Sudan University of Science and Technology

College of Graduate Studies

A Comparative Study between Processed and Foam Purified Gum Arabic Var. Senegal

دراسة مقارنة بين الصمغ العربي المجهز والمنقى رغوياً

A Thesis Submitted in Partial Fulfillment for the Requirements of a Master Degree in Chemistry

By:

Sara Suliman Elmubark Mohamed

Supervisor:

Prof. Mohammed El Mubark Osman

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الاستهلال

قال تعالى: (اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ)

سورة النور الآية (35)

Dedication

I dedicate this work to:

The soul of my father Suliman,

My mother

My husband and children

My brothers and sisters

Acknowledgment

I would like to express my deepest thanks to Allah Almighty for the great support I got during my whole life and especially in this study.

I am greatly indebted to my supervisor **Prof. Mohammed ElMubark Osman** for his keen guidance, encouragement and concern. Also I wish to thank him for never failing to be there when needed. It was through his invaluable advice I have been able to present this thesis.

Thanks are due to the staff of the chemistry department, Sudan University of Science and Technology for technical support, colleagues and friends.

Abstract

Physicochemical properties of *Acacia Senegal var Senegal* gum purified by foaming and processed gum (ELNsr) samples were studied. The results showed that the moisture content, ash, nitrogen, protein, viscosity, specific optical rotation and pH were (10.74% and 10.86%), (3.84% and 3.55%), (0.25% and 0.27%), (2.35% and 2.30%), (14.5 cm^3 g^{-1} and 14.8 cm^3 g^{-1}), (-33.61° and -30.75°) and (4.9 and 4.5)] respectively.

The gum produced by foaming consists of arabinose (33%), rhamnose (13.5%) and galactose (37%). The total sugar contents reaches (83.5%). On other hand, the processed sample consists of arabinose (30.5%), rhamnose (11.5%) and galactose (35%). The total sugar contents reaches (77%).

مستخلص البحث

تمت دراسة الخصائص الفيزوكيميائية لرغوة صمغ الهشاب والصمغ الصناعى (النصر) و كانت نتائج محتوى الرطوبة، الرماد، الناتروجين، البروتين، اللزوجة، الدوران الضوئى النوعى والأس الهيدروجينى كالآتى: (10.74% و 10.86%) ، (3.84% و 3.55%)، (0.25% و 0.27%)، (2.35% و 2.30%)، (14.5 سم^3 جم$^{-1}$ و 14.8 سم^3 جم$^{-1}$)، (-33.61^o و -30.75^o) و(4.9 و 4.5) على التوالى.

أحتوى الصمغ المصنع من رغوة صمغ الهشاب على أرابينوس (33%)، رحمنوز (13.5%) وغالاكتوز (37%). والنسبة الكلية لمحتوى السكر هى (83.5٪)، بينما أحتوى الصمغ الصناعى (النصر) على أرابينوس (30.5%)، رحمنوز (11.5%) وغالاكتوز (35%). والنسبة الكلية لمحتوى السكر هى (77٪).

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Introduction

1. Introduction

The Sudanese major gums of economic importance are in the order of gum arabic gum Talha and *polyacantha* gum. Gum arabic, sometimes known as the dried gummy exudation of *Acacia*. (Elgaili *et al*., 2015). *Acacia polyacantha* (family *Leguninosae*) exudates are closely related, and can be distinguished from *A. senegal var Acacia Senegal* exudates by differences in physicochemical characteristics. The two species, *Acacia senegal var Acacia senegal* and *Acacia polyacantha* belong to the same group known as *Acacia senegal var Acacia senegal* complex. All gum exudates, from this group of *Acacia* species, have a laevorotatory (-ve) specific optical rotation in contrast to the *Acacia seyal* complex which produce gum exudates, that have a dextrorotary (+ve) specific optical rotation, other structural, botanical characteristics are noticeable even within the same species (Elgaili *et al*., 2015).

Gum arabic is a dried exudate obtained from the stems and branches of *Acacia senegal var Acacia senegal* or *Acacia seyal* (fam. Leguminosae) (Hamza, 1990).

Gum arabic consists mainly of high-molecular weight polysaccharides and their calcium, magnesium and potassium salts, which, on hydrolysis, yield arabinose, galactose, rhamnose and glucuronic acid. Items of commerce may contain extraneous materials such as sand and pieces of bark, which must be removed before use in food. It is a branched molecule with protein content of about 2.0–2.5 (Hamza, 1990).

In Sudan, the term gum arabic is used in a wider context to include two types of gum which are produced and marketed, but which are, nevertheless, clearly separated in both national statistics and trade: "hashab" (from *A. Senegal*) and "Talha" (from *A. Seyal*). In a still wider sense, gum arabic is often taken to mean the gum from any *Acacia* species (and is sometimes referred to as "Acacia gum"). "Gum arabic" from Zimbabwe, for example, is derived from *A. karroo* (Hamza, 1990).

In practice, therefore, and although most internationally traded gum arabic comes from *A. Senegal*, the term "gum arabic" cannot be taken as implying a particular botanical source. In a few cases, so-called gum arabic may not even have been collected from *Acacia* species, but may originate from *Combretum*, *Albizia* or some other genus (Hamza, 1990).

1.1 *Acacia senegal var. senegal*

1.1.1 Botanical classification

Class: Equisetopsida

Subclass: Magnoliidae

Superorder: Rosanae

Order: Fabales

Family: Leguminosae/Fabaceae -Mimosoideae

Genus: *Acacia*

1.1.2 Description of the tree and gum

Acacia senegal var. senegal is a small to medium sized thorny tree, with a stem, which is irregular in form and often highly branched. In leaf, like many other *Acacias*, it has a dense, spreading crown. In common with other members of the *A. senegal var. Senegal* complex, it has characteristic sets of prickles on the branches, usually in threes with the middle one hooked downward and the lateral ones curved upward. The bark is not papery or peeling. The tree is deciduous, droops its leaves in November in the Sudan (FAO, 2007).

Gum arabic (*A. senegal*) *var. Senegal* is a pale white to orange-brown solid, which breaks with a glassy fracture. The best grades are in the form of whole, spheroidal tears of varying size with a matt surface texture. When ground, the pieces are paler and have a glassy appearance. Gum from (*A. seyal*) is more brittle than the hard tears of gum arabic (*A. Senegal*) *var. Senegal*.

