SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF GRADUATE STUDIES

Successive Ethanol Fractionation of the Gum from *Acacia seyal* Var.*seyal* And Their Physio-chemical Properties

التجزئة المتتابعة بالإيثانول لصمغ الاكيشياسيال صنف السيال وخصائصها الفيزيوكيميائية

A Thesis Submitted in Partial Fulfillment for the Requirement ofof

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By

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إستهلال

(قالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا

إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ)

سورة البقرة (الآية 32)

DEDICATION

To my parents, my husband, my brothers and sisters

With love

ACKNOWLEDGEMENT

First of all I would like to thank Allah Almighty, for giving me strength to complete this work.

I would like to express my deepest and sincerest gratitude to my supervisor, Dr. Mohamed El Mubarak Osman , for his guidance and understanding. His wide knowledge and logical way of thinking have been of great value to succeed this work.

I would also like to express thanks and gratitude to the staff of Nopec natural gums factory and (QC Lab) team for their technical support, and I send my gratitude to my friends and colleagues for their help and guidance.

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Abstract

*Acacia Seyal*var*Seyal* gum was fractionated by precipitation using ethanol, the total percent yield was 87%.The phesio-chemical properties were determined for the gum and its fractions .

The results showedthat, the moisture was (9.16)% for Talha sample and in ranges from (4.63-6.41)% for fractions , the total ash of Talha gum and its fractions were between (3.18-3.62)% , the results of optical rotation were (45-50) , the intrinsic viscosity values were (9.82-13.30)cm^3/g , PH (4.62-5.95) , the protein content was calculated using coefficient factor 6.6 , it found in the rang (1.32-2.72)% .

المستخلص

تمت تجزئة صمغ الإكيشيا سيال صنف السيال بإستخدام الترسيب التجزيئي بالإيثانول والحصول علي نسة كلية87% .تم تقدير الخصائص الفيزيو- كيميائية للصمغ وأجزائة.

أظهرت النتائج ان الرطوبة لصمغ الطلح (9.16) %, ولأجزائة في المدي (4.63-6.41)%, محتوي الرماد بين (3.18-3.62)%, الدوران الضوئي في المدي (45-50), اللزوجه الذاتيه بين (9.82-13.3)سم^3/جم, درجة الحموضة بين(4.62-5.95) , تم حساب محتوي البروتين باستخدام ثابت 6.6 وكانت النتائج في المدي (1.32-2.72)% .

Chapter one

Introduction

1. Introduction and literature

1.1 Definition of Gums

Gums are polysaccharides with fractions of proteins, can be classified as salts of an uranic acid which on hydrolysis give a mixture of sugars which vary in composition.[1]

1.1.1 Formation of gums

The most common theories suggest that gums are formed as a natural phenomenon of the plant in which internal plant tissues disintegrate through a process called gummosis. This in turn form cavities, which exudes transformed carbohydrates called gums. Secondly it is caused as a result of injury to the bark or stem. Thirdly, some others attribute to fungi and bacteria attack. Majority of the gums are exuded from the stem. Only few gums are obtained from roots, leaves and other parts of the plant. These gums on heating decompose completely without melting.large number of families notable among them are *Leguminosae* and Sterculiaceae. Other important gum yielding families are Anacardiaceae, *Combretaceae*, *Meliaceae*, *Rosaceae* and *Rutaceae*.[1]

1.1.2 Gum Arabic

Gum Arabic is the oldest and best known of all tree gum exudates and has been used as an article of commerce for over 5000 years. It is the exudation from certain *Acacia* trees, which occurs in a wide belt of semi-arid land stretching across sub-Saharan Africa. There are more than 1200 species of *Acacia*, which has been classified into three major groups: *subgenous Acacia (Gummiferae)* containing 120-130 species: *Subgenous Aculeiferum* (*Vulgares*) with 180-190 species: and subgenous *Phyllodinae* with more than 900 species. In Sudan more than thirty distinct *Acacia* species are found. Around twenty species are gums producing sources.[2]

