter is from μ to mm, the particle diameter is larger than the wavelength of light, and the light scatter from the

edge of the particle at an angle which is dependent on the size of the particle. Larger particles scatter light at relatively smaller angle than light scattered from smaller particles. Moreover, the sample of the small particles has more surface area, and the total surface area depends on both the size and shape of the particles. There is inevitably size distribution so this is very important concept.

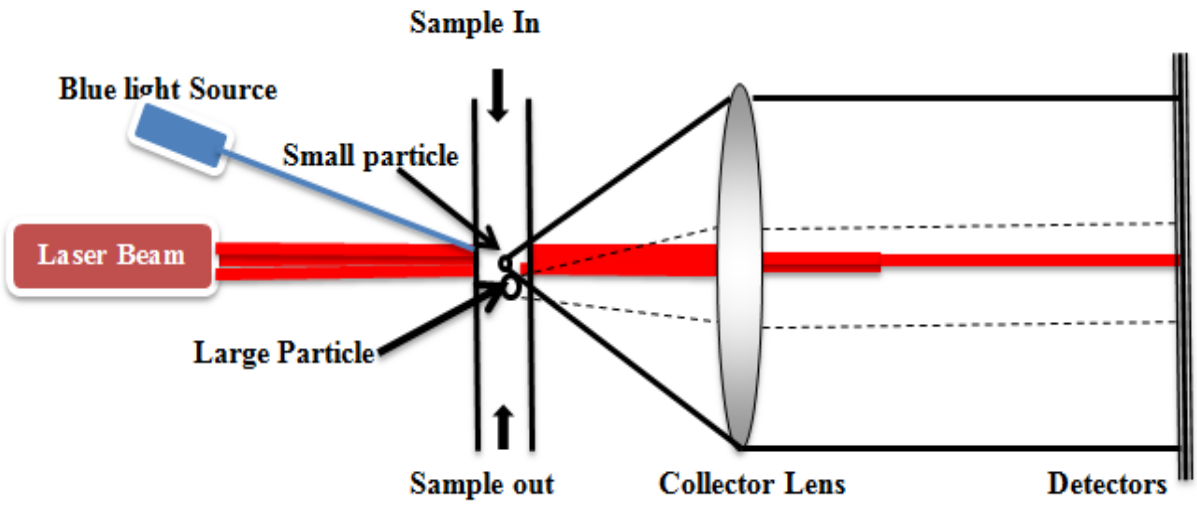

Fig. 1.16. Basic system of laser diffraction for particle size analysis.

1.5. Rheology

1.5.1. Definition:

Rheology is the study of the flow of matter, primarily in liquid state, but also as soft-solid or solids under conditions in which they respond with plastic flow rather than deforming elastically in response to any applied force. It is the branch of physics which deals with the deformation and flow of materials, both solid and liquid. (Wikipedia).

1.5.2. Applications:

Rheology has applications in materials science engineering, geophysics, physiology, human biology and pharmaceutics. Materials science is utilized in the production of many industrially important substances such as cement, paint and chocolate, which have complex characteristics.

In addition, plasticity theory has been similarly important for the design of metal forming process. The science of rheology and the characterization of viscoelastic properties in the production and use of polymeric materials has been critical for the production of many products for use in both the industrial and military sectors.

Study of flow properties of liquids is important for pharmaceutics working in the manufacturing of several dosage forms, such as simple liquid, ointments creams, pastes, etc. The flow behavior of liquid under applied stress is of great relevance in the field of pharmacy. Flow properties are used as important quality control tools to maintain the superiority of the product and reduce batch to batch variations, (Wikipedia).

1.5.3. Background:

It is important in the manufacture and processing of food products such as chees and gelato. Thickening agents are substances which, when added to an agents mixture, increase its viscosity without modifying in other properties such as taste., also it govern the product development, design and evaluation of the process and also affect flow behavior of the products (Bolmstedt, 2000) . The rheological properties of *Acacia* gums play a significant role in the food industry as they govern

the product development, design and evaluation of the process and also affect flow behaviour of the products (Bolmstedt, 2000). Rheological properties of dilute *Acacia gum* solution have not been considered in great details since the Newtonian character is assumed a priori. At concentrations between 10 - 25% acacia gum dispersion exhibits more pronounced shear-thinning behaviour than dispersions with concentrations in the range 30-50%. (Sanchez, *etal* 2002). Non-Newtonian behaviour of gum solutions is induced by the presence of molecular associations (Li, *etal* 2011). The occurance of AGP micelles endows the gum Arabic solution with Non-Newtonian and shear thinning behavior (Li, *etal* 2011) . The flow of gum solutions shows nearly Newtonian flow for 50% concentration for *A. senegal* var *senegal* and 50% for *A. seyal* var. *seyal* (Daoub,2016). The apparent viscosity values increase when gum concentration rises, for gum exudates from *A.senegal* (Mothè, *etal* 1999) . *Acacia nilotica* gum solutions exhibit a high viscosity at low shear rates and a Newtonian region at high shear rate similar to that found for *A.senegal* (Elhag, 2018).

Viscoelastic properties of arabic gum dispersions reveals a predominant liquid- like behavior. Mechanical spectra obtained at 6 wt % AG concentration by oscillatory testing reveals that the viscous-like modulus is high than the elastic-like modulus, after 120 min rest the dynamic mechanical spectra shows a typical gel-like behavior (Sanchez, 2002). The effect of temperature in the dynamic rheological behaviour of *A.senegal* var. *senegal* and *A. seyal* var. *seyal* , at temperature less than or equal 60C reflects a viscous behaviour and at temperature 70C the behaviour becomes elastic at low frequency region for *A.seyal* var. *seyal* while *A senegal* var. *senegal* has completely different behavior (Elhag, 2018).

1.5.4. Continuum Mechanics Classification:

Rheology concerned with extending continuum mechanics to characterize the flow of materials and also concerned micro or nano establishes predictions of mechanical behavior based on the structure of the materials . It classify into the following:

Table 1.4. Classification of Continuous materials:

<table>
<tr><td rowspan="3">The study of the physics of continuous materials</td><td rowspan="3">Solid mechanics: The study of the physics of continuum materials with the defined rest shape</td><td colspan="2">Elasticity</td></tr>
<tr><td>Plasticity</td><td rowspan="2">Rheology</td></tr>
<tr><td>Non-Newtonian fluid</td></tr>
<tr><td rowspan="2"></td><td rowspan="2">Fluid mechanics: The study of the physics of continuum materials which deform when subjected to a force.</td><td colspan="2">Newtonian fluid</td></tr>
</table>

.1.5.5. Definitions:

- **Elasticity**: Describes materials that return to their rest shape after applied stresses are removed.
- **Plasticity**: describes materials that permanently deform after sufficient applied stress.

1.5.6. Classification of liquids:

There are, two classes of liquids:

1.5.6.1 Newtonian fluids:

Flows Newton's hypothesis considered to be perfectly viscous, because the relation between the shear rate and shear stress are constant. That the viscosity of the liquid remains constant at all possible shear rates for a given temperature, e.g. pure water, oils, and organic solvents. Because of their purity and the lack of dispersion, Newtonian fluids are much easier to measure. They are not very common.

1.5.6.2 Non-Newtonian fluids:

Most liquids do not have a constant ratio between their shear rate and shear stress. These fluids can be unpredictable in how their shear stress changes according to shear rates, as the shear rate increase, depending on the fluid's own characteristics. As a result, the viscosity of the material is highly variable. Non-Newtonian fluids will have apparent viscosities that depend entirely on the specific experimental conditions, which is classified into two groups:

1.5.6.2.1. Power Low Fluids:

Categorized base on how viscosity is affected by the shear

- If the viscosity is increase as shear increases, this is dilatant or shears thickening fluid fig.1.17, e.g. candies, sand, water mixture clay, slurries.
- If the viscosity decreases as the shear increases, this is a pseudo plastic or shear thinning fluid. Include: solution suspensions, gels, mayonnaise, paints emulsions and dispersions.

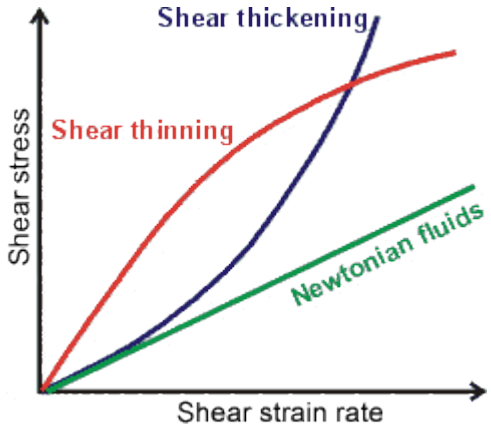

Fig. 1.17. Comparison of Newtonian, shear-thinning and shear-thickening fluids

1.5.6.2.2. Time dependent fluids:

will change over time.

- If the viscosity increases as time increases, this is rheopetic fluid. Fluids that include solvents that evaporates, such as adhesives of coatings.
- As time increases, however, other fluids will decrease in viscosity if the shear rate is held constant. These are thixotropic, and they can return to their original internal structure before the shear. As such, these materials sometimes give a false high viscosity rate when first measured, because some of the fluid has retained viscosity while in other parts of the fluid, the viscosity has decreased significantly.

1.5.7. Dynamic Modulus:

Viscoelasticity is studied, using dynamic mechanical analysis, applying a small oscillatory stress and measuring the resulting strain.

- Pure elastic material has stress and strain in phase, so that the response of one caused by the other is immediate.

- In purely viscous material, strain lags stress by a 90^o phase lag.
- Viscoelastic materials exhibit behavior somewhere in the middle of these two types of material, exhibiting some lag in strain.

1.5.8. Viscoelastic Parameters (Wikipeadia):

Rheology polymer is characterized by:

1.5.8.1. The Complex Modulus: Present the relations between the oscillating stress and strain – measure of materials overall resistance to deformation:

G = Stress / strain

G = G ` + L-G\\

$L^{-2} = -1$

1.5.8.2. The Elastic (storage) Modulus:

Measure of elasticity of material. The ability of the material to store energy

$$G` = \frac{\text{Stress}}{Strain} \text{ Cos } \ominus$$

1.5.8.3. The Viscous (Loss) Modulus:

The ability of the material to dissipate energy – energy lost as heat.

$$G`` = \frac{\text{Stress}}{Strain} \text{ Sin } \ominus$$

1.5.8.4. Tan Delta

Measure of material damping such as vibration or sound damping:

Tan G = G``/G`

1.5.9. Parameters for Rheological Properties:

Table 1.5. Parameters for Rheological Properties

	Classical Extremes	
Ideal solid	External force	Ideal fluid
Steel		Water
Strong structure		Weak structure
Rigidity		Fluidity
Retains/recovers		Flow
Form stores energy		Losses forum Dissipates energy
Purely elastic		Purely viscous
Elasticity		Viscosity
Storage modulus		Loss modulus
	Real behavior [Energy + time] [viscoelastic material	

1.5.10. Oscillatory Frequency Sweep:

One of the most fundamental properties of any materials is its elastic susceptibility, or its response to a shear excitation. Example: The primary different between a fluid and solid is their contrasting response to an applied shear stain, solid store mechanical energy and are elastic (G`) whereas fluid dissipate mechanical energy and are viscous (G``).

Many materials are viscoelastic the both store and dissipate energy with the relative proportions depending on frequency.

The elastic susceptibility is parameterized by the complex shear modulus G(ω) which determine the stress induced in a material upon application of an oscillatory shear strain at frequency (ω) (Ferry,1980).

1.5.11. Rheology Analysis and Viscoelasticity, Viscosity Test:

The instrument can test sample ranging from very thin to very thick liquids and from ultra-fragile colloidal structures to high-rigid solid.The following rheological testing procedures are available:

- Viscosity, shear rate sweep.
- Shear stress and shear rate sweeps in either continuous stepped-ramp or steady-state (equilibrium) mode.
- Viscosity, temperature ramps.
- Thixotropy testing: Either up-down ramps or three steps methods.
- Oscillation stress or strain amplitude sweeps.
- Oscillation frequency sweeps.
- Oscillation temperature ramps (continuous) or sweeps.
- Creep/Relaxation test.

1.6. Objectives:

The objectives of this study are:

1- To study physico-chemical properties of *acacia tortillis* var. *spirocarpa* gum from different location.

2- To investigate certain applications (emulsifications, rheology) properties.

3- To compare characteristic of *Acacia tortilis with other* varieties *raddiana* and *tortilis.*

Chapter Two

Materials and Methods

Chapter Two

2. Materials and Methods

2.1. Materials:

Twenty four samples of *A. tortilis* var. *spirocarpa* crude gum, naturally exudate, were collected from two different locations Khartoum and Gezira states by the author Table 2.1. Authenticity of the resource was confirmed by Dr Elshaikh A. Elshaikh, from the National Forestry Corporation. Each sample was taken from different tree.

Table 2.1. Description of Samples

Code	State	Area	Forest	Season	Soil	Rainfall
Sample from A_1 to A_{10}	Khartoum	Sharq Elneel	Abushmal	Apr., May, Jun 2014	Mixed	400mm
Composite A_{11} Composite A_{12}	Khartoum	Sharq Elneel	Abushmal	Apr., May, Jun 2014	Mixed	400mm
Sample from T_1 to T_{10}	Gezira	Rofaa	Tayba	Jan 2018	Mixed	400mm
Composite T_{11} Composite T_{12}	Gezira	Rofaa	Tayba	Jan 2018	Mixed	400mm

The collected gum was relatively pure; the impurities such as wood pieces, sand, tree leaf were carefully removed. The samples were air dried for ten days and ground using a mortar and pestle then kept in closed plastic containers for further physicochemical analysis.