Senegal Shrubs or small trees 2-12m high bark yellow to light brown or grey, rough, fissuring or flaking, young branches with horizontal slit- like lenticels, stipules non. spine scent, prickles at nodes in threes, 2 lateral pointing upward or forward and one central pointing down ward or back ward, falcate, 4-7mm long, pinnae 2-6 pairs, 0.5-3cm long, leaflets 8-18 pairs, linear to elliptic oblong, 1-6x 0.5-2 cm. inflorescence spectate, 2-10 cm long on peduncles 0, 7-2cm long, flower white or cream, sessile, sepals 2x0.7 mm, pubescent, petals 2.5 x 0.3 mm glabrous, stamens 4-7 mm long, glandular. Fruit flat straight oblong membraneous dehiscent pods 3-24 x 1-3.3 cm pale brown to straw-coloured, seeds vertical in pog, orbicular, compressed, 8-12 mm across, yellow or pale brown, areoles crescent – shaped. Centrals, 1.5- 6 x2.5 – 5 mm fanciless 7.5 mm long. flowers Nov-Feb, fruits Jan - April On sandy and clay plains in short grass savanna forming a continuous belt from east to west in central Sudan, more common one the western sand plains of Kordofan and Darfur as pure stands associated with Amelliteca (Hamza, 1990).

Figure (1.1): ***Acacia Senegal var. Senegal*, a. flowering branch, b. seed, c. flower, d. fruit, e. prickles (Hamza, 1990)**

1.1.3 Distribution

Acacia senegal var. senegal has a wide distribution and remarkable adaptability. It is essentially a semi-arid zone species, but it is both drought, frost resistant, and can grow with a rainfall of between 100 and 800 mm per year. It grows across Africa, from Senegal to Ethiopia, through Mali, Nigeria, Chad and Sudan, to mention only the major producing areas (Hamza, 1990). It is also found in the Middle East, Yemen, India and Pakistan. In the Sudan, particularly in the Kordofan and Darfour provinces, the species is uniform and found in pure stands giving the Sudan an important advantage of being the most important producer of this type of Gum Arabic. In other producing countries, *Acacia Senegal var. Senegal* often found mixed with other species (Hamza, 1990).

1.1.4 Quality of gum Arabic

The quality of gum arabic as received by the importer depends on the source. Gum arabic (hashab) from Sudan is the highest quality and sets the standard by which other "gum arabics" are judged (Hamza, 1990). Not only does Sudanese gum come from a species (*A. Senegal Var. Senegal*) which intrinsically produces a high quality exudate with superior technical performance, but the collection, cleaning, sorting and handling of it up to the point of export is well organized and highly efficient (Hamza, 1990).

Within Sudan, gum arabic from the Kordofan region has the highest reputation, and traders and end-users in importing countries often refer to "Kordofan gum" when indicating their preferences (Hamza, 1990).

Gum talha from Sudan (produced from *A. seyal*) is intrinsically a poor quality gum than hashab, it has inferior emulsifying properties and even light-coloured samples of whole gum sometimes form dark solutions in water due to the presence of tannins and other impurities. It is more friable than hashab (Hamza, 1990).

1.1.5 Supply sources "geographical distribution

The gum belt referred to earlier occurs as a broad band across Sub- Saharan Africa Figure (1.2) from Mauritania, Senegal and Mali in the west, through Burkina Faso, Niger, northern parts of Nigeria and Chad to Sudan, Eritrea, Ethiopia and Somalia in the east, and northern parts of Uganda and Kenya. Most of these countries appear in the trade statistics as sources of gum arabic, although they differ greatly in terms of the quantities which are involved.

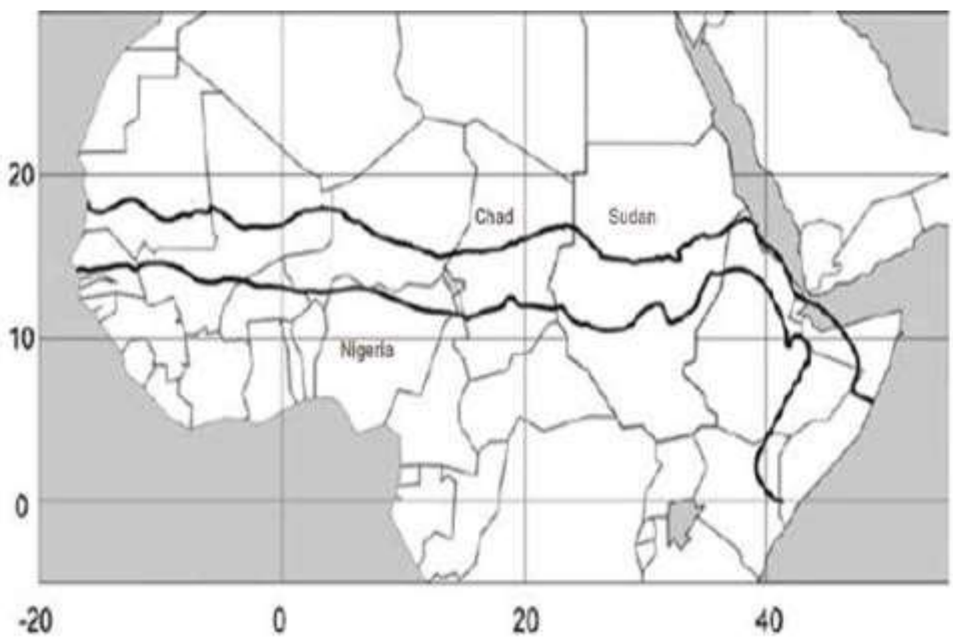

Figure (1.2): The African Gum Arabic Belt

Sudan is the world's biggest producer of gum arabic, and since very little is consumed domestically it is also the main source of gum in international trade. A few countries which have gum-yielding *Acacias* produce gum for the local market, but not in sufficient quantities to enable exports to be made (FAO, 2007). Two such examples are Zimbabwe and South Africa, which produce gum arabic from *A. karroo*. Outside Africa, India produces small amounts of gum, similar in quality to gum talha, but a proportion of its exports of gum arabic consists either of re-exports of African gum or locally produced gum ghatt (From. *Anogeissus latifolia*) misclassified as gum Arabic (FAO, 2007).

1.1.6 Structure of plant gums

Gum nodules contain polysaccharide material of complex nature usually contaminated with impurities such as bark fragments, entrapped dust and insects. Inert pertinacious material and a few amounts of terpenoid resins can also be present. Gums are polyuronides; the uronic acid residues may carry acetyl or methyl groups and, generally, occur at least in part as methyl groups or as metallic salts. The hexose residues are present in the pyranose configuration, while the pentose residues occur in the furanose (Stephen *et al*., 1955 and 1983) beside the foregoing gums, *Sterculia termentosa* gum contains rhminose, galactose and probably galacturonic acid, *Olibanum*gum was found to be of an arabino-galactan and polysaccharide containing galactose and galactouronic acid (Elkhatem *et al*., 1956). It was noted that the gum was very heterogeneous and it has been described as heteropolymolecular, i.e. having either a variation in monomer composition and/or a variation in the mode of linking and branching of the monomer unites, in addition to distribution in molecular weight (Lewis and Smith, 1957; Dermyn, 1962 and Stoddart, 1966). According to Philips (1988) and Williams (1989), fractionation hydrophobic revealed that *Acacia Senegal var. Senegal* gum consists of at least three distinct components. Fraction 2 (arabinogalactan) AG, fraction 1 (Gelpermeatachronology) GPC and fraction 3 (galactoprotein) GP. But even those contain a range of different molecular weight components revealing the polydiverse nature of the gum (Osman, 1994).