Sudan is the world's largest producer of gum Arabic, with production reaching 40 000 tons in 1996. According to the Journal Official of France, the Food Chemical Codex, the US Pharmacopoeia and the Birtish Pharmacopeia gum Arabic is defined as the dried gummy exudation obtained from the stems and branches of *A. senegal*(L) *Willdenow* or closely related species of *Acacia*, family *leguminosae*. While the USA Food and Drug Administration defined it as the gum originating from 'various species of the *Acacia*. Gum Arabic has been evaluated by Joint Expert Committee for Food Additives (JECFA) on several occasions, namely in 1978,1982, 1986,1990 and 1995. Attempts have been made recently, by the Food and Agriculture Organization (FAO), Joint Committee for Food Additives (1996) and the European Union (EU,1996), to achieve a uniform definition. '*Acacia* gum(EU)/Gum Arabic (JECFA), is a dried exudation obtained from the stems and branches of natural strains of *Acacia Senegal* (L) or closely related species of *Acacia* (family *Leguminosae*)'. It consist mainly of high molecular mass polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid'. Toxicological monographs were prepared. And the definition refers to *A.senegal* and *A.seyal*. Other *Acacia* species not included.[3]

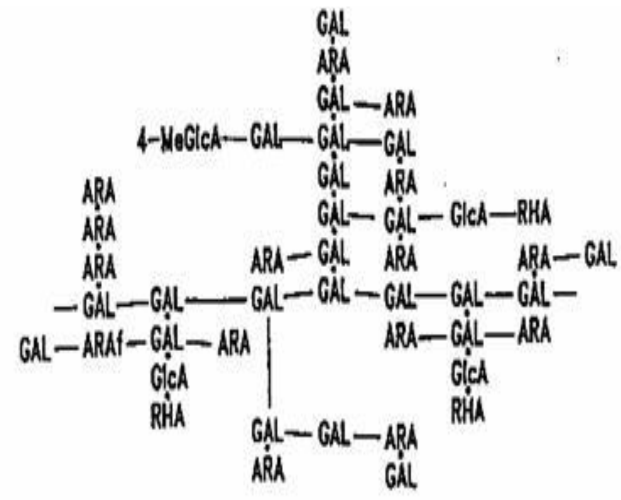
1.1.3 The occurrence and origin of gum in plants

Products from plants such as gums, resins and several other materials were classified as minor forest products. Gums and resins, presently, form an important, and widely, used group of non-wood forest products. Stress conditions such as heat, poorness of soil, drought and other hostile environmental conditions promote the production of gums. Gums are produced by a large number of plant families. The market of gums has declined over the years due to several reasons. The main reasons of this decline are the unscientific and brutal methods of tapping, over exploitation leading to the death of the tapped trees and erratic supply of these products in the market (FAO, 2007).

Commercial tapping of gum is done by peeling or making deep cuts on the stems. On account of injurious and wasteful tapping and overexploitation the populations of gum producing plants have markedly declined.(2)

1.1.4 Chemical composition and structure of gums

The uncrystallizable properties of gums, rendering their purification difficult and uncertain, that has been the cause of their chemistry being uncertain for so many years. Nitrogen is not regarded as an essential constituent, considered as impurity. Natural gums (gums obtained from plants) are hydrophilic carbohydrate polymers of high molecular weights, generally composed of monosaccharide units joined by glucocidic bonds. Until the last century, gums were simply considered as carbohydrate, substances similar to sugar, starch and cellulose, and the formula $C_{12}H_{22}O11$ or $(C_6H_{10}O5)n$ assigned to them. **(Figure 1.1)**, gums were found not to be only carbohydrates but complex acids built up of a nucleus acid combined with several of the less common sugars **(Figure 1.2)**. The chemical compositions vary slightly with source, climate, season, age of the tree, etc. The chemical properties of the gum of a plant, taken at different seasons, may not always be the same due to the variation in the proportion of the sugars unit or an nucleus acid to form the natural complex gum acid and variation in the proportions of the complex acids in the mixture that constitute the natural gum. On hydrolysis with dilute mineral acids, the gums form various sugars such as the pentose's, arabinose, xylose, and the hexose, galactose. Not all the gum is converted into sugars but usually about 20% resists treatment. Gums, therefore, consist of glucosidal acids of high molecular weight. In most gums the acids combined with calcium, potassium or magnesium in the form of salts, but in some gums present in the free state . Basic gum substance believed to be as an individual chemical compound, is "arabin" from gum Arabic. Gums were complex acids built up of Arabic acid[5].