2.2. Methods of Analysis of *A. tortilis* var. *spirocarpa Gum:*

2.2.1. Determination of Moisture Content:

10g of sample of *A. tortilis* var. *spirocarpa* gum were weighed by a moisture analyzer and heated at 120°C. After 15 minutes the loss on drying of the sample was automatically calculated.

2.2.2. Determination of Ash Content:

The total ash of test samples was determined according to FAO paper No. (44). A crucible was heated at 550°C cooled in a desiccators and weighed (w_1). Accurately one gram of sample was weighed in the crucible (w_2) and ignited in muffle furnace at 550°C for 6 hours, and cooled in desiccators and weight (w_3). Then the total ash % was calculated from the following relation (AOAC, 1990)

$$\text{Ash\%} = \frac{(\text{weight of ash in grams})\text{X } 100}{\text{weight of sample in grams}}$$

2.2.3. pH Value:

The pH value was determined for 1% aqueous solution, using a calibrated pH-meter at room temperature (3505 Jenway).

2.2.4. Determination of Specific Optical Rotation

1% solution were prepared from the dry samples using distilled water, the gum solutions were mixed on a roller mixer until the sample completely dissolved, and then filtered through Wattmann No. 42 filter paper. Then loaded into the sample holder without trapping air bubbles.

Optical rotation was measured using digital polarimeter equipped with 250mm tube filled with the test solution at room temperature. Specific rotation was calculated by the following relation:

Specific optical rotation $[\alpha]_D^L = a\ \frac{100}{CL}$

Where: α is the observed angle of rotation of the solution in circular degrees, C is the grams of substance per 100 ml of solution, and L is the length of the solution in decimeters (Moffit and Young, 1956).

2.2.5. Determination of Total Nitrogen and Protein:

0.2 gram of sample was weighed and transferred to Kjeldahl digestion flask. One Kjeldahl tablet (copper sulfate) was added to sample. Then 30 ml of concentrated nitrogen free, sulfuric acid were added. The flask was then mounted in the digesting heating system which was heated at 245°C and capped with aerated manifold. The solution was then heated at the above temperature and a clear pale yellowish–green color was obtained which indicates the completion of the digestion. The flask was then allowed to cool to room temperature. This content was quantitatively transferred to Kjeldahl distillation apparatus. 15 ml of sodium hydroxide 0.2N were added.

The steam distillation of ammonia was connected. The released ammonia was absorbed in 10ml of 2% boric acid. Back titration of the generated borate was then carried out versus 0.01M NaCl using methyl red as indicator. Blank set of experiment was carried in the same way.

The nitrogen content percentage of sample was calculated:

$$\text{Nitrogen \%} = \frac{14.01 \text{ X M X volume of titrant X } 100}{\text{Weight of sample (grams)}}$$

The protein content was then estimated from the determined nitrogen value using nitrogen conversion factor of 6.7 due to (Abduelrahman, 2011) as follows:

Protein content = N% x 6.7

2.2.6. Determination of Acid Equivalent Weight and Total Uronic Acid:

2.5ml of (3% w/v) of gum sample were introduced on top of a column packed with amperlite 120 and allowed to elute under gravity action. The elution rate was adjusted to be one drop per 2 second. The eluent and washing were collected and titrated against standard 0.1M solution of sodium hydroxide using phenolphthalein indicator (2drop were added), phenolphthalein indicator was prepared as 2% solution in ethanol. Column length and diameter were 15/25 and the height of the resin in the column was 20 cm.

$$\text{Acid equivalent weight} = \frac{\text{Weight of sample X } 1000}{\text{Volume of the titrant X M of alkaline}}$$

$$\text{Total uronic acid \%} = \frac{\text{Molar mass of uronic acid X } 100}{\text{Acid equivalent weight}}$$

2.2.7. Determination of Sugar Composition:

2.2.7.1. Sample Preparation:

The samples were hydrolyzed to liberate the sugar residues. Sample was weighed out (100mg, taking into account the moisture content) and added to $10cm^3$ of 4% H_2SO_4 and incubated at 100°C for 6 hours. Following this, 1g of $BaCO_3$, was added to the solution and left overnight (minimum of 12 hours) to neutralize the

solution. After $BaCO_3$ treatment (universal indicator strips) were used to ensure that the sample was neutral before proceeding to the next stage. The solution was then centrifuged at 2500 rpm for 10 minutes to allow the barium sulfate (formed from neutralizing the H_2SO_4) to settle. The supernatant was removed and filtered through a 0.45 μm Whattmann nylon filter and then diluted 1:1 with 70/30 acetonitrile buffer. This constituted the final solution of which 1ml was put in a vial (filtered via 0.45 μm filter) prior to injection into HPLC column.(Randall, *etal* 1989)

2.2.7.2. Method:

Stock concentrations of 5mg cm^{-3} for each sugar were made up by hydrating in 70/30 acetonitrile buffer for 2 hours. Dilutions of the stock solution was diluted to six different concentrations for each sugar over a range of 2.5 – 0.5 mg cm^{-3}. This allowed six levels for the calibration curve and average of 3 replicates for each level was used to ensure accuracy. This calibration allowed the determination of the unknown sugar content for the gum sample. The concentration of each sugar was calculated by peak height an expressed as a % of the total sugar content.

2.2.8. Determination of Cationic Composition of *A. tortilis* var. *spirocarpa Gum*:

0.5g gum sample was dissolved in conc. HCl acid; 1% solution was prepared.1cm^3 was aspired into ICP system. Inductive coupled plasma – optical emission ICP-OES Spectrometer - Plasma Quant PQ 9000.

2.2.9. Determination of Absolute Viscosity:

Labeled LV or RV spindle sets, these comprise simple shafts ending in a disk or cylinder. 25% w/v of a sample taken in 100 ml suitable container and place under the viscometer which was then lowered to dip the spindle into the sample up to an immersion marks on the spindle shaft, set at 100rpm at 25^oC measurement

was taken after 60s. The dip-in spindle was suitable for comparative testing of the viscosity of free-flowing fluids.

2.2.10. Determination of Calorific Value:

The calorimeter (IKA® Cl Fig. 2.1, was used to determine the calorific value of solid and liquid materials according to national and international standards (e.g. DIN51900, BS1016 T5, ISO1928 and ASTM5468, 5865,4809).

The system was calibrated using benzoic acid tabs, 1g(2Tabs), Fig. 2.10., of cal.val. 26461J/g, RSD 0.03%, and LOT SZBD2180V, at 19°C, gas pressure at 30 bars, and the pump flow of 2700 rpm. 0.5g of *A. tortilis* var *spirocarpa* gum samples were places into a plastic bag C12A, big bag, Fig. 2.1. with cross cal.val. The samples were combusted in an oxygen atmosphere. The calorific value of the sample was calculated, and the net cal.val., was calculated by adding moisture content calorific value.

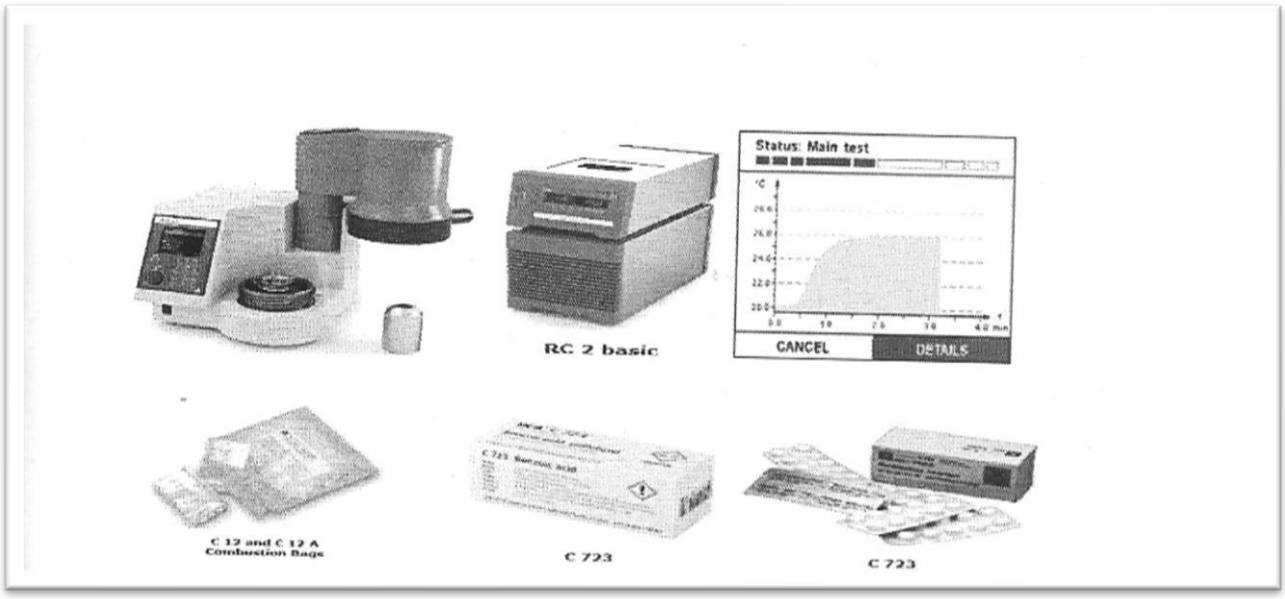

Fig. 2.1. Calorimeter IKA®CI System, Plastic Bags, and Standard Benzonic Acid

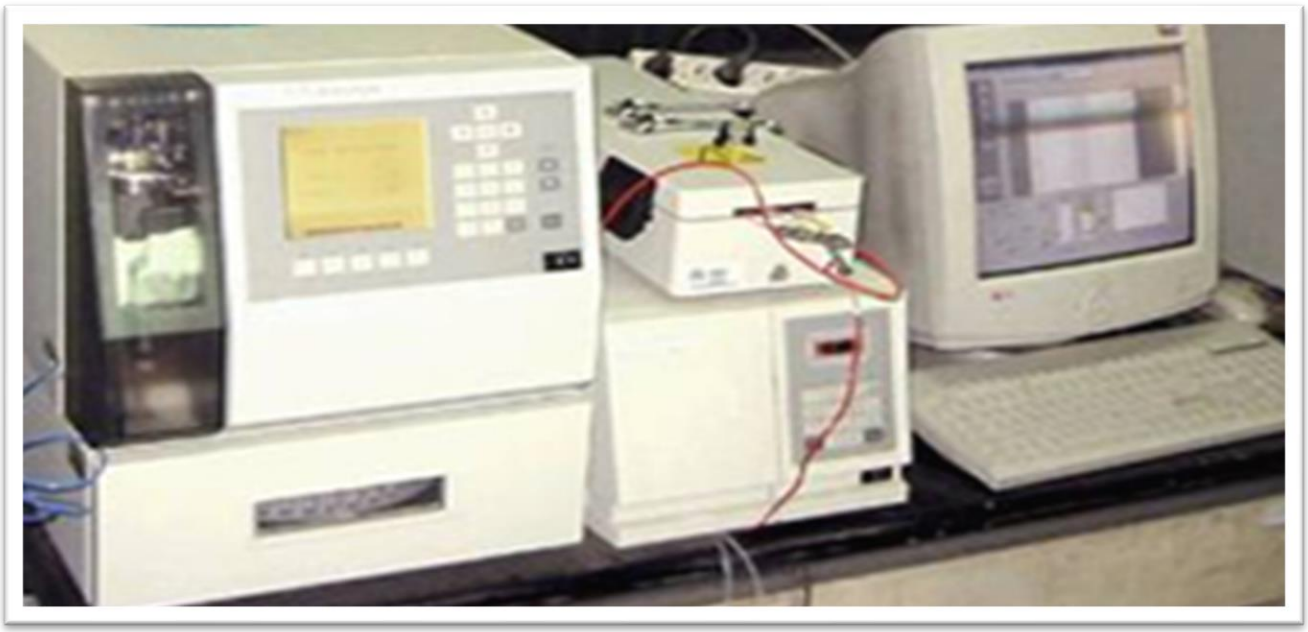

Fig. 2.2. Gel Permeation Chromatography setup

Gel permeation Chromatography(GPC) : Separates gum into three fractions.

Lights Scattering (LS) Detector: Provides the absolute molecular weight (M_w) and size (radius of gyration R_g.) of macromolecular in solution.

Refractive Index (RI) Detector: used to determine the concentration of the macromolecular at each slice.

Ultraviolet(UV) Detector: used to determine protein component of the gum, amount of protein in fractionated gum.

GPC is used to:

- To Investigate aggregation and degradation of protein.
- To qualify branching fractional gum linear or branched sample.
- The weight average molecular mass and molecular mass distribution of the sample.
- To estimate a quantitative relationship between molecular weight and elution volume.

2.3. Fractionation and Molecular Weight Determination:

2.3.1. Instrumentation:

Gel permeation chromatography coupled to a multi-angle laser light scattering detector (GPC – MALLS) Fig. 2.2 . System was used to determine the molecular weight and molecular weight distribution. Loading sample injector equipped with 10ml sample loop. The system utilizes waters solvent (Division of Millipore, USA). Delivery system Model 6000A connected to a column containing Superpose (Amersharm Biosciences) (10x300mm), manual Rheodyne Model 7125 syringe. The column elute was monitored by three detectors, refractive, index (RI) Wyatt Optilab DSP interferometric refractometer operated at 633nm (Wyatt Technology Corporation, USA), multi-angle laser light scattering photometer DAWN EOS using the He– Ne laser at 690nm (Wyatt Technology Corporation, USA), and an Agilent 1100 series G1314A, UV detector or (214nm, Agilent Technologies) (Al-Assaf, *etal* 2005).