Fraction 1 containing 88% of the total has only small amount of protein content. Fraction 2 represents 10% of the total and had 12% protein content.

Fraction 3 resembles 1.24% of the total but contains almost 50% of protein AGP are responsible for the emulsifying properties of gum Arabic (Williams, 1989, and Phillips, 1988). No mention has been made to detailed comparison between the structures of gums from different species of trees, but is believed that D- galactose and uronic acid residues generally constitute the backbone of gum polysaccharide with 1- 3 and 1-6 linkages predominating side chain are characterized by the presence of D xylopyranose, L-arabinose, and L- arabino-furanose linkage (Elnour, 2007; Alaa, 2015).

1.1.7 Theories of gums formation

There are many theories of gum formation theories and functions which have been formulated to explain the phenomena of gummosis, formation of gum exudates is pathological condition resulting from microbial (fungal and bacterial) infection of injured tree, natural factor that tends to lessen the vitality of the trees, such as poor soil, lack of moisture, and the weather improve gum yields. Other considers the production of gum to metabolic process in the plant with quantity and quality produced being function of environmental condition, some believed that the gum formed as a defense mechanism to seal off the wound to prevent desiccation other proposed that the starch might undergo transition into gum. (the latter is refuted by Anderson and Dea 1968), as the enzyme system necessary to transform starch into highly branched arabinoglactan with galactose, arabinose, rhamnose, glucuronic acid and its 4-o-methyl ether are complex, further (Anderson and Dea, 1968) found that the starch was not represent in tissues of excited branches and therefor proposed that gums have a hemicelluloses types, highly branched arabinoglactan precursor in which rhamnose glucronic acid and 4-o-methyl glucuronic acid are the peripheral groups

(Omer, 2004).

1.1.8 Chemical structure of gums Arabic

Gum Arabic is branched, neutral or slightly acidic, complex polysaccharide obtained as a mixed calcium, magnesium, and potassium salt. The backbone consists of 1,3-linked b-d-galactopyranosyl units. The side chains are composed of two to five 1,3-linked b-d-galactopyranosyl units, joined to the main chain by 1,6-linkages. Both themain and the side chains contain units of a-1-arabinofuranosly, a-1-rhamnopyranosyle, dglucuronopyranosyl, and 4-O-methyl-b-d-glucuronopyranosyl, the latter two mostlyas end-units (Anderson and stoddart, 1966). They further analyses the product by methylation and gelpermeation chromatography and found that the uronic acid and the rhamnose residues eliminated first which proved that they are located at the periphery of the molecule and the core was consisted of a β1,3-galactopyranose chain with branches linked through 1, 6 position. Also found that the protein component was associated with the high molecular weight fraction and lower molecular mass fraction was virtually exclusively polysaccharides.

Figure (1.3) shows the Monosaccharides in gum Arabic (Churms *et al.*, 1983) used computer modeling to analyze the previous data and proposed the structure illustrated in Figure (1.4) (Churms *et al.*,1983). Subjected the gum to smith degradation leaving the reaction to reach completion after each stage of degradation procedure. They obtained different values for the composition and size of themolecule of each degradation product than those previously obtained by (Anderson, 1966b), and proposed amore regular structure than the previous one proposing that the galactan core consisted of 13β-, 3-D-galactopyranosyl residues Figure (1.4) having two branches which give single repeating subunits having molecular mass of 8×10^3 within the molecule.

As the whole gum was found to have molecular weight of 560,000 thus it was proposed that the molecule consists of 64 of these subunits and that they were symmetrically arranged in their structural studies of gum Arabic using A 25.182 MHz ^{13}C-NMR (Alaa, 2015).

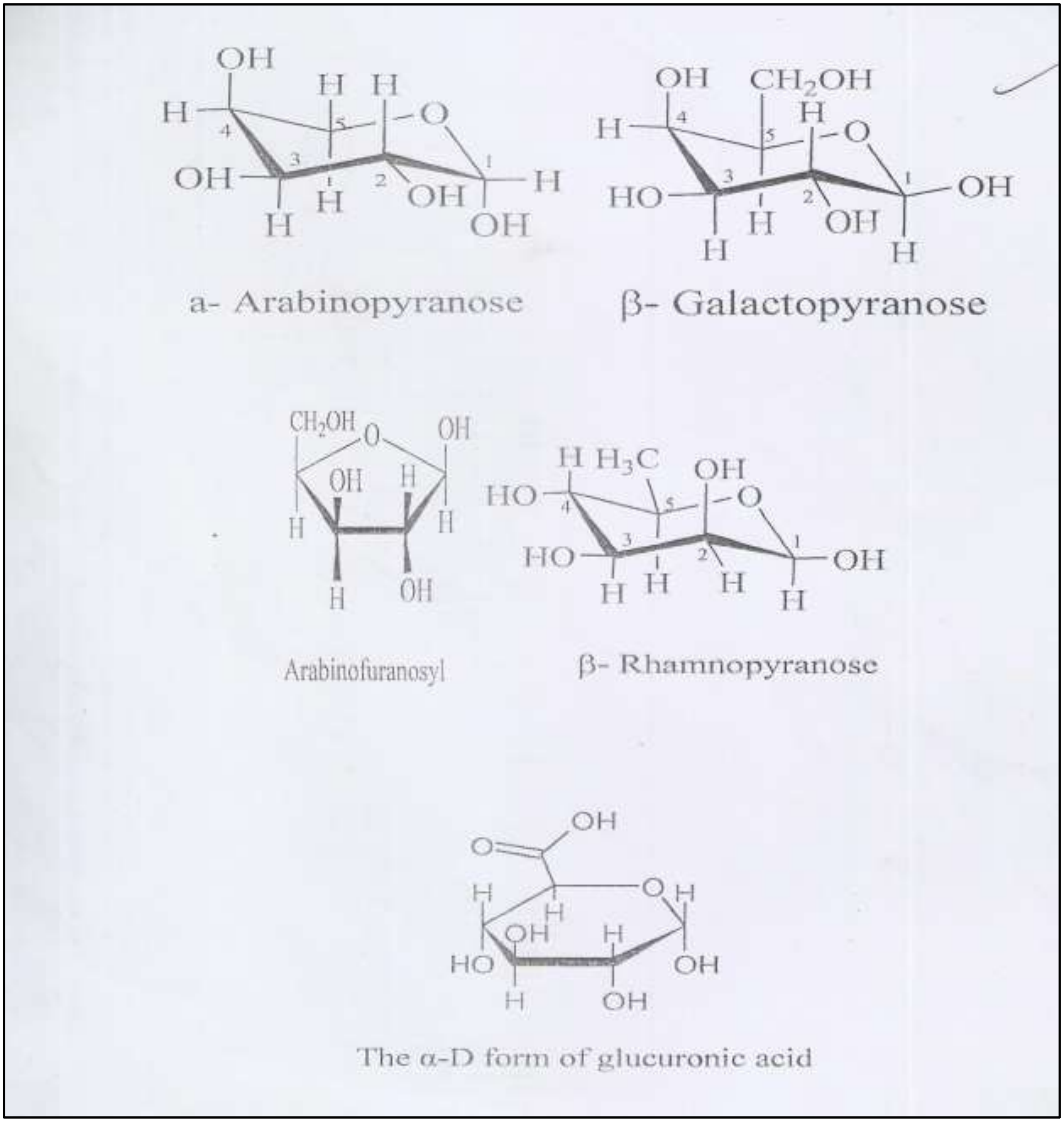

Figure (1.3): Structural of carbohydrates units of gum molecule (Alaa, 2015)