GAL = Galactose, ARA = Arabinose, GlcA = Glucuronic Acid

RHA = Rhamnose, 4-MeGlcA = 4-O-methylglucuronic Acid

Figure (1.1): The structure of gum Arabic[16]

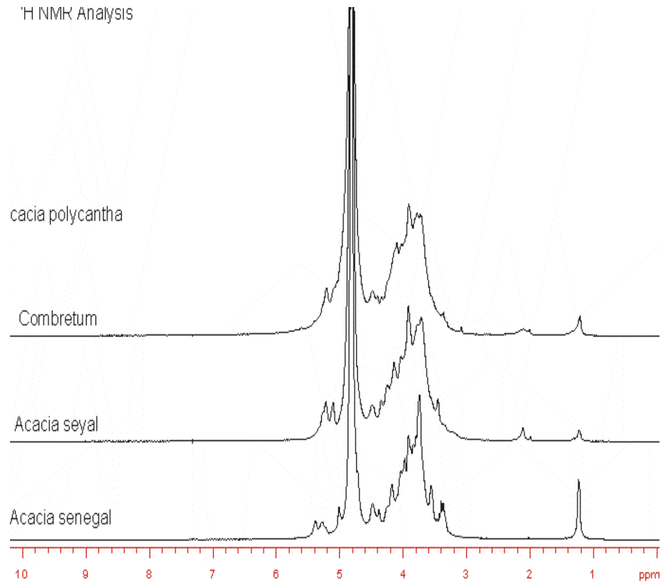


Figure (1.2): 1H-NMR structure of *Acacia senegal*, *Acacia seyalCombretum and Acacia polycantha*[5]

1.1.5 Fractionation of Gums

Fractionation is one of the most important methods of analysis; the techniques for fractionating poly dispersed polymer are divided in to preparative and analytical methods. (4)

1.1.5.1 Preparative fractionation

This method is the simplest; it involves the addition of precipitants to an aqueous solution of fractions having different solubility's. Co precipitation may occur . Different fractions of different solubility's of the gum can be collected . The gum can also be fractionated by careful precipitation with near saturated sodium sulphate solution and complex formation using complexing agents e.g. cupric acetate.

The fractions can be obtained by fractional precipitation of gums using hydrophobic affinity chromatography. (4)

1.1.5.2 Analytical fractionation

Analytical fractionation yields information about the distribution of molecular masses in a polymer by separating it into discrete Sephadex or Biogel. Gum arabic is a highly heterogeneous material, but was separated into three major fractions by hydrophobic affinity chromatography. Gum Arabic was fractionated by hydrophobic interaction chromatography to yield four fractions, all of which had a similar carbohydrate composition, but differed in their protein content, amino acid composition and molecular mass distribution. [5]

1.1.5.3 Gel permeation chromatography

(GPC) is widely used to determine the molecular mass distribution of macromolecules. GPC coupled on line with absolute molecular weight determining device such as a laser light scattering photometer and a concentration sensitive detector such as refractive index or ultraviolet are currently the best available techniques for the quick and absolute determination of polymers molecular weights and their distribution.[5]

1.1.5.4 Enzymatic fractionation

The degradation of gum polysaccharides by bacteria was studied by measuring viscosity, pH and short chain fatty acids (SCFA).Guar gum, locust been gum and gum tragacanth were completely degraded with their viscosities diminished and SCFA produced. The extent of degradation and Pattern of end products may be related to the chemical linkages and the tertiary structure of the molecule. Fractionation studies showed that the degree of hydrolysis of polysaccharides (gum Arabic) to reducing sugars depends upon the strain of bacteria.[6]

1.1.5.5 Immunological fractionation

Immunological fractionation is useful tools, which can be used for separation and qualitative of polysaccharide of gums. The site of interaction is the Arabino-galactan protein (AGP). This anti AGP interaction showed fractionation to contain epitopes characteristic of an arabinogalactan protein complex. [6]

1.1.5.6 Electrophoresis

Electrophoresis may be defined as the migration of charged species in one direction in solution under influence of an electric field. This method offers some criterion of hetero-homogeneity. However, the electrophoresis technique employing polysaccharides solution in molar potassium hydroxide is capable of resolving certain mixture of structurally different polysaccharides. This technique might be applicable to characterize and fractionate a limited class of polysaccharides. The main advantages of such methods are, in assessing polymer homogeneity, its speed and also the fact that very little material is used.[4]

1.1.6 *Acacia seyal*var*seyal* (talha)

Subgenus: *Acacia*

Series: *Gummiferae*

Tree 3-17m high.spread irregular. Bark powdery.