RI provides an accurate concentration profile, MALLS enable absolute molecular mass and radius of gyration (R_g.), and the UV detects the proteinaceous components of the gum (Katayama, *etal* 2006). The data was processed by the Astra for Windows Software (version 4.90.07, Wyatt Technology Corporation).

2.3.2. Sample Preparation:

0.02g gum in 10ml of 0.2M NaCl (based on dry weight) was prepared, and hydrated by roller mixer (SRT9. Stuart Scientific, UK) overnight. The solution was then centrifuged for 10 minutes at speed of 3000rpm. Using Megafuge 1.0R (Herdeus SEPATECH, Germany). Centrifuge, then filtered using 0.45 μm nylon filter (Whatmann, 13mm) prior to injection into GPC-MALLS System.

2.4. Emulsification Properties: Materials and Methods:

Fig. 2.3. Homogenizer Vacuum Emulsifying Mixer

2.4.1. Materials:

- *Acacia tortilis* var. *spirocarpa* gum (10% w/w)
- Citric acid 0.12%.
- Sodium benzoate 0.13%.
- Octanoic/Decanoic acid triglyceride oil (ODO) 10%.

2.4.2. Emulsion Preparation:

Distilled water was added to about 8g of the gum sample (based on dry weight) in glass bottle. The total weight became about 40g (conc. of 20% w/w) gum solution. The sample were dissolved using a roller mixer (SRT9 - Stuard Scientific, UK) and centrifuged using (Megafuge 1.0R), Heraeus SEPATECH) for 10 min. at a

speed of 2500 rpm to remove insoluble particles. Then the solution was filtered using 100 μ mesh.

0.52 ml of (10% w/v) sodium benzoate solution and 0.48 ml of 10% (w/v) citric acid solution were added. 15-71 ml, and 15-73 ml of distilled water were added, then 42g of ODO oil (10%) was added to the prepared gum solution(20g) to give a total of 40g and final concentration of 10%. The mixed solution was homogenized for 3 min. using a POLYTRON (PT2100, KINEMA TICA AC) homogenizer at 2200 rpm Fig 2.3. Impeller (PTDA 21.9 mm tip diameter) was used as dispersing tool. The pre-emulsified mixture was homogenized using a high – pressure Nano Vater (NV30-FA, MITSUBISHI GDT 1000). It was passed twice at 75 MPa. The final emulsion kept in closed glass universals, and the emulsion was measured as prepared.

2.4.3. Accelerated Temperature Stress Test:

The prepared emulsion sample was placed at 60°C in the vacuum oven (GALLENKAMP-OVA031XX1.5) for 3 and 7 days and the droplet size was measure.

2.4.4. Droplet Size Analysis:

The droplet size distribution of the emulsions was measures using Masterizer 3000, a Laser diffraction particle size analyzer (Malvern Instruments).

Distilled water was used as dispersant and the refractive index of oil phase (ODO) was set at 1.45. Emulsification stability of samples after accelerated test was evaluated by droplet size change. The droplet size was described by the volume median diameter (VMD). The ratio of the droplet of 1 μ or more and 2 μ or more were calculated.

2.5. Rheology

The following rheological testing were achieved for *A. tortilis* var. *spirocarpa*.

- Viscosity, shear rate sweeps.
- Oscillation frequency sweeps.

A rheometer is a laboratory device used to measure the way in which liquid, suspension or slurry flows in response to applied forces. It is used for those fluids which cannot be defined by single value of viscosity and therefore, require more parameters to be set and measured them in the case for a viscometer (Wikipedia).

2.5.1. Determination of Rheological Properties:

2.5.1.1. Solutions preparation:

(50 % w/w) based on dry weight gum solutions were prepared in water containing (0.005% w/v) NaN_3 as a preservative. The solutions agitated on tube roller mixer (SRTg. Stuart Scientific, UK) overnight to ensure that the sample is fully dissolved. The solution was centrifuged for 10 minutes at 3000 rpm using (Megafuge 1.0R, Heraeus SEPATECH, Germany) centrifuge. One dilution 25% (w/v) was prepared from stock solution centrifuged and solved at 4°C prior to investigation of their rheological behavior.

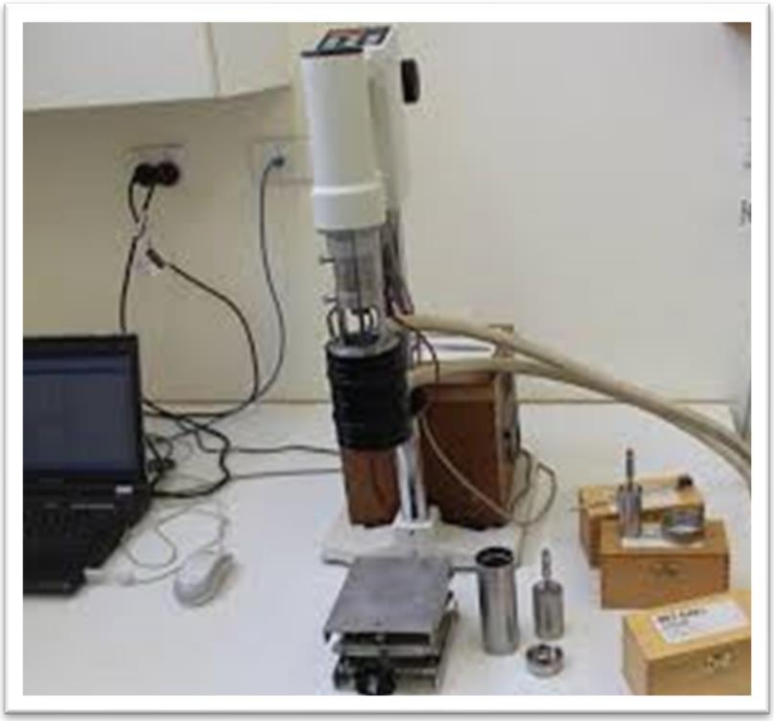

Fig. 2.4. Rheology and Pipe-Loop Testing

2.5.1.2. Rheological Measurements:

Rheological measurements were carried out using KINEXUS pro (Malvern Instruments) fitted with cone and plate geometry with a cone diameter of 40 mm and an angle of 2° Fig 2.5. Steady shear viscosity curve were measured for gum solutions 25% and 50% w/v both upon shear rate ramp-up (from 0.01 to 10000s^{-1}), and subsequent shear rate ramp-down (from 10000 back to 0.01s^{-1}). Dynamic rheological measurements to determine the elastic modulus (G`). Viscous modulus (G``) and dynamic viscosity, were performed in the frequency range of (0.1-10 Hz). The linear viscoelastic region was assessed at (1 Hz). The temperature of the samples was controlled within 0.1°C using a Petlier element. The rheometer control and data

processing was done by computer software (Rheology Advantage Data Analysis program). In all experiments, samples were covered with a solvent trap to prevent evaporation.

Fig. 2.5. Rotational Rheology

Chapter Three:
Results and Discussion

Chapter Three

3. Results and Discussion

3.1. Chemical Characteristics:

The study was carried out on the excudated gum of *Acacia tortilis* ssp. *spirocarpa* var. *spirocarpa* collected from Abushmal Forest, Sharq Elneel Locality Khartoum State, and from Tayba Forest – Gezira State. The results were summarized at Table 3.1. , then it was compared with the physicochemical properties of *A. tortilis* var. *raddiana* gum from Abushmal Forest, Sharq Elneel Khartoum State and from Algetaina, White Nile State, done by (Abduelrahman, 2011), and *A. tortilis* var. *tortilis* gum from Algetaina – White Nile State and Wadbnda – Northen Kordofan State which was studied by (Alnour , 2014).

3.1.1. Moisture Content:

The moisture which is determine the hardness of the gum since the variability of densities and the amount of air entrapped during nodule formation, the mean value was (11.6 to 10.7 %) for Abushmal sample and the range of (11.3 to 9.7%) for Tayba samples. The mean values were (10.9 to 10.7) for Abushmal and Tayba samples respectively, Table 3.1. , while that reported by Karamalla (1999) was 6.4% .

For var. *raddiana*, the moisture content was ranged between (12.9 to 11.0 %) for Algetaina samples ranged between (12.5 to 10.6 %) for Abushmal samples. The mean values were (11.9 to 11.7%) for Algetaina and Abushmal samples respectively.

For var. *tortilis*, the moisture content ranged between (12.3 to 10.9%) for Algetaina samples and between (12.4 to 6.0 %) for Wadbnda samples and the mean values were (11.4 to 10.8%) for Algetaina and Wadbnda respectively.

So it can be considered that the moisture content means for the three species ranged from 10.7% for var. *spirocarpa* to 11.9% for var. *raddiana.*

3.1.2. Ash Content:

The study showed that the ash content for Abushmal samples for var. *spirocarpa* gum ranged between (2.9 to 1.2%) and for Tayba samples between (3.7 to 1.5%) . The mean values were (2.9 and 2.5%) for Abushmal and Tayba . Table 3.1. .

For var. *raddiana*, the ash contents ranged between (2.4 to 1.5%) for Algetaina samples and between (2.6 to 1.2%) for Abushmal samples, while the mean values were (1.80 to 1.78%) for Algetaina and Abushmal respectively.

For var. *torilis*, the ash contents ranged between (2.45 to 1.15%) for Algetaina samples and between (2.35 to 1.10%) for Wadbnda samples. The mean values were (1.67 and 1.61%) for Algetaina and Wadbnda samples respectively.

So the ash content mean was varied for the three varieties between the lowest value 1.6% for var. *tortilits* to 2.5 % for var. *spirocarpa* gum.

3.1.3. pH Value:

The pH values for *A. tortilis* gums indicate slight acidity. For var. *spirocarpa* gum the pH was found to be within range (6.6 to 4.4) for Abushmal samples and within (4.7 to 4.2) for Tayba samples. The mean values were (5.8 to 4.6) for Abushmal and Tayba samples respectively. Table 3.1. . For var. *raddiana*, the pH

values ranged between (6.4 to 6.0) for Algetaina and between (6.3 to 5.7) for Abushmal. While the mean values between (5.95 to 6.2) for Algetaina and Abushmal respectively.

For var. *tortilis*, the pH values ranged between (6.2 to 5.0) for Algetaina and between (6.21 to 5.0) for Wadbnda. While the mean values between (6.2 and 5.4) for Algetaina and Wadbnda respectively.

The total mean range for the three varieties between 4.6 the lowest value for var. *spirocarpa*, that means more acidity, while the higher value was 6.2 less acidity for var. *raddiana* and var. *tortilis*. This is agreed with the result of *A .tortilis* pH is 6.5 (Bisht, *etal* 2013) There was insignificant difference between the three varieties.

3.1.4. Specific Optical Rotation:

Acacia species were classified into Subgenus *Acacia* (Gummiferae), and subgenus Aculieferum (Vulgares) . *A. tortilis* varieties have a positive specific rotation, so it belongs to Gummiferae while *A. senegal* has negative value, so it belongs to Vulgares.

Table 3.1. showed highest value of dextrorotary optical rotation of *A. tortilis* var. *spirocarpa* which ranged between (+92.0 to + 70.0) for Abushmal samples and between (+88.5 to +71.0) for Tayba samples and the mean values were (+80.3 to +76.6) for Abushmal and Tayba Forest while that recorded to (Al-assaf *etal*.2005) was +65.

For var. *raddiana* the optical rotations were in range between (+91.6 to +79.4) for Algetaina and (+91.2 to +78.3) for Abushmal sample. The mean values were (+86.4 and +85.4) for Algetaina and Abushmal . For var. *tortilis*, the optical rotations were in range of (+91.1 to +78.8) for Algetaina and were (+83.90 to +82.81) for

Algetaina and Abushmal. The ranged means for the three varieties from +76.6 for var. *spirocarpa* to +86.4 for var. *raddiana*.

Table 3.1. Physicochemical Characterization of *Acacia tortilis* var. *spirocarpa* Gum from Abushmal (A) and Tayba(T) Forests.