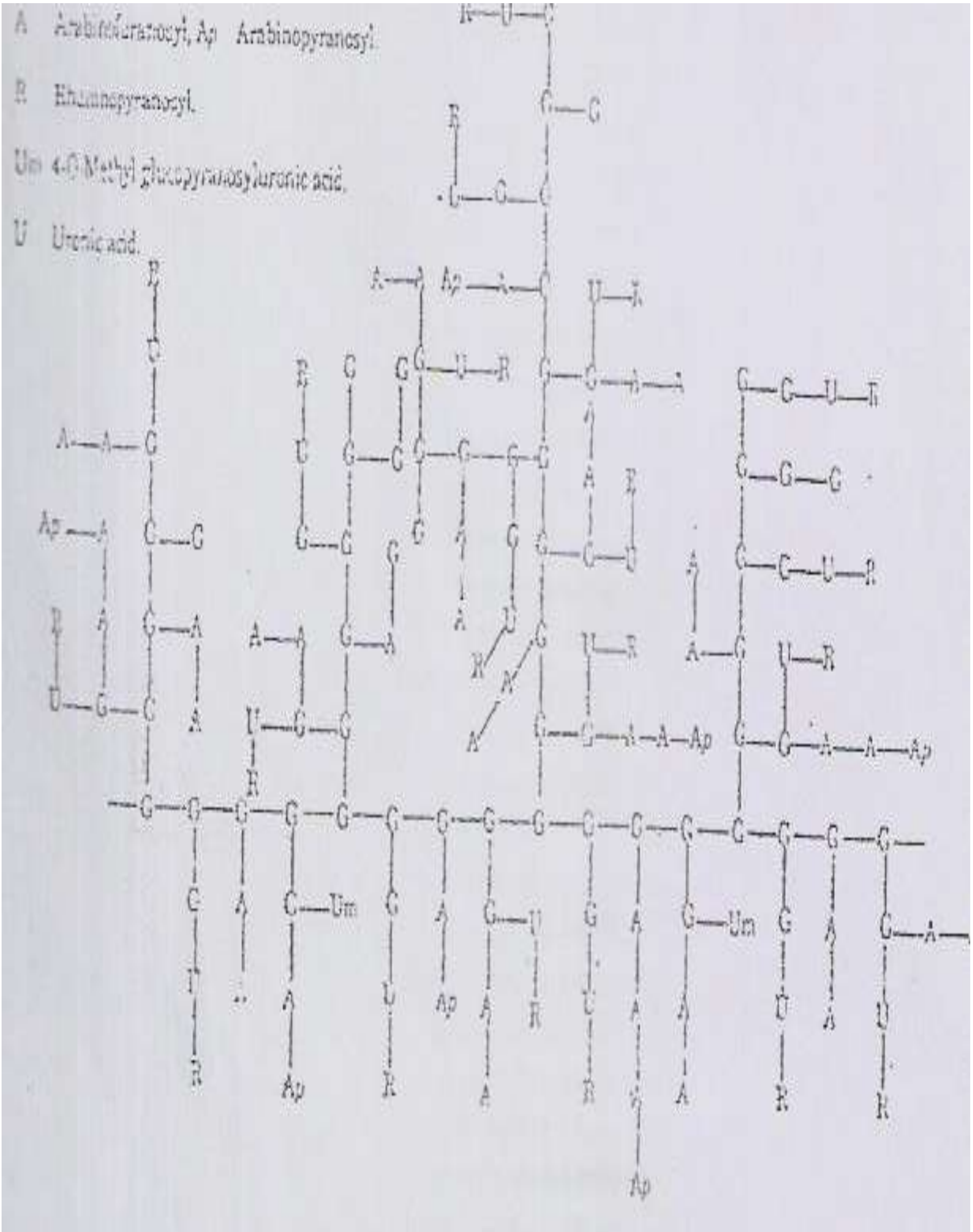

Figure (1.4): The proposed structural of *Acacia Senegal var Senegal* gum (Churms *et al*., 1983)

1.1.9 Applications of plant gums

The solubility and viscosity of gum are the most fundamental properties, which make it unique among polysaccharides, the majority of gums dissolve in water at different concentrations, and such properties are exploited in many applications (Alaa, 2015).

1.1.10 Applications in the food industry

Gums for their high viscosity in solutions and inability to crystallize, are particularly suited to serve in foodstuff such as: thickeners for beverages, stabilizers for oil and water emulsions and as wider application where function is to prevent agglomeration and setting of minute particles. They are also used to incorporate flavors in confectionery such as pastilles and gum drops, and the preparation of lozenges. The role of gum Arabic in confectionary products is usually either to prevent crystallization of sugar or to act as an emulsifier (Glicksman *et al.,* 1973).

1.1.11 Pharmaceutical and cosmetic applications

Gums are used as a suspending and emulsifying or binding agents in pharmaceutical industries, it has been used in tablet manufacturing, where it functions as a binding agent or as a coating prior to sugar coating, sometimes in combination with other gums, *A. polyacantha* gum used to act as general health tonic as antidote for snake bite, and cure for venereal diseases. A preparation from the bark is used for general stomach disorders (Voget, 1995).

1.1.12 Paints and coating composition application

The hydrophilic colloids and modified cellulose find application in paint industry because of their stabilizing effect on paint emulsions, waxes and numerous others products. (Grady and Gamble, 1938) treated pigments with water soluble hydrocolloids such as gum Arabic to add controllable chemotropic properties to paints. The gum also finds application in coating composition developed non glare coating based on a water soluble dye dissolved in gum Arabic solutions.

Due to its adhesive properties gum have been used in the manufacturing of adhesives for postage stamps and also in the formulations of paints and inks. Gum may serve as a source of monosaccharide, as e.g. mesquite gum serve as a source of L-arabinose (51%) because of its easier hydrolysis, and availability of the gum in large quantities. The mesquite gum can be dialyzed by addition of ethanol (White, 1947 and Hudson, 1951), or alternatively, isolated by crystallization from methanol after removal of acidic oligosaccharides on ion exchange resin or precipitated by barium salts. Gums are widely used in textile industries to impart luster to certain materials (silk), as thickeners for colors and mordant in calico printing (Omer, 2004).