Habitat: dark cracking clay. Found often on higher slopes of the rivers and valleys in addition to the hard clay plains of Central Sudan. Also in clay depression areas where water is accumulating. It is distributed all over the Sudan. More than 70% of the Sudanese gum production comes from *Acacia seyal*, which is prevalent in the southwestern part of the country and in the Nile region. These trees are not tapped and only natural exudates are collected and sold as talha gum. The gum is inferior to hashab and is reported to be structurally different to *A.senegal* gum .With specific optical rotation of +41 to +61, a nitrogen content of 0.14% (W/V) and a tannin content of 1.9%. As such, gum talha did not satisfy the 1990 specification for food grade gum Arabic[2]

1.1.6.1 Clasification of TalhaGum

Acacia seyal is less valued than *Acacia Senegal* due to its poor emulsification properties therefore, it is considered to be in the second class and an inferior quality gum. [3]

1.1.7 Uses of 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.[6]

1.1.7.1 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. [6]

1.1.7.2 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. [7]

1.1.7.3 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. Gamble and Grady (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 Horne *et al*. (1953) developed non glare coating based on a water soluble dye dissolved in gum Arabic solutions.[8]

1.1.7.4 Textiles application:-

Gums can be using in the melting yarn chips process. They added to make the yarn stronger and increase its tensile strength. These days, many textile manufacturers use a modified starch mixed with Gum Arabic.[8]

1.1.7.5 Gum Prints application

Gums have been combined with a sensitizer and a soluble pigment, applied to paper, and exposed through a negative under a powerful light source. This can produce beautiful prints only surpassed by adding further layers of Gum pigments It is also possible to print colour separated black-white negatives to produce gorgeous true colour prints. [8]

1.1.7.6 Water Colours application

The essential ingredients in water colours are pigments, a binding agent (usually Gum Arabic), and water. When combined these three components create transparent water colour. Gum Arabic acts as the binder for both water colour and paints. Pigments are ground up and a liquid Gum Arabic solution is added to produce paint that is more opaque and which imparts a dusty quality to the surface. Gum Arabicis resoluble once it has dried; therefore it can be stored in cakes. Occasionally oxgall (a wetting agent) is added to water colour to aid the even pigment. [9]

1.1.7.7 Petroleum and gas industry

Gum is used as a component in drilling fluids, removing calcareous deposits, acidizing wells and secondary recovery of oils[17]

1.1.7.8 Environmental Protection and Soil Improvement:-

Talha gum plants are environmental protection and soil improvement important as they:

1. Fix nitrogen into the soil
2. It reduces desertification
3. It enhances soil moisture conservation
4. It reduces soil erosion

Any meaningful a forestation programme of Sudan and Sahel (Semiarid) Zones should prominently feature the use of Gum[8]

1.1.7.9 Other industrial uses

Due to its adhesive properties gum have been used in them an ufacturing of adhesives for postage stamps and also in the formulations of paints and inks. Gum may serve as a source of monosaccharide. Gums are widely used in textile industries to impart luster to certain materials (silk), as thickeners for colors and mordant in calico printing [4]

1.1.8 Physiochemical properties of gums

Physical properties of gum are of prime importance in determining their uses and commercial value. The gum, from the same species when collected from the plants growing under different climatic conditions or collected from the same plant at different seasons of the year, differs from one to another. The age of the gum when collected, may affect some of its physical properties. Storage temperatures and treatment of the gum after collection e.g. drying and bleaching in the sun also affect the physical properties of gums. Color and viscosity are probably the two most important factors in assessing the value of a gum, but good solubility is also important.(Table 1.1) [4]

Table (1.1): Chemical properties of Gum Arabic *(A.sengal)* and Talha Gum *(A.sayal)*..[14,15,19]

Characteristics	Identification
Total ash	4%
Acid insoluble ash	0.5%
Acid insoluble matter	1%
Arsenic	Not more than 10mg/l (or <= 10ppm)
Lead	Not more than 5mg/l (or 3 ppm)
Starch or dextrin	No reddish or bluish colour should be produced when a boiled solution (1 in 50 solution) is tested with iodine

1.1.8.1 Solubility

Gums are either water soluble or absorb water and swell up or disperse in cold water to give a viscous solution or gel, They can be classified into three categories with regard to their water solubility:-