No.	Moisture % Content		Ash % Contents		pH		Optical Rotation	
	A	T	A	T	A	T	A	T
1	11.5	9.9	2.6	2.3	5.8	4.6	+88.0	+85.5
2	10.9	10.5	2.5	3.5	6.6	4.7	+92.0	+71.0
3	10.7	10.8	2.3	2.4	6.2	4.5	+82.5	+76.0
4	11.0	11.7	2.5	2.1	4.4	4.6	+69.5	+77.5
5	11.1	10.1	2.7	3.3	6.2	4.6	+73.0	+78.5
6	11.1	9.7	1.8	2.9	5.1	4.4	+80.0	+84.5
7	10.7	10.5	1.2	2.1	5.2	4.8	+80.5	+82.0
8	10.8	9.9	1.5	1.5	6.6	4.4	+74.5	+88.5
9	11.6	12.4	2.5	3.7	5.7	4.4	+73.0	+76.0
10	10.8	11.1	2.7	1.5	5.4	4.6	+70.0	+77.0
11	10.8	11.3	2.4	1.5	6.1	4.2	+72.5	+79.0
12	10.9	10.6	2.9	2.7	5.7	4.6	+85.0	+88.0
Mean	11.0	10.7	2.3	2.45	5.75	4.6	+76.6	+80.3

3.1.5. Nitrogen Content and Protein Estimation:

Nitrogen and protein content of *A. tortilis* var. *spirocarpa* plays a responsible role for formation of arabino glactan protein complex and their role in emulsification properties of the gum. Table 3.2. showed the nitrogen content of var.*spirocarpa of* Abushmal samples (2.2%) and Tayba samples (1.4 %) and means values were (2.2 and 1.4 %) for Abushmal and Tayba respectively, while that recorded by (Al-Assaf *etal* 2005) was 1.27% and by Karamalla,(1999) was 1.4

For var. *raddiana,* Algetaina ranged from (1.9 to 1.5%) and Abushmal ranged from (1.9 to 1.4 %) and the mean values (1.7 and 1.6%) for Algetaina and Abushmal respectively, and that recorded by (Al-Assaf *etal*. 2005) was 0.38%. For var. *tortilis* Algetaina samples ranged from (1.9 to 1.3 %) and Wadbnda samples from (1.9 to 1.2 %) and the mean values were (1.5 and 1.46%) for Algetaina and Wadbnda respectively. The nitrogen content means for the three varieties range from1.4% for var.*spirocarpa to* 2.2% for *spirocarpa* . Verma, (2016) recorded the nitrogen content for *A. tortilis* was 0.99%.

The estimated protein for var. *spirocarpa* were (13.8%) for Abushmal samples and (8.4 %) for Tayba samples and the mean values were (13.8 and 8.4%) for Abushmal and Tayba samples .

For var. *raddiana* samples of Algetaina ranged from (12.0 to 9.0%) while Abushmal ranged from (12.1 to 8.8%.) the mean values were (11.2 and 11.0%) for Algetaina and Abushmal samples. For var. *tortilis* samples Algetaina ranged from (12.9 to 9.7%) and Wadbnda ranged from (12.70 to 9.5%), and the mean values were (11.1 and 10.9%) for Algetaina and Wadbnda . The protein means for the three varieties were 8.8% for var. *spirocarpa,* 11.24% for var. *raddiana* the highest value. While it was recorded for *A. tortilis* varieties as 6.18%. (Verma ,2016) The values

of nitrogen and protein are high for the three varieties comparing with other acacias gum.

3.1.6. Sugar Composition:

Table 3.2. showed the percentage of the value of rhamnose, arabinose and galactose for *A. tortilis* var. *spirocarpa* gum. The percentage values were found to be range between (4.5-2.9), (43.4 - 40.3), (17.1 to 13.9) respectively for Abushmal samples. The mean values were 3.8, 42.6, 14.5 respectively. For Tayba samples ranged between (5.1- 2.6), (45.4- 43.9), (17.5- 14.0) in the same order.The mean values were 3.7, 43.0, 15.2 respectively.

For var. *raddiana*, the mean values were 1.6, 48.9, 8.7 respectively for Algetaina and 1.8, 46.6, 9.2, respectively for Abushmal samples.For var. *tortilis* the mean values were 1.63, 46.80, 9.36 respectively for Algetaina, and 1.83, 44.56, 10.05 respectively for Wadbnda. Comparing the three varieties, all of them have low rhamnose and high arabinose content. The lowest values (1.6) rhamnose for var. *raddiana* and the highet (3.7) for var. *spirocarpa*. while arabinose the lowest value (43.0) for var. *spirocarpa*. and (48.9) highest value for var. *raddiana*. For galactose the lowest value (8.7) for *raddiana* and highest value (15.2) for *spirocarpa.*While that recorded for Churms, *etal 1*986 for *Acacia tortilis* galactose 23, Arabinose 66, rhamnose less than 13.

The low percentage of rhamnose and high percentage of galactose and arabinose is a typical characteristics of gum belonging to gummiferae series according to Bentham classification(1875) ,(Anderson,1974).

Since the core of the arabino galactan (AG) macro-molecules present in gum (which consist ~98% for the gum by weight) is believed to consist of a backbone of

β-1,3 linked galactose residues. The increased arabinose content recorded for *A. tortilis* gum samples suggest that it possesses more and longer branches, which may account for its less compact structure (Al-Assaf *etal*. 2005).

Table 3.2. Sugar Composition, Nitrogen and Protein% of *A. tortilis* var. *spirocarpa* Gum Abushmal (A) and Tayba (T) Forests.

No	Rhamnose %		Arabinose %		Galactose %		Nitrogen%		Proteins%	
	A	T	A	T	A	T	A	T	A	T
1	4.5	5.1	40.3	43.9	17.1	14.0	2.20	1.40	13.80	8.78
2	4.1	2.6	43.4	39.8	13.4	14.2	2.21	1.40	13.81	8.76
3	2.9	3.6	44.3	45.4	13.0	17.5	2.20	1.40	13.80	8.77
Mean	3.8	3.7	42.6	43.0	14.5	15.2	2.2	1.40	13.8	8.7

3.1.7. Acid Equivalent Weight and Total Uronic Acid:

Table 3.3. showed the acid equivalent weight of a composite sample of *A. tortilis* var. *spirocarpa* Abushmal Forest (A) which was found to be 2376.1 and composite sample for Tayba Forest (T) was 2301.5. The total uronic acid for Abushmal was 8.17% and for Tayba was 8.43%.

For var. *raddiana*, the acid equivalent weight were 2142 for Algetaina and 2142 for Abushmal. The total uronic acid was 9.1% for Algetaina and 9.1 for Abushmal. For var. *tortilis* for one sample 2142 for Algetaina and 2329 for Wadbnda and the uronic acid was (9.1 and 10.4 %) respectively. The three varieties ranged from 2142 for *raddiana* sample to 2376.1 for *spirocarpa* for acid equivalent weight, and the uronic acid ranged from (9.1 to 8.2%) respectively.

Table 3.3. Acid Equivalent Weight & Uronic Acid % for *A. torilis* var. *spirocarpa* Composite Samples Gum Abushmal (A) andTayba (T) Forests.

Analyzer	Composite A	Composite T
Acid equivalent weight	2376.1	2310.5
Uronic Acid %	8.2	8.4

3.1.8. Cationic Composition:

The major part of *Acacia* gums is composed by minerals (3 - 5%). The concentration and nature of ions are important since they are supposed to impact the charge density of arabinose – glactan protein in the hydration, solubility and compactness of AGP as well as in the stabilization of colloidal suspension.

The cationic composition was studied using inductive coupled plasma technique ICP, the results showed that calcium was the major element in both composite samples Tayba and Abushmal with (8718-7727ppm) , then potassium (3320-1087ppm), magnesium (1820-1330ppm) and sodium (98.9-42.3ppm). The rest elements namely Fe, Al, Mn, Co, Ni, Cr were found as traces, while the heavy metals Pb, Zn, Cu, As were traces, Table3.4.

For var. *raddiana* using atomic photometer , the study took on consideration the test of some elements and the result in the mannar Fe>Cr>Cd>Mn. For var. *tortilis* using atomic photometer the order of the elements in the mannaer Ca>K>Mg>Na, Fe>Cd>Cr>Mn with very low values. *A. tortilis* have been proposed to be a source of calcium the diet that may be particularly important insectivorous gum feeders.

Table 3.4. Cationic Composition for *Acacia tortilis* var. *spirocarpa* Gum.

Element	Composite A	Composite B
Al	5.8	4.9
Ca	8718	7727
Cu	2.4	1.6
Fe	13.9	6.4
K	3320	1087
Mg	1827	1330
Mn	11.5	5.1
Na	94.9	42.3

3.1.9. Absolute Viscosity:

Absolute (Brookfield viscosity) for *A. tortilis* var. *spirocarpa* was found to be 23 cps and 45 cps for Abushmal and Tayba respectively, at 100 rpm at 25°C after 60s. Table 3.5.

The viscosity of *senegal* and *seyal* range between (86.0 – 109 cps), (57-172 cps) respectively at 60s.100 rpm after 5 minutes. It can be seen that there was a variable in viscosity among samples from the same species for all gum types.

Table 3.5. Absolute Viscosity for composite samples Gum (Abushmal and (Tayba forests).

	Abushmal (A)	Tayba (T)
Viscosity cps	23	45
% Viscosity	39.1%	76.5%

3.1.10. Calorific Value:

The calorific value of *Acacia tortilis* var. *spirocarpa* was identical to the values obtained by (Elhag, 2018) for *A. tortilis* var. *raddiana* ,*A tortilis* var tortilis, *A. senegal*, *A. seyal*, Table 3.6 and Table 3.7, Fig. 3.1. and Fig. 3.2.

Table 3.6. Calorific Values for Composite Samples Gum for *Acacia tortilis* varieties=*spirocarpa, raddiana, tortilis*

Varieties	Wt. of Sample/g	Cal. Value of Sample Kcal/g
Spirocarpa	0.5069	4.030
Raddiana	0.5269	4.062
Tortilis	0.5775	4.20

Fig. 3.1. Calorific Values for Composite Samples Gum for *Acacia tortilis* varieties *spirocarpa, raddiana, tortilis.*

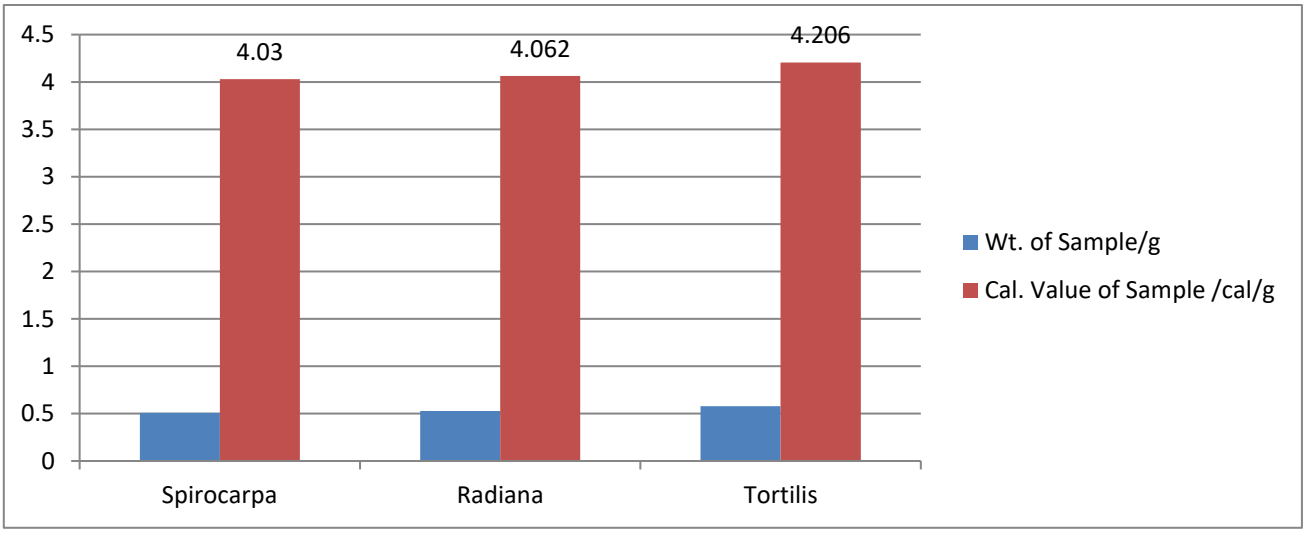

Table 3.7. Calorific Values for Composite Samples Gum for *A.senegal* and *A. seyal* (Elhag,2018).

Vareities.	Wt. of Sample/g	Cal. Value of Sample Kcal/g
Senegal	0.5	4.099
seyal	0.51	3.958

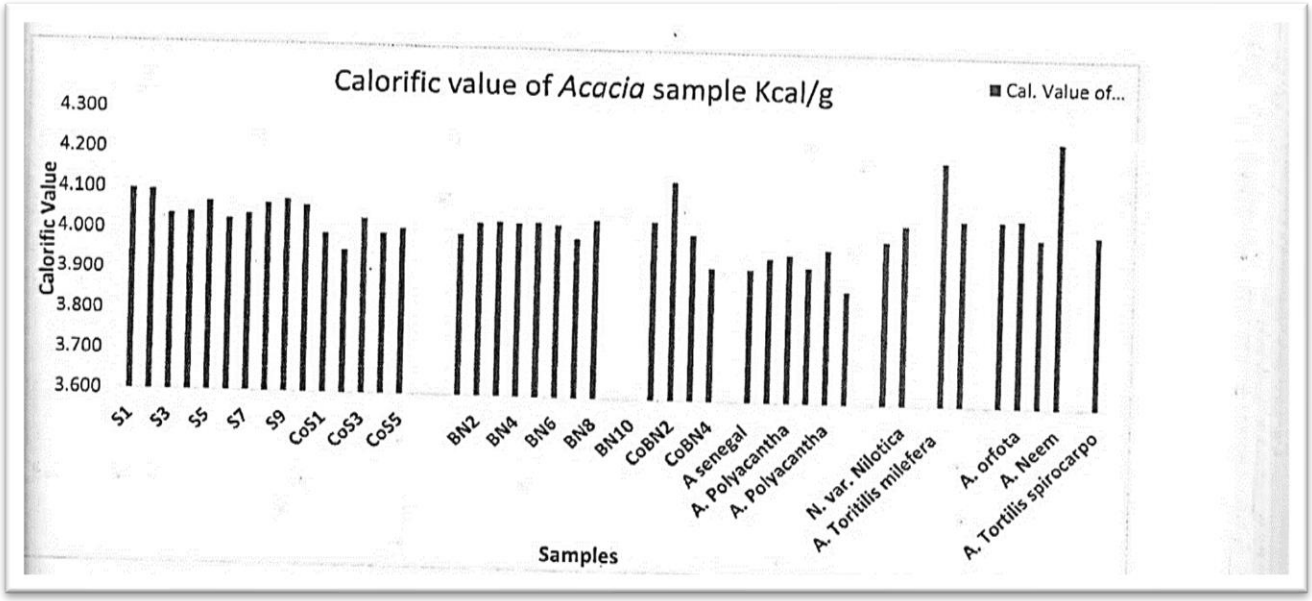

Fig 3.2. Calorific Values of *Acacia* samples Kcal/g (Elhag,2018)

3.2.Molecular Weight

3.2.1. Molecular Weight and Molecular Weight Distribution:

Two composite samples of *A. tortilis* var. *spirocarpa* gum were analyzed using the technique of gel permeation chromatography (GPC) which gave a typical elution behaviors .