1.1.13 Physicochemical properties of *Acacia senegal* gum

The physical properties of the natural gum are most important in determining their commercial value and their use. These properties vary with gums different botanical source, and even substantial differences in gum from the same species when collected from plants growing under different climatic conditions or even when collected from the plant at different season of the year (Hirst *et al.*, 1958). The physical properties may also be affected by the age of the tree and treatment of the gum after collection such as washing, drying, sun bleaching and storage temperature.

1.1.13.1 Solubility

Gums can be classified into three categories with regard to their solubilities:

1. Entirely soluble gums: e.g. *A. senegal*, *A. seyal*.
2. Partially soluble gums: e.g. *Gatti* gum.
3. Insoluble gums: e.g. *Tragacanth* gum (Omer, 2004).

1.1.13.2 Colour

The colours of gums vary from water- white (colourless) through shades of yellow to black. The best grades of gum are almost colourless with slight traces of yellow; some possess pink likes (Siddig, 2003). On the other hand dark or even black gums sometimes occur e.g. mesquite gum. There are also the pale rose pinks, darker pink and yellowish gums. The pink colour is probably due to the presence of different quantities of tannin materials (Omer, 2004).

1.1.13.3 Shape

Natural gums are exuded in a variety of shapes and forms: usually the fragments are irregularly globular or tear globular or tear shaped. The best known being the tear or drop shape of various grades of gum Arabic. Other shapes are flakes or threat like ribbons with gum *tragacanth*. The surface is perfectly smooth when fresh but may become rough or crusty, covered with small cracks (Omer, 2004).

1.1.13.4 Moisture

The hardness of gum would be determined by moisture content. The moisture content of good quality gum does not exceed 15 and 10% for granular and spray dried material respectively (FAO, 1999). The moisture content is weight lost due to the evaporation of water (Person, 1970). It shows the hardness of the gum and hence variability of densities, the amount of densities, and the amount of the air entrapped during formation. (Omer 2004) recently, reported that the moisture content of *A. Senegal* gum to be around 12.1 %.

1.1.13.5 Ash

In a study of 800 authentic formulations of gum from *Acacia Senegal* var *senegal* collected from 32 different localities of the gum producing belt of the Sudan, showed that the type of the soil had no significant effect on the ash content of the gum (Karamalla *et al.,* 1998). (Anderson *et al.,* 1983) found that the value of ash content of commercial gum arabic to be 4.4%. Later, (Anderson *et al.*, 1991) reported 3.6% Ash content for Sudanese formulations. FAO (1999) reported that the ash content of gum Arabic did not exceed more than 4%.

1.1.13.6 Nitrogen and protein content

Gum arabic is a polymer with about 3% protein (Anderson *et al*., 1991). The protein fraction is responsible for the emulsification properties of the gum. The role of nitrogen and nitrogenous component in the structure, physicochemical properties and functionality of gum arabic was recently subjected to intensive investigation. Structurally the "Wattle blossom" model (Fincher *et al*., 1983) depends on the nitrogenous component (Figure 1.5).

An adsorbed layer of protein at oil /water interface provides the primary stabilizing structure in many food colloids (Dickinson, 1994). According to (Dickinson et al., 1988), the variability in the emulsifying properties of gums from different Acacia species is dependent not only on the total protein (on polypeptide content) but also on the distribution of the protein-peptide between the low and high molecular weight fractions and on the molecular accessibility of the protein peptide for absorption according to (Dickinson, 1994). The United States pharmacopoeia and European Union specification defined the minimum standards of the protein content for good quality gum arabic as 3%. (Anderson, 1986) found that the average nitrogen content for commercial *Acacia senegal Acacia senegal* gum formulations to be 0.37%.

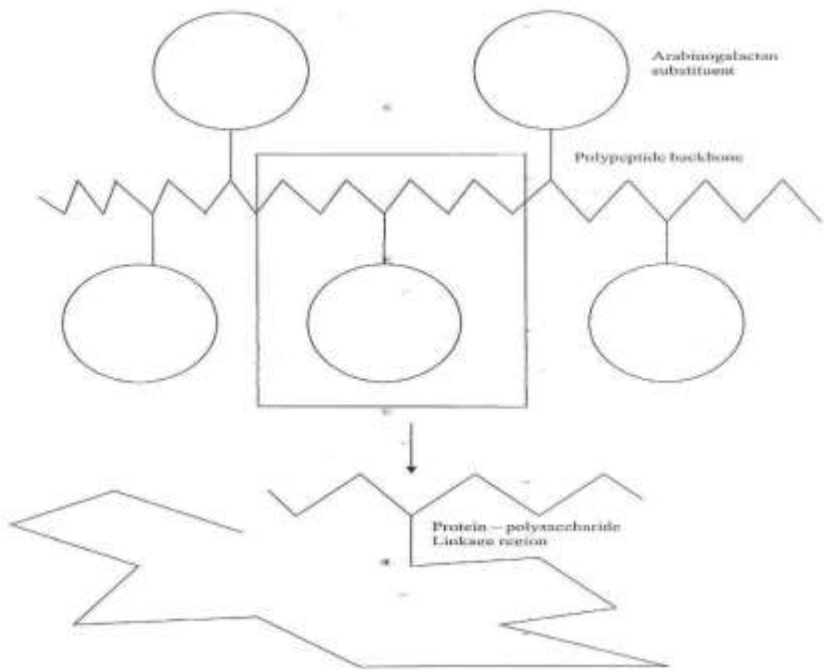

Figure (1.5): The Wattle–Blossom model for *Acacia Senegal* gum as proposed by (Fincher *et al.*, 1983)

Investigations of protein in *Acacia Senegal* gum have been carried out by (Akiyama *et al.*, 1984), they reported that gum Arabic contained 2.0% protein and they established that amino acid of gum arabic is rich in hydroxyproline and serine while alanine content is low. (Anderson *et al.*, 1985) described gum arabic as a proteinaceous polysaccharide with a protein content ranging from 1.5 to 3.0%. And concluded that the variation was mainly due to different localities. They reported the value of 0.23- 0.58% nitrogen for commercial formulations. Osman (1998) reported 0.33- 0.36% nitrogen (2.14-2.16% protein for *Acacia Senegal var Senegal* gum) and (Jurasak *et* *al.,* 1993) in a chemo metric study for different Acacia species reported 0.27-038% nitrogen for commercial samples of Gum arabic from Sudan. Awad Elkarium (1994) reported that the average nitrogen contents of different commercial grades are around 0.28%. (Karamalla *et al.,* 1998) reported that the average nitrogen content of different commercial grades is around 0.33%.