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

Gums are generally insoluble in oils or organic solvents such as Hydrocarbons, ether or alcohols.[5]

1.1.8.2Smell, Taste and Toxicity

True gums are generally scentless and tasteless but some are slightly sweet according to botanical origin. Gum Arabic occurs as non-toxic, tasteless and odorless material.[5]

1.1.8.3Colour

Colour is mainly due to the presence of impurities of some form or other, but the coloring matter in gums has been little investigated. Colour is of great importance in the commercial valuation of gums, a strong preference being always shown for those that are light colored The colour of gums verity, depending on the climate.[13]

1.1.8.4Moisture contents

Moisture content is the measure of the weight lost due to the evaporation of water. It is helps to determine the dry weight of the gum. [9]

For granular Gum Arabic, it is not more than 15 % (105C^o, 5hr) and not more than 10% (104c, hr) for spray-dried material. Ungrounded samples should be powdered to pass through a No.40 sieve and mixed well before weighing .[10]

1.1.8.5 Ash content

Ash content is measure of inorganic residue remaining after removal of organic matter by burning. the inorganic residue exists as elements explaining that, the type of the soil (clay or sand) affect the ash content significantly .

the values of 1.94 –3.61% for individual nodules of *A.seyal* gum. The range of 1.6–3.9 % ash content.[10]

1.1.8 .6 Optical rotation

Specific rotation **[α]** is a property of a chiral chemical lcompound. It is defined as the change in orientation of monochromatic plane-polarized light, as the light passes through a sample of a compound in solution. Compounds which rotate light clockwise are said to be dextrorotary, and correspond to positive specific rotation values, while compounds which rotate light anticlockwise are said to be levorotary, and correspond to negative values. If a compound is able to rotate plane-polarized light, it is said to be “optically active”. Specific rotation is an intensive property, distinguishing it from the more general phenomenon of optical rotation. The observed rotation (**α**) of a sample of a compound can be used to quantify the enantiomeric excess of that compound, provided that the specific rotation **[α]** for the enantiopure compound is known. The variance of specific rotation with wavelength (a) phenomenon known as optical rotatory dispersion can be used to find the absolute configuration of a molecule.[20]

1.1.8.7 Nitrogen and protein content

Protein content in gums effects on emulsifying behavior of a gums and the best emulsion capacity and stability is found in gums with highest nitrogen content.[9]

1.1.8.8Acidity

Gums are slightly acidic in water their natural pH which range from 4.20 to 4.80[18]

1.1.8.9Viscosity

The viscosity of a gums solution may have a complicated variation with composition, due to the possibility of H-bond among the solute and solvent molecules, the more hydroxyl groups make 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 gums solutions is inversely proportional to temperature, varies with the pH and decreases with time. Gums has very low viscosity at high concentrations .Intrinsic viscosity or limiting viscosity number (I.U.P.A.C), [η], represents the most crucial "physico-chemical fingerprint” to distinguish *Acacia sayal* from other *Acacia* gum. Intrinsic viscosity of gum Arabic decreases due to autohydrolysis, UV radiation and mild acid hydrolysis. The coefficient of intrinsic viscosity in poises i.e. dynes cm^{-2}sec org $cm^{-1}sec^{-1}$ units . In the viscosity measurements, the time required for a fixed volume of liquid to flow is measured and if the solution and solvent have the same density, the flow time (t) is proportional to the viscosity (η):

$$\text{Relative viscosity } \eta r = \eta sol / \eta solv = tsol / tsolv \quad (1)$$

$$\text{Specific viscosity } \eta sp = \eta r - 1 \quad (2)$$

$$\text{Reduced viscosity } \eta red = \eta sp / c \quad (3)$$

$$\text{Inherent viscosity } \eta inh = Ln\ (\eta r / c) \quad (4)$$

$$\text{Intrinsic viscosity } [\eta] = Lim\ (\eta sp / c)c = 0 \quad (5)$$

Where c is the concentration of the polymer in g/mL of the solution. The unit of intrinsic viscosity is (mL/g) or (cm3/g) depending upon the concentration unit of the solution. The intrinsic viscosity is also called the limiting viscosity number. The plot of ηsp/c versus c or 1/c lnηr versus c often gives a straight line, the intercept of which is [η].[6]

