بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Characterization, Functional Applications and Cytotoxicity of the *Piliostigma reticulatum* gum

التوصيف، التطبيقات الوظيفية وسلامة إستخدام صمغ الخروب

A Thesis submitted in candidature for the degree of Ph.D in Chemistry

BY:

Rugaia Abow Mustafa Abow

(B.Sc. honors, M.Sc., Chemistry)

Supervised by:

Prof. Mohammed Elmubark Osman

Dr. Elfatih Ahmed Hassan

November, 2017

الإستهلال

قال تعالى :

بسم الله الرحمن الرحيم

" يَرْفَعْ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ "

صدق الله العظيم

سورة المجادلة، الآية (11)

DEDICATION

To the soul of my parents,

my husband

***my daughters* Aya and Alaa**

my brothers and sisters

ACKNOWLEDGEMENT

Praise is due to Allah the most Gracious the most Merciful for giving me health to accomplish this work.

I wish to express my deepest gratitude and thanks to my supervisor Prof. Mohammed Elmubark Osman and co- supervisor Dr. Elfatih Ahmed Hassan, for their encouragement, support, and guidance throughout this work.

Special thanks are due to the late Prof. Amin M. Baraka, University of Cairo (Egypt) for hosting me in his laboratory to do my practical research and providing me with invaluable help, advice and endless support.

I would like to thank Dr. Altaf Halim Basta Makkar , National Research Center Cairo (Egypt), for her invaluable technical support.

Thanks and appreciation is due to Mr. Mohamed Abd Al rasool Bassi for his invaluable help in collecting samples of *Piliostigma reticulatum* pods.

I would like to thank my husband, Medani Suleman Mohammed who has shared, with me. I am fully appreciative and deeply grateful to him as he extended his help in every possible way he could.

Finally, I would like to acknowledge Alfashir University and the financial support of the Ministry of Higher Education And Scientific Research, Sudan.

ABSTRACT

Thirty samples of mature dry seeds of *Piliostigma reticulatum* (Carob) trees were collected from the areas around Nyala town – southern Darfur state, Sudan, during the seasons 2014, 2015 and 2016. The seeds were dehusked; the germ and gum were separated manually. Physicochemical characterization and toxicity of the gum and germ samples were studied . Rheological, emulsification and some thickening application properties of *Piliostigma reticulatum* gum were also studied. The results obtained, showed mean values for the moisture (4.03%) , ash (3.09%) , water holding capacity (2.64 %) , nitrogen content (2.75%) and protein content(17.19%) for the gum , while for the germ the moisture

(5.6%) , ash (7.21 %) , Water holding capacity(1.93%) , nitrogen content(10.94%) and protein content (68.38 %). Both gum and germ are insoluble, in cold-water and organic solvents. The solubility at 60 °C of gum sample in H_2O, 0 .1 M Na_2CO_3 and 0.5 M $NaHCO_3$ were 8.2%, 48.9% and 80% respectively, and gelled using 0.5M$Na_2B_4O_7$ and 0.1 M NaOH. Whereas that of germ sample in H_2O , 0 .1 M Na_2CO_3 , 0.5 M $NaHCO_3$, 0.5 M$Na_2B_4O_7$, 0.1 M NaOH , 0.5 M NaOH and 1M NaOH were 22.7%, 53.5% ,60% ,81% ,46.5% , 69% and 74% respectively . Elemental analysis shows that, both gum and germ are rich in potassium, calcium, phosphorus and silicon, but have low contents of sodium, zinc and manganese. Percent averages of sugar composition of the gum is mannose (42.58%) , galactose (20.27%) and ribose(1.79 %) , while percent sugar composition of germ is glucose (3.4%) , fructose(6.9%) and sucrose (5.6%) . Amino acid content of gum and germ were determined .The results showed the presence of fifteen amino acids namely, Asparagine , Threnine , Serine , Glutamine , Proline , Glysine , Alanine , Valine , Methionine , Leucine , Tyrosine ,

Phenylalanine , Histidine , Lysine and Arginine. Rheological properties of the gum reveal a non-Newtonian behavior. At low concentrations 1, 2 and 3% (w/w) at 25 °C the behaviour is shear thinning while at concentration 5 % (w/w) the behaviour is shear- thickening. The viscosity at different temperatures was measured at concentration 3% (w/w), the result showed that the viscosity was decreased as temperature increased. Rheological study properties of the Corn Starch – *Piliostigma reticulatum* gum mixture result showed that shear thinning behaviour. Emulsification studies of *Piliostigma reticulatum* gum showed that the gum has less emulsifying stability and larger droplet size. Thickening application studies of gum in Yogurt and Baking, results showed that in Yogurt it is good thickening and stabilizing agent and improve the quality of bread .Toxicological study of *Piliostigma reticulatum* gum and germ on two types of cancer human cells lines, was undertaken to assess the safety of using the germ and gum as food additives. The results showed that *Piliostigma reticulatum* germ is harmful and the gum has no toxic effect.

المستخلص

جمعت ثلاثون عينة من البذور الجافة من ثلاثين شجر الخروب من بعض المناطق حول مدنية نيالا – ولاية جنوب دارفور بالسودان خلال المواسم 2014، 2015 و 2016 . تم نزع القشرة الخارجية للبذور وفصلت كل من الصمغ و بروتين . لقد تمت دراسة و توصيف الخصائص الفيزيوكيميائية و تحديد السّمية لعينات الصمغ والبروتين. و كذلك الخصائص الريولوجية ، الخصائص الاستحلابيه وخصائص تغليظ القوام للصمغ . أظهرت النتائج بأن قيم متوسط كل من الرطوبه (4.03%)،الرماد (3.09%) ، المحتوى المائ (2.64%)، محتوي النتروجين (2.75%) و محتوي البروتين(17.19%) لصمغ الخروب . و لبروتين الخروب متوسط كل من الرطوبه (5.6 %)، الرماد (7.21%) ، المحتوى المائ (1.93%) ، محتوي النتروجين (10.94 %) و محتوي البروتين (68.38 %) . اظهرت الدراسة ان كلا من الصمغ و البروتين لا يذوبان فى الماء البارد ولاالمذيبات العضوية .كما اظهرت النتائج ان ذوبانية الصمغ عند (° 60) درجة مئوية في كل من H_2O ، Na_2CO_3 0.1M و $NaHCO_3$ 0.5 كانت 8.2%, 48.9% و 80% علي التوالي ، وتكونت جل فى كل من NaOH 0.1M و $Na_2B_4O_7$ 0.5 بينما لبروتين الخروب فى كل من H_2O ، M 0.1 Na_2CO_3 ، $NaHCO_3$ 0.5M و $Na_2B_4O_7$ 0.5، NaOH 0.1 M ، NaOH 0.5M و NaOH 1M كانت 22.7% ، 53.5% ، 60% ، 81% ، 46.5% ، 69% و 74% على التوالى . تمت دراسة قيم العناصر للعينات حيث أبانت بان كلا من الصمغ و البروتين مصدر غنى بكل من البوتاسيوم ، الكالسيوم ، الفسفور و السيلكون وبكميات قليلة من عناصر الصوديوم ، الزنك و المنجيز. أظهرت دراسة محتوى المواد الهيدروكربونية أن النسبة المئوية المتوسطة لمحتوى السكر لصمغ الخروب كل من المانوز(42.58%) ، الجالاكتوز(20.27%) و الرابيوز(1.79%) و لبروتين الخروب كل من الجلكوز(3.4%)، الفركتوز(6.9 %) و السكروز(5.6%) . أظهرت دراسة محتوى الاحماض الامينية حيث أبانت بان كلا من الصمغ و البروتين يحتويان على خمس عشرة من الاحماض الامينية كل من اسبارجين ، ثيرنين ، سيرين، جلتوماين ، برولين ، جلاسين ، الانين ، فالين ، ميثونين ، ليوسين ، تيروسين ، فانيلالنين ، هستدين ، لوسين وارجنين. اظهرت الدراسة الريولوجية للصمغ أنه يسلك سلوكاً غير نيوتوني. عند التراكيز المنخفضة 1، 2، 3 % (وزن/ وزن) عند (°25) درجة مئوية ، أن الصمغ يسلك سلوكا (قص استرقاقي) حيث ينخفض معامل اللزوجة بالنسبة الي معدل القص ، أما عند تركيز 5% (وزن/ وزن) (قص استغلاظي) . كما أبرزت الدراسة أيضا عند قياس اللزوجة مع اختلاف فى درجات الحرارة لتركيز 3% من الصمغ بأنه عند ازدياد درجة الحرارة تقل اللزوجة . كما اظهرت الدراسة الريولوجية لخليط صمغ الخروب مع نشا الذرة أنه يسلك سلوكا غير نيوتونى (قص استرقاقي).

اظهرت دراسة خصائص الصمغ الاستحلابيه أنه يتمتع بثباتية استحلابية اقل كما أن مستحلبات صمغ الخروب تمتلك قطرات اكبر حجماً .

اظهرت دراسة خصائص تغليظ القوام فى كل من الزبادى والخبز، وعند إضافة صمغ الخروب الى الزبادى تبينت أنه عامل تغليظ وتثبيت جيد ، وعند إضافته الى الخبز (الرغيف) ادى الى تحسين الرغيف.

تمت دراسة سّمية بروتين و صمغ الخروب حيث أُ ختبر على نوعين من الخلايا السرطانية أظهرت النتائج أن بروتين الخروب ضار و صمغ الخروب ليس له تاثير سام.

.

List of contents

Chapter Tow

Materials and Methods

Chapter Three

Results and Discussion

Chapter Five

Emulsification and Some Thickening Application Properties of *Piliostigma reticulatum* gum

Chapter Six

Toxicological study of *Piliostigma reticulum* gum and germ

List of Tables

List of Figures

List of Abbreviations

LBG	Locust Bean Gum
JECFA	The joint Expert Committee of Food additives of the FAO/WHO
FAO	Food and Agriculture Organization of the United Nation
FAD	Food and Drug Administration
HPLC	High Performance Liquid Chromatography
IC_{50}	The half maximal inhibitory concentration
LD_{50}	The half maximal lethal dose
GRAS	Generally recognized as safe
ADI	Acceptable Daily Intake
DMEM	Dulbecco's Modified Eagle Medium
PBC	Phosphate buffer saline

Chapter one

1 Introduction and Literature Review

1.1 General introduction

Plant gums are generally the exudates from various plants formed as protection mechanism to cover wounds on the bark of trees to prevent attack by microorganisms shortly after injury (Mantell , 1965).These exudates are usually viscous fluids that ooze on the surface of the trees bark at the point of injury and gradually dry to hard glassy nodules. Plant gums are safe for use as pharmaceutical substances and food additives apart from various industrial applications. Natural gums are incorporated in a very diverse range of food formulations to impart avid variety of characteristics to food products. Thus, gums are used as stabilizing, suspending, gelling, emulsifying, thickening, binding and coating agents. Further, many plant gums have been modified in order to improve the properties for application as matrix for controlled drug delivery ,tissue reconstruction ,pH and electrical sensitive hydro gels and also to remove heavy metals from effluents , (Verbeken ,*et a*l., 2003). Gums are hydrophilic carbohydrate polymers of high molecular weight, generally composed of monosaccharide units joined by glycosidic bonds. Natural gums are of plant origin and are found either in the intracellular parts of the plant or as extracellular exudates, produced as a result of injury to the plant. At the site of injury, as a defense mechanism an aqueous gum solution is exuded to seal the wound, preventing the infection and dehydration of the plant. These exudates dry on contact with air and sun light to form hard lumps that can be easily collected (Silva, *et al.,* 2007). Gums are class of substances organic in nature and related to sugars and carbohydrates. They are un crystallizable and are usually composed of

carbon, hydrogen and oxygen. Gums have the characteristic property of forming viscous solutions and mucilage either by dissolving in water or absorbing their own volume of water .Gums are colloids, amorphous hydrophilic and organic – solvent phobic (Awouda , 1973). Gums are polyuronides, containing D- glucuronic acid residues. In addition, hexoses, pentose and methylpentoses have been isolated from different gum hydrolysates, some gums contain acidic components and others are neutral.

1 .2 Objectives

• To isolate gum and germ from *P .reticulatum* seeds.

• To characterize isolated components of gum and germ.

• To study the rheological behavior of *P. reticulatum* gum.

• To study the emulsification and some thickening application properties of *P.reticulatum* gum.

• To undertake toxicological evaluation of *P.reticulatum* gum and germ examine the safety of using gum in food and pharmaceutical applications.

1. 3 Review of plant gums

Plant gums are high molecular weight polymeric compounds, composed mainly of polysaccharides capable of processing colloid properties in appropriate solvents (Glicksman , 1973). Gums are either hydrophobic or hydrophilic. Hydrophobic gums are insoluble in water and include resins, rubber ….ect, where as hydrophilic gums are water soluble, and can be subdivided into natural, semi synthetic and synthetic gums (Glicksman, 1973). Natural gums are those derived from plant and animals. Naturals gums of plant origin seem to be associated with plant life processes , formation of plant gums natural gummy exudates , occurs when the tree is

in an unhealthy condition (Meer.*et al.*,1980) as a result of evaporation on the surface of soft droplet – like tears and diffusion of the aqueous portion from the inside to the outside of the tear . One particle aspect, which is imperfectly understood, is the extent to which micro-organisms are active in producing trees, and whether the gum itself is formed to prevent such bacterial infection or is a result of that infection: but some explain the formation of gums as a defense mechanism to seal off the wounds caused by insects or micro – organisms to prevent desiccation (Blunt, H.S.I, 1926).

1.3.1The gum belt

The gum belt refers to abroad band , situated at altitude of between 4° and 16° north ,stretching across sub – Saharan Africa from Mauritania in the west ,through Senegal and Mali , Burkina Faso , Niger north , north Nigeria to Sudan, Eritrea , Ethiopia , Kenya , Somalia and northern Uganda in the east. Most of these countries appear in the statistics as sources of gums, although they differ greatly in terms of quantities involved (A. A. SAtti, 2011).

1.3.2 *Acacia* gums

The exuded from species of the genus *Acacia* have been important commercial material since ancient times, the genus *Acacia* is one of the largest and most complex in the plant kingdom and botanical specialists agree that 1000 different *Acacia* species have been identified (Kordofanian,1989) of the 90 *Acacia* species studied to date , some are rare and other do not yield gum sufficiently copiously to be of poor solubility , poor colour and astringent taste and therefore appear to be of little possible commercial interest. At the present time, it appears that *Acacia Senegal* wild (synverek) is the source of 80% the *Acacia* gum, commercially, marketed with the *Acacia Seyal* and members of its

complex providing some 10%; while others probably contribute the balance (D.M.W Anderson, 1977).The origin of plant gum is still uncertain, but some authorities believed it to be starch granules present in the cell. They are extremely complex polysaccharide and occur naturally as salt especially of Ca, Mg and in some cases a proportion of hydroxyl groups are acetylated or mentholated (Aspinal, 1970).

1.3.3 Non *Acacia* gums in Sudan

Others , gum yielding , species widely distributed in Sudan is *Albizia amara , Combretum glutinosum , Boswillia papyrifera etc: , B.papyrifera* is the source of frankincense or gum olibanum grows , naturally, on the rocky ground of the hilly parts of central Sudan, Blue Nile (Jebel Garri), Kordofanian (Nuba mountains), Darfur (Zalinge, Jebel Marra).It is used widely as incense in the holly place and temples,(Hamza Mohamed Elmin ,1990)

1.3.4 Classification of gums according to their source

Gum exudates from certain *Acacia* (family *Leguminosae*) trees. The genus is second largest within the *Leguminosae* family and contains more than thousand species of *Acacia* that a summary of their botanical classification following Bentham (Bentham, 1875; Vassal, 1972 and Ross, 1993). Gum may be classified according to their sources (Table1.1).

Table 1.1: Classification of gums according to their source

Source	Gum
Tree exudates	
• *Acacia sengal; Acacia seyal* and other *Acacia* of African origins • *Astragalusurens* (Iran/Turkey) • *Anogeissus latifolia*	Gum Arabic Gum tragacanth Gum *karaya*, *Gum ghatti*
Seaweed extracts	
• Gelidicum and Gracilar species • Euchemacottoni;*Euchemaspinosum*; *Choandus crispus* and *Gignrtina* species • *Laminariahyperborean*; *Macrocystispurifera* and *Ascophyiius nodosum*	Agar Carrageenan Alginate
Plant extracts • Peels of various citrus fruits and apples pommace	Pectins
Seed and roots gums • *Cyamopsis tetragonoloba* • *Ceratonia sp* • *Cesalainia sp* • *sqAmorphophallus konjac*	Guar gum Locust bean gum Tara gum Konjac gum
Microbial gums • *Xanthamonas camestris* • *Auromonas sp*	Xanthan gum Gellan gum
Cellulose gums • Cellulose pulps and cotton linters	Sodiumcarboxymethylcellulose, Methylcellulose

1. 4 Seeds gums

Plant-derived polysaccharides are excellent stabilizing and thickening agents and are common components of processed foods. For example, locust (Carob) bean gum is listed as generally recognized as safe (GRAS) for use in food up to a maximum level of 0.75% (21 C.F.R. **8** 184.1343). It is also approved for use as a thickening agent in Europe (EEC No. E410). Guar gum is listed as GRAS for use in food up to a maximum level of 2% (21 C.F.R. **0** 1 84.13). Both are used in various foods including baked goods, cheese, and jam jellies (Hallagan J.B *et al* ., 1997).

1. 4.1 Galactomannans

Galactomannans are hydrocolloids used in the food industry, which come from a plant origin. These are widely used due to their ability to alter rheological properties of aqueous solutions, such as thickening and gelling behavior. The most common galactomannans are from the Carob tree (*Ceratonia siliqua*), from the guar plant (*Cyamopsis tetragonoloba*) and the Tara shrub (*Cesalpinia spinosa*), though the first two are used more extensively. All three types of galactomannans have identical backbone composed of linear (1, 4) -β-D- mannan chains with varying concentrations of (1,6) linked -D- galactose side units. Each galactomannan can be identified by the mannose - galactose ratios. Different quantities of galactose lead to different physical and chemical properties of each galactomannan. All three galactomannans mentioned are capable of interacting with other hydrocolloids such as agar-agar, Danish agar, Carrageenan and xanthan gum in order to form three dimensional structures (Meer, 1977 and Wielinga, 2000).

1. 4.1.1 Chemistry and structure of galactomannans

Galactomannans polysaccharide with higher molecular weight, the chemical structure consists of a polymeric mannose chain branched with galactose units .LBG is very similar to others, the main difference being the level of galactose side chains present. The range extent from fenugreek, which completely substituted to *Ivory nut mannan* which is essentially un substituted.