1.1.13.7 Specific optical rotation

The optical activity of organic molecules (saccharides and carbohydrates) is related to their structure and a characteristic property of the substance (Stevens *et al.,* 1987). The gum of natural origin, e.g. *A. Senegal var Senegal* gum, has the property of rotating the plane of the polarized light. The direction of the rotation, as well as the magnitude is considered as a diagnostic parameter (Biswas *et al.,* 2000). *Acacia Senegal var Senegal* gum gives a negative optical rotation ranging between -20° to -34°. The Specific optical rotation is used to differentiate between *A. Senegal var Senegal* and other botanically related *Acacia* gums. (Anderson and Stoddart, 1966b) reported that the specific rotation for electrodialysed *Acacia Senegal var Senegal* gum was -31°, 5°. Pure gum from *A. senegal var Senegal* has specific optical rotation of -27° to 30° (Tioback, 1922). Certain variation in the degree of the optical rotation (-27° to -32°) has been noticed by (Anderson, 1968) (Karamalla *et al.* 1998)

found that the mean of the specific optical rotation of commercial *A. Senegal var Senegal* gum was -30.54°. The optical rotation is not affected by both auto hydrolysis and variation, while mild acidic hydrolysis has a significant effect on optical rotation (Barron, 1991).

1.1.13.8 Acidity and pH measurements

Comparative studies Show that gum from *Acacia Senegal var Senegal* has higher content of rhamnose (12-14%) and lower arabinose content (24-29%) compared to rhamnose and arabinose of other *Acacias* (Karamalla *et al.,* 1998).

The main content of gum arabic is arabian (acid substance), and when it was decomposed it gave arabinose (Karamalla *et al.,* 1998). Thus gum arabic is called arabic acid therefore, the gum solution is slightly acidic with pH 4.66, as reported by (Karamalla *et al.,* 1998).

1.1.14.9 Viscosity

The viscosity of liquid is its resistance, to shearing, to stirring or flow through a capillary tube (Bancraft, 1932).

Studies of flow of gum solutions play an important role in identification and characterization of their molecular structure. Since viscosity involves the size and the shape of the macromolecule, it was considered as one of the most important analytical and commercial parameter (Anderson *et al.,* 1969). The viscosity of a solution may have a complicated variation with composition, due to the possibility of hydrogen bonding among the solute and solvent molecules (Pimentel e*t al.,* 1960). More hydroxyl groups makes high viscosities, because a network of hydrogen bonds is formed between the molecules, this network extends throughout the liquid, thus making flow difficult. The viscosity of gum solutions is inversely proportional to temperature. They also found that the viscosity of gum Arabic solutions changes with pH, but they found a maximum viscosity at pH 6-7. Viscosity can be explained in different terms such as relative viscosity, specific viscosity, reduced viscosity, inherent viscosity and intrinsic; it is also represented as kinematics or dynamic viscosity. It can be used to determine the molecular weight of *Acacia Senegal var Senegal* gum (Anderson and Dea, 1971). Karamalla, (1999) showed that wide variations in values for intrinsic viscosity and viscosity average were obtained indicating that such parameters cannot be used as qualifying Indices.

1.1.13.10 Equivalent weight and Uronic Acid

Titrable acidity represents the acid equivalent weight of the gum, from which the uronic acid content could be determined (Karamalla, 1965, Anderson *et al.*, 1983). (Karamalla *et al.*, 1998), assessed the potentials of new parameters such as equivalent weight and total uronic acid content as additional qualifying indices. They found that the mean values for gum of *Acacia Senegal var Senegal* for the equivalent weight was 1436 and for uronic acid was 13.71%.

1.1.13.11 Molecular weight

The molecular weight of the polymers can be determined from physical measurement or by application of chemical methods. The applications of chemical methods require that the structure of the polymer should contain well known number of functional groups per molecule and they invariably occur as end groups. The end group analysis method gives an approximately number of molecules in a given weight of sample; they yield the average number of molecules for polymeric materials. This method becomes insensitive at high molecular weight, as the fraction of end groups becomes too small to be measured with precision (Meyer, 1971). This is due to the fact that fraudulent 14 sources of the end groups not considered in the assumed reaction mechanism steadily become consequential as the molecular weight increases and the number of end groups diminishes to such an extent their quantities determination is not feasible. Those reactions confine frequent application of chemical methods to condensation polymers with average molecular weight seldom exceeding 2.5×10^3 (Flory, 1953). Physical methods frequently used for establishing polymer molecular weight are osmometry, polymer viscosity, measurement of coefficient of diffusion, ultracentrifugation and light scattering. One of the most recent advanced methods is light scattering (LS), which provides an absolute method for polymer molecular weight and size measurement. LS are rapid, accurate and requires small amount of sample. The molecular weight of gums varies greatly in values due to gum heterogeneity as well as variation in techniques used to separate, purify and determine the molecular weight. A 3.0 × 10^3 was reported by (Saverbon 1953) using centrifugal method. Using the light scattering technique gave higher values reported a Mw =1.0×106, up to Mw = 5.8 × 105 and (Fenyo, 1988) reported a range of 4.0 × 106 to 2.2 × 106. Recently GPC coupled on line to multi angle laser light scattering (MALLS) has been demonstrated to be a very powerful method for characterizing highlypolydisperse polymer systems and the molecular weight of *A. Senegal var Senegal* gum was found to be equivalent to 5.4 × 105 (Picton, 2000).

1.2 Foam fractionation process

Foam fractionation is a separation technique in which surface-active solutes are either concentrated from a dilute solution or separated from a mixture by preferential adsorption at a gas liquid interface created by sparking an inert gas through the solution (Narsimhan, 2000). These gas bubbles entrain the surfactant solution and form stable foam with a large gas liquid interfacial area. As the foam moves through the column, the surfactant solution tends to drain due to gravity and capillary forces. This results in a decrease in the amount of liquid in the foam. The reduction in the entrained liquid is first associated with the bubbles forming the closest spherical packing, after which they will deform to a dodecahedral shape and then possibly coalesce. Consequently, there is an increase in the gas liquid interfacial area per unit volume of the liquid (Narsimhan, 2000). The surfactant tends to adsorb preferentially at the gas liquid interface. At the top of the column, the foam is sent to a foam breaker where the foam is broken either mechanically or chemically. This results in either enrichment or concentration of more surface-active protein because of the recovery of adsorbed protein from the gas liquid interface into the bulk entrained liquid (Narsimhan, 2000). In the case of a dilute solution of a single protein, the extent of enrichment would depend upon the relative amount of adsorbed protein compared to that in the bulk entrained liquid. In the case of a mixture of proteins in solution, the separation of a protein from the mixture would depend upon the extent of preferential adsorption of that protein at the gas liquid interface (Narsimhan, 2000). Since the adsorption isotherm usually leads to a much higher proportion of adsorbed protein at very low bulk concentrations, foam concentration is very effective for extremely dilute solutions. Because of the presence of hydrophilic and hydrophobic functional groups, proteins are surface active. Therefore, foam-based separations are viable for separation of protein solutions. Foam based separation has been applied to various proteins and enzymes (Narsimhan, 2000).