1.1.8.10 Rheological properties

Some gums exhibit typical Newtonian be haviour up to 40% concentration. Above this level solution assume Pseudo plastic characteristics. Rheological be haviour also denoted by increase in viscosity with increase in shearing stress. [2]

1.1.8.11 Viscosity-Molecular weight determination

The methods used to determine the molar mass M are either relative methods which require calibration with samples of known molar mass and include viscosity and osmometry or absolute methods which yield weight averages as light scattering. The relationship between the viscosity

and molecular weight is complicated with other factors as concentration, shape of polymer molecules and reaction of the molecules with solvent.
The intrinsic viscosity [η] is the quantity usually correlated with molecular weight by Mark- Houwink equation:-

$$[\eta] = KM^{a} \qquad (6)$$

Where K is a constant for a given polymer – solvent system, and α depends on the shape of the molecules and the stiffness of the polymer chains. α and K can be found from two fractions of polymer of known molecular weights by the measurement of their viscosities. Determination of α and K provided that the fractions have narrow molecular weight distributions .
Determination of the viscosity and molecular weight of the fractions was done in 0.35M NaCl at PH10 to which 0.25% of the sodium salt of ethylene di amine tetra acetic acid was added as a sequestrated. The intrinsic viscosity in this solvent was nearly as low as in 0.04M HCl. The Staudinger's constants K and α were calculated. Values deduced were:-

$$K = 1.33 \times 10^{-2} \text{and } \alpha = 0.54$$

The intrinsic viscosity may be given as a function of **:**

$$[\eta] = K \quad (7)$$

The degree of branching for the polymer is obtained from the ratio on $M_{\mathbf{w}}$ ⁄ $M_{\mathbf{n}}$ application of statistical model of Erlander and French. The equivalent weight of the gum acid is 1000 to 12000 .Chemical characteristics of gum from *acacia Senegal* presented average molecular mass as 380,000 .Recently, series of studies on numerous gum Arabic samples from a variety of sources were reported, utilizing gel permeation chromatography coupled to multi angle laser light scattering , refractive index and UV detectors. It showed extensive variation in the molecular weight distributions of the various distributions of the various components between individual samples, even those supplied from a common vendor. Variations are traced to the origin, type and age of tree, and even to possible effects of processing conditions such as spray drying.[7]

1.2 objective

To successively fractionate gum Talha (*Acacia seyal* var *seyal*) using ethanol.

To determine the physiochemical properties of Talha gum and its fractions.

Chapter Two

Materials and Methods

Materials and Methods

2.1.1 Materials

-Authenticated gum sample from *Acacia seyal* (Figure 2.1) was kindly supplied by NOPEC natural gums company from season(2015 – 2016) from Aldaeen .

Figure (2.1) :Talha Gum

2.1.2 Purification of crude gum

The gum samples used in this work were relatively pure, however, impurities such as wood particles and sand particles were carefully removed by hand. Then each sample was reduced to a fine powder using a mortar and pestle and kept in labeled self-sealed polyethylene bags.

2.2 Fractionation of Gum Arabic

Solutions of 25% (w/v) gum *Acacia seyal* were prepared by hydrating the appropriate amount of gum overnight in distilled water. Then absolute ethanol, 99% was add (50ml portions) with continuous stirring until fraction (I) was separated. Fraction (I) was removed by decantation, dried at air, weighed and kept in a plastic container for further analysis. Ethanol was added to fraction (I) solution to separate fraction(II) which was treated as described in fraction(I). The same procedure was used to separate fraction (III) , fraction (lV) and fraction(V).(2)

2.2.1 Fractionation yield %

The dried fraction samples obtained by previous fractionation method were weighed. The weight of each fraction was calculated as w/ w % from the total weight of the gum sample.(5)

2.3 Physio-chemical determinations

2.3.1 Determination of moisture content

Accurately 0.5 gram of sample and the fractions were weighed in a clean preheated and weighed Petri dish. Then it was dried in an oven(at 105^{o}C)for 12 hours to a constant weight. Moisture content was then calculated as a percentage of the initial weight.(5)

2.3.2 Determination of total ash

0.5 g of powdered sample and the fractions were weighed in a dry porcelain crucible. The crucible was ignited in a furnace at 550°C for 6 hrs, until free from carbon. It was then cooled in a desiccator and weighed. The procedure was repeated to a constant weight of a carbon free ash. The percentages of the total ash of dry samples were calculated the results in table 3.1.(9)