Galactomannan substitution levels

Ivory nut mannan	no galactose
Carob bean gum	1 galactose: 4 mannose
Tara gum	1 galactose: 3 mannose
Guar gum	1 galactose: 2 mannose
Fenugreek gum	1 galactose: 1 mannose

The level of substitution has major impact on the properties of the gums as polymannan can associate and self cross link. Hence *Ivory nut mannan* can be completely insoluble whereas *fenugreek* shows the properties of a simple random coil. Carob bean gum shows intermediate properties in that it is soluble but shows substantial aggregation in solution. (Mc Cleary BV *et al* .,1983).

1 .4 .2 *Cassia* gum

Cassia gum (trade name Diagum SR^{TM}), flour from the endosperm of *Cassia tora* and *Cassia obtusifolia*, is well suited for use in the manufacture of gels, frequently in combination with other colloids, and has several food applications. It is used to achieve a variety of functional effects, including thickening, emulsification, foam stabilization, moisture retention, and texture improvement. Besides the aforementioned technical

applications, the main application of *Cassi*a gum is likely to be to create a range of soft to firm gels in synergism with kappa-carrageenan or xanthan. *Cassia* gum is used at concentrations comparable to locust (Carob) bean gum (Hallagan J.B *et al*., 1997).

Common usual name: *Cassia* gum (Diagum SR^{TM})

Applicable conditions of use:

As discussed further below, based on the extant data and experts

Cassia gum is deemed by Noveon to be GRAS for use in the applications set forth in the Table 1.2.

1. 4.2.1 Natural sources of *Cassia* gum

Cassia gum **(CAS** No. 110 78-30-1) (trade name Diagum SR^{TM}) is the flour from the purified endosperm of the seeds of *Cassia tora* (C. *tora)* and *Cassia obtusifolia* (C. *obtusifolia),* which belong to the *leguminosae* family. C. *tora* and *C. obtusifolia* closely resemble one another but have been classified by some botanists as distinct species. C. *tora* and *C. obtusifolia* grow wild in subtropical regions of the world, and C. *tora* is occasionally cultivated. C. *tora* and *C. obtusifolia* are annual ruderal plants that ripen after approximately 100 days.

1.4.2.2 Chemical structure and physical properties of *Cassia* gum

Cassia gum is comprised of at least 75% polysaccharide consisting primarily of a linear chain of 1, 4- β -D- mannopyranose units with 1, 6 linked α -D – galactopyranose units. The ratio of mannose to galactose is 5: 1. The following composition was revealed in the sugar analysis

Conducted using HPLC : mannose (77.2-78.9%), galactose (15.7-14.7%) and glucose (7.1-6.3%). Like most polysaccharides, the following formula applies: $(C_6H_{10}O_5)_n$ H_2O. The structure, ratio of mannose to galactose units, and molecular weight of *Cassia* gum are outlined in Table 1.3. For comparison purposes, also contains structural properties of several other

gums that are commonly used in food. And Physical properties of *Cassia* gum (Diagum SR ^{TM}) presented in Table 1.4.

Table 1.2: Applications of *Cassia* gum in Foods of maximum concentration ,ppm, (Leimgruber R. and Marbot H.(1991).

Foods	Maximum concentration (ppm)	Intended uses
Baked Goods – cheese –crème filling in pastries and other cheese-crème filled desserts	5,000	Water retention
Soups, Sauces, Seasonings-Dry soup mixes only	2,000 - 5,000	Thickening agent
Salad Dressings-Selected oil-free dressings only	3,000 - 5,000	Thickening agent
Meat and Poultry Products-Sausages, corned beef and canned poultry meats only	3,000	Texture improvement and water retention

Table 1 .3: Structural properties of Galactomannan gums

Substance	Structure	Mannose:Galactose	Molecular Weight
Cassia gum[1] (CAS No.1 1078-30-1)	1,4- β-D-mannopyranose units with 1,6 – α - D-galactopyranose units attached to every fifth mannose	5 :1	200,000- 300,000
Guar gum [2] (CAS No.9000 – 30- 0)	1,4- β -D-mannopyranose units with 1,6- - α -D-galactopyranose units attached to every alternate mannose	2:1	220,000-250,000
Locust (Carob) bean gum[3] (CAS No.9000 – 40- 2)	1 ,4- β -D-mannopyranose units with 1,6- α -D-galactopyranose units attached to every fourth or fifth mannose	4:1	3 10,000

Table 1.3 Continued

Tara gum[4] (CAS No.39300 – 88 – 4)	1,4- β -D-mannopyranose units with side chains of 1,6- α -D-galactopyranose attached to approximately every third unit	Approximately 3**:** 1	Not reported

[1] CAS Registry Number for "galactomannan".

[2,3] Klose and Glicksman, 1968.

[4]NTP, 1982b.

Table 1.4: Physical properties of *Cassia* gum (Digum SR ™)

Appearance	Off-white to pal beige powder without lumps
Odor	Neutral to slightly fruity
Bulk density	0.6 kg/l
Particle size (sieve analysis)	<250 µm(average) not less than 100% 100 µm (average) not less than 60%
Solubility	Disperses well in cold water and forms a colloidal solution
pH (1 %)	6.5-8.5

1.4.2.3 Specifications for *Cassia* gum and related galactomannans

Cassia gum is related to locust (Carob) bean gum and guar gum in terms of structure and chemical properties, although in terms of functionality *Cassia* is more comparable to locust bean gum than to guar gum.

Cassia galactomannan can be differentiated from other galactomannans by electrophoresis. Based on the molecular weights of the structurally related polygalactomannans , guar gum and locust (Carob) bean gum (Klose and Glicksman, 1968; Leung and Foster, 1996), the molecular weight of *Cassia* gum is estimated to be between 200,000 and 300,000 (Denkler , 1997). The specifications of *Cassia* gum and related gums are listed in Table 1.5.

Table 1.5: Specifications and purity of Galactomannan gums use in foods

	***Cassia* gum**	**Locust (Carob) gum[5]**	**Guar gum[6]**
	Proposed limits	**FCC limits**	**FCC limits**
Galactomannan	≥ 70%	≥75%	≥70%
Moisture	≤ 12%	NR [11]	NR
Ash	≤ 1.2%	≤1.2%	≤ 1.5%
Proteins	≤ 7%	≤7%	≤10%
Fat	≤ 2%	NR	NR
Total anthraquinones	max. 10ppm	NR	NR
Heavy Metals:	≤0.002%	≤ 0.002%	≤ 0.002%
Lead	≤ 5mg pb/kg	≤5mg pb/kg	≤5mg pb/kg
Arsenic	≤ 3mg As/kg	≤ 3mg As/kg	≤ 3 mg As/kg

[5, 6] Food Chemicals Codex, 1996 And *"NR"* means not reported

1.4.3 Guar gum

Guar gum is found in the endosperm of the seeds of the guar plant, *Cyamopsis tetragonoloba*, which is milled in order to obtain guar gum (Meer1977; Wielinga , 2000).Guar gum is a neutral hydrocolloid with linear chains of consists of 1,4- β - D- mannopyranose units with 1,6- α - D- galactopyranose units attached to every alternate mannose. The ratio of mannose to galactose is 2:1(Klose and Glicksman , 1968). The molecular weight of guar gum is between 220,000 and 300,000 (Hoyt, 1966). Guar gum has a higher degree of galactose substitution (40%) than locust bean gum 20-23% (Maier, 1992). The galactose content of galactomannans has been studied to show that it strongly influences the behavior of each hydrocolloid. Low galactose content leads to stronger synergistic interactions with other hydrocolloids as well as a stronger gelling capacity independently based upon interactions of smooth areas of the mannan backbone (Dea , 1977 and Mc Cleary ,1985). The higher galactose content leads to prevention of strong cohesion of the main backbone, so no extensive junction zones or crystalline regions can be formed. Another factor that influences physical behavior of the galactomannan is the distribution of galactose units along the mannan backbone. Guar gum is evenly substituted, which means there are no smooth and hairy regions of the mannan backbone. On average, for every two molecules of mannose, a galactose side unit is attached (Meer, 1977). A two to one mannose-galactose ratio leaves small galactose uninhibited mannose areas, which has been shown to have lesser functionality (Mc Cleary ,1979 and Launayn , 1986) Whereas locust bean gum, on average, has a four to one ratio, which should exhibit greater functionality(Richardson , 1998) reported that two galactomannans, with the same average galactose content but with different mannose-galactose ratios, would exhibit different degrees of functionality(Richardson ,1998). The galactomannan

with the broader distribution of galactose units would be more functional because they contain a greater proportion of chains with lower galactose content (Mc Cleary, 1979 and Launay, 1986).

1.4. 3.1 Functions and Applications of guar gum

Guar gum is used as a thickener and stabilizer in the food industry as a result f its hydration and water-binding properties. It is used as a stabilizer at a concentration of 3.0% in ice cream, ice pops, and sherbet. It improves the body, texture, chewiness, and heat-shock resistance by binding free water (Wielinga, 2000). Guar gum is also used in cheese products. In cold-pack cheese it is used at a concentration of 3.0% in order to prevent syneresis and weeping. In soft cheeses it is added to increase the yield of curd and to give the curds a better texture. Concentrations of 25-.35 are added to pasteurized cheeses in addition to locust bean gum and act as a stabilizer. Guar gum is also added to dessert and Pastry products, such as pie fillings, icings, cake and donut mixes. It is added to pie fillings to thicken but prevent shrinking and cracking of the filling (Meer, 1977 and Wielinga , 2000). In icing it is added to absorb free water, which prevents stickiness as well as not sticking to packaging materials. Meat and gravy sauces contain guar gum since it allows for reduction of the total solids in the product. Another use for guar gum is in canned meat products. It is used in conjunction with agar to prevent fat migration during storage as well as controlling syneresis. As a thickener it is sometimes added to salad dressings, pickle and relish sauces.

Guar gum can also be used in dietetic beverages or low carbohydrate products due to its suspending ability and improving body of thin and watery products. (Meer, 1977).An advantage of guar gum is its cold water solubility which allows viscous pseudoplastic solutions to form when hydrated in cold water (Deis , 2001).

Its viscosity is dependent upon factors such as time, temperature, concentration, pH, ionic strength, and type of agitation. Maximum viscosity is reached during the temperature range of 25-40ºC, with higher temperatures increasing the rate at which maximum viscosity is achieved. However, too high a temperature will degrade the gum and normal function will not be carried out. Guar gum is stable over a wide range of pH, with its optimal rate of hydration between pH 7.5-9.33. The maximum viscosity will remain stable between the pH range of 1 to 10.5.Another advantage of guar gum is its ability to be compatible with salts over a wide range of electrolyte concentrations. For instance, guar gum with borate ions, the borate ions act as cross-linking agents with guar gum to form structural gels .It is also a good emulsifier due to the amount of galactose substituent. Guar gum exhibits stability during freeze-thaw cycles as it is able to retard ice crystal growth by slowing mass transfer across solid and liquid interfaces (Chaplin, 2003).Though guar gum is easily hydrated and is an economical stabilizer and thickener; it has some limitations as well. Unlike locust bean gum, it does not form gels. Guar gum is stable over a wide pH range, however if both temperature and pH are at extreme points, it could lead to degradation. For instance, at a pH 3 And temperature of 50ºC, guar gum starts to degrade.

1.4.4Tara gum

Tara gum like Guar gum and locust bean gum, Tara gum is derived from the endosperm of legume shrub (*Cesalpinia spinosa*). Tara gum is a relatively new food additive so there is less data on it, the chemical structure of Tara gum consists of 1, 4-β -D- mannopyranose units with side chains of 1,6- α -D- galactopyranose attached to approximately every third (NTP, 1982b).

1.4 .5 Carob bean gum

The Carob tree grows in many parts of the word, in some countries such as Italy there are regional variations in the naming. The most well known names internationally are Carob and Locust bean. The Carob has a very uniform seed size, about 200g and was as a standard weight in medieval times by jewelers. It has been perpetuated until this day as the unit of gold measurement. The Carob tree has been recognized as a valuable resource for many centuries. Its origins lie in the Middle East where it grows in dry areas with poor soils. The Carob tree is native lands to the Mediterranean basin whereas Arabs moved the tree along the North African coast and into Spain and Portugal. The history of the Carob tree in the USA is somewhat clearer and more recent being introduced in to the USA by the patents office in 1854.The Carob tree is important in the Mediterranean basin as a crop plant that can be grown on marginal lands where few others crops can be grown economically.(Mc Cleary Bv *et al* ., 1983). The Carob is scientifically called *Ceratonia Siliqua* and is leguminous plant (Irwin and Barnab , 1981). The Carob tree is important in the Mediterranean basin as crop that can be grown on marginal lands where few other crops can be grown economically. Traditionally Carob tree are inter planted with olives, grapes and nuts. The Carob pods with their high sugar content are then used as cattle fodder. More recently the major interest in the Carob tree has been the extraction of the gum from the seeds (Mc Cleary BV *et al* ., 1983).

1.4.5.1 Description of Carob bean gum

Carob bean gum, also known as Locust bean gum, and sometimes referred as “Carob bean gum” or “Carob gum”. However, the gum should not be confused with Carob powder, a flavoring made from roasted Carob beans that can be used as substitute for chocolate. (M c Cleary *et al*., 1983).

Carob bean gum is obtained from the endosperm of seed of the Carob (Locust) tree, *Ceratonia Siliqua*, family leguminosae. The leaves have a thick waxy coating that prevents excessive moisture loss in semi arid climates. In July of alternating years the leaves are shed and replaced with new (Batlle and Tous,1997).The flowers of the Carob are small and numerous, arranged in a twisting manner down the inflorescence in numbers of 15 to 20. They are only found on old wood and inflorescences are between 6 and 12 cm in length. The Carob tree is a large evergreen tree and its fruit is a long brown pod containing very hard brown seeds, the kernels. The seeds are dehusked by treating the kernels with dilute sulfuric acid or with thermal mechanical treatment, elimination of the germ followed by milling and screening of the endosperm (native Carob gum). The gum may be washed with ethanol or iso propanol to control the microbiological load (washed Carob bean gum). Carob bean gum is mainly consisting of the high molecular weight (approximately 50,000 – 3,000,000) polysaccharides composed of galactomannans. The gum is a white to yellowish white, nearly odorless powder. Carob bean gum is insoluble in most organic solvents including ethanol. it is partially soluble in water at ambient temperature and soluble in hot water . It is needs heating to above 85° for 10 minutes for complete solubility. The gum may be precipitated from aqueous solution by some electrolytes, and, in particular, polyvalent ones such as lead acetate, phosphotungstic acid and tannic acid. Solutions containing greater than 0.3% gum are gelled by addition of borate at pH 7.5 or greater. The gel reversed by decreasing the pH below 7.0, by heating, or addition of Manito asequestrant for borate (Puhan Z, Wielinga MW, unpublished report of INEC).

1.4.5.2 Composition of Carob bean gum

Carob bean gum is comprised of a high molecular weight polysaccharide composed of galactomannan consisting of linear chain of (1 - 4) – linked-

β – D- mannose units with (1 - 6) - α- D - galactose residues as side chains, Figure 1.1 shows structure of polysaccharide of Carob bean gum .Standard texts report the molecular weight as approximately 310,000. However the INEC Technical Committee reports that the standard method using the consumption of sodium chloride is unsuitable and recommends a gel permeation chromatography method. This provides a molecular weight rang of 50,000 to 300,000. The mannose: galactose ratio of Carob bean gum is approximately 4:1. The mannose and galactose content has been reported as 73-86% and 27- 14% respectively. The commercial samples of Carob bean gum contain approximately 5-12 % moisture, 1.7 - 5 % acid –soluble ash, 0.4- 1.0 ash and 3-7 protein.(Philips,G.O ., *et al* 1995).

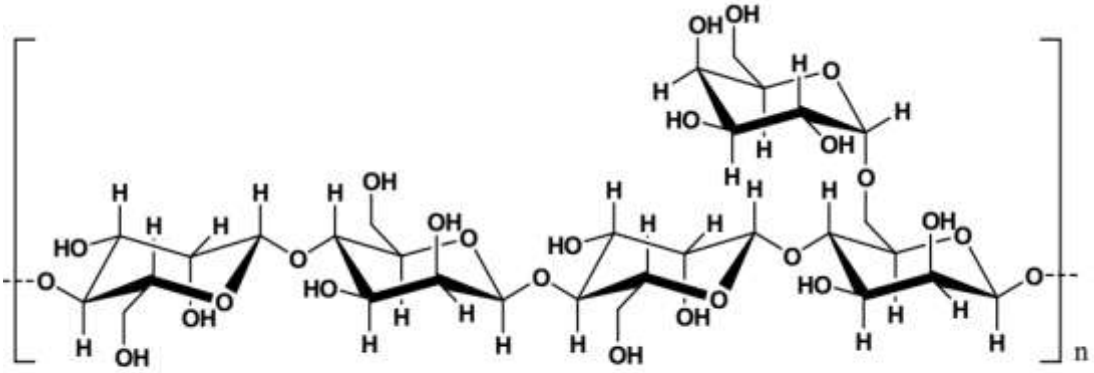


Figure 1.1: Structure of polysaccharide of Carob bean gum

1.4. 5.3 Carob bean gum properties

Carob bean gum or locust bean gum (LBG) comes in variety of forms, basically they can be divided into high grade , industrial and technical (Wielinga, 1989) .A basic composition of the different grades can be seen in acid insoluble residues gives an indication of how well the skin has been removed from the seed. Hence the lower the acid insoluble the better and protein gives an idea of how well the germ has been separated from

the endosperm. Hence the lower the protein levels the better.LBG is only partially soluble in cold water. The mannan sections of the polymer chain can bind together to form a crystalline region which is thermodynamically more stable than the solution state. Hence even when in solution at ambient temperature there is a tendency for the polymer chains to which to aggregate. This makes the accurate measurement of molecular weight difficult due to the presence of aggregated species in solution. However it also has some advantages. The aggregation can be increased by reductions in water activity and reduction in solution temperature which ultimately forms a3D network and gel. The synergy of LBG with kappa carrageenan is the basis of the majority of non gelatin dessert jellies as well as most of the jelly used in canned pet foods. The texture of a LBG/Carrageenan gel can be manipulated to be close to gelatin and higher melting temperature is a positive advantage in warmer countries where refrigeration is not present in every household. It is also known that the melting point of the synergistic gel can be manipulated by altering the molecular weight of the LBG. This probably has more use in terms of aiding processing than it actually does in terms of providing a melt in the mouth sensation. Carob bean gum is compatible with xanthan gum and forms gel. LBG has several functional properties that it's used as thickener, stabilizer, emulsifier, gelling agent. (Wilelinga MW, 1990). There have been several

functional properties that's its users are looking for , one of the largest food uses of LBG in cream cheese where it is used to bind water and produce a spreadable texture without imparting sliminess(Batista MT *et al* .,1996)

1.4.5.4 Food categories and use level of Carob bean gum

In the United States, Carob bean gum is listed for use as a stabilizer and thickener in the following foods (Survey of Industry on the use of Food Additives, 1997). Table 1.6 shows Food category maximum use level (%) of Carob bean gum. In the 1977, survey of the United States food industry, Carob bean gum was reported as being used as a firming agent, humectants, stabilizer, and thickener in the foods which shows in Table 1.7.