The LS detector showed two peaks. The first peak has a high response which was corresponded to high molecular weight content (AGP). The second was broader

with lower response and it accounted for AG and GP contents. The RI detector (concentration detector) showed three peaks , peak1for the AGP which give lower response peak than that of LS indicating mass1(< 2%), also it showed peak 2, which consist (AG + GP) with higher mass 2 (98%).

The UV response showed three peaks: Peak1, was for AGP which contained protein and carbohydrate attached to it. Peak2 weak and appeared after the (AGP) peak and corresponded to the AG. Peaks3 strong and eluted before the total volume and it corresponded to the (GP). GP peak was not detected on LS separately, LS gave two mass% and two R_g. , GP interfered with AG peak on RI detector chart , Fig. 3.3. and Fig. 3.4. . The result weight average molecular weight, molecular distribution and the radius of gyration of two composite samples are given in Table 3.10. A small variation was found in their weight average molecular weight. The molecular weight (M_w) was measured for the whole gum and the eluent three peaks which corresponds to the arabinogalactan-protein (AGP), arabinogalactan (AG) and glycoprotein (GP) as fractions identified by the ultraviolet profile. The three fractions of gum differ in their size and protein content. Most of the percent mass of the gum (98.28%) contain a large amount of protein and has a molecular weight of $3.87x10^5$ for (AG + GP) fraction . The other fraction the percent mass (< 0.2%) contain less amount of protein and has a molecular weight of (4.49×10^6) for (AGP) molecule. Radius of gyration (R_g) for the whole gum was ranged (21-35 nm) which gives rise to a very compact molecular structure for the two samples For AGP the R_g. was (40-53nm) which have the highest radius of gyration throughout the size range considered, suggesting a less tightly packing as compared with the whole gum.

Table 3.8. Molecular Weight , Mass Recovery and Radius of Gyration for *A. torilis* var. *spirocarpa* Gum Composite Samples.

Sample	Whole gum		AGP			AG + GP	
	M_Wx 10^5	R_g. Nm	M_Wx10^6	%mass	R_g.nm	M_W	%mass
S_1	4.59	21	4.05	1.84	40	3.89	98.16
S_2	4.62	35	4.49	1.80	53	3.87	98.20

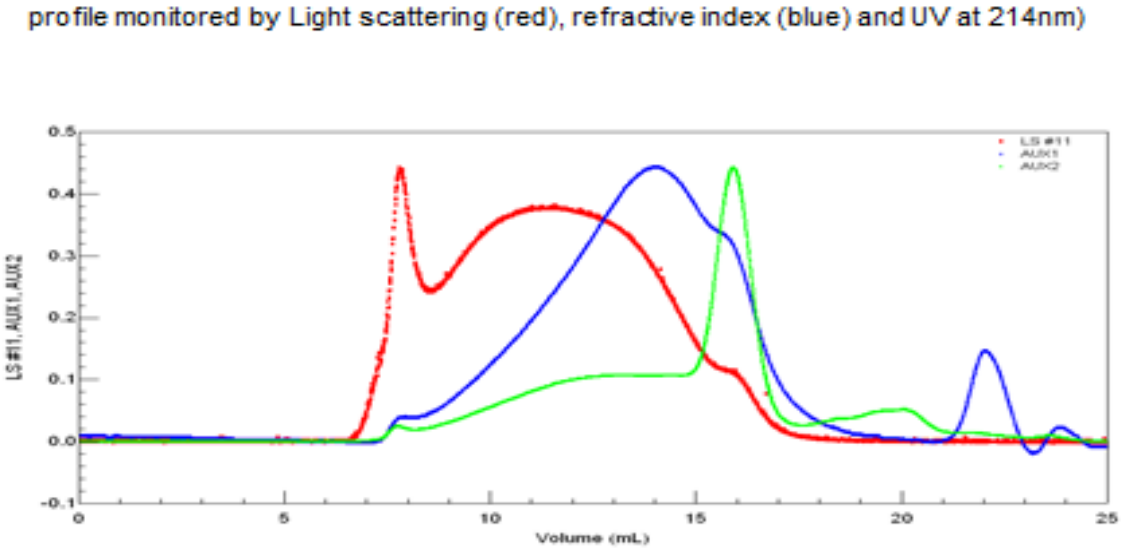

Fig. 3.3. Elution Profile of *A. tortilis* var. *spirocarpa* Sample 1 (composite)

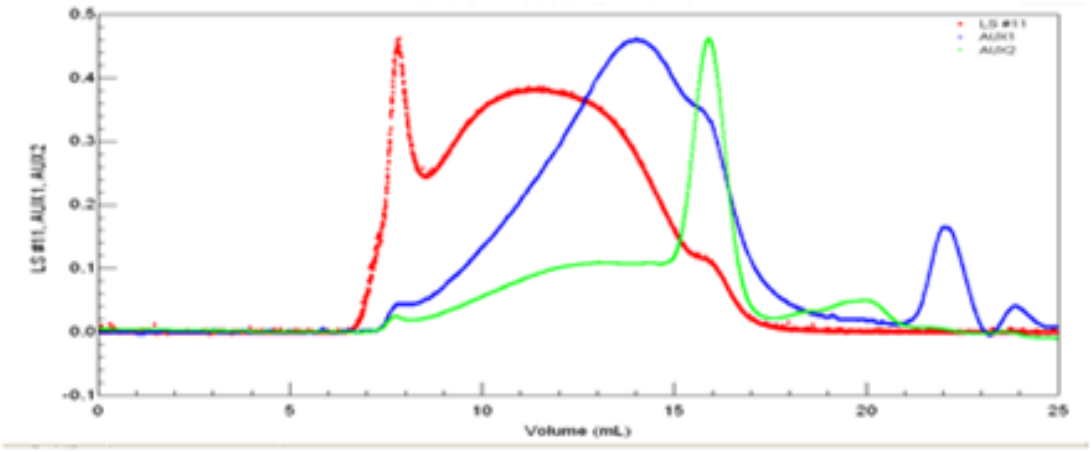

Fig. 3.4. Elution Profile of *A. torilis* var. *spirocarpa* sample 2(composite).

3.2.2. Comparison between *A. tortilis* varieties in Molecular Weight:

Table 3.9 showed the molecular weight of the whole gum slightly differs from that of (AG + GP) fractions, the difference in weight assumed to the rest components of the gum, the author suggested the small fractions for peptides and amino acids due to appearance of a peak in UV curve which was concerned with protein distribution, after the volume elution.

Table (3.9): Molecular Weight Fractions of *A. tortilis* var. *spirocarpa* Gum*:*

No.	M_w. of whole gum	M_w. of AGP	M_w (AG + GP)
S_1	4.50 x 10^5	4.40 x10^6	3.89 x10^5
S_2	4.62 x 10^5	4.49 x 10^6	3.89 x 10^5

Where : (S_1 and S_2) composite samples 1and 2

Table 3.10 showed the molecular weight of the AGP for both *spirocarpa* and *raddiana* are slighty different , and the molecular weight of the sum of AG + GP for *raddiana* equal $3.66x10^5$ which was nearly equal to that of *spirocarpa* $3.89x10^5$. So the molecular weight of AG and GP separately for *spirocarpa* was nearly equal to that of *raddiana* AG (~$1.07x10^5$), GP (~$2.59x\ 10^5$).

Table 3.10 Molecular Weight Fractions of *A.tortilis* var *raddiana* gum (Abdelrahman, 2011)

No.	M_w. of whole gum	M_w. of AGP	M_w AG + M_w GP
S_3	$13.3x10^5$	$4.40\ x10^6$	$1.07x10^5 + 2.59\ x10^5$

Table 3.11 there was a similarity in the values of molecular weight of the AGP and in molecular weight of (AG + GP) between *spirocarpa* and *tortilis.*

Table 3.11 Molecular weight Fractions of *A. tortilis* var. *tortilis* Gum (Alnour, 2014)

No.	M_w. of whole gum	M_w. of AGP	Mw of(AG+GP)
S_4	$11.52x10^5$	$4.24\ x10^6$	$3.48\ x\ 10^5$

The radius of gyration for *spirocarpa* for the whole gum within the range (21 – 35nm), for *raddiana* was (~29.1nm) and for *tortilis* was (22.3- 30.3nm), indicating the compactness of the three varieties molecule. The R_g of AGP complex, for *spirocarpa* ranged (35-53nm), for *raddiana* (45.6nm) and *tortilis* (38.3-50.0nm), indicating more branched molecule in the three varieties.

The percentage mass for *spirocarpa*, the AGP between (1.84 – 1.80) and for (AG + GP) the mass range (98.16 – 98.20) Table 3.8. For *raddiana* the percentage mass for AGP was (7.4) and for (AG + GP) was (78 + 9.0). For *tortilis* the percentage mass for AGP was in range (8.98 – 7.49) and for (AG + GP) equal (91.02 + 92.5).

AGP molecule is more branched than the whole gum , which it can be explained by: wattle blossom model for AGP and gum predicts a few large polysaccharide substituents along the polypeptide backbone of a spheroidal macromolecule (Fincher *etal*.,1983) and through the ion strength (gum composed minerals (3-5%) they are supposed to impact the charge density of AGP which plays an important role in hydration. The crystal break down and AGP branched with high radius of gyration.

Twisted hairy rope model also explained the long branching of AGP.The gum is simple glycol protein predicts a repeating polysaccharide-peptide subunits of about 7 kilodalton. the small poly saccharide substituents will maximize intramolecular hydrogen bonding if alignated along the long axis of the molecule forming highly branched molecule. (Qi *etal*.1991)

On the hydration of the gum there are two possible types of polypeptides: glycoprotein (GP) and arabinoglactan protein(AGP) , the AGP spreads in a network and makes high radius of gyration molecule.. the author attributed this behavior to the presence of microaggregate in acacia gum dispersion. During the hydration and mechanical treatment (3000rpm) the gum dissociated into AG and GP fractions be in activated state then the molecules associated again to form the gum in a shape of AGP molecule highly branched through hydrogen bonding to be a huge molecule. depends on the technique and the duration that spend before analysis. The percent

mass of AGP is minor value comparing with that of AG+GP major value revealed that AG and GP are previously exist after dissociation of gum then AGP was formed.

3.2.3. Gel Permeation Chromatography Profile of *A.senegal* var *senegal*:

Fig.3.5. showed the MALLS profile obtained for *A.senegal* var *senegal* sample.It showed one sharp peak with high molecular weight corresponding to AGP molecule (M_W :$4.45x10^6$) the second peak with less molecular weight ($2.89x10^5$) refered to AG and GP fraction, the percentage mass of AGP is (11.16%) .the weight average molecular for the whole gum was found to be ($4.20x10^5$) which agreed with the value obtained by (Anderson *etal*.1967) ($5.8x10^5$) .The highest molecular mass fraction is about (88.84%) .The radius of gyration for AGP was 33nm and for the whole gum was 33nm.

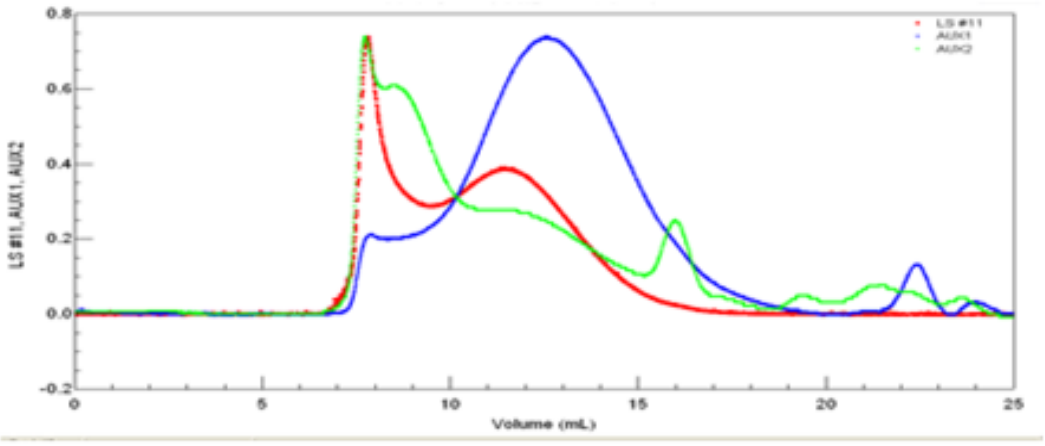

Fig. 3.5. Elution Profile of *A.senegal* var *senegal* Sample

3.2.3.1. Comparison between *A. spirocarpa* and *A. senegal* in Molecular Weight:

Table 3.12 showed the molecular weights of the AGP and the whole gum for *spirocarpa* and *senegal* in these samples were slightly different. , although *spirocarpa* belongs to sub-genus Gummiferae and *senegal* to Acudeiferum , but there was a different in M_w of (AG +GP), which the author assumed to the difference in the amount of protein between *spirocarpa* and *senegal* contents, revealed by Kjeldahl method showed protein content for *spirocarpa* was ranged (13-8%) Table3.2 , while that of *senegal* was (1.5-2.0%).