2.3.3 pH of the solutions

The pH of 5% solutions of sample and the fractions were determined on a Digital pH meter " JENWAY ,pH Meter |, model 3505". On the measuring procedure, the gum solution was placed on to a magnetic stirrer and the foam layer was removed from the surface of the solution. The measurement was done on a calibrated pH meter at room temperature the results in table 3.1.[9]

2.3.4 Specific optical rotations of the solutions

1.00 g/L aqueous solution was prepared (using a magnetic stirrer) from gum sample and each of the fractions ample. The optical rotation of the aqueous solution was determined on automatic polarimeter "ATAGOO , Model POLAX-L" using 1 dm polarimeter tube, according to the International Commission for Uniform Methods of Sugar Analysis (ICUMSA). The readings for these solutions (no foam layer and air bubbles) were recorded at room temperature. The specific angular rotation for a sample can be determined by using the equation:

SOR = α/C*L

Where:

SOR = The specific optical rotation in angular degrees per dm and per g $/cm^3$**.**

α = The optical rotation in angular degrees

C = the concentration of the solution in g / $cm^{3.}$

L = the length of the polarimeter tube in dm. [5]

2.3.5 Nitrogen (Protein) content

Nitrogen was determined using khjeldahal method . Accurately weighed 0.2 gram of gum samples were taken in triplicates in khjeldahal digestion flasks then khjeldahal tablet (Copper sulphate-potassium sulphate) along with 3.5 mls of concentrated nitrogen free sulfuric acid were added to each flask, The flasks and contents were then heated over an electric heater until the solution attained a clear blue color and the walls of the flask were free from carbonized materials. The contents of the flask were then transferred to a steam distillation , and 15 mls of 40% sodium hydroxide solution were added, and distillations were carried out. The distillate was then collected in 10 mls of 2% boric acid solution with three drops of methyl red indicator, and titrated against (0.01) N HCl. The same procedure was carried out for fraction samples.

N %= (M1 –MS2)×× 1 0N0×0 14.01 × 100

Where:

M1: mls of HCl that neutralized the sample distillate

M2: mls of HCl that neutralized the blank distillate

N : Normality of HCl titrate (0.01).

S : Sample weight (0.2g).

The reactions involved in these steps are as follow:

$$\text{Sample} + H_2SO_4 \text{ (conc.)} + \text{Catalyst} + \text{heat} \rightarrow (NH_4)_2SO_4.$$

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O.$$

$$NH_3 + H_3BO_3 \rightarrow NH_4 + H_2BO_3$$

The protein content was determined by multiplying nitrogen percent by the factor 6.66, the results in table 3.1 . [19]

2.3.6 Determination of intrinsic viscosity

3% aqueous solution was prepared from *A.cacia seyal* var *seyal* gum sample and from each of the fraction samples in 100 ml, 1 M, sodium chloride. The flow times were recorded for the successive dilutions (3% to 0.5%, to give different concentrations) using the Ubbelohde capillary viscometer in a constant temperature bath (room temperature). The intrinsic viscosities of these solutions were determined from their flow times. The results in table 3.1

Chapter Three

Results and Discussion

Results and Discussion

3.1 Total yield percent

Tables (3.1) shows the calculated percentage yields of the five isolated fractions for Talha gum sample, the total recovery for fractions was 87% . The study shows that Talha gum could be separated in to five and three successive fractions of different yields which supported the previous works. (7) The fractionation is an extraction technique from the sample which depends on the nature and composition. These yields of the fractions are based on the solubility, with the less soluble fraction being the first to precipitate out from solution.

3.1.1 Physicochemical characterizations

Table (3.1) show that physicochemical characterization studies which included the determination of the total yield percent, moisture content, ash content, pH, specific rotation, nitrogen content, protein content, intrinsic viscosity were done for Talha sample and its five successive fractions.