Table 1.6: Food category and maximum use level (%) of Carob bean gum

Food Category	Maximum Use Level (%)
Baked goodsand baking mixes	0.15%
Non–alcoholic beverages and beverage bases	0.25%
Cheeses	0.8%
Gelatins , pudding and fillings	0.75%
Jams and Jellies	0.75%
All others foods	0.50%

Table 1.7: Food category and mean use level (%) of Carob bean gum

Food Category	Mean Use Level (%)
Milk products	
Chocolate milk	0.00015
Yogurt	0.039
Sour cream	0.073
Cheese	
Processed cheese	0.32
Cottage cheese	0.016
Cream cheese	0.46
Frozen dairy desserts	
Ice cream	0.055
Sherbets	0.10
Shakes and malts	0.071
Fruits and Fruit juices	0.093
Fruit drink and ades	0.00057

1. 5 Botanical classification of *Piliostigma reticulatum* (DC) Hochst

Family: Caesalpiniaceae (Lequminosae – Caesalpinioideae)

Synonyms: Bauhinia reticulate DC, Bauhinia glabra A .chev, Bauhinia glauca A.chev (Hamza Mohamed Elmin , 1990).

Vernacular names:

Camel's Foot, Monkey bread, Abefe , Kalgo ,Semellier and Musacanca

(Djuma , 2003)

Common name: Carob

1 .5.1 Description of *Piliostigma reticulatum* tree

Every green shrub, occasionally a small tree with bush spherical crown, few big knotty trees can be found. Bark dark grey to brown, fibrous corky, slash dark red. Leaves grey- green, glabrous, 6 – 12 cm long, 4-8 cm wide; Flowers dioeciou , clustered in short hairy racemes , axillary measuring 4-5 cm. Petals white with pink stripes(Hamza Mohamed Elmin , 1990). Figure1.2 shows *Piliostigma reticulatum* tree. Fruit along, hard, straight, but mostly twisted pod, glabrous or weakly pubcent, ligneous, brown, indehiscent up to 25 cm long and 5 cm wide and persisting, many seeded. (Hamza Mohamed Elmin , 1990) Figure 1.3 shows *Piliostigma reticulatum* branch with fruit pods .Seeds are compressed and slightly along with dimensions of 8mm to 10mm long by 7mm to 8mm wide by 3mm to 5 mm thick, each seed is covered by shiny brown and very hard tested which accounts for 30 to 33% of seed weight(Batlle and Tous , 1997).

Figure1.2: *Piliostigma reticulatum* tree

Figure 1.3: *Piliostigma reticulatum* branch with fruit pods

1.5.2 Origin and geographic distribution of *P.reticulatum tree*

Piliostigma reticulatum occur in the Sahel – Sudanian region from Senegal and Mauritania eastward to Sudan (Baumer ,M., 1983).There are two species in the Sudan *Piliostigma reticulatum* (DC) Hochst and *Piliostigma Thonnigii* (Schum).Its occurs in the southern parts of center Sudan, running from east of south Kassala , south Blue Nile, south Kordafan , south Darfur (Jabel Mara) and extending southward Bahr Elghzal ,upper Nile and Equatorial . *Piliostigma reticulatum* (DC) Hochst much – branched trees up to 8m high, in the drier savanna areas of central Sudan in south Kordfan and south Darfur (Hamza Mohamed Elmin, 1990). Figure 1.4shows geographic distribution of *Piliostigma reticulatum* tree

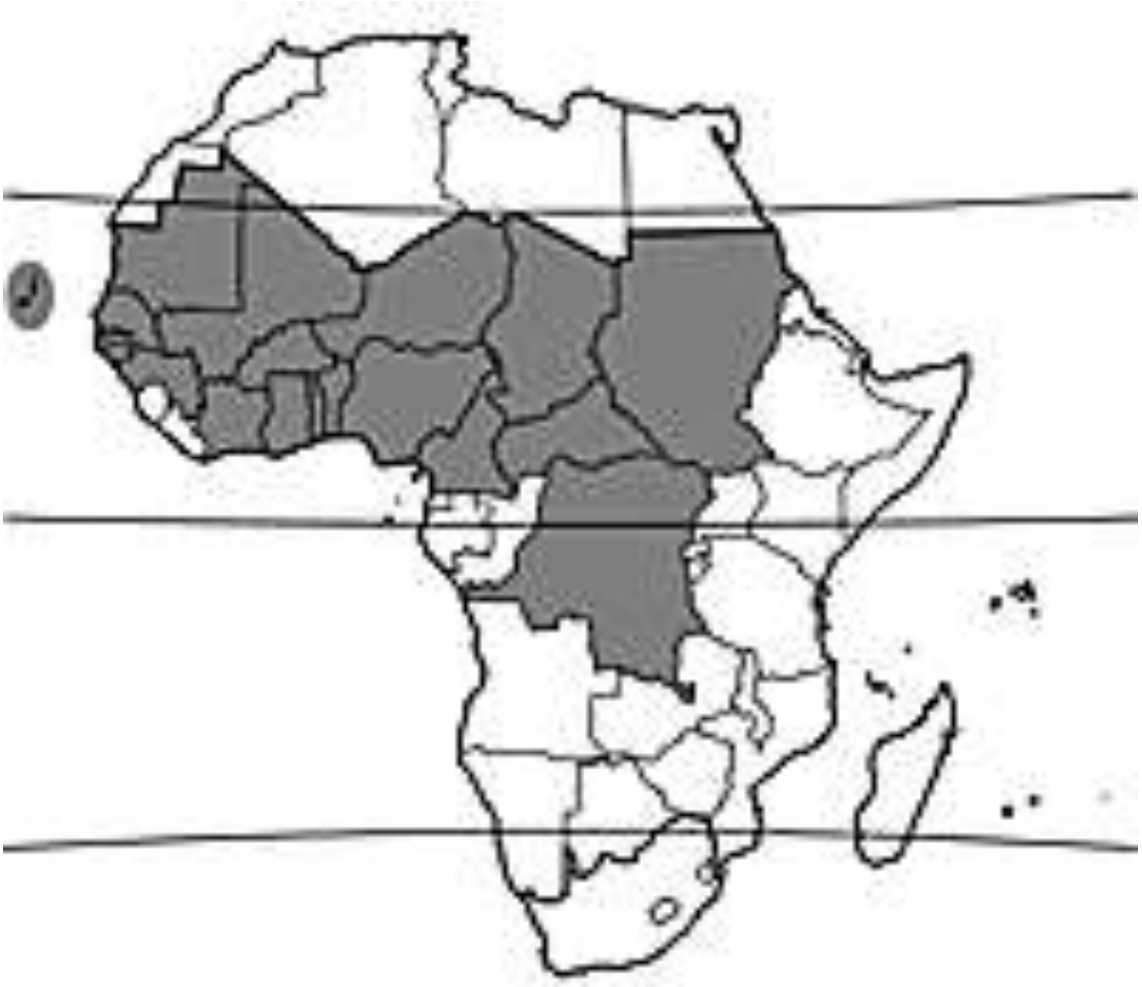


Figure 1.4 : Geographic distribution of *Piliostigma reticulatum* tree

1 .5 .3 Growth and development of *P. reticulatum* tree

The growth of *Piliostigma reticulatum* is recorded to be slow- flowering in the dry season. In drier areas *Piliostigma reticulatum* is semi-deciduous, losing most of it is leaves at the end of the dry season but more humid zones the leaves are often persistent. The plant resprouts after the stem has been cut. Animals eating the fruits contribute to dispersal of the seeds (Diack, *et al*; 2000).

1 .5.4 Ecology of *P. reticulatum* tree

Piliostigma reticulatum occurs from sea level 2000m altitude in areas annual rain fall of (200-)400 – 1000 mm , mainly on heavy and poorly drained soils , but also on sandy soils – it is a pioneer species in wood

land ,wooded scrubland, wood grass land . Valleys and disturbed habitats such as cultivated fields fallows and roadsides . The species is common and locally abundant. (Baumer , M., 1983).

1.5.5 Propagation and planting of *P. reticulatum* tree

Piliostigma reticulatum propagated by seed, one kg contains 11000 to 14500seeds. Germination is poor (Hamza Mohemed Elmin ,1990). But can be improved by soking the seed successively in 90% H_2SO_4 for 30 minutes and in water for 24 hours, or by soaking them in hot water over night in98 % HCl for 90 minutes. Due to fast growth of the root system, seeding can be planted out already at 5-7 weeks after sowing.

1.5.6 Management of *P. reticulatum* tree

The tree is spared during land preparation and maintained in ogroforestry system. The density of the species in parkland is very variable.

1 .5 .7 Harvesting of *P. reticulatum* tree

The bark is harvested by cutting the branches. It is hard to remove the bark when the stem is dry, for this reason it is recommended to harvest the bark as soon as the stem is cut. The bark is stripped off, dried and used as rope, mature fruits and young leaves are directly harvested from the plant for various uses.(Baumer , M.,1983).

1 .5 .8 Handling after harvest of *P. reticulatum* tree

Piliostigma reticulatum is not explanted for industrial fiber production. The park is directly collected from cut branches in the field; sometimes collectors take the whole stem to their houses before removing the bark. The remaining wood is later use as fuel.

1.5.9 Genetic resources of *P. reticulatum* tree

Piliostigma reticulatum has a wide distribution and is common and locally abundant in its distribution area, it is not treated by genetic erosion.

1 .5 .10 Prospects of *P.reticulatum* tree

Piliostigma reticulatum is a valuable multipurpose plant yielding a wide range of useful products .It is becoming Integration of *Piliostigma reticulatum* in the traditional agro forestry system in semi-arid countries is important for sustainable use of the species. Further research on the domestication potential of the species is worthwhile. The antimicrobial and anti- inflammatory properties warrant further research for pharmaceutical uses, (Baumer , M .,1983)

1 .5.11 Uses of *P .reticulatum* tree

Drinks are prepared from the leaves and from pounded and boiled pods. The bark is used for cordage, tying roof rafters of granaries, huts or houses, and it is used in the production of articles such as baskets, chairs, mats, arrows and masks. Farmers use the bark to tie firewood gathered in the bush or to tie up sheep and pigs to houses during the rainy season. Branches leaves and pound pods for cattle; sheep and goats prefer the pods. Bark contains up to 18% tannin. Wood reddish, darkening to brown, attacked by various insects and termites, fuel wood .A red dye is obtained from the pounded roots, a blue dye from seeds and pods. Various applications in local medicine, the leaves (with a slightly acid taste) are said to be effective in curing colds if prepared as tea and used to wrap foods. The stems are used as toothbrushes; the bark is astringent and has curative properties for diarrhea and dysentery. Leaves and bark are used as a haemostatic, antiseptic and treatment for ulcers, boils, wounds and syphilitic cancer (Hamza Mohamed Elmin , 1990).The tree provides poles

and the wood is used for making tool handles, household utensils ,stools, masks and other small articles , it is also used as tinder ,as fuel wood and for making charcoal, fruits and roots are cooked and eaten . The boiled leaves are added to cereal porridge to make it more acid and keep it edible for several days. The plant is considered to contribute to soil improvement and farmers use the leaves for mulching .In addition *Piliostigma reticulatum* provides shade during agricultural activities, e.g.in nurseries. (VonMaydell , H.-J., 1986).

The species is extensively used in traditional medicine; the roots are used for the treatment of gonorrhea, hookworm, a scites and dropsy. Root decoctions are used in preparations against liver and gall complaints, and are drunk as antidote for plant poison. Root decoctions or macerations are taken or used in vapour baths against cough, diarrhea, constipation, stomach –ache and muscular pain. The sap of ground roots is applied on swellings in dislocations, and taken against painful pregnancy and nausea. The bark is widely used on wounds, cuts, ulcers and sores as astringent, haemostatic and cicatrisant. Bark decoctions are used for washing wounds and as a mouthwash and for treatment of fever, colds, stomach –ache, indigestion and diarrhea or bouillon is drunk against gonorrhea. The leaves are used against fever and as a tranquillizer, and for the treatment of range of ailments colds, bronchitis, headache, rheumatism, ophthalmic, toothache, mumps, syphilis, vertigo and epilepsy. Leaf preparations are often applied on wounds, ulcers and sores; they are considered haemostatic, antiseptic and cicatrisant. Boiled leaves are rubbed in against lumbago. Leafs infusions are used in drinks or baths as a sedative and anti- rachitic for new – born children , and to stimulate their appetite, young leaves and flower buds are given against rickets in babies , kwashiorkor and anorexia. The bark , root , pod , young steam or leaves have been used for treating leprosy , smallpox , coughs , ulcer , heart pain

, gingivitis , snake bite ,dysentery , fever , wounds and a variety of closely related disease conditions (Burkill , H .M ., 1995) . The fruit is used as laxative and for the treatment of wounds, sores, ringworm, headache, encephalitis, bronchitis, cough, liver problems and indigestion. Powdered fruits are applied on the skin for the treatment of wounds and skin problems, and crushed fruits against cough. In traditional veterinary medicine the powdered root is put into drinking – water for the treatment of diarrhea in cattle. In Nigeria powdered seeds are added to brewery waste and given to animals against trypanosomiasis.(Neuwinger ,H.D.,2000).

1. 6 *Piliostigma reticulatum* gum

Piliostigma reticulatum gum also known as Carob gum .It is obtained from the endosperm of seed of the Carob (*Piliostigma reticulatum*) tree, it is a thickening agent and a gelling agent used in food technology. Carob gum is a galactomannan vegetable gum extracted from the seeds of the Carob tree; the long pods that grow on the tree are used to make this gum. The pods are kibbled to separate the seed from the pulp; the seeds have their skins removed by an acid treatment. The deshusked seed is then spilt and gently milled. This causes the brittle germ to break up while not affecting the more robust endosperm, the two are separated by sieving, and the separated endosperm can then be milled to produce the final Carob (*Piliostigma reticulatum)* gum powder (Diack, M *et al*., 2000).

1. 6. 1Chemistry and structure of the gum

Carobs seed are used to produce *Piliostigma reticulatum* (Carob) gum, the chemical structure of Carob gum consists of linked sugar units – specifically two- hexose sugars (sugars containing six carbon atoms) , mannose and galactose .It is known as galactomannan polysaccharide , the main chain consist of (1-4) linked β– D- mannose residues , and the

side chain are (1- 6) linked α – D - galactose (Mc Cleary BV *et al* ., 1983). This structure can effect in the properties, this structure is similar to that of galactomannan gums, but the UN even side, chain distribution make it is less soluble and viscous.

1. 6.2 Production of the *Piliostigma reticulatum* gum

When *Piliostigma reticulatum* (Carob) pods arrive at the processor they are stored in ventilated areas to allow their moisture to settle down to about 8%, this improves their storage life. The first operation is kibbling the pods to separate the seeds from the pulp. The pulp is then ground to various sizes or dried and fine milled to produce Carob powder for the food industry. The seeds have their skins removed by either an acid treatment where sulphurirc acid at room temperature is used to carbonize the outer skin which can then be removed by a combination of washing and brushing .Or by a rosting process where the skins are roasted so the literally peel off .

The acid process generally produces whiter products but is not so easy to handle, the deskinned seeds is then spilt and gently milled, and this causes the brittle germ to break up while not affecting the more robust endosperm. The separated endosperm can then be milled to produce the final *P.reticulatum* gum powder. They are major differences in the properties of gums from different regions but these differences have not been adequately assessed by academia. The Carob pod can be split in two fractions pulp and seed. Carob pulp varies in properties depending on the harvesting time, cultivar and farming practices (Puhn and wielinga ,1996).

The protein content is fairly low on digestibility due to being bound to the fiber and tannin content of the pulp, the pulp can also be dried and ground to powder where it is used as cocoa substitute. Carob chocolate is amide flavor than dark chocolate, more akin to milk chocolate but is lower in

calories and free from the caffeine and the bromine that is found in coca. Pulp can also be extracted to from sweet syrup that is popular as a drink in some countries; Carob pulp has also been used in fermentation processes to produce both proteins and alcohol. The endosperm contains the polysaccharide know as galactomannan, the seed coating contains higher level of antioxidants which could explaining the empericical obevervation that technical grade Carob gum with it is higher level of seed coating is more retort stable in applications like pet food than much cleaner food grade gum. The antioxidant properties of the seed at also offer the potential as a new food ingredient. (Batista *et al*;1996). In order to separate the germ from the endosperm, the whole seed excluding the tests is milled so that the endosperm remains in large scale like pieces and the germ is turned into affine powder (Batlle and Tous ,1997). The can be achieved due to differences in friability of two fractions, the germ and endosperm. The endosperm goes through another milling step to produce affine powder that is sold under the trade name Carob gum (Battle and Tous , 1997 ; Hoefler ,2004).

1. 6.3 *Piliostigma reticulatum* gum Properties

Piliostigma reticulatum gum insoluble in cold water. The Mann sections of the polymer chain can bind together to form a crystalline region which is thermodynamically more stable than the solution at ambient temperature there is a tendency for the polymer chains to witch to aggregate. This makes the accurate measurement of molecular weight difficult due to the presence of aggregated species in solution. However it also has some advantages, the aggregation can be increased by reductions in water activity and reduction in solution temperature. (Mc Cleary BV *et al*; 1983).

1.6.3.1 Temperature (gels and melts)

Viscosity of gum thickened solution decreases as temperature increases. *P.reticulatum* gum does not gel by itself so gelling and melting of the formed gel depends on the others hydrocolloid.

1.6.3.2 Texture

By itself, liquids thicked comparable to those thickened by LBG or guar gum or another hydrocolloid providing a viscous short – textured solution, it really shines when used with other ingredients to prevent synersis or improve gel texture.