Table 3.12 Molecular Weight Fractions of *A.spirocarpa* and *A.senegal*

No.	M_w. of whole gum	M_w. of AGP	M_w (AG + GP)
Spirocarpa	4.59×10^5	4.05×10^6	3.89×10^5
Senegal	4.20×10^5	4.45×10^6	2.89×10^5

Fig3.6. , Fig3.7 , Fig. 3.8 showed LS, RI, UV, profiles separately. For UV profile, *spirocarpa* has different peak . The peak of GP was higher than that of AGP in *A. spirocarpa*, indicating the protein was concentrated at the GP in contrast to *A. senegal* Fig. 3.8. the protein was concentrated at AGP . From the R_g. values *spirocarpa* molecule was more compact than *senegal*, and the AGP in *spirocarpa* was more branched than *senegal* Table 3.13 . The peak height (peak 1) for *spirocarpa* taken by LS detector for the AGP was higher three times comparing with *senegal* . Fig 3.6

Table 3.13. Comparison between varieties *spirocarpa* and *senegal* in Radius of Gyration and Percent Mass

R_g	*Spirocarpa*	*senegal*
Whole gum	21	33
AGP	40	33
% Mass		
AGP	1.84	11.16
(AG+GP)	98.16	88.84

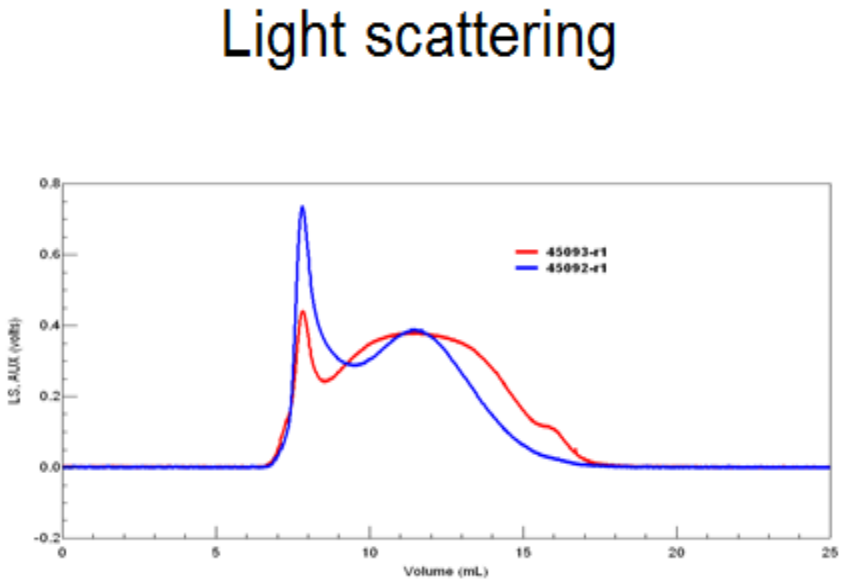

Fig. 3.6. Light Scattering Profile for *A*.var. *spirocarpa* and *A*.var *senegal*

Blue line= *spirocarpa*, red line= *senegal*

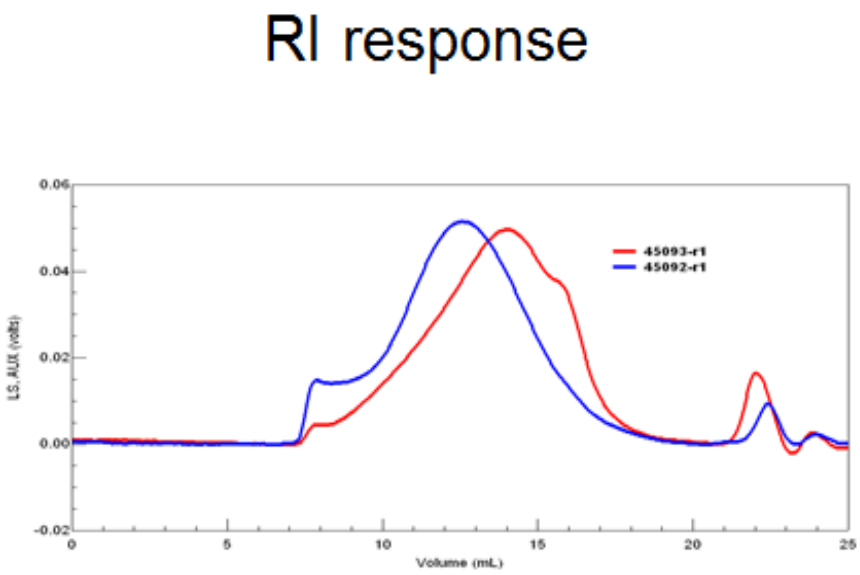

Fig. 3.7. Refractive Index Profile for *A.* var. *spirocarpa* and *A.*var *senegal*

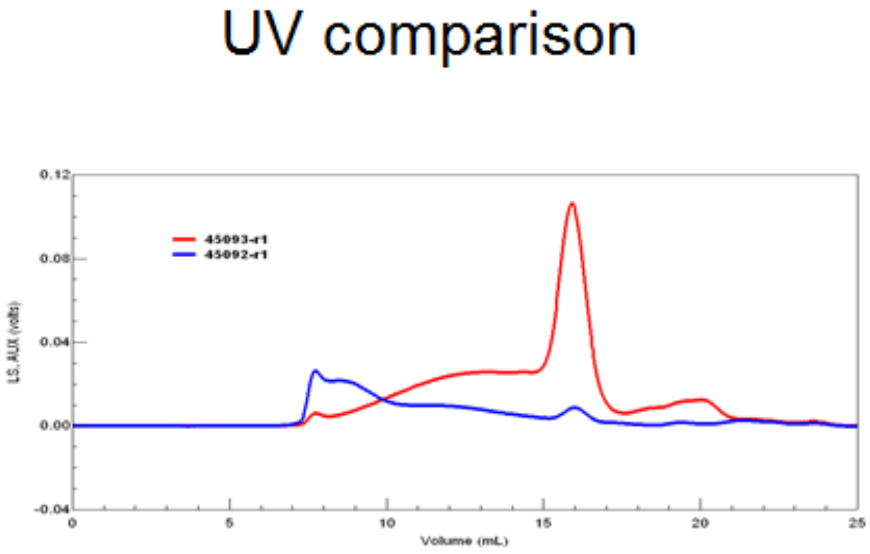

Fig. 3.8. Ultra Violet Profile for *Acacia* var. *spirocarpa* and *Acacia* var.*senegal*

3.3. Emulsification Properties:

3.3.1. Emulsion of *Acacia tortilis* var. *spirocarpa Gum:*

Emulsion of oil ODO/water was unstable, due to the creaming that becomes significant when droplet size was greater than 0.5 μ . *Acacia tortilis* var. *spirocapa* gum was used as stabilizer and emulsifier for the emulsion.

Galactouronic acid is the most effective in increasing emulsifying stability, galactose displays enhanced role in stability, protein also improves emulsifying stability. These are the content of gum in a reasonable amount.

The surface activity of the gum has its molecular origin in either (i) non-polar character of groups attached to the hydrophilic poly saccharide back bone or (i) the presence of a protein component linked covalently or physically to the poly saccharide (Dickinson, 2017). The emulsion stabilization is attributed predominantly to the carbohydrate protein, contributing to viscosity, steric, and electrostatic effects. The amount of protein which is covalently linked with highly branched polysaccharide structures is the arabino galatan protein (AGP) fraction that is critical in establishing the emulsifying properties of *acacia* gum. The AGP – rich fraction of gum arabic is a dissolved on the oil-water interface. It has been shown to be responsible for the emulsifying properties of gum. (Dickinson , 2003)

This effective emulsifier was rapidly reduces the interfacial tension at the freshly formed o/w interface, binded strongly to the interface once adsorbed, and protected newly formed droplets against flocculation and coalescence.

Citric acid was used to adjust pH at 4 which increase the solubility of AGP and greatly improved stable AGP film surrounding the oil droplet and gave highly surface active.

The weight of AGP is related to interfacial surface area mean. Increasingly concentration of surfactant AGP reduce flocculation, by reinforce the entropic barrier, and reinforce the electrostatic barriers. Increasing the interfacial film thickness, reduces coalescence and so prevents creaming.

The emulsion was more stable, because the emulsificated particles were smaller and the particles homogenized to small size < 1nm using high pressure Nano Vater. In order to achieve effective disaggregation of the gum, it was passed twice at 75 MPa. Several breakdown processes may occur on storage, depending on particle size distribution. The temperature alters emulsifiers solubility, and the surfactant moves away from oil/water interface thus destablishing of the emulsion can occur.

The emulsion was accelerated to stress temperature 60°C and stored for 3 days then for 7 days at 60 °C. Table 3.14. , Fig.3.9, fig.3.10 , fig.3.11 showed the span, specific surface area, surface weight mean, volume weight area, cumulative volume median diameters for two concentrations (5%, 10% w/w), for three samples (fresh sample, sample stored for 3 days at 60°C, sample stored for 7 days at 60°C) were measured by using laser diffraction Malvern Mastersizer 3000. The analysed samples range between fresh and 7 days stored at 60°C, the distribution width was in the range of (2.557 – 4.218) for 5% concentration and range of (2.876 – 3.274) for 10% concentration. Specific surface area ranges from (68.28 – 88.570) for 5% and from (68.850 – 60.978) for 10% concentration the change was decreased wih increasing concentration.The interfacial activity attributed to the presence of the

proteinaceous moiety and the specific high molar mass of AGP concentration (Al-assaf, *etal 2006*, Randall, *etal* 1989).the higher the protein content, the higher is surface activity especially when used at higher concentration(Mahfoudhi, *etal* 2014). The span change very little from the fresh sample to that be stored for 7 days at 60°C, this clearly showed that the span ranged from (1.386 to 2.326%) in fresh emulsion and (1.081 to 3.479%) for emulsion stored for 7 days at 60^{o}C.

Droplet size distribution expressed by polydispersity which is in a good uniformity varied between 1.2 to 1.1% for 5% and from 2.3-3.5% for 10% concentration. Acceleration and storage, conjoined increase in droplet size distribution, graduall smaller change was observed from the fresh sample to that be stored for 7 das at 60C. The mean droplet diameter was expressed as the volume mean diameter D4,3 (emulsion droplet volumetric diamer,Vogler,2012) . On doubling concentration of AGP produce excellent results and multiply AGP farthermore, the emulsion form large droplet size but stable (Han, *etal* 2019). The stability of emulsion obviously decreased with the storge duration but destabilization was impaired as the AGP protein concentration increased (Aphibanthamakit, *etal* 2020). There is acombined effect between high Mw protein rich AGP and total gum concentration on droplet diameter (Aphibanthamkit, *etal* 2020). The molecule AGP with Mw upper than 10^6 g.mol protein poor and AGP lower Mw than10^6 g.mol protein rich, simultaneously affect the stability of emulsion(Ramdall *etal* 1989, Renard, *etal* 2006)the well distribution of protein between AGP-rich and AGP-poor protein affect the proper stability.

Table 3.14.: Physicochemical Parameters of Laser Diffraction for *A. tortilis* var. *spirocarpa* Gum

Time/Parameter	**Concentration**	**Fresh emulsion**	**3 days @** 60°C	**7 days @** 60°C
Span	5%	2.557	4.711	4.218
	10%	2.876	3.539	3.274
Specific surface area m^2/kg	5%	68.270	89.860	88.570
	10%	68.850	61.710	60.978
$d_{(4,3)}$	5%	0.480	0.515	0.459
	10%	0.243	0.475	0.309
$d_{(3,2)}$	5%	0.0925	0.070	0.071
	10%	0.0916	0.102	0.104
$d_{(0.1)}$	5%	0.0295	0.0238	0.0241
	10%	0.0382	0.0416	0.0426
$d_{(0.5)\ VMD}$	5%	0.376	0.247	0.266
	10%	0.164	0.196	0.196
$d_{(0.9)}$	5%	0.991	1.190	1.140
	10%	0.510	0.735	0.683

d (4.3) = volume weight mean (De Bracker mean)

d (3.2) = surface area mean (Sauter mean diameter)

d (0.1) = 10% of the population lies below the D_{10}.

d (0.5) = The volume median diameter, half the population lies below this value.

d (0.9) = 90% of the distribution lies below D_{90}.