Table 3.1:- Phesiochemicalcharactrization of Talha Gum sample and its five successive fractions

Samples	Fractionation yield%	Moisture content%	Total Ash%	Specific optical rotation	Intrinsic viscosity(g/l)	pH	Nitrogen content%	Protein content%
Talha sample	_	9.16	3.22	50	11.43	4.62	0.20	1.32
Fraction 1	28	4.63	3.18	47.5	10.65	5.46	0.22	1.45
Fraction 2	21	5.89	3.22	50	13.30	**5.49**	0.31	2.05
Fraction 3	16	6.41	3.50	45	11.00	**5.95**	0.70	4.62
Fracyion 4	12	4.63	3.48	50	10.24	**6.26**	0.64	4.23
Fraction 5	10	5.30	3.62	50	9.82	**6.13**	**0.71**	4.72

3.1.2 Moisure content

The water content showed significant variations between Taha sample and the fractions. The value for Taha sample was 9.16% which agrees with JECFA specification of *Acacia Seyal* var seyal gum , The successive fractions showed moisture content ranges between (4.63 – 6.41)%.

Moisture content facilitates the solubility of hydrophilic carbohydrates and hydrophobic proteins in gum . The solubility of the gums and their fractions in polar solvent (water) indicates that they are polar compounds or contain terminal polar groups. The approximate similarity of the values within the samples shows that they have same polarity and thus same strength of interaction with water molecules.

3.1.3 Total Ash%

The total ash is a parameter of the amount of impurities in the sample .Talha sample shown a value of 3.22% similar to fraction (2), the other fractions gave values of total ash between (3.1-3.62) this result agree with other workers, [3,8] and similar to the average value of 2.7%.[3] There is evidence that there are no great differences in the ash content for the whole gum samples due to possession of the same inorganic impurities from the same soil.

3.1.4 Specific optical rotation

The results of specific optical rotation of Talha gum and its successive fractions showed that, Talha gum has optical activity and it is dextrorotary that means it contains chiral carbon centre.

There were small difference between values of talha sample and its fractions , three fractions (2,4,5) and talha sample gave same value of optical rotation which was (50%), fraction (1) and (3) showed values (47.5 , 45.0) respectively .This results agree with JECA for specification of Talha gum. The values of specific rotation for the fractions were near to the values of previous study.[3]

The similarity of the values for the whole gums indicates the same number and type of the saccharides and carbohydrates in the gum which arrange in the same manner to give the optical activity.

3.1.5 Intrinsic viscosity (ml/g)

The results pointed out that , the intrinsic viscosity of fraction 2 was higher than the whole gum samples which was (13.3)ml/g , Talha sample gave value of (11.43)ml/g and other fractions between(13.30 - 9.82)ml/g The variation of viscosity from sample to sample may be due to the difference in geographical location, and the proportion of the sample compositions. The viscosity of a solution varies with composition due to the possibility of H-bond among the gum and water molecules, the more hydroxyl groups make high viscosities. A network of hydrogen bonds is formed between the molecules which extend throughout the liquid, thus making flow difficult. So the whole gums possess more hydroxyl groups which make high viscosities and these decrease gradually during the successive fractionation.

3.1.6 pH

The pH value of Talha sample was (4.62) and for the successive fractions between (6.26 – 5.46) this values agree with the results (5.83 – 5.73) which reported in literature. [2]

The results detailed above show that, *Acacia seyal* and their fractions are slightly acidic in water as shown by their pH values, due to the presence of carboxylic group attached to individual monosaccharide units [15]. It is observed that the whole gums are polysaccharides that contain more acidic groups which decrease in the successive fractions. The chemical composition is responsible for the acidic characteristic, thus the whole gums differ in composition from the fractionated products. The variation in acid number is influenced by the source, age of the sample and inorganic impurities.

3.1.7 Nitrogn and protin content

The protein content was calculated using nitrogen conversion factor (6.6) , the highest protein content showed in fractions (3,4) with values (4.62 , 4.23 , 4.72)% respectively, fraction (1) has lowest percent of protein (1.32) , this make agreement with literature.[3]

The slight difference in the nitrogen (protein) content of the whole gum samples is according to the type. The wide difference within the fractions is considered as one of the very useful parameters in distinguishing the fractions. The results across the successive fractions are due to the variation of amino groups ($-NH_2$) of amino acid residues and the peptide linkages (-NHCO-).[5] This study shows that the third , fourth and fifth fractions are protein-rich-components of the whole gum (glycoproteins) and have good emulsion properties

3.2 Conclusion

The last three fractions contain protein more than three times than parent gum

fraction (II) shows the highest intrinsic viscosity.

All fractions show similar specific optical rotation and ash content,

The total recovery for the fractions was 87%.

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