1.6.3.3 Appearance

Cloudy, though modified versions of Carob gum can be transparent.

1. 6.3.4Mouth feel

When used alone, gives a smooth thick and acidic moth feel, when combined with another hydrocolloid gum that synergistic with, a strong and rigid gel forms. At higher loading levels, the gel can be firm and elastic.

1 .6.3.5 Hydration

Hot soluble at above 80 °C (176°F) . Mix dispersed with other ingredients and boil for three mintes to fully hydrate . Salts ,sugar and other ingredients that compete for water can slow the hydration rate.

1.7 *Piliostigma reticulatum* Germ

The germ is separate from the endosperm of the seed of *Piliostigma reticulatum* tree; the germ is much more brittle and reduces in size easily when compared to the endosperm. The germ is used as protein supplementation in both food and feed (Dakia *et al*., 2007). The

P.reticulatum germ is created as byproduct of *P.reticulatum* gum production. The proteins form aggregates linked via non – covalent and disulfide bonding, these proteins have a well balanced amino acid content with an essential amino acids presents (Dakia *et al*.,2007). Carob germ flour has been traditionally used as a protein additive in animal feeds and foods for human consummation because of it are well balanced amino acid content (Bengoechea *et al*., 2008).

Chapter Two

2Materials and methods

2.1Materials

2.1.1 Fruit pods samples

Piliostigma reticulatum fruit pods were collected from Southern Darfur State (Nyala areas) during seasons 2014 ,2015and 2016.The fruit pods - were dehusked , *Piliostigma reticulatum* seeds were removed for each season. The dried seeds were then packed in plastic bags and stored in dry place until the extraction process.

2.1.2 Extraction of *Piliostigma reticulatum* gum

The dried seeds Figure 2.1 were kept in 60% H_2SO_4 (Merck, K38346531) solution for 30 minutes and then in the water for 24 h. The soft coat was then peeled off using the tip of the finger and then separated the endosperm manually from the germ and dried them at room temperature (Dakia *et al*., 2008). The endosperms Figure 2 .2wereground to obtain *Piliostigma reticulatum* gum and also the germ Figure 2.3 was ground to obtain *Piliostigma reticulatum* germ and kept them in labeled plastic containers for analysis.

Figure 2.1:*P.reticulatum* seeds

Figure 2.2: *P .reticulatum* gum

Figure 2.3:***P. reticulatum* germ**

2.2 Methods of analysis

2.2.1 Determination of moisture content

Moisture content of the gum and germ samples was determined according to (AOAC, 1990) method. One gram of each sample was accurately weighted in a clean, dry reweighted shallow weighing dish. The weighted dish and its contents were dried in an oven at 105^{o}C for five hours, then cooled in a desiccators and reweighed. The loss on drying was calculated as follows:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (2.1)$$

W_1 = Original weight of sample (g).

W_2 = Weight of sample after drying (g).

2.2.2 Determination of ash content

Accurately tow grams of the dried samples were weighted on dry porcelain crucible and ignited at 550^{0}C in a muffle furnace until free from carbon, cooled in a desiccator and weighed. Then the total ash % was calculated as follows: (FAO, 1998)

$$\text{Ash (\%)} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots... (2.2)$$

Where:

W_1 = Weight of the empty crucible (g).

W_2 = Weight of the crucible + the sample (g).

W_3 = Weight of the crucible + ash (g).

2 .2.3 Water Holding Capacity (WHC)

Water – holding capacity (WHC) was determined following the method of (Chua *et al.*, 1979). Briefly, 1g of each sample was weighed and then stirred into 10 ml distilled water for 1min in a vortex (Thermolyne

vortexer). These fibrous suspensions were centrifuged at 2200 xg for 30 min and supernatant the volume of measured. Water – holding capacity was expressed as g of water held per g of sample.

2.2.4 Nitrogen and protein Content

Kjeldahl method was used to determine the total nitrogen in gum and germ samples according to (AOAC, 1990). The method consists of three basic steps: (1) digestion of the sample in sulfuric acid with a catalyst, which results in conversion of nitrogen to ammonia, (2) distillation of the ammonia into a trapping solution, and (3) quantification of the ammonia by titration with a standard solution. The reactions involved in these steps can be shown as follows:

- Sample + H_2SO_4 (conc.) catalyst +Heat $\rightarrow$ $(NH_4)_2SO_4$
- $(NH_4)_2SO_4$ + 2 Na OH $\rightarrow$ $2NH_3 + Na_2SO_4 + 2H_2O$
- $NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^-$
- $H_2BO_3^- + HCl \rightarrow H_3BO_3 + Cl^-$

2.2.4.1 Method

0.5 gram of each sample (in duplicate) was weighed and transferred to Kjeldahl digestion flasks and Kjeldahl tablet (copper sulphate-potassium sulphate catalyst) was added to each. 10 cm^3 concentrated, nitrogen free, sulphuric acid was added. The tube was then mounted in the digestion heating system which was previously set to 240^oC and capped with an aerated manifold. The solution was then heated at the above temperature until a clear pale yellowish-green color was observed which indicates the completion of the digestion. The tubes were then allowed to attain room temperature. Their contents were quantitatively transferred to Kjeldahl distillation apparatus followed by addition of distilled water and 30% (w/v)

sodium hydroxide. Steam distillation was then started and the released ammonia was absorbed in 25 cm^3 of 2% boric acid. Back titration of the generated borate was then carried out versus, 0.02M, hydrochloric acid using methyl red as an indicator. Blank titration was carried in the same way

$$\%N = \frac{14.01\ x\ M\ x\ (\ volume\ of\ titrant - volume\ of\ blank)x\ 100}{weight\ of\ sample\ (grams)}$$

Where:

M is the morality of hydrochloric acid.

Protein content was calculated using nitrogen conversion factor as follows:

% protein = % N x 6.25

2.2.5 Solubility and pH Measurement

1g of each sample was stirred in 100ml of water or electrolyte solution, and then it was placed on hot and stir plate and heated it at 60°C for 5 min (Hoefler, 2004). Then let it to cool and filter it by filter paper and washed by deionized water, then let these filter papers to dry at room temperature and weighed them to record their weight.

Solubility % = wt. of soluble /1x 100

2.2.6 Determination of elements composition

Atomic absorption spectrometry is a technique which can be applied effectively to determine about 70 elements. It is based on the absorption of radiations by the atoms of a particular element in the ground state, raising them to exited states. Excitation is produced by radiation energy at a wavelength equivalent to the energy needed to lift an atom from its ground state to higher level, the energizing radiation is thus absorbed and the amount of absorption is directly dependent on the population of the ground state

atoms in the flame. The sample solution is aspirated in the gaseous state by vaporization and dissociation of molecules. A hollow cathode lamp, which consists of a cathode of the element of interest or coated with it and anode at a low pressure of neon or argon, is used as a source of radiation. A monochromatic is used in conjunction with the hollow cathode lamp to isolate the desired spectrum. The radiation that finally reaches the detector system is amplified.

2.2.6.1Method

1 gram of each sample was placed in a well-glazed porcelain dish. Started in a cold furnace, and then heated to 550^0C, the temperature was maintained for 4 hours. The sample was cooled and 10 ml of 3N HCl were added. The dish was covered with watch glass, and the sample was boiled gently for 10 minutes. The sample was cooled, filtered into a 100 cm^3 volumetric flask, and diluted to the volume with deionized water.

Atomic absorption spectrometer (SensAA-Dual-GBC Scientific equipment) was used to determine the elements.

2.2.7 Determination of sugar composition

HPLC is widely considered to be a technique mainly for biotechnological, biomedical, biochemical research, and for the pharmaceutical industry, is as well widely used in a lot of fields such as cosmetics, energy, environmental, and food industries (Marcrae, 1985).

2. 2.7.1 Sample preparation

The samples were hydrolyzed to liberate the sugar residues. Sample was weighed out (0.5g, taking into account the moisture content) and added to 10 cm^3 of 4% H_2SO_4 and incubated at 100 ^{0}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 Sulphate (formed from neutralizing the H_2SO_4) to settle. The supernatant was removed and filtered through a 0.45 μm Whatman 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.

2.2.7.2Method

The purpose of analyzing the gum and germ samples by HPLC was to determine the relative concentration of each sugar residue present in the sample, namely galactose , mannose and ribose for gum and glucose, sucrose and fructose for germ, before analysis of the gum and germ samples. Stock concentrations of 5 mg cm^{-3} for each sugar were made up by hydrating in 70/30 a cetonitrile/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 the unknown sugar content for the gum and germ samples. The concentration of each sugar was calculated by peak height and expressed as a % of the total sugar content

2.2.8Amino acid analysis

The acid hydrolyzed amino acids by amide bond breakage were determined according to (Pellet and Yong., 1980).

2.2.8.1 Procedure

From each dry wheat sample, 1g was defatted using diethyl ether and 0.4 g was hydrolyzed in sealed evacuated pyrex test tube using 5 ml of 6N hydrochloric acid at 110° C for 24 hrs. At the end of the period, hydrolysate was transferred quantitavely to containers and water bath.

Distilled water (5 ml) was added to the hydrolysate and then evaporated to dryness to remove the excess of hydrochloric acid and then further addition of distilled water till complete removal of excess hydrochloric acid and samples were dried till dry film was obtained. The obtained dry film was dissolved in a known volume of sample dilution buffer (0.1 N Sodium acetate buffer, pH 2.2) and the solution was filtered through a 0.45 mm membrane filter and the samples stored frozen in a sealed vials until fractionation of the amino acids by amino acid analyzer (LC 3000 Eppendorf, Central Lab of Desert Research Center, Egypt).

2.2.8.2 Separation of amino acids by amino acid analyzer

Samples of amino acid were transferred to the devices plastic capsules and then injected into an amino acid analyzer and separation performed using a hydrolysate column Eppendorf LC 3000 - (250 x 4.6) and its temperature is 47°C by elution with a series of buffers over the pH range 3.3 to 11 (flow rate 0.2 ml/ min). Ninhydrin is used for the detection of amino a cids at λ 440 for proline and 750 nm for other amino acids through an oxidative decarboxylation reaction to the amino acids with ninhydrin to give ruhemann's purple a compound detected by the lamp of spectrophotometer .The peak area and percentage of each amino acid were calculated using an external standard concentration 2.5 μ mole amino acid by computer software and the concentration of each amino acids was calculated as mg/ g dry weight.

Chapter Three

3 Results and Discussion

3.1 Physicochemical characterization of gum and germ of *Piliostigma reticulatum*

Considering the solubility of *Piliostigma reticulatum* gum and germ , the samples were not soluble in water at room temperature they were need heating to obtained solubility. The gum is type of polysaccharides and germ is type of protein.

Table (4.1) shows the moisture %, the ash%, the water holding capacity %, nitrogen % and protein % of *Piliostigma reticulatum* gum, germ and related galactomannan gums. The average moisture content of *Piliostigma reticulatum* gum sample was 4.03 %, whereas that of germ sample 5.6 %, they had a low moisture content which may be due to dry state of collection. The average ash content of gum sample 3.09% , this was the higher value than for others galactomannan gums (Klose and Glicksman, 1968 , Food Chemicals Codex, 1996) ,whereas the average ash content of germ sample 7.21 % , this was the higher value than for Locust bean germ (Bengoechea *et al* ., 2008). The average water holding capacity of the gum sample 2.64%, this was lower than which reported for polysaccharide gum (Galla , N *et al* ., 2010) , whereas that of germ sample 1.93% this was lower than what were reported for other flours (Lin , M *et al* ., 1974).

The average Nitrogen content of gum sample was 2.75% whereas that of germ sample was 10.94 %. The average protein content of the gum sample was 17.19% , this value is higher than for others galactomannan gums (Klose and Glicksman, 1968 , Food Chemicals Codex, 1996) , whereas

that of germ sample was 68.38 % , This value is higher than for Locust bean germ (Bengoechea *et al* ., 2008).

Table 3 .1: Physical composition (%)of gum, germ of *Piliostigma reticulatum and* related galactomannan gums

Treat	*P.reticulatum* gum	Carob (bean) [1] gum	*Guar*[2]gum	*Cassia*[3]gum	*P.reticulatum* germ	Carob (bean)[4]germ
Moisture %	4.03	NR	NR	≤ 12	5.6	5.76
Ash %	3.09	≤ 1.2	≤ 1.5	≤ 1.2	7.21	6.34
Water holding capacity%	2.64	NR	NR	NR	1.93	NR
Nitrogen %	2.75	NR	NR	NR	10.94	NR
Protein%	17.19	≤ 7	≤ 10	≤ 7	68.38	48.2

[1,2]Food Chemicals Codex, 1996, [3] Klose and Glicksman, 1968,

[4] Bengoechea *et al*., 2008 and *NR:* not reported

3.2 The Solubility of gum and germ of *Piliostigma reticulatum*

Table (3.2) shows the solubility of gum and germ of *Piliostigma reticulatum* at 60 C°, the *Piliostigma reticulatum* gum and germ samples are insoluble at room temperature, and it is soluble at 60°C (Hoefler, 2004). The results showed that the solubility of gum sample in H_2O , 0 .1 M Na_2CO_3 and 0.5 M $NaHCO_3$were found to be 8.2% , 48.9% and 80% respectively, and gelled by using 0.5M$Na_2B_4O_7$ and 0.1 M NaOH .Whereas that of germ sample in H_2O, 0 .1 M Na_2CO_3 , 0.5 M $NaHCO_3$,

0.5 M$Na_2B_4O_7$,0.1 M NaOH , 0.5 M NaOH and 1M NaOH were found 22.7%, 53.5% ,60% ,81% ,46.5% , 69% and 74% respectively. Table 3. 3 shows the solubility of the gum and germ samples in some organic solvents , from result data shows that the gum and germ samples are insoluble in organic solvents , this was agreement for solubility of Carob bean gum and plant polysaccharides gum (Torio, M. *et al* ., 2006).

Table (3.2): Solubility of the gum and germ of *Piliostigma reticulatum*

Solvents	Gum	Germ	pH: gum germ
H_2O	8.2%	22. 7%	6.28 5.97
0.5 M $Na_2B_4O_7$	Gel	81 %	8.28 8.18
0 .1 M Na_2CO_3	48.9%	53.5%	10.59 10.55
0. 5 M $NaHCO_3$	80 %	60%	8 .53 8.37
0.1 M NaOH	Gel	46.5 %	12 .53 12.41
0.5 M NaOH	Gel	69%	12.80 12.64
1M NaOH	Gel	74%	12.94 12.82

Table (3.3): Solubility of the gum and germ in some organic solvents

Solvents	Gum	Germ
Ethanol	Insoluble	Insoluble
Acetone	Insoluble	Insoluble

3. 3 The elemental contents of gum and germ of *Piliostigma reticulatum*

Table (3.4) shows The element contents (%) of gum and germ of *Piliostigma reticulatum* , the gum and germ samples has recorded higher values for the elements potassium, calcium , phosphorus and silicon except for sodium, zinc , manganese and copper, which ,though is below measured levels . These values were high when comparable with values reported for some legumes (Elegbede JA, 1998).

Table (3.4): Elemental content (%) of gum and germ of *Piliostigma reticulatum*

Elemental	Gum	Germ
Potassium	13.56	31.36
Calcium	14.36	12.81
Phosphorus	2.51	13.04
Sodium	0.5	0.4
Zinc	0.30	0.09
Silicon	8.91	2.69
Manganese	0.0006	0.07
Iron	2.55	0.32
Copper	0.0003	0.04

3.4The sugar compositions of gum and germ of *Piliostigma reticulatum*

Tables (3.5) shows the average values of the sugar compositions of *Piliostigma reticulatum* gum samples and related galactomannan gums .The most abundant sugars yielded after hydrolysis are: mannose, galactose and ribose for gum sample and glucose, fructose and sucrose for germ sample.The result showed that *Piliostigma reticulatum* gum contain mannose (42.58%) , galactose (20.27%) and ribose (1.79%) , comparing these result with those of Carob bean gum and *Cassia* gum showed that both contain mannose and galactose and which are different of amounts, both Carob bean and *Piliostigma reticulatum* gums contain ribose sugar while *Cassia* gum contain glucose sugar.

Table (3.6) shows the average values of sugar compositions after hydrolysis of germ samples, the result showed presence of sugar namely glucose (3.4 %), fructose (6.9 %) and sucrose (5.6%).

Table (3.5): Sugar compositions of *Piliostigma reticulatum* gum and related galactomannan gums

Sugar content%	*Piliostigma reticulatum* gum	Carobbean[5] gum	*Cassia* gum[6]
Mannose	42.58	73- 85	77. 2 – 78
Galactose	20.27	27- 29	15.7 – 14.7
Ribose	1.79	0.5	-
Glucose	-	-	7.1 – 6.3

5 Philips,G.O.,*etal*.,1995and[6]CAS RegistryNumber for “galactomannan”.

Table (3.6): Sugar compositions of *Piliostigma reticulatum* germ

Sugar content %	*Piliostigma reticulatum* germ
Glucose	3.4
Fructose	6.9
Sucrose	5.6

3. 5Amino acid contents of gum and germ of *Piliostigma reticulatum*

Table (3.7) shows amino acid content of gum and germ of *Piliostigma reticulatum* .The analysis showed that fifteen amino acids were determined in gum and germ (Asparagine , Threnine , Serine , Glutamine , Proline , Glysine , Alanine , Valine , Methionine , Leucine , Tyrosine , Phenylalanine , Histidine , Lysine and Arginine).The germ had amino acid contain in high concentration than the gum, all the known amino acids. Germ can be considered as a ‘‘complete protein’’ in which all the essential amino acids are present (Dakia *et al*., 2007).

Table (3.7): Amino Acid compositions of *Piliostigma reticulatum* gum and germ

Compound	gum(mg /g)	germ(mg /g)
Asparagine	7.32	12.27
Threnine	2.17	3.89
Serine	2.27	5.31
Glutamine	16.07	34.79
Proline	5.20	10.19
Glysine	4.47	9.57
Alanine	5.29	8.32
Valine	4.39	7.73
Methionine	0.76	1.8
Leucine	6.32	11.13
Tyrosine	2.70	10.66
Phenylalanine	3.38	6.72
Histidine	3.02	6.60
Lysine	4	7.48
Arginine	4.27	10.21

Chapter Four

4 Rheological properties of *Piliostigma reticulatum* gum

4.1 General introduction

The rheological characteristics of a fluid are important in evaluating the ability of a fluid to perform a specific function. Many materials in use today are disperse systems where one substance (often particulate) is dispersed in another phase. These material types include adhesives, agrochemicals, cement, ceramics, colloids, cosmetics and personal care formulations, food and drink, mining and mineral slurries, paints, inks and surface coatings, pharmaceuticals and polymer systems. For example In the inks industry, the understanding of rheology and particle properties allows solid pigment content to be changed in different formulations whilst maintaining the critical rheological characteristics required for optimized printing. In the cement industry, the understanding of rheology and particle properties, such as the aggregate morphology, allows flow behavior during processing and application to be controlled. In the Cosmetics and Personal Care industries, it is essential to understand the relationship between rheology and particle properties to provide the optimum balance in terms of formulation, consumer acceptance and application performance. The physical properties of the dispersed particles, such as the average particle size, the size distribution, the charge on the particles and even the shape of the particles all help influence the overall(bulk) materials properties such as the rheology.(Braun *et al.*, 2000).