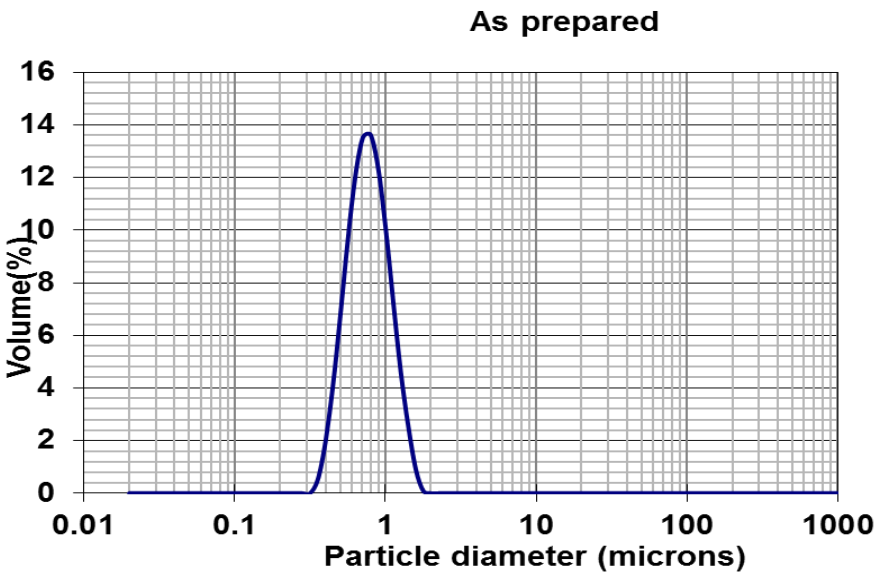

Fig. 3.9. Particle Size Distribution of *A. tortilis* var. *spirocarpa* Prepared Emulsion (composite sample)

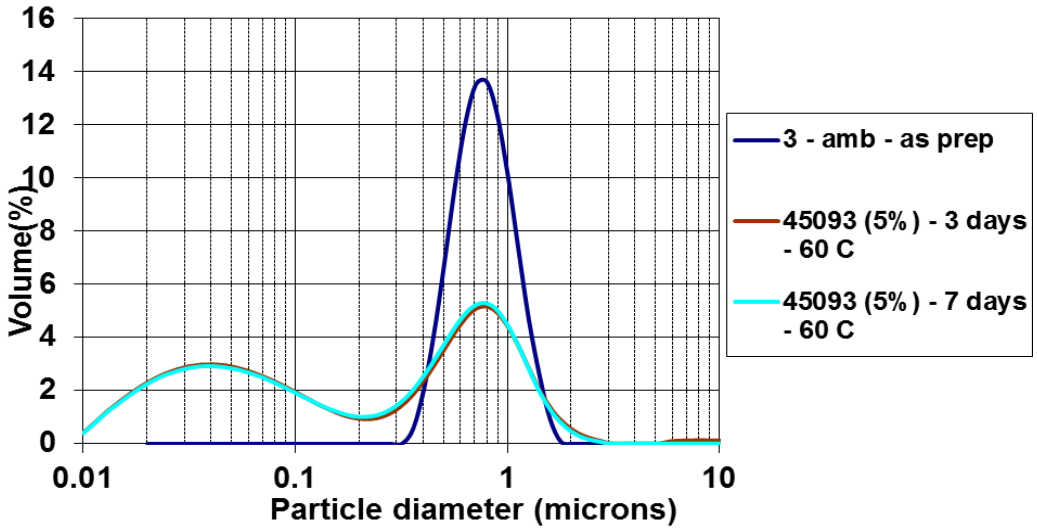

Fig. 3.10. Particle Size Distribution of *A. tortilis* var. *spirocarpa* Prepared Emulsion and Stored for 3-7 days 5%Conc. (composite sample)

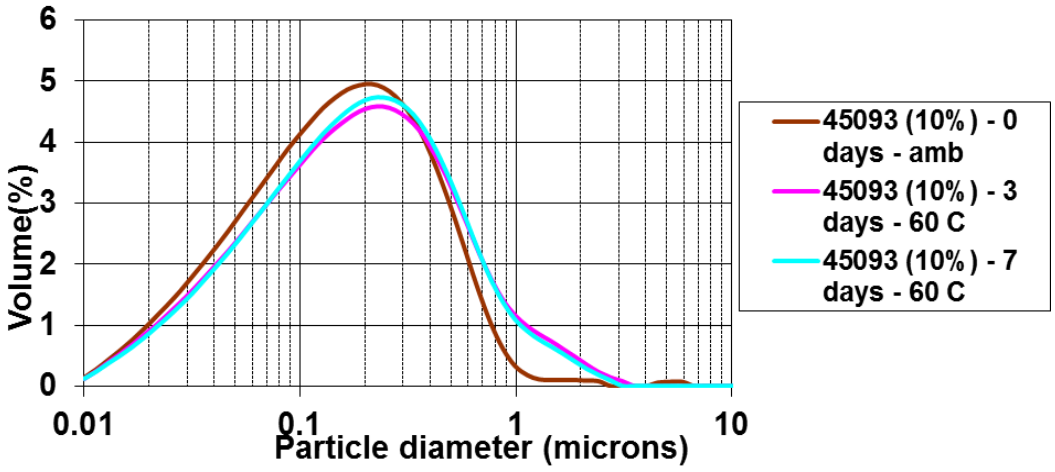

Fig. 3.11. Particle Size Distribution of *A. tortilis* var. *spirocarpa* Prepared Emulsion and Stored at 3-7 days 10%Conc. (composite sample)

3.3.2. Measurement of Particle size Distribution :.

The instrument Master sizer 3000 is capable of measuring particles with a size distribution range from 0.01 to 1000 μm.The particle size is distributing in three decades.The cumulative droplet distribution percentile diameters d_{10}, d_{50}, and d_{90} represent the size, in micrometers below which (10, 50, 90%) respectively the particle size falls.

The VMD is the volume median diameters ($d_{0.5}$), for both concentrations, the measurement shows a very small change from fresh sample to that be stored at 7 days 60°C ranged (0.37– 0.266) for concentration 5%, and (0.164– 0.196) for 10% concentration Table 3.14. and Fig. 3.12. .The majority of particles have size between 0.3 to 0.7 μ .On acceleration, small particles tend to coalesce with others forming particles with size less than 0.7 μ .The particles smaller than 1 μ , which is difficult to overcome the Van Der Waals forces and get particle seperates.

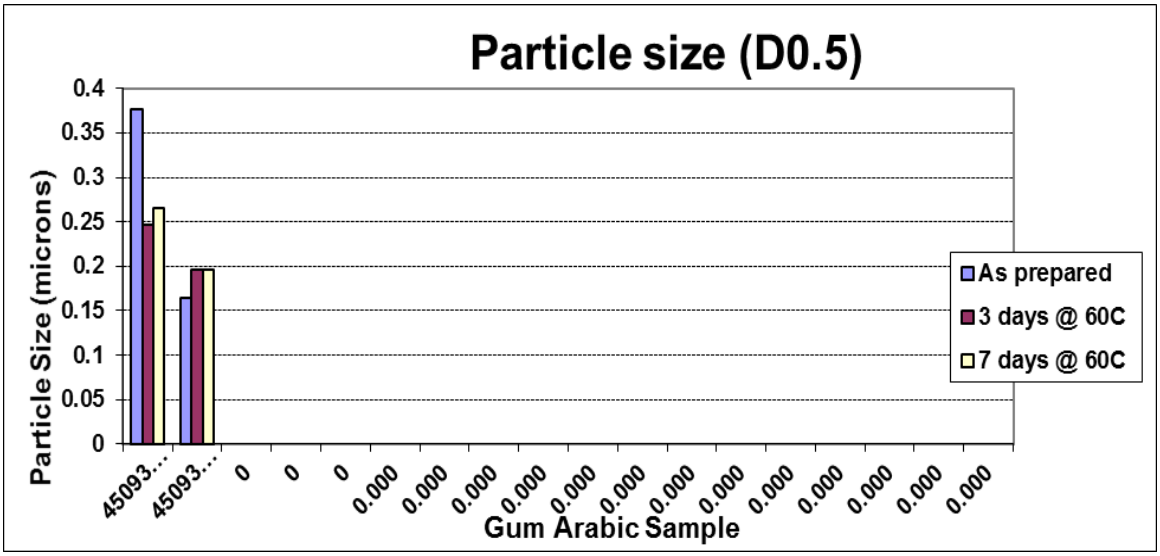

Fig.3.12. Particle Size VMD Diagram

3.3.3. Emulsion stability Index (ESI):

Stability of the emulsion was determined for (fresh sample, 3 days at 60°C sample, 7 days at 60°C) sample, by measuring the change in particle size of emulsions based on initial and final particle size using particle size analyzer. ESI was calculated according to Phillips Hdrocolloid Research Center (PHRC) grading system using the equation:

$ESI = d_{0.5\ (as\ prepared)} + (d_{0.5\ (3\ days\ @\ 60°C)} - d_{0.5(as\ prepared)} + (d_{0.5\ (7\ days\ @\ 60°C)} - d_{0.5(as\ prepared)})$, where $d_{0.5}$ is the value median diameter.

The gum samples which showed a change of 0.7μ or less were classified as grade 1 very good emulsifier. And that shown a change between 0.7-0.85 μ were classified as grade 2 good emulsifiers. A change (> 0.85) was allocated grade 3 (poor emulsifier). So that *A. tortilis* var. *spirocarpa* was classified as very good emulsifier, Table 3.15 The eumulsion was stable although it was stored for 3-7days and the temperature was elevated to 60°C . The particle size measurement indicated

that most particles falls in the range >1 μ and <2 μ . Table 3.16, Fig. 3.13 and Fig. 3.14. In the three samples fresh, 3 days at 60°C and 7 days at 60°C.

Table 3.15. Emulsion Stability Index (ESI μ) of *A. tortilis* var. *spirocarpa* Gum

Concentration	ESI	Grade
5%	0.137	1
10%	0.228	1

Table 3.16. Particle Size Diameter of *A. tortilis* var. *spirocarpa*

Particle size	Concentration	Fresh	3 days @ 60°C	7 days @ 60°C
> 1 μ (% > 1 μ)	5%	9.71	15.71	14.66
	10%	1.28	6.01	4.81
> 2 μ (% > 2 μ)	5%	0.78	1.73	0.870
	10%	0.43	1.51	0.750

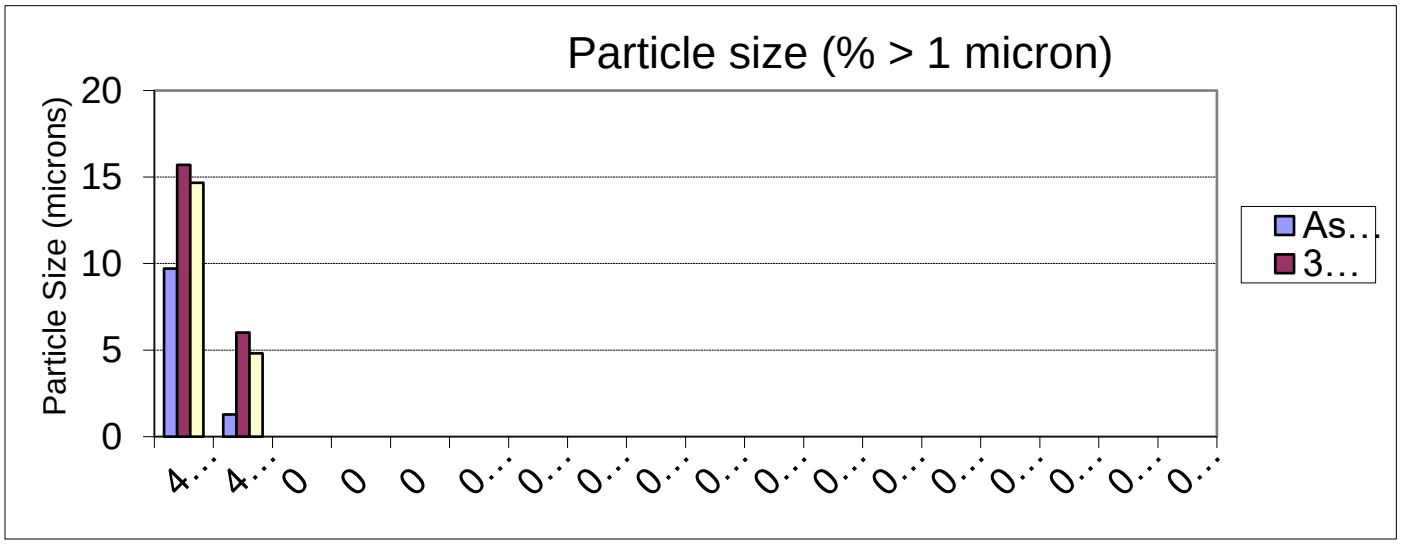

Fig. 3.13. Particle Size (%>1micron) Diagram

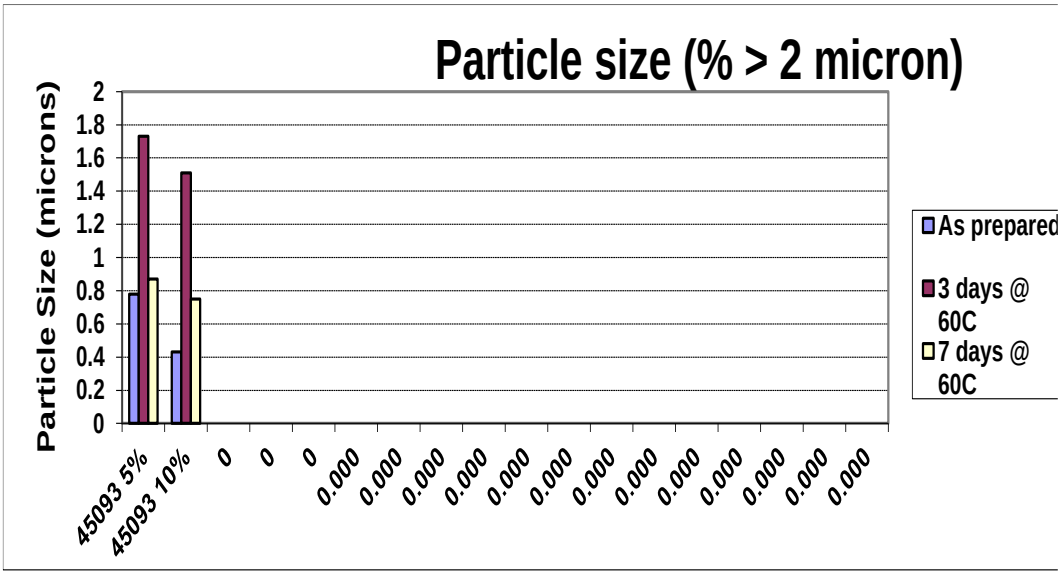

Fig. 3.14. Particle Size (%<2micron) Diagram

3.3.4. Comparison between *Acacia tortilis* varieties in Emulsification:

The result obtained from *A. tortilis* var. *spirocarpa* in a good agreement with that of *A. tortilis* var. *raddiana* (Abduelrahman, 2011), and *A. tortilis* var. *tortilis* (Alnour, 2014).