1.2.1 Performance characteristics of foam fractionation

To evaluate the performance of the separation the following criteria are considered. Enrichment (Ef) is defined as the ratio of foam concentration to that of initial feed.

$$\mathbf{Ef} = \frac{\textbf{Concentration of protein in the foam}}{\textbf{Concentration of protein in the initial solution}}$$

On the other hand, the recovery of protein ratio (PR) is the fraction of feed protein recovered in the foam. It determines the efficiency of the process and is given by:

$$\mathbf{PR} = \frac{\mathbf{k_d}}{(\mathbf{K_d} + \mathbf{V_r} / \mathbf{V_f})} \times \mathbf{100}$$

Where K_d is the distribution coefficient, defined as the ratio of protein concentration in foam to that of the residual solution, V_r and V_f are the respective volumes of the residue and foam after separation.

The residual ratio (RR) is also considered as a measure of the residual concentration with respect to the original feed concentration:

RR = Concentration of protein in the residual solution Concentration of protein in the initial solution:

$$PR = \frac{\textbf{Concentration of protein in the residual solution}}{\textbf{Concentration of protein in the initial solution}}$$

The volume of the foam produced is also a measure of the performance as this relates to the loss of liquid from the initial solution

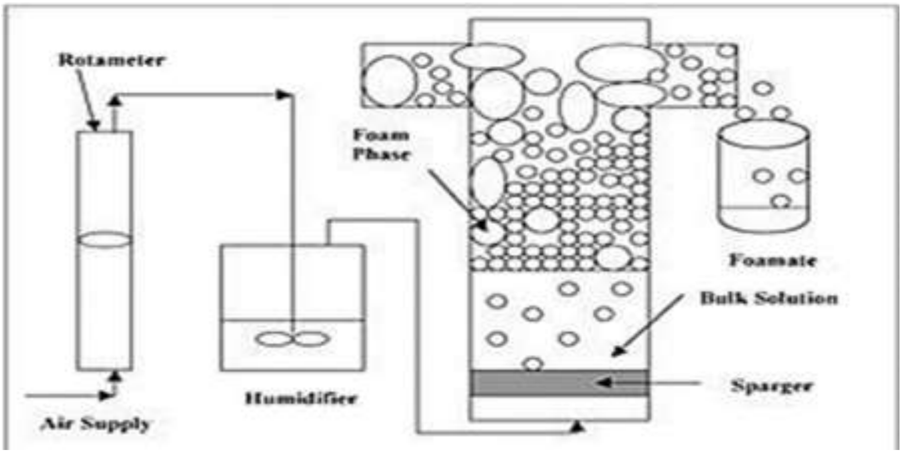

Figure (1.6): Diagram of laboratory batch foam – apparatus (Narsimhan, 2000)

Figure (1.6a): Foam fractionation process when gas start passing

Figure (1.6b): Foam fractionation process after gas passing

1.3 Objectives

The objectives of this study is:

- To purified *Acacia senegal* gum by foaming.
- To compare the physiochemical properties of gum foam and processed gum (ElNsr) sample.

Chapter Two

Materials and Methods

Chapter two
Materials and methods

2.1 Materials

Crude *Acacia senegal var senegal* gum was collected from Western Sudan in July, 2016. Processed *Acacia Senegal* (AlNasr) was bought from the local market Omdurman-Sudan.

2.2 Sample preparation and treatment

The crude gum was ground by using mortar and pestle to fine powder and kept in clean and dry plastic containers.

2.3 Chemicals and materials

- Distilled water.
- Kjeldahl tablet
- Sulphuric acid
- Hydrochloric acid
- Boric acid
- Methyl red
- Phenolphthalein

2.4 Apparatus and Instruments

- Porcelain.
- Beaker
- Measuring cylinder
- Weight bottle
- Sensitive balance
- Hot air oven
- Thermostatic water bath
- pH meter.

- Viscometer
- Polarimeter.
- Mortar and pestle.
- HPLC-15950, SHIMADZU.

2.5 Methods analysis

2.5.1 Foaming purification of *Acacia Senegal var Senegal* gum

30 g of *Acacia Senegal* gum was dissolved in 300 ml of distilled water, the solution was then blown with air using an aerating pump till foaming stops. The foam was collected on a Petri dish and left to dry at room temperature. After that the yield was taken to determine the physicochemical properties.

Figure (2.1): *Acacia senegal var senegal* gum

2.5.2 Determination of moisture content

0.5 gram from each fraction were accurately weighed in glass dishes and heated at 105°C in oven for 6 hours to a the initial weight from the following relationship:

Moisture % = $(w_2/w_1) \times 100$

Where w_2 = weight of sample after heating

w_1 = weight of sample before heating

2.5.3 Determination of ash content

The total ash of test sample of each sample 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 grams 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 weighed (w_3). Then the total ash % s calculated from the following relation.

Ash % = (weight of Ash in grams / weight of sample in grams) × 100

2.5.4 Determination of total nitrogen and protein

0.5 gram of each sample (in duplicate) were weighed, and transferred to Kjeldahl digestion flasks, one Kjeldahl tablet (copper sulphate – potassium sulphate catalyst) was added to each. Then 10 ml of concentrated (nitrogen free) sulphuric acid were added. The flask was then mounted in the digestion heating system which was heated at 245°C, and capped with aerated manifold. The solution was then heated at the above temperature until a clear pale yellowish – green color was obtained which indicates the completion of the digestion. The flasks were then allowed to cool to room temperature, this content was quantitatively transferred to Kjeldahl distillation apparatus, and the steam distillation of the ammonia was commenced the released ammonia was observed in 25 ml of 2% was commenced. The released ammonia was observed in 25 ml of 2% boric acid. Back titration of the generated borate was then carried out versus, 0.02 M, HCl using methyl red as indicator. Blank set of experiment was carried in the same way.

The nitrogen content percentage of the samples was calculated from the relation;

N% = 14.01 × M × (volume of titrant – volume of blank) / weight of sample (grams)

Where M is the molarity of HCl.

Protein content of sample was calculated using nitrogen

Conversion factor (NCF) of 6.7 as follows

Protein % = N% × 6.7

2.5.5 Determination of intrinsic viscosity

An aqueous solution (1%) was prepared from each sample of the whole gum sample and the fractions and the rate of flow recorded for successive dilutions using a capillary viscometer (shott Gerate type 50 120/11) in a constant temperature bath at 30°C. the intrinsic viscosity was obtained by extrapolation of reduced viscosity against concentration back to zero concentration.