4.1.1Rheology

Rheology is the science of material deformation and flow. It relates to the relationship between stress, strain and time. It would commonly use the words lubricate, spread, squeeze, as non-technical examples of rheological responses. As force is applied to an object it can do one of several things: It can cause the object to be strained or ruptured, or it can make the object move, flow or accelerate. Rheology describes material response to the forces of deformation.(Goodwin *et al*., 2008). Historically, the origins of Rheology can be traced back to a Pre-Socratic Greek metaphysician named Heraclitus. In the 5^{th} Century B.C., Heraclitus was noted for documenting "Everything Flows."At the turn of the 17th century, renowned English mathematician and rheologist Sir Isaac Newton started to define rheology as a formal science. When a force is applied to a liquid, the liquid will flow to relieve the strain from this force. Different systems will resist this flow more than others and the measurement of this resistance is a measure of the viscosity of the system (Han, 2007). Newton first introduced a basic model for the flow measurement of a liquid between two parallel plates and this is represented in Figure (4.1).Using this model a number of common rheology terms can be defined as follows:

Shear Stress (τ): the force required to move a given area of the fluid, τ = Force/Area = F/ A (Nm^{-2} or Pascals (Pa)).

Shear Rate (γ): the rate of movement of the fluid between the plates. It is determined by dividing the velocity difference between the plates by the distance between them. This can also be called the velocity gradient, $\gamma = dv/dx$ (s^{-1})

Viscosity: this is expressed mathematically as, η =shear stress/shear rate and is the measurement of the resistance to flow of a fluid. Pascal. Second (Pa.s) is the basic unit of viscosity, but Poise or cent poise (cp = one hundredth of a Poise) is often used and one cp is equivalent to a millpascal-second, mPa.s. When quoting viscosities the shear rate (or measurement method/equipment used) should be stated together with the temperature at which the measurement was taken. Rheological measurements are generally represented by a plot of log η versus logγ. The logarithm is taken to enable the viscosity shear rate data to be presented over many orders of magnitudes (Goodwin *et al*., 2008).

4.1.2 Rheological Classifications

There are two types of fluids: Newtonian and Non-Newtonian.

4.1.2.1 Newtonian Fluids

Examples include water, certain oils and dilute resin solutions. This is the simplest type of fluid flow where the materials viscosity is constant and independent of the shear rate. The rate of deformation is directly proportional to the applied force. This is shown in the two different plot types in Figure (4.2). Newtonian liquids are so called because they follow the law of viscosity as defined by Sir Isaac Newton.

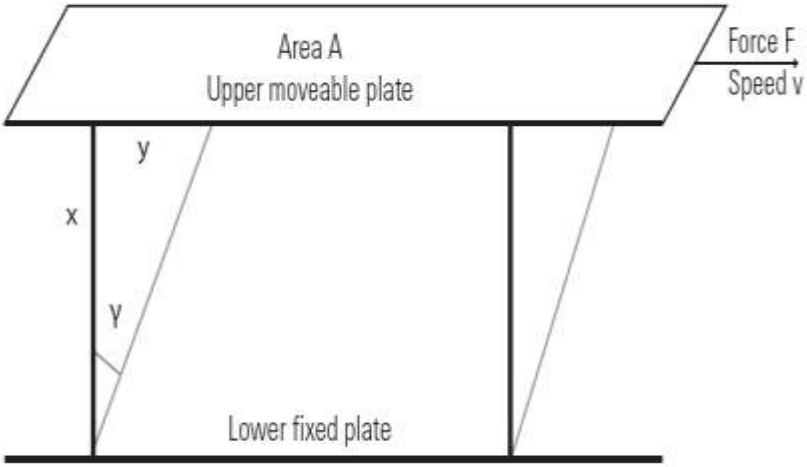


Figure 4.1: Diagram for rheological definitions

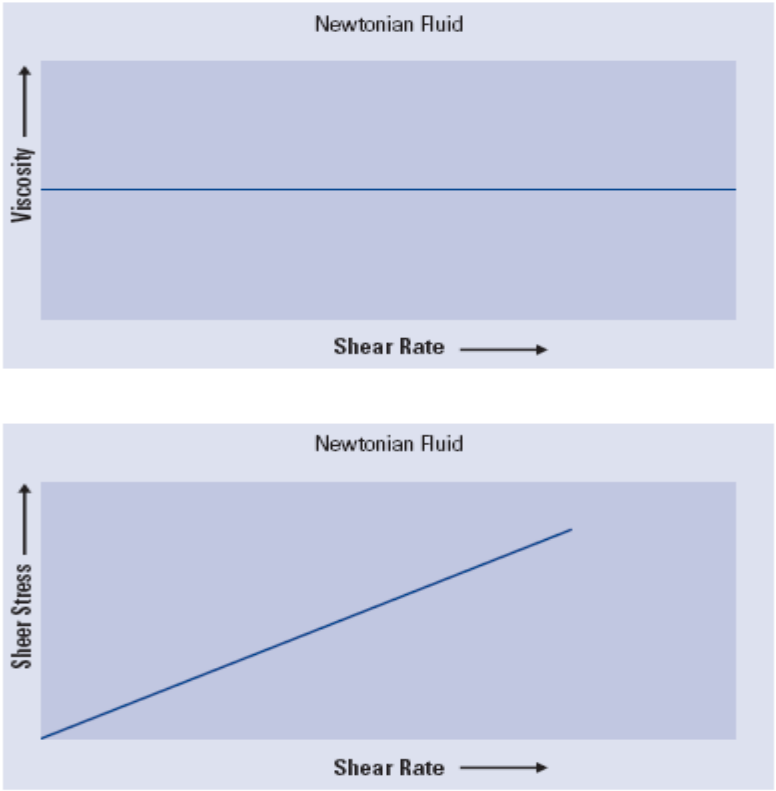


Figure 4.2: Diagram for viscosity of a simple Newtonian fluid

4.1.2.2 Non-Newtonian Systems

Many systems show behavior that deviates from that of simple Newtonian flow and examples of this are defined below:

4.1.2.2.1 Pseudo plasticity

A large number of liquids show a large decrease in viscosity when the shear rate is increased and this type of flow is defined as pseudo plastic or shear thinning behavior, shown in Figure (4.3).

Emulsions, suspensions and dispersions are typically pseudoplastic as are many paint, ink and adhesive systems. At higher shear rates the flow behavior becomes more linear (Newtonian). A related type of flow behavior is that of plastic materials, which initially resist deformation until a certain yield stress is reached. Beyond this point the flow is then that of a pseudoplastic fluid. Pseudoplastic behavior is portrayed below in two different plot types

4.1.2.2.2 Dilatancy

Dilatant fluids show the opposite type of behavior to pseudoplastic systems i.e. they show an increase in viscosity as the shear rate increases (shear thickening) is represented in the plot below in Figure (4.4).

It is seen in highly concentrated suspensions or slurries. This type of behavior is relatively uncommon.

4.1.2.2.3 Thixotropy

For thixotropic liquids the viscosity decreases over time for a given shear rate until a minimum is reached, Figure (4.5). Once the shearing force is stopped the viscosity recovers over time. This is typical of the behavior observed in gelled paints applied to a vertical surface, where the viscosity reduces under the shear of the brush or roller and then recovers to prevent sagging.

These are text book definitions; in common usage pseudoplastic and thixotropic may be used interchangeably or in combination. They are also not mutually exclusive.

4.1.2.2.4 Rheopexy

Rheopectic fluids show the opposite behavior to thixotropic fluids i.e. they increase in viscosity over time for a given shear force until a maximum is reached followed by a viscosity reduction over time when the shear is stopped. This type of behavior is extremely rare (Han, 2007).

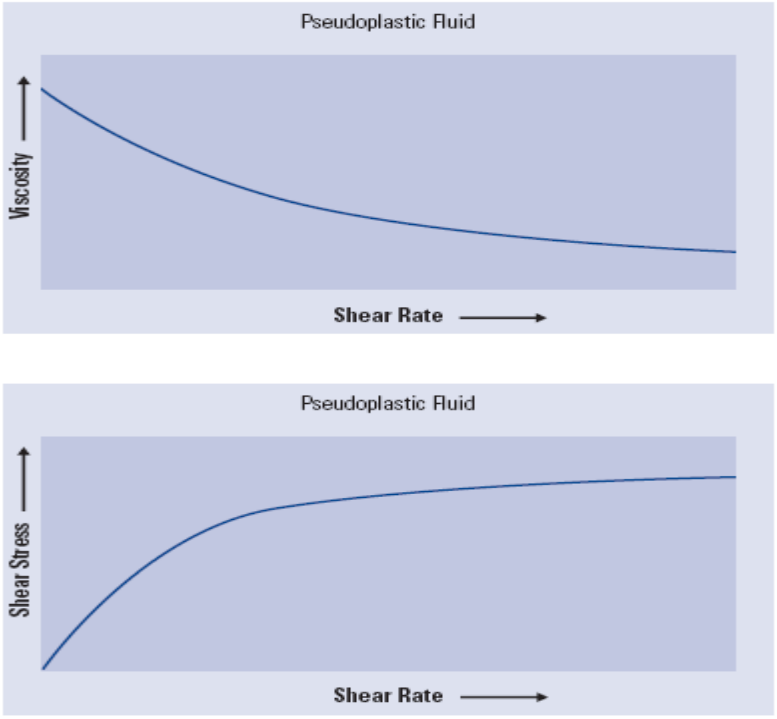


Figure 4.3: Diagram for viscosity of Pseudoplastic fluid

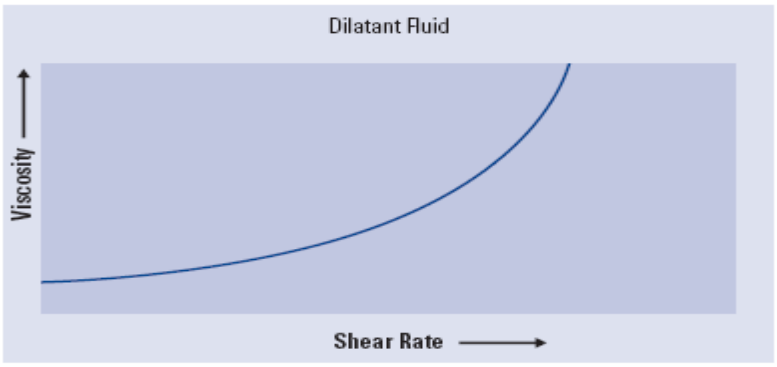


Figure 4.4: Diagram for viscosity of Dilatant fluid

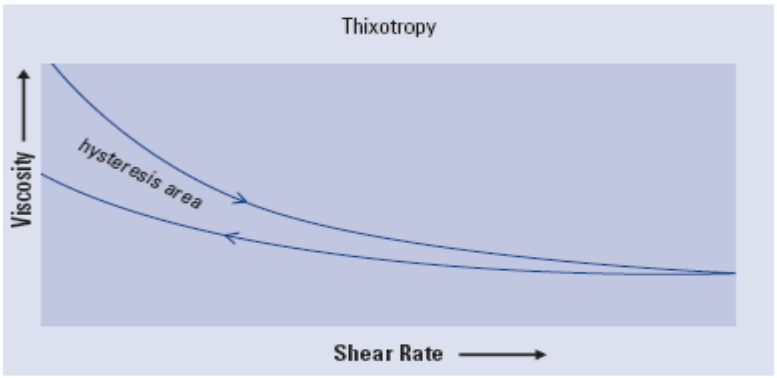


Figure 4.5: Diagram for viscosity of Thixotropy fluid

4.1.3 Measurement Apparatus

There are two common methods used for rheometric measurements on fluid systems: capillary (or tube) and rotational. Measurement devices can be grouped into one of two general classifications. Viscometers are devices used principally for the measurement of viscosity, while rheometers are devices used for the measurement of rheological properties of materials, typically fluids and melts. These instruments impose a specific stress field or deformation to the fluid, and monitor the resultant deformation or stress. Instruments can be run in steady flow or oscillatory flow, in both shear and extension (Goodwin *et al*., 2008)

4.1.3.1 Rotational Rheometer

High-precision, continuously-variable-shear instruments in which the test fluid is sheared between rotating cylinders, cones, or plates, under controlled-stress or controlled- shear rate conditions, are termed rotational rheometers. The basic rotational system consists of four parts: (i) a measurement tool with a well-defined geometry, (ii) a device to apply constant torque or rotation speed to the tool over a wide range of shear stressor shear rate values, (iii) a device to determine the stress or shear rate response, and (iv) some means of temperature control for the test fluid and tool. Depending on the design specifications, rheometers may also include built-in corrections or compensations for inertia, drift, and temperature fluctuations during measurement. Most rheometers are based on the relative rotation about a common axis of one of three tool geometries: concentric cylinder, cone and plate, or parallel plates.

In the concentric cylinder (also called Couette or Coaxial) geometry, either the inner, outer, or both cylinders may rotate, depending on instrument design. The test fluid is maintained in the annulus between the cylinder surfaces. This tool geometry comes in several configurations, of which the three most commonly encountered are illustrated in Figure (4.6).

The double-gap configuration is useful for low viscosity fluids, as it increases the total area, and therefore the viscous drag, on the rotating inner cylinder, and generally increases the accuracy of the measurement. The cone and hollow cavity configurations are specifically designed to reduce or account for end effects. In addition, to prevent slippage, the inner cylinder surface is sometimes serrated or otherwise roughened. The concentric cylinder geometry is typically used for the analysis of fluid suspensions.

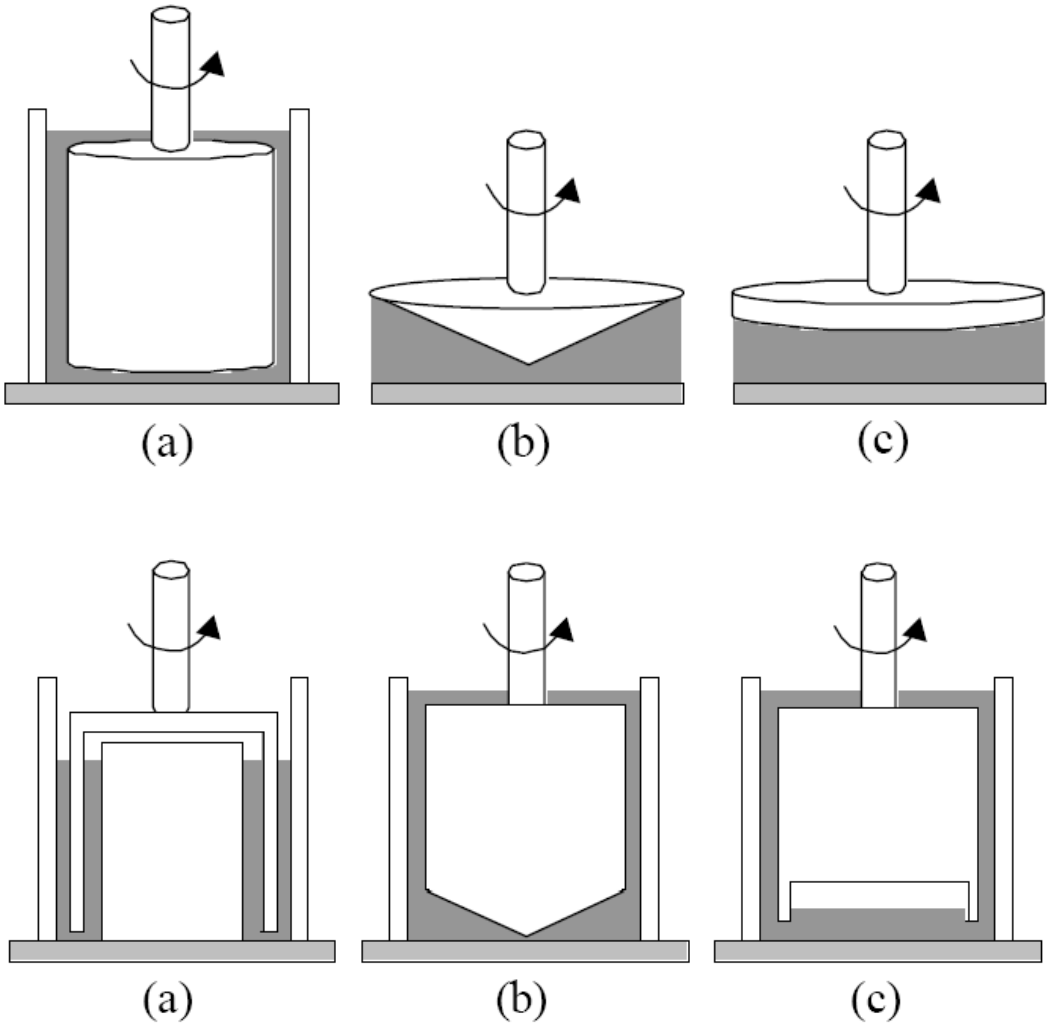


Figure 4. 6: **Schematic diagram of basic tool geometries for the rotational rheometer: (a) concentric cylinder, (b) cone and plate, (c) parallel plate**

The cone and plate geometry consists of an inverted cone in near contact with a lower plate. The cone is usually designed with an angle of less than 4^0. Either the upper or lower surface may rotate depending on instrument design.

The parallel plate geometry can be considered a simplified version of the cone and plate, having an angle of 0^0. The test fluid is constrained in the narrow gap between the two surfaces. Cone and plate and parallel plate measurement tools are most often used for highly viscous pastes, gels, and concentrated suspensions (A.A.SAtti, 2011).

4.1.4 Rheology 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 concrete , paint and chocolate which have complex flow characteristics. In addition, plasticity theory has been similarly important for the design of metal forming processes.