The span, specific surface area measurement, the total range of samples (fresh – 7 days at 60°C) were nearly similar, Table 3.17. The $d_{0.5}$ also had semi similar range and ESI for the three species < 0.1μ all of them had grade 1 as very good emulsifiers.

Table 3.17. *A. tortilis*: Comparison between the three varieties in Emulsion Parameters:

Properties	*Spirocarpa*	*raddiana*	*Tortilis*
Span range	2.533-4.218	1.386-2.326	1.386-2.326
	2.876-3.274	1.080-3.479	1.080-3.479
Specific surface area	68.27-88.570	34.57-51.0	34.57-51.0
	68.85-60.978	27.4-45.6	27.4-45.6
$d_{.0.5}$	0.376-0.266	0.207-0.215	0.207-0.215
	0.266	0.209	0.209
ESI grading	Grade 1	Grade 1	Grade 1

3.4. Rheological Properties:

3.4.1. Viscosity Profile:

In the following graphs of gum solution, Fig. 3.15. and Fig. 3.16. , the viscosity – shear rate relationship were illustrated at concentrations (25-50% - w/w) for *A. tortilis* var. *spirocarpa* composite gum from Abushmal Forest – Khartoum State. The shear rate intervals (0.1-1 small flow), (1-10 moderated), (10-100 high flow).

Fig. 3.15. concentration (25% w/w): The profile start at viscosity around (5Pa.s) at shear rate of(0.1 1/s) and then the viscosity decreased with increasing shear rate to(0.8Pa.s) at a shear rate around (80 1/s). The viscosity attains a plateau known as the low shear (zero-shear rate) or first Newtonian Plateau at (0.6-0.7 1/s) shear rate and viscosity around (1.7-1.4 Pa.s).The curve showed non-Newtonian fluid, the viscosity was not a fixed value but was depenended upon the degree of shear which was exposed to, the type was shear-thinning dominates.

Fig. 3.16. concentration (50% w/w): The profile start with viscosity around (11 Pa.s) at shear rate of(0.1 1/s). It is high viscosity comparing to above concentration, result from increasing concentration. The curve start with a nearly Newtonian fluid until the viscosity reached around (4 Pa.s) with shear rate (1.5 1/s), then the ratio between shear stress and shear strain be constant until shear rate was (80 1/s). So the viscosity was Newtonian fluid. The graph has showed the clear – zero – viscosity plateau around (0.63-0.79 1/s) shear rate and viscosity around (4.9-4.6 Pa.s) .

3.4.2 Steady Shear Flow:

The decrease in viscosity with increasing shear rate is a behaviour of typical of polysaccharide system which attributed to the disentanglement of macromolecular chains under shear (Mothè and Rao,1999).

At concentration (25% w/w):

Gum solution, at low flow rates, molecules were long and this has effectively large cross sections due to their tumbling in solution, this assumed to existence of AGP molecules which have high R_g value see Table 3.8 , AGP less compact and oriented at different directions and highly branched , so it was resist the flow of the solution but on increasing shear rate, at zero- shear- rate the molecules the flow giving much smaller effective cross-sections and hence much lower viscosity due to degradation of AGP (oblate ellipsoid particle with a central intricate network, Sanchez, *etal*.2008, Renard, *etal* 2012) to primary particles and elongated particles , the small fragments supposed to be AG and GP molecules (GP = 9nm , ring-like shapes, Renard, *etal* 2014) , more compact molecules, their orientation were not affected the flow of solution so much and the viscosity decreased with increasing shear strain rate. So the viscosity was non Newtonian and with shear thinning behavior.

At concentration (50%w/w):

The density of AGP molecules was high, and the applied shear rate was not directly propotion with the amount of AGP molecules, so increasing of shear rate was not affect the AGP and the viscosity was Newtonian behavior.

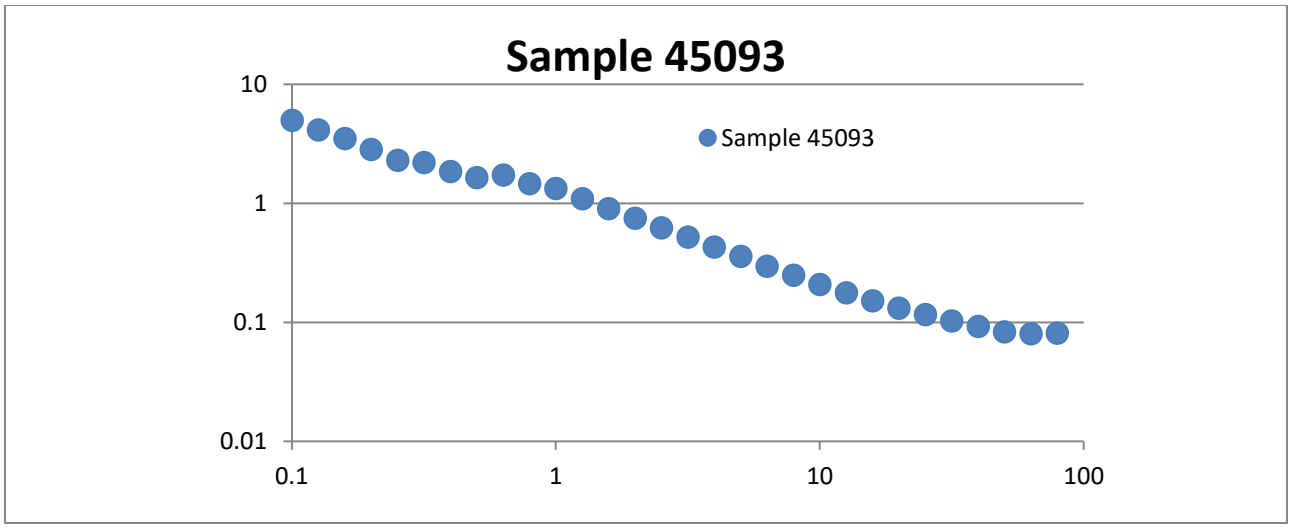

Fig. 3.15. Shear Rate- Viscosity Profile for *A. tortilis* var. *spirocarpa*

(25%w/w)

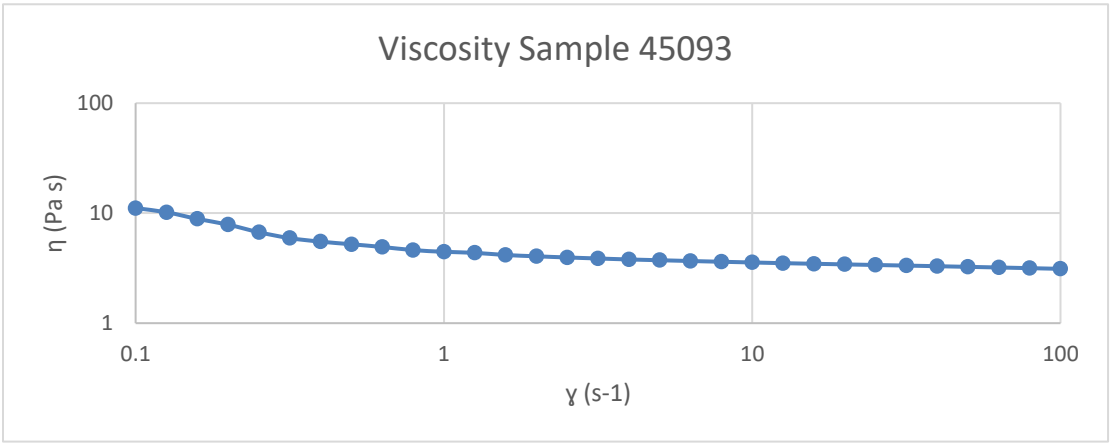

Fig. 3.16. Shear Rate –viscosity profile for *A.tortilis* var. *spirocarpa* (50%w/w)

The steady shear flow for *A.tortilis* var. *raddiana* (Abduelrahman, 2011), and , *A. tortilis* var.*tortilis* (Alnour, 2014), showed very clear thinning behavior.

3.4.3. Oscillatory Rheological Properties:

Fig. 3.17. showed the spectra of the gum solution. The plot is of storage modulus G` and loss modulus G`` and viscosity η against frequency f(w) at

concentration (50%w/w) on applying small stress (0.1-1.003 Pa). The viscosity curve crosslink G`curve at frequency(0.15Hz, 0.9 rad/s)and then crossolink G``curve at frequency(0.3Hz,1.9 rad/s), the dynamic viscosity($\eta = G``/ \omega$) ω the angular frequency , then equal 2.1Pa.s and at frequency (6.28 rad/s) equal (10.4 Pa.s), the micro structure in the gum due to AGP micelles which give it the elastic nature, on increasing the frequency AGP start to degradate at dynamic viscosity to give AG and GP micelles the viscosity curve fall down as shear thinning with increasing frequency and the solution start to increase the property of viscous-like

Initially, G` was observed to be higher than G``,due to strong molecular association at the lowest accessible frequencies (0.1-1Hz , 0.628 – 6.28 rad/s) the response was solid-like. Both moduli were increased with increasing frequency. Near the gel point the steady state frequency (1.9Hz ,11.9 rad/s) there was a crosslink junction(G_C) and frequency(Wc) were found to be 121.9Pa and 2.5 Hz,21.7rad/s respectively, which is considered as a measure of the polymer solution relaxation time. Both moduli became constant and frequency independent. Then G`` was increased more than G`, independent of frequency.

The phase shift δ (0.59) with respect to applied strain deformation - lied between that of solid and liquid ($0< \delta > \Pi/2$), the angle 31^o was less than 45^o so the solution was viscoelastic solid. Complex modulus G*(Pa) started from low value 29.8 to high value 381 indicated the polymer gradual change from soft-solid to hard-solid present rigidity and intergrity of the material internal structure resulting from flocculation and interaction of dispersed particles or droplets, or cross-linking and entanglement of dissolved polymer. Complex (G*= G`/ G``). (G* >1) so solution prone to hard solid .

phase shift δ (0.59) G* 29.8 to 381

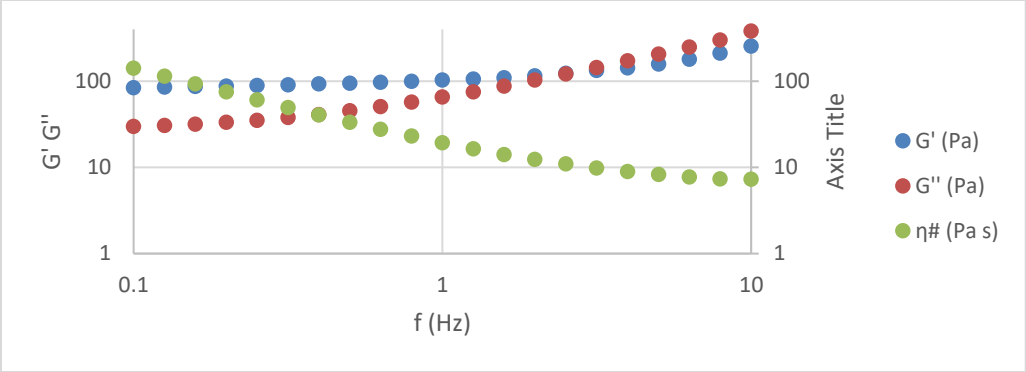

Fig. 3.17 Dynamic Modulus for *A. tortilis* var. *spirocarpa*

3.4.4. Comparison between *Acacia* varieties in Rheology:

For the oscillation measurement for var. *raddiana* G` was higher than G`` refered to certain rigidity in the gum structure and exhibts solid- like behaviour.

Conclusion:

- The physicochemical study of *A. tortilis* var. *spirocarpa* gum showed that it has lower (Rha) and higher (Ara) content, a dextrorotary optical rotation , also it has higher nitrogen and protein content.
- The emulsification study exhibited the gum of *A. tortilis* var *spirocarpa* is grade 1.
- The rheological properties study showed Newtonian flow behaviour at high concentration and also exhibited solid -like behaviour.
- There are insignificant difference between samples from different locations.
- There are slightly difference between *Acacia tortilis* varients *spirocarpa* . *raddiana*. *tortilis i*n physicochemical propertis and functionality. Therefore it can be marketed as one species.

Recommendations:

- The study of the prebiotic properties of the *Acacia tortilis* var. *spirocarpa* gum.
- Fractionate the gum into carbohytdrate and protein component and study the physiochemical and functional properties of the fractions.
- The study of thermodynamic properties of the gum.

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