2.5.6 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 dissolve, and then filtered through watmann No.42 filter paper. Then loaded into the sample holder without trapping air bubbles. Optical rotation was measured using digital polarimeter equipped with 250 mm tube filled with the test solution t room temperature. Specific rotation was calculated by the following relation;

$[\alpha]^{t}_{D} = \alpha \bullet 100/Cl$

Where α is the observed rotation of the solution in circular degrees, C is the grams of substance per 1 ml of solution, and l is the length of the solution in decimeter.

2.5.7 Determination of pH value

The pH was determined for 1% aqueous solution of each sample, using a pH-Meter- Corning 555 at room temperature.

2.5.8 Determination of sugar composition

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

Before analysis of the gum samples, calibration curves of these sugars were prepared. Stock concentrations of 5 mg cm^{-3} for each sugar were made up by hydrating in 70/30 acetonitrile/buffer for 2 hours. Dilutions of the stock solution achieved 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 an average of 3 replicates for each level was used to ensure accuracy. This calibration allowed the determination of sugar content for the gum samples. The concentration of each sugar was calculated by peak height and expressed as a % of the total sugar content.

Chapter Three

Results and Discussion

Chapter three

Results and discussion

Table (3.1): Physicochemical properties of the *Acacia Senegal var Senegal* gum foam purified and processed gum (ELNasr)

Physicochemical properties	Foam purified gum	ElNasr processed gum
Moisture content (%)	10.74	10.86
Ash content (%)	3.84	3.55
Nitrogen content (%)	0.25	0.27
Protein content (%)	2.35	2.30
Intrinsic viscosity (cm^3g^{-1})	14.5	14.8
Specific optical rotation (°)	-33.61	-30.75
pH value	4.9	4.5

3.1 Moisture content

Moisture content of the gum determines the hardness of the gum and hence the variability of densities and the amount of air entrapped during nodule formation. It can be determined by measuring the weight lost after evaporation of water. The moisture content values of *Acacia Senegal var Senegal* foam gum and processed gum (ELNasr) samples in Table (3.1) were (10.74% and 10.86%) which showed similarity of moisture content values (11.11%, 11.01% and 11.01%) that studied by (Omer, 2006; Abdelrahman, 2008; Younes, 2009), but it less than that value (12%) reported by (Fathia *et al*., 2016).

3.2 Ash content

The ash content indicates the presence of inorganic elements existing in salt form. The ash content values of *Acacia Senegal var Senegal* foam gum and processed gum (ELNasr) samples in Table (3.1) were (3.84% and 3.55%) was more than that reported by (Omer, 2006; Abdelrahman, 2008) (3.27% and 3.32%), but it less than that values (4.89% and 4.30%) studied by (Younes, 2009; Fathia *et al*., 2016) as respectively.

3.3 Nitrogen content

The nitrogen content values of *Acacia Senegal var Senegal* foam gum and processed gum (ELNasr) samples in Table (3.1) were (0.25% and 0.27%) was less than that reported by (Omer, 2006; Abdelrahman, 2008; Younes, 2009) (0.37%, 0.35% and 0.35%) as respectively, but it showed similarity to that studied by (Fathia *et al*., 2016) (0.28%).

3.4 Protein

The protein content values of *Acacia Senegal var Senegal* foam gum and processed gum (ELNasr) samples in Table (3.1) were (2.35% and 2.30%) which showed similarity to that studied by (Omer, 2006; Abdelrahman, 2008; Younes, 2009) (2.3%, 2.4% and 2.3%) as respectively, but it was to that reported by (Fathia *et al*., 2016) (1.85%).

3.5 Viscosity

The viscosity of a liquid is its resistance to shearing, to stirring or to flow through a capillary tube. Viscosity was considered as one of the most important analytical and commercial parameters, since it is a factor involving the size and the shape of the macro – molecule. Viscosity can be presented in many terms such as relative viscosity, specific viscosity, reduced viscosity, inherent viscosity and intrinsic viscosity. It is also presented as kinematic or dynamic viscosity. Solutions viscosities are useful in understanding the behavior of some polymers. The viscosity values of *Acacia Senegal var Senegal* foam gum and processed gum (ELNasr) samples in Table (3.1) were (14.5 cm^3g^{-1} and 14.8 cm^3g^{-1}) which showed similarity to that reported by (Omer, 2006; Abdelrahman, 2008) were 14.6 cm^3g^{-1} and 14.7

cm^3g^{-1} respectively, but it was more than that studied by (Fathia *et al*., 2016) (10 cm^3g^{-1}). Furthermore, it was less than that reported by (Younes, 2009) (18.9 cm^3g^{-1}).

3.6 Specific optical rotation

The optical activity of organic molecules (saccharrides and carbohydrates) is related to their structure and is a characteristics property of the substance, and thus the specific rotation is considered as the most important criterion of purity and identity of any type of gum. The specific optical rotation values of *Acacia Senegal var Senegal* foam gum and processed gum (ELNasr) samples in Table (3.1) were (-33.61° and -30.75°) which showed similarity to that reported by (Omer, 2006; Abdelrahman, 2008; Younes, 2009) (-32°, -31.5° and -30°) as respectively.

3.7 pH

The hydrogen ion concentration plays great importance in the chemistry and industry of the gums. The change in the concentration of hydrogen ion may determine the solubility of gum and the precipitation of protein, therefore functional properties of a gum may be affected by change in pH for example viscosity and emulsifying power. Crude gum Arabic is slightly acidic because of the presence of few free carboxyl groups of its constituent acidic residues, D-glucuronic acid and its 4-O-methy1 derivatives. The pH values of *Acacia Senegal var Senegal* foam gum and processed gum (ELNasr) samples in Table (3.1) were (4.9 and 4.5) which were similar to that reported by (Younes, 2009; Fathia *et al*., 2016) (4.8 and 4.53) as respectively.

Table (3.2): Sugar content of the *Acacia senegal var senegal* gum foam purified and processed gum (ELNasr)

Type of sugars	Foam purified gum	ElNasr processed gum
Arabinose (%)	33.0	30.5
Rhamnose (%)	13.5	11.5
Galactose (%)	37.0	35.0
Total sugar contents (%)	83.5	77

3.8 Determination of sugar contents

The sugar composition of *Acacia senegal var senegal* foam purified gum and processed gum (ELNasr) samples in Table (3.2) were measured using HPLC, the results showed that the foam purified gum consist of arabinose, rhamnose and galactose of *Acacia senegal var senegal* foam gum were (33%, 13.5% and 37%) respectively, the total sugar contents was (83.5%) it was more than that studied by (Fathia *et al*., 2016) (79%), but the sugar contents, arabinose, rhamnose and galactose of processed gum (ELNasr) were similar compositions was obtained, the total sugar contents was (77%) it was less than that studied by (Fathia *et al*., 2016) (79%).

3.9 Conclusion

From all the data obtained it is clear that the *Acacia senegal var senegal* foam purified gum and processed gum (ELNasr) samples investigated are similar, but the solubility of processed gum (ElNsr) was more than foam purified gum. The results were similar and difference with the previous studies.

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