The science of rheology and the characterization of viscoelastic properties in the production and use of polymeric materials have 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 pharmacists working in the manufacture of several dosage forms, such as simple liquids, ointments, creams, pastes etc. The flow behavior of liquids 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.

One of the largest application areas is thin films and coatings, which can be produced on a piece of substrate by spin coating or dip coating. Other methods include spraying, electrophoresis, inkjet printing or roll coating. Optical coatings, protective and decorative coatings, and electro-optic components can be applied to glass, metal and other types of substrates with these methods. With the viscosity of a sol adjusted into a proper range, both optical quality glass fiber and refractory ceramic fiber can be drawn which are used for fiber optic sensors and thermal insulation, respectively. The mechanisms of hydrolysis and condensation, and the rheological factors that bias the structure toward linear or branched structures are the most critical issues of sol-gel science and technology.

Rheology is very important in Geophysics. Geophysics includes the flow of molten lava and debris flows (fluid mudslides). Also included in this disciplinary branch are solid Earth materials which only exhibit flow over extended time scales. Those

that display viscous behavior are known as rheids , e.g. Granite can do a plastic flow with a vanishingly small yield stress, (i.e. a viscous flow). Long term creep experiments (~ 10 years) indicate that the viscosity of granite under ambient conditions is on the order of 10^{20} poises. In Physiology, the rheometry has a very important role. Physiology includes the study of many bodily fluids that have complex structure and composition, and thus exhibit a wide range of viscoelastic flow characteristics. In particular there is a specialist study of blood flow called hemorheology. This is the study of flow properties of blood and its elements (plasma and formed elements, including red blood cells, white bloodcells and platelets). Blood viscosity is determined by plasma viscosity, hematocrit (volume fraction of red blood cell, which constitutes 99.9% of the cellular elements) and mechanical behavior of red blood cells. Therefore, red blood cell mechanics is the major determinant of flow properties of blood. Food rheology is important in the manufacture and processing of food products. Food rheology is the study of the rheological properties of food, that is, the consistency and flow of food under tightly specified conditions. The consistency, degree of fluidity, and other mechanical properties are important in understanding how long food can be stored, how stable it will remain, and in determining food texture. The acceptability of food products to the consumer is often determined by food texture, such as how spreadable and creamy a food product is. Food rheology is important in quality control during food manufacture and processing. Thickening agents, or thickeners, are substances which, when added to an aqueous mixture, increase its viscosity without substantially modifying its other properties, such as taste. They provide body, increase stability, and improve suspension of added ingredients. Thickening agents are often used as food additives and in cosmetics and personally giene products. Some thickening agents are gelling agents, forming a gel. The agents are materials used to thicken and stabilize liquid solutions, emulsions, and

suspensions. They dissolve in the liquid phase as a colloid mixture that forms a weakly cohesive internal structure. Food thickeners frequently are based on either polysaccharides (starches, vegetable gums, and pectin), or proteins (Braun *et al*., 2000 and Schramm, 2000).

4.2 Experimental

Piliostigma reticulatum gum is not water soluble. The rheological properties measured only for viscous solutions, it needs to heating to obtained soluble solution.

4. 2 .1Procedure of samples prepared by heated in water bath at 80°C

Piliostigma reticulatum gum solutions were prepared at different concentration (w/w) by dispersing the 1, 2, 3 and5g of gum sample in distilled water, under slow -stirring at room temperature .The mixture was then heated in a water bath at 80 °C for 15 min followed by stirring by a magnetic stirrer at room temperature for 90 min for complete hydration (Farahnaky, A. *et al.*, 2013).

4.2.2 Procedure of Starch powder – *Piliostigma reticulatum* gum mixture

Starch powder was dispersed in the previously prepared aqueous gum solution (0.35%).Starch concentrations were within the range 4% (w/w). The corn starch/gum dispersions were heated from 30to 90°C, kept at this temperature for 15 min, and then cooled (Doublier, 1981).

4.3 Rheological measurements

Rheological measurements were carried out using: BROOKFILED (Rheometer DPTA1315108, Model DV- 111 programmable Rheometer) which it is shown in Figure 4.7. The apparent viscosity was measured for samples, the rheometer control and data processing was done by computer software (Rheology Advantage

Data Analysis Program, TA) under the following experimental condition at temperature (25°C±0.1). The apparent viscosity was calculation using the formula

$\eta = \tau/D$

Where $\eta$is the apparent viscosity (cp), τ is the shear stress (dyne /cm^2), D is the rate of shear S^{-1} (A. ragheb *et al*., 1989).

Figure (4.7): BROOKFILED (Rheometer DPTA1315108, Model DV- 111 programmable Rheometer)

4.4 Results and discussion

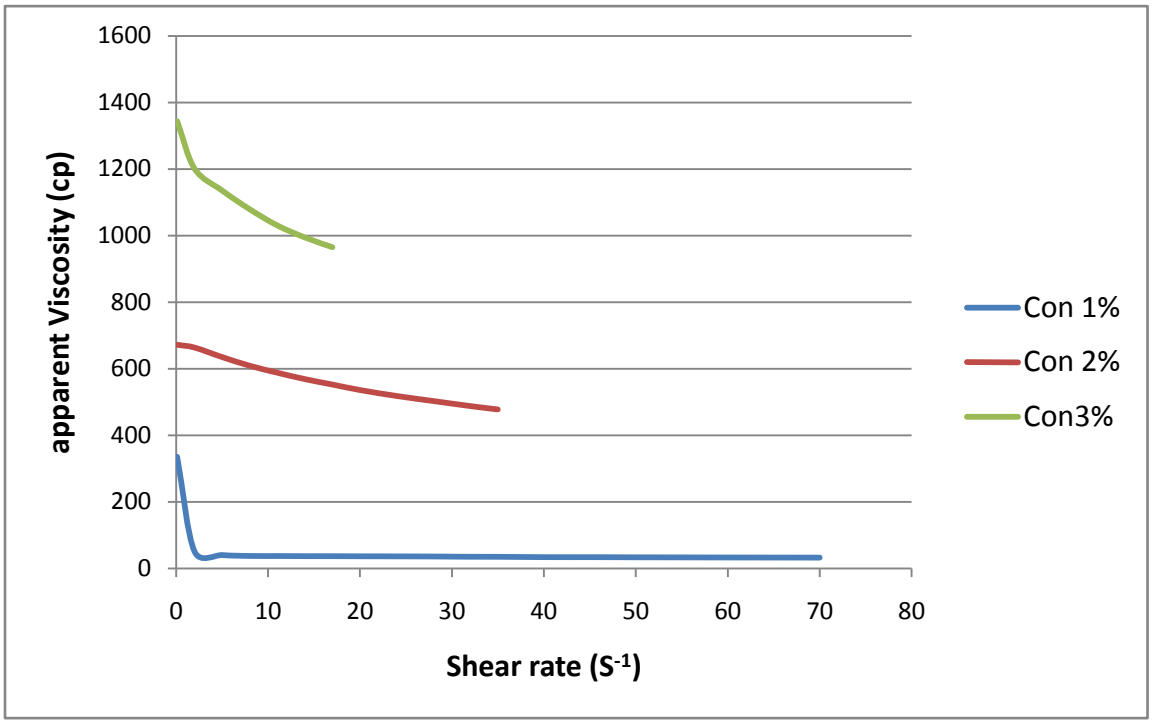
4.4.1 Rheological properties of *Piliostigma reticulatum* gum

Figures (5.8) and (5.9) show the rheological properties of *Piliostigma reticulatum* gum at different concentration 1%, 2% and 3% (w/w). Al most all *Piliostigma*

reticulatum gum solutions showed shear thinning flow behavior, the apparent viscosity decreased with increasing shear rate at 25°C. Characterized by non – Newtonian pseudoplastic with yield point (plasticliquid), at any concentration of three samples, the Rheometer stopped automatically at a specific shear rate. Figures(5.10) and (5.11) show the variation of apparent viscosity with shear rate for the concentration (5%) , the Figures show the shear thickening behaviour of concentration 5% of *Piliostigma reticulatum* gum sample where the apparent viscosity increased when the shear rate is increased at 25° C . The measured viscosity is stopped, automatically, at shear rate 0.70 S^{-1}.

The shear-thickening behaviour as considered before may be attributed to the fact that: the rate of forced disentanglement as the shear rate is increased, becomes less than the rate at which new entanglements can be formed and this decreases the freedom of movement of individual chains (Mezger , 2002).

Piliostigma reticulatum gum as seed galactomannan, they are extracted from plant seeds are in seed gum group and they are biocompatible polymers (Pazur , J.H , *et al*., 1986) . Forecaster the rheological properties of *P.reticulatum* gum were similar to properties of Locust (Carob) bean gum and theses results are in agreement with the report of the shear thinning behavior of Locust (Carob) bean gum solutions has been reported in previous studies (Stittikijyothin , W, *et al* ., 2005 , Wu.Y , *et al*., 2009 and Bourbon, A. L , *et al* .,2010) .Shear thinning of Locust(Carob) bean gum solutions might be due to modifications of macro molecules organization in the solution as the shear rate changes,i.e with increasing shear rate , the molecular entanglements are destroyed , so the molecules align in the direction of flow and viscosity decreases (Dakia ,P.A *et al* ., 2008 and Farahnaky ,A , *et al* ., 2013) .



Figure(4.8)Apparent viscosity versus Shear rate profile of *Piliostigma reticulatum* gum of concentrations of 1%,2% and 3%

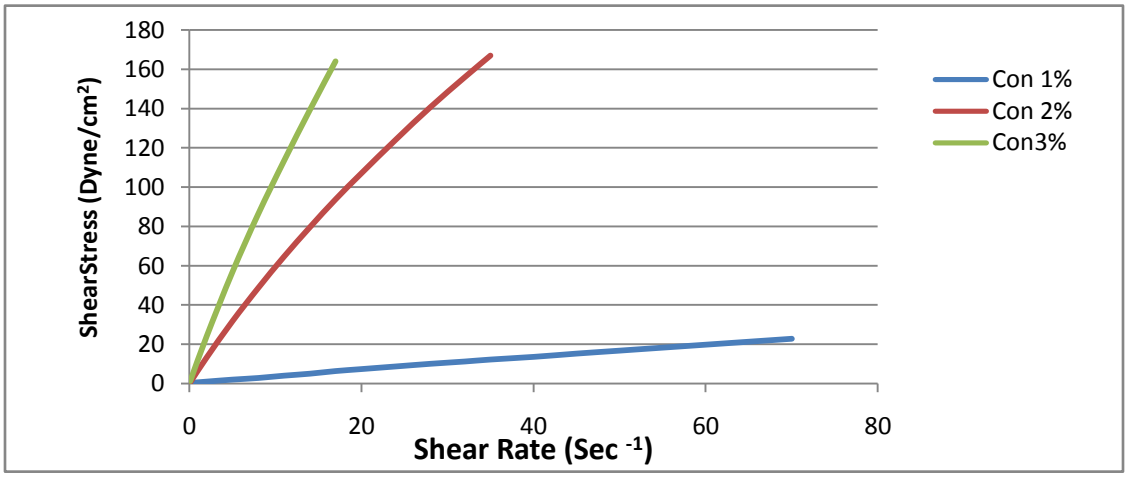


Figure (4.9) Shear stress versus shear rate for *P.reticulatum* gum of concentration 1, 2 and 3%

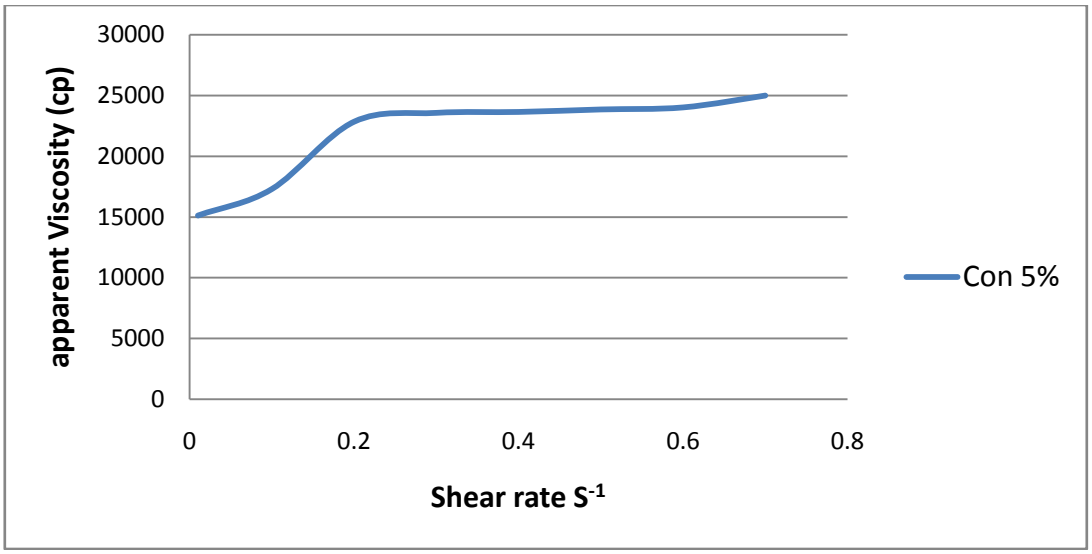


Figure (4.10) Apparent viscosity versus shear rate profile for concentration 5% of *Piliostigma reticulatum* gum at 25 C°

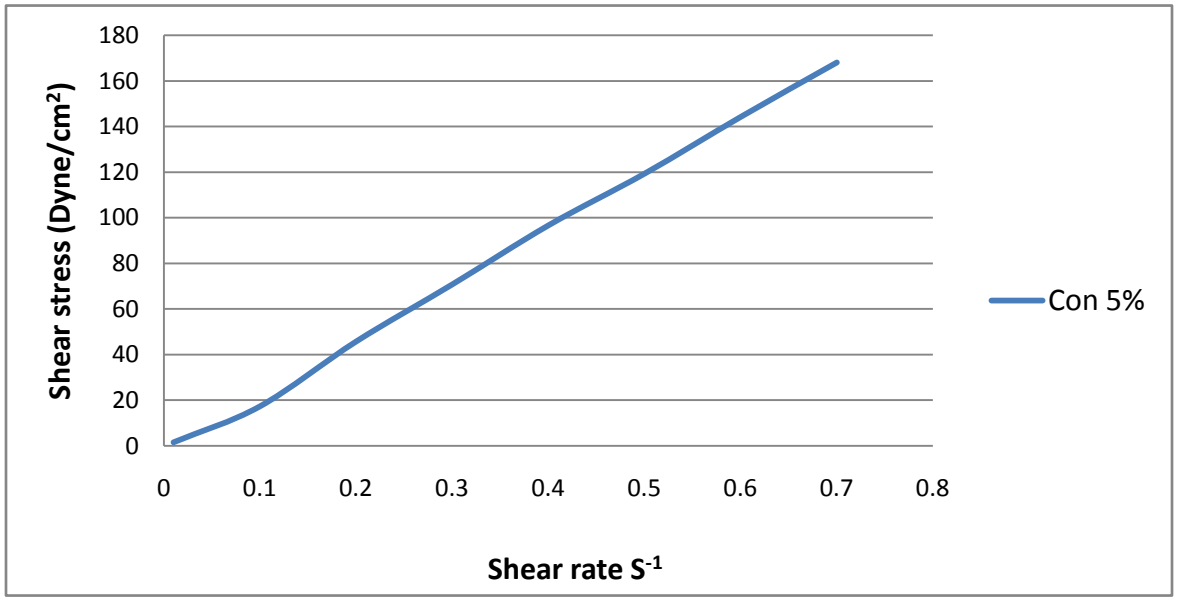


Figure (4.11) Shear stress versus shear rate for *Piliostigma reticulatum* gum at 25 C° of concentration5%

Figure 4.12 shows the viscosity at different temperature for concentration (3%) of gum sample, the result showed the viscosity decreased as the temperature increased, this result was similar to properties of Locust (Carob) bean gum and in agreement with the report in previous studies (Dea , I.C.M., and Morrison ,A., 1975) .

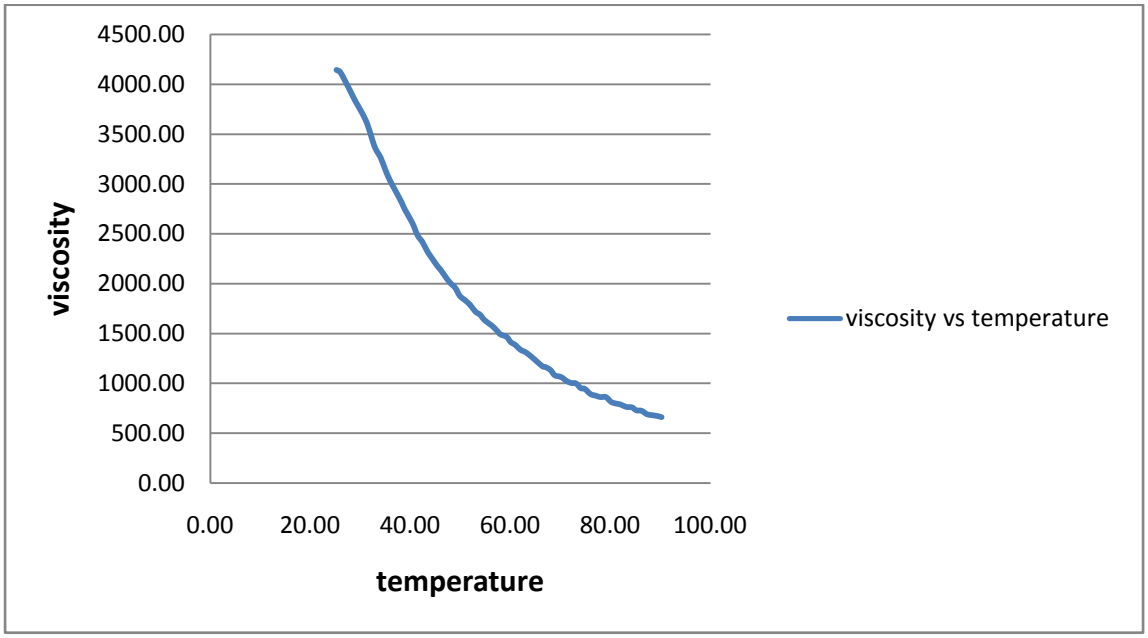


Figure (4.12) Viscosity versus temperature of *Piliostigma reticulatum* gum for concentration 3%

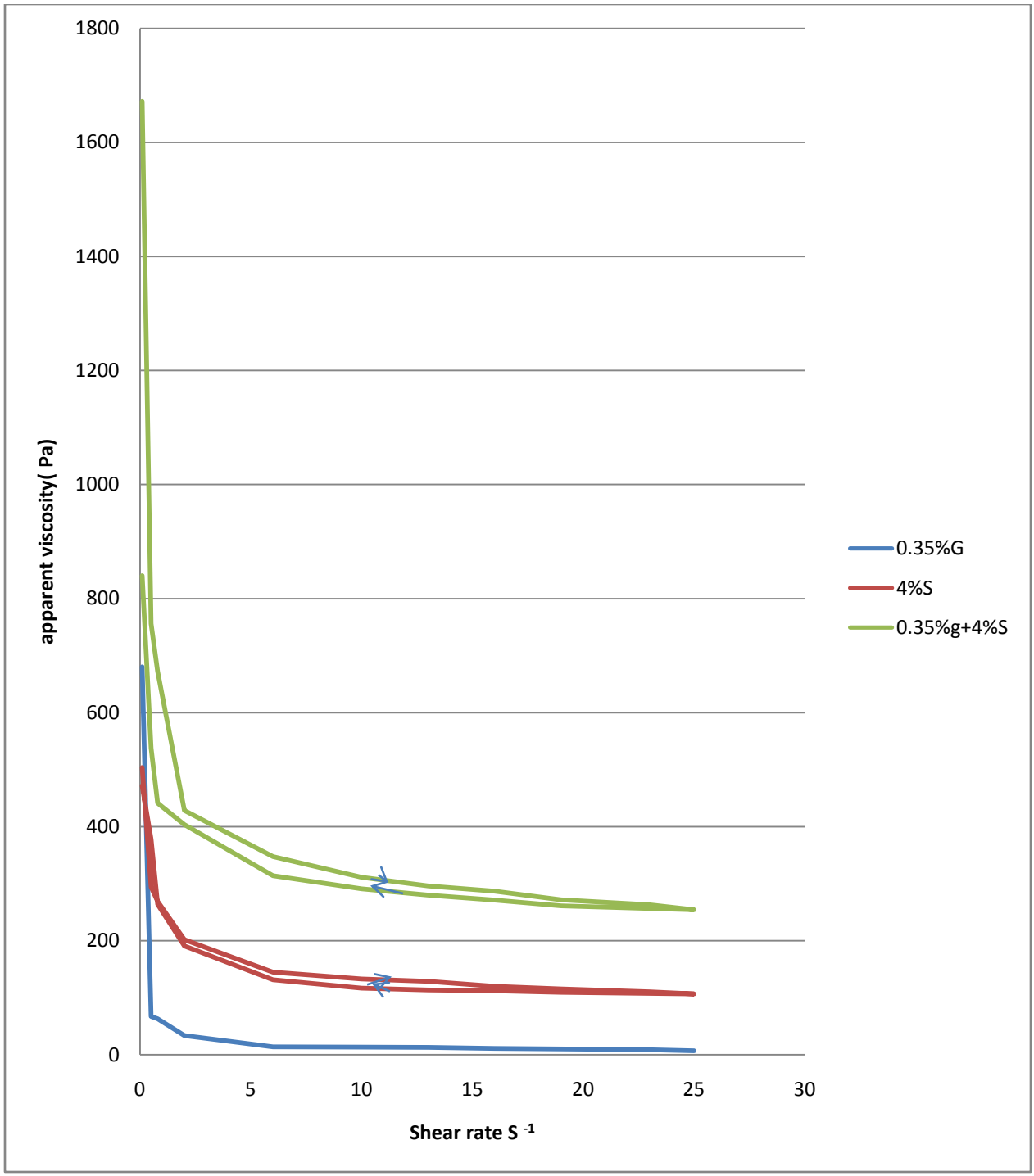
4.4.2 A Rheological properties of the Corn Starch – *Piliostigma reticulatum* gum mixture

Viscosity measurements (on starch paste 4%, gum solution 0.35% and blend (0.35% gum + 4% Corn starch) . Measurements were carried out at 25° C. Figure 4.13 shows flow curves of 4% corn starch paste , the 0.35% *Piliostigma reticulatum* gum solution and 4% starch +0.35% *Piliostigma reticulatum* gum

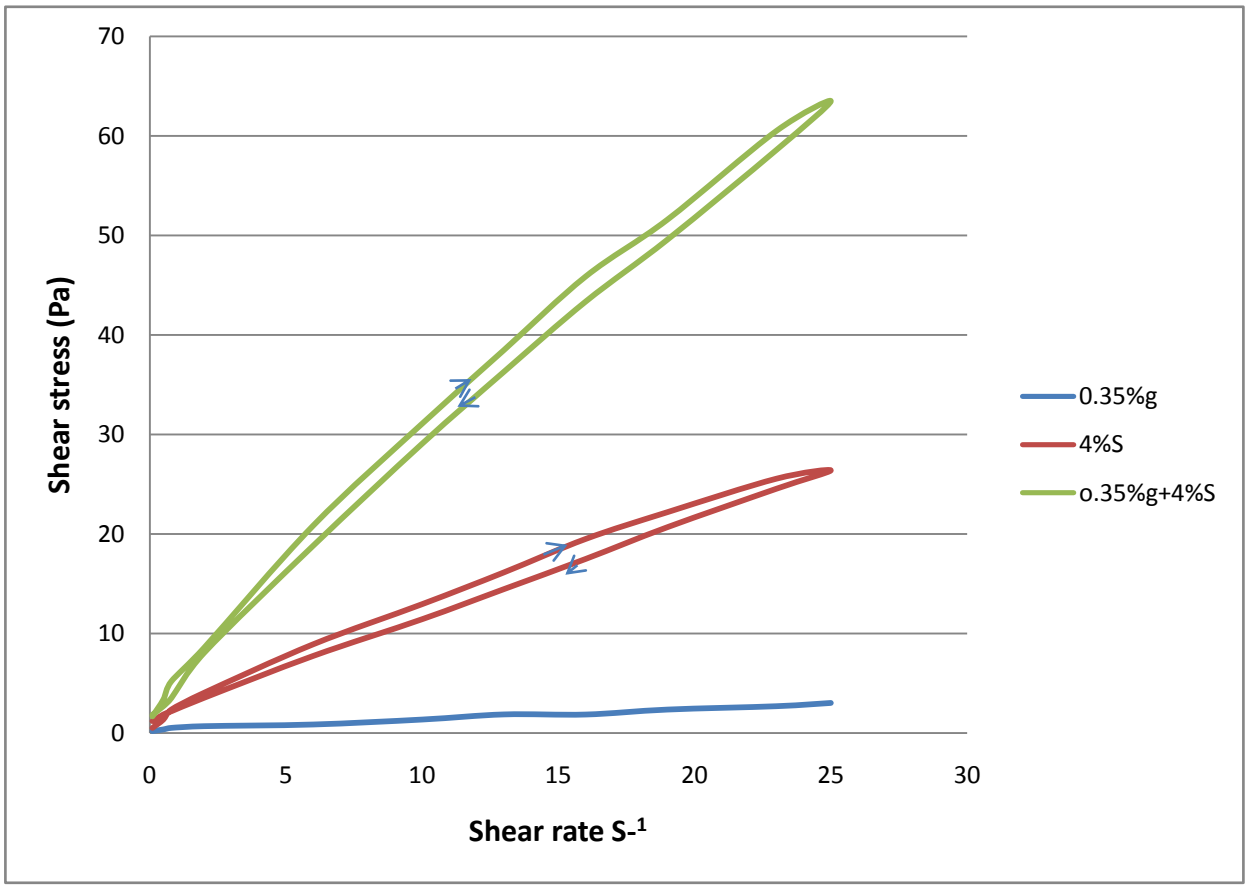
mixture . Curve starch4% is pronounced shear – thinning behavior and thixotropic loop (Doublier, 1981) .Similarly, curves *P.reticulatum* gum and the blend were usually displayed by *Piliostigma reticulatum* gum solution with a shear – thinning behavior at this concentration and no alone but with a higher apparent viscosity than starch .Thixoxtropy appears to be less pronounced in the presence of the hydrocolloid, whereas the apparent viscosity is more important for the blends. This is related to the absence of thioxtropy in *Piliostigma reticulatum* gum.

Figure 4.14 flow curves obtained in the same experiment. The curve of starch alone displays the classical tendency to a yield stress at low shear rates (Evans and Haisman , 1979 and Wong and leliever ,1982).Existence of such a yield stress is typical of suspensions and has been ascribed to the presence of highly deformable swollen particles in the medium .The flow curve of *Piliostigma reticulatum* gum is indicating a shear – thinning behavior , the Newtonian region displayed by such

Type of solution at high shear rate cannot be evidenced due to low sensitivity of the viscometer. The flow curves of the blends seem to exhibit both types of behaviors, resemble curve for *Piliostigma reticulatum* alone, in contrast to low shear rates where a tendency to a yield stress is exhibited. Also give some evidence that cereal starch paste can be described as suspension of swollen particles dispersed in a macromocular medium (Doubiller *et al.*, 1987).



Figur (4.13)Apparent viscosity versus shear rate profile of*P.retculatum gum 0.35%* , corn starch 4%and corn starcch 4% + 0.35 *P.retculatum* gum concentration



Figure(4.14)Shearing stress versus Shear rate of *P. reticulatum* gum 0.35% , corn starch 4% and corn starch 4% +0.35% gum concentration

Chapter Five

5 Emulsification and Some Thickening Application Properties of *Piliostigma reticulatum* gum

5.1 Emulsification properties of *Piliostigma reticulatum* gum

Emulsions are by definition, dispersions of a liquid phase in another, which exhibit certain stability, in most cases thanks to the presence of an adsorbed surfactant or an emulsifier at interface. Emulsion systems are either oil-in-water (O/W) or water-in-oil (W/O) (Laughlin, 1994).

As a general trend, it can be said that oil and water alone produce a two-phase system which leads to an unstable dispersion upon stirring, due to the higher interfacial tension, and therefore a higher Gibbs free energy. To reduce this interfacial energy, the system will seek to minimize the interfacial area between the oil and the water. In the absence of surfactants, this is achieved by coalescence of the oil droplets, to give separated layers of oil and water. The presence of adsorbed surfactant molecules lowers the interfacial tension and hence the driving force for coalescence is reduced, and consequently the time of separation is increased. Thus, although emulsion tend to be regarded as thermodynamically unstable, it is possible by judicious use of surfactants, to control the kinetics of destabilization and to produce emulsions with, lengthy shelf lives. However, the large interfacial area can be taken advantage of an emulsion in some applications and has been successfully used as media for organic synthesis (Holmberg, 2006).As far as the phase behaviour is concerned, the added surfactant may also tends to reduce the oil-water immiscibility gap, just as it would do with an increase in temperature, but this time through a so-called solubilization process associated with the presence of micelles and other self-association structures(Salager , 2006).

The process of converting two immiscible liquids to an emulsion is known as homogenization which is usually carried out by a mechanical device called a homogenizer. The Tendency for emulsion droplet to break up during homogenization depends on the strength of the interfacial force that hold the droplet together compared to the strength of disruptive force provided by the homogenizer. In the absence of any applied external forces, emulsion droplet tends to adopt spherical shape to minimize the contact area between the oil and the water phases. Changing the shape of the droplet, or breaking it into smaller ones, will increase the interfacial area and therefore requires a higher Gibbs free energy. The nature of the disruptive forces that act on droplet during homogenization depends on the flow condition and on the type of homogenizer used to create the emulsion. Since emulsions are, highly, dynamic systems, the droplets, continuously, move around and, frequently, collide with each other. The droplet-droplet collisions depend on the intense mechanical agitation of the emulsion by the homogenizer. If the droplets are not protected by sufficient amount of strong emulsifier membrane, they will tend to coalesce immediately after the disruption of an emulsion during homogenization. To prevent coalescence from occurring, it is necessary to form a sufficient concentrated emulsifier membrane around a droplet before it has time to collide with its neighbors. The size of the droplets produced during homogenization therefore depends on the time taken for the emulsifier to be adsorbed on to the surface of the droplets compared to the time taken for droplet-droplet collisions. If the adsorption time is greater than the collision time, the droplets will be, rapidly, coated with emulsifier and hence stable emulsions formed. On the other hand if adsorption time is less than the collision time, the coating with the emulsifier won't be sufficient and the droplets will rapidly coalesce (Casimir *et al.*, 2008).

Generally, an emulsion stabilizer material may be defined as any ingredient that can be used to enhance the stability of an emulsion and may therefore be either an emulsifier or thickening agent (Khachatryan , 2003).Emulsifiers are surface-active material that adsorb to the surface of freshly formed droplets during homogenization, forming a protective membrane that prevents the droplets from coming close enough together to aggregate. In addition to the mechanism of reducing the interfacial tension between oil and water phases, emulsifier may act through the mechanism of covering the oil droplets with a charged layer to create a physical barrier preventing flocculation (Walstra , 1986). Stabilizers such as thickening agent usually stabilize emulsions by increasing the viscosity of the continuous phase and hence retarding the movement of the droplets of the dispersed phase (Glicksman, 1973). Others factors increasing the stability of emulsions are : decreasing the droplet size of the dispersed phase and equating the density of dispersed phase with the density of the continuous phase.

Various emulsifiers and stabilizers are used for obtaining a stable emulsion with a good shelf life. Polysaccharides are among the most widely used in the food industry to stabilize oil in water emulsions and control their rheological properties (Paraskevopoulos *et al*., 1997 and Phillips and Williams, 2000). They are generally odorless, colour less, and tasteless, have low energy value and digestibility. The majority of them show little surface activity and their incorporation into oil-in-water emulsions is aimed inhibiting droplet creaming by increasing the viscosity of the aqueous phase thus preserving the desired textural properties of the emulsion (Glicksman, 1973).

5.1.1 Emulsification properties of Hydrocolloids

Emulsion stability is measure of the rate at which an emulsion creams, flocculates or coalesces. The rate of these changes can measure by determining the size and distribution of the oil droplets in the emulsion. Stokes law states that the velocity at which a droplet moves is proportional to the square of its radius. The stability of an emulsion to gravitational separation can therefore be enhanced by reducing the size of droplets (Mc Clements, 1999). Hydrocolloid gums are mostly hydrophilic polymers, which do not exhibit significant surface-activity (Dickinson and Stains by, 1988). However, as a stabilizer in food emulsions, some gums were found to migrate slowly to air – water and oil – water interfaces and exhibit some surface and interfacial activities (Reichman , 1992; Grover , 1993 and Garti , 1999) . Hydrocolloid gums, although water soluble rigid and very hydrophilic, can precipitate adsorb on to oil droplets and satirically stabilize emulsions against flocculation and coalescence. The main stabilizers used are xanthan gum, galactomannans, propylene glycol alginate, pectin and carboxymethyl cellulose. By using a mphiphilic polysaccharides such as gum arabic or propylene glycol alginate. Others emulsifiers used frequently in the food industry are a mphiphilic proteins and phospholipids monoglycerides, such as sucrose esters of fatty acids, or salts of fatty acids (Krog , 1990). The structure of the emulsifier may be described in terms of hydrophilic lipophilic balance as the most common case (i.e. one side of the emulsifier molecule is polar moiety and the other side is non polar (Davis, 1994). Fenugreek gum has a mannose backbone with galactose units attached at an average ratio of 1:1. It was found to adsorb onto the oil interface forming a relatively thick interfacial film (Garti *et al*., 1997 and Garti, 1999). Purified Fenugreek gum reduced surface tension and exhibited interfacial activity and was able to form an oil – in – water emulsion having small

droplet sizes (2-3 μm) and long –term stability (Garti *et al*., 1997).concluded that the interfacial activity observed in galactomannan gums was not related to the presence of residual protein. Methyl cellulose dispersions exhibit inverse solubility characteristics., that is they form gels when heated and return to their original liquid viscosity when cooled .In general both high viscosity and small oil droplet size contribute to the stability of emulsions. By delaying the hydration of methyl cellulose at a high temperature, the low viscosity mixture allows the production of an emulsion with smaller oil droplets (Grover ,1993), suggested that the intrinsic surface activity of methyl cellulose coupled with interfacial dehydration caused the polymer concentration at the air – water interface to increase at the expense of polymer dissolved in the bulk phase .This surface thermal gelation prevent oil droplets from coalescence and promotes the formation of emulsions having unusual stability at elevated temperatures (Gaonkar , 1991 and Dow Chemical Company , 1999) evaluated the emulsification activity of methyl cellulose and alginate , and concluded that the chemical nature of the functional groups in theses gums was very important in providing stability to the oil / water emulsions. In preliminary study, Fenugreek gum was shown to produce a very stable oil- in-water emulsion when assayed by centrifugation and storage time procedures.

5.1.2 Application of Emulsion

Emulsions can be used in variety of fields:

5.1.2.1 In Food

Oil- in water emulsions are common in food; it can be used in:

• Creams (foam) in espresso – coffee oil in water (brewed coffee) , un stable emulsion

•Mayonnaise and Hollandaise sauce – these are oil – in water emulsions that are stabilized with egg yolk lecithin, or with others types of food additives, such as sodium stearoyl lactylate

• Homogenized milk – an emulsion of milk fat in water and milk proteins

Water – in- oil emulsion s are less common in food but still exist:

•Butter – an emulsion of water in butterfat

•Vinaigrette – an emulsion of vegetable oil in vinegar .If this is prepared using only oil and vinegar (i.e., without an emulsifier), an un stable emulsion results (Mason *et al*., 2006)

5.1.2.2 In medicine

In pharmaceutics, hairstyling, personal hygiene and cosmetics, emulsions are frequently used. These are usually oil and water emulsion, but which is dispersed and which is continues depends in many cases on the pharmaceutical formation. Theses emulsions may be called crèmes ,ointments , liniments (balms) , pastes , films , or liquid , depending in mostly on their oil – to – water ratios , others additives , and their intended route of administration . The first 5are topical dosage forms, and may be used on the surface of the skin, transversally, ophthalmic ally, rectally, or vaginally. A highly liquid emulsion may also be used orally, or may be injected in some cases. Popular medications occurring in emulsion form include calamine lotion, cod liver oil, polysporin cream, Canesten, and Fleet. Micro emulsions are used to deliver vaccines and kill microbes. Typical emulsions used in these techniques are nanoemulsions of soybean oil, which particles that are 400 – 600 nm in diameter. The process is not chemical, as with others types of

antimicrobial treatments, but mechanical. The smaller the droplet, the greater the surface tension, and thus the greater the force required tomerge with others lipids. The oil is emulsified with detergents using a high – shear mixer to stabilize the emulsion, so when they encounter the lipids in the cell membrane or envelope of bacteria or viruses, they force the lipids to merge with themselves. On a mass scale, this effectively disintegrates the membrane and kills the pathogen. The soybean oil emulsion dose not harms normal human cells, or the cells of most other higher organisms, with the exceptions of sperm cells and blood cells, which are vulnerable to nanoemulsions due to the peculiarities of their membrane structures. For this reason, theses nanoemulsions are not currently used intravenously. The most effective application of this type of nanoemulsion is for the disinfection of surface .Some types of nanoemulsions have been shown to effectively destroy HIV-I and tuberculosis pathogens on non- porous surfaces.

5. 1.2.3 In firefighting

Emulsifying agents are effective at extinguishing fires on small, thin – layer spills of flammable liquid (Class B fires). Such agents encapsulate the fuel in a fuel – water emulsion, thereby trapping the flammable vapors in the water phase. This emulsion is achieved by applying an aqueous surfactant solution to the fuel through a high – pressure nozzle. Emulsifiers are not effective at extinguishing large fires involving bulk /deep liquid fuels, because the amount of emulsifier agent needed for extinguishment is a function of the volume of the fuel, whereas other agent such as aqueous film – forming foam (AFFF) need cover only the surface of the fuel to achieve vapor mitigation (Silvestre *et al*.; 1999).

5.1.3 Emulsion preparation

The gum were dispersed in distilled water at level of 1%, 1.5% and 2% (w/w) with 0.05% Sodium benzoate as preservative. The samples were heated to 80° C in a water bath and then thoroughly mixed for 3h.(X. Huang *et al* .,2001).The emulsion were prepared by slowly mixing 5 ml of Olive Oil in to the gum samples , then emulsifying the mixtures with Sonicator up 950 a cculab,time 5 min , pulser ratio 50%.

5.1.4 Particle size distribution

Particle size distribution of emulsions were analyzed using Particle Size and Zeta Potential analysis (Particle Sizing Systems Inc .Santa Barbara ,Calif ., USA), presented in Figure(5.1).Distilled water was used as dispersant, the prepared samples were kept closed in glass vessel at 25°Cand particle size distribution of the emulsions were determined .

Figure (5.1): Particle Size and Zeta Potential analysis Instrument

5.1.5 Results and discussion

The particle size distribution of the emulsion droplets size was determined on the different concentration (1%, 1.5% and 2%) of *Piliostigma reticulatum* gum emulsions for freshly prepared and after storage for 3 and 7 days at 25° C ,using Particle Size and Zeta Potential analysis (Particle Sizing Systems Inc .Santa Barbara , Calif ., USA) . The resulting expressed as a cumulative distribution, the particle size distribution where the cumulative distribution 50% is known as the median droplet diameter.

Tables (5.1) to (5.3) and Figures (5.2) to (5.4) show the particle size cumulative distribution of *Piliostigma reticulatum* emulsions for 1%, 1.5% and 2% concentration. Results show that the *P.reticulatum* gum emulsion of all concentration has large particle droplet size and increased of all parameters from (25%) to (99%) of any conditions. The emulsion of 1% has a very large particle droplet size with distribution 50% (11556.1nm) and decreased during storage from 3 to 7 days at at25°C. The emulsion of 1.5% has droplet particle size with distribution 50% (1229.7nm), increased from 3 days and decreased for 7 days. The emulsion of 2% has droplet particle size with distribution 50% (1574.2nm) , increased from storage time 3 to 7 days at25°C. From the presented results showed change was found in most emulsions during the incubation for 3 to 7 days at25°C, mean that the *Piliostigma reticulatum* gum emulsions less stability.

Table 5.1: The particle size cumulative distribution in (nm) of *Piliostigma reticulatum* gum emulsion of concentration 1% at different conditions

Condition	25%	50%	75%	80%	90%	99%
Freshly Prepared	3191.1	11556.1	24065.6	29256.1	47893.2	96153.1
After 3days at25°C	801.4	1301.4	1841.5	2016.8	2577.3	4707.1
After 7days at25° C	637.7	1105.0	1615.3	1785.2	2339.2	4544.3

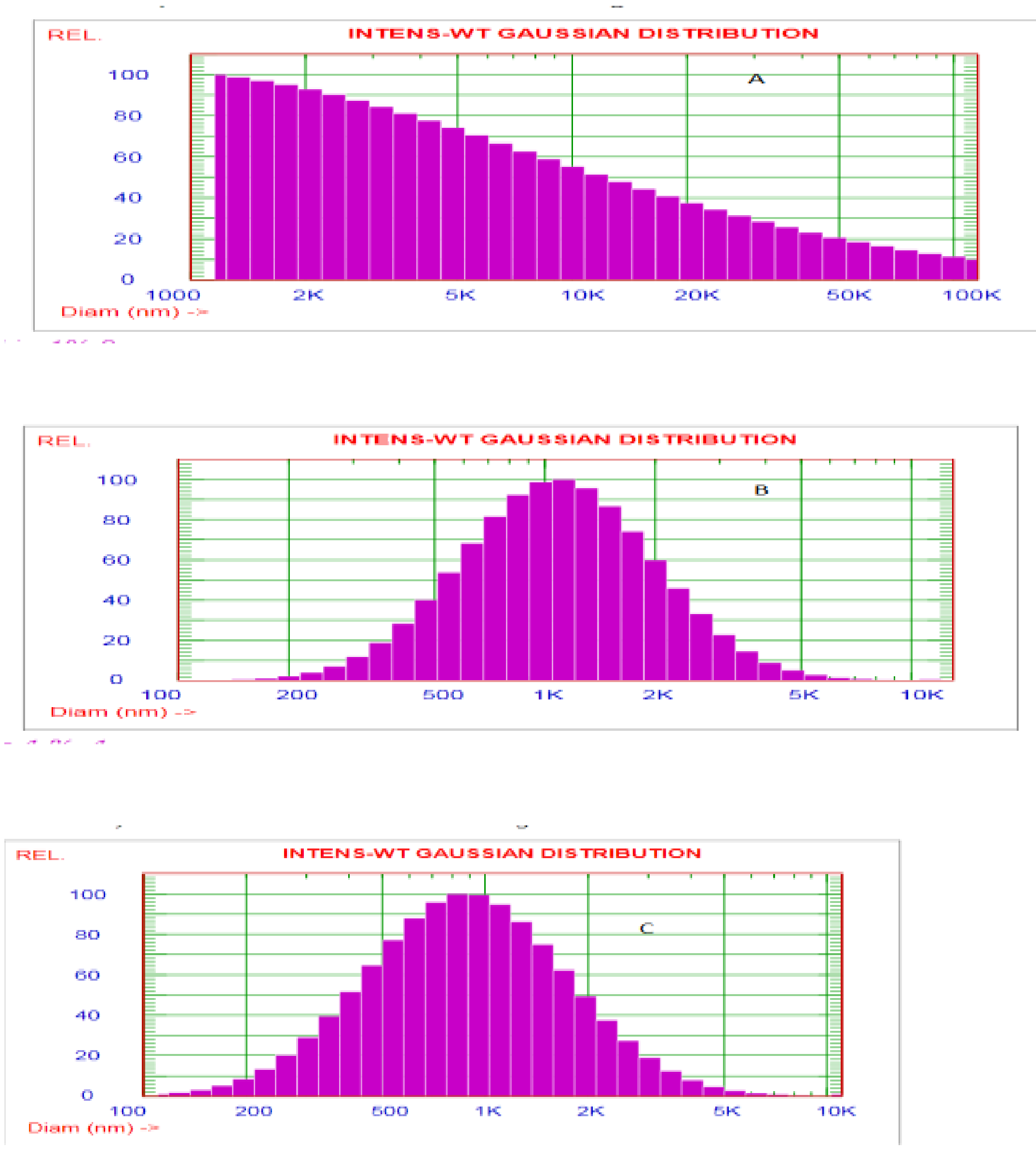
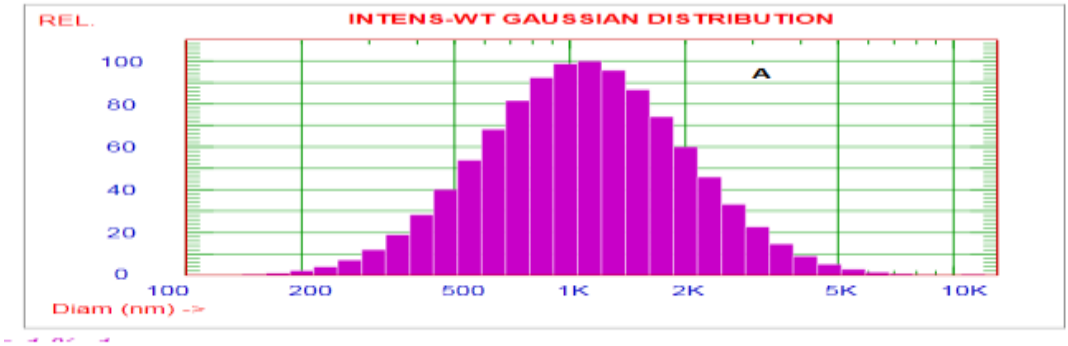


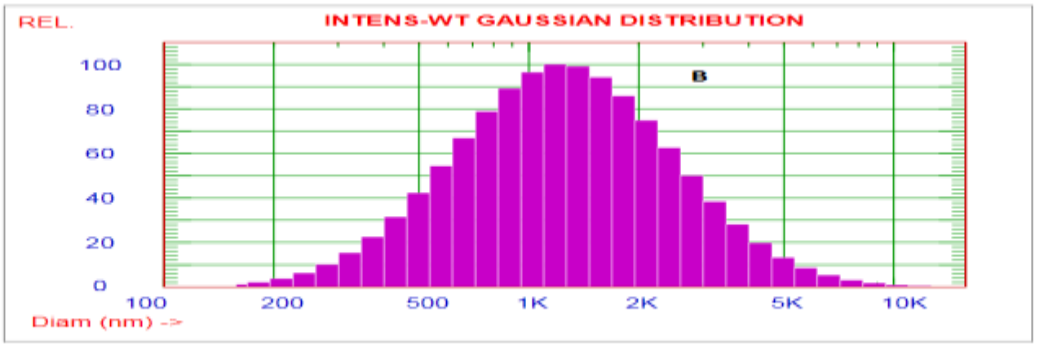
Figure (5.2) Particle size distribution of 1% *P. reticulatum* emulsion as

(A) Freshly prepared, (B) after 3 days (C) after 7 days at 25 ° C

Table 5.2: **The particle size cumulative distribution in (nm) of *Piliostigma reticulatum* gum emulsion of concentration 1.5 % at different conditions**

Condition	25%	50%	75%	80%	90%	99%
Freshly Prepared	738.3	1229.7	1762.6	1937.4	2500.4	4680.7
After 3days at25°C	916.6	1441.0	2004.6	2185.1	2756.4	4872.6
After 7days at25° C	570.2	1076.2	1637.7	1830.5	2474.7	5161.6





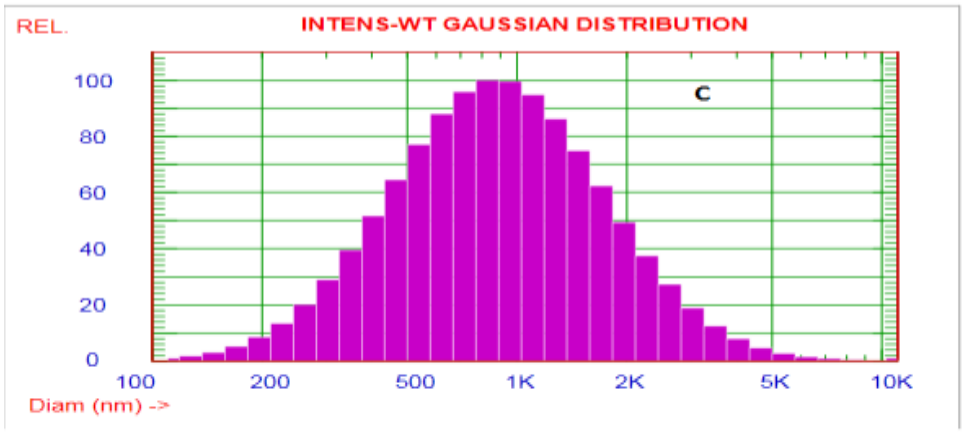
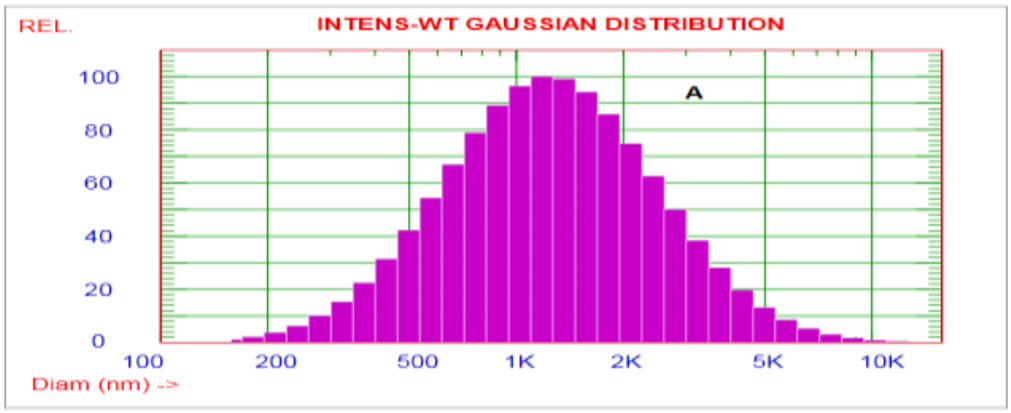
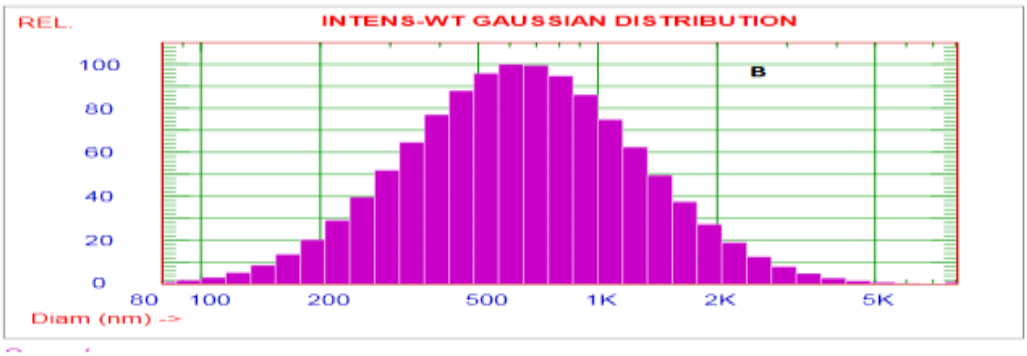


Figure (5.3) Particle size distribution of 1.5% *P. reticulatum* gum emulsion as (A) Freshly prepared, (B) after 3days (C) after 7days at25 ° C

Table 5.3: The particle size cumulative distribution in (nm) of *Piliostigma reticulatum* gum emulsion of concentration 2 % at different conditions

Condition	25%	50%	75%	80%	90%	99%
Freshly Prepared	893.0	1574.2	2320.3	2570.2	3389.4	6685.8
After 3days at25°C	2291.6	4113.1	6114.8	6789.8	9013.9	18060.0
After 7days at25° C	4503.9	6351.6	8303.9	8899.8	10716.9	16854.4





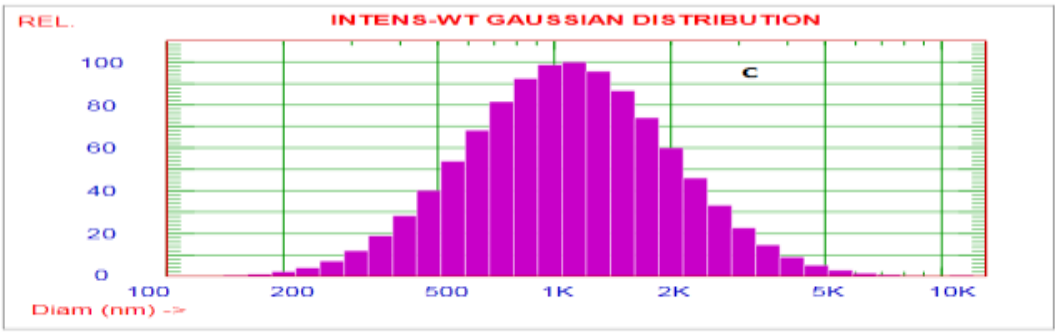


Figure (5.4) : Particle size distribution of 2 % *P.reticulatum* gum emulsion as (A) freshly prepared (B) after 3 days (C) after 7 days at 25 ° C

5.2Thickening Application Properties of *Piliostigma reticulatum* gum

Polysaccharides gums are used in dairy products such as ice creams, packed milk, Chocolate milk , processed baby foods , soft drink , fruit juices and Yogurt , also used in the backing industry for its comparatively low water absorption properties and its favorable adhesive properties in glazes and toppings

(Nussinovitch , 1997).

5.2.1 Yogurt experimental

Yogurts (and other cultured milk desserts) are produced by fermentation of milk by The addition of certain types of bacteria, e.g. Streptococcus thermopiles and Lactobacillus bulgaricus . Generally a yogurt is classified as two types: The two main types of yoghurt are set and stirred Yoghurt. The main difference between them is that set yoghurt is more or less semi-solid with the coagulum remaining intact and is usually packaged in cup-like packages. In its production the milk is inoculated, put in packages and sealed before fermentation. Stirred Yoghurt, on the other hand, is a more liquid product obtained by fermenting milk base in large tanks, the curd is broken by stirring and the product chilled and packaged in bottles (Lee and Lucey, 2004).Yoghurt is available in many varieties including plain, flavored, mixed with fruit purees and whole or sliced fruit. Fluid yoghurt drinks, soft or hard-frozen in various flavors and frozen yoghurt sticks are also commercially available (Lutchmedial *et al.*, 2004). Stirred Yogurt, which is fermented in bulk before packing, and Set Yogurt which is fermented in the consumer pack. Before addition of the starter culture, referred to as "inoculation", a number of ingredients may be added to the milk .Milk powder and other milk solids non-fat such as whey powder (where permitted). This contributes to the structure of the yogurt, giving a richer, creamier "mouth feel". Food hydrocolloids,

or food gums, have high molecular weights when compared to carbohydrate ingredients, such as sugar or corn syrup (Kuntz, 2002). Food gums are usually added to food systems products for specific purposes, such as thickening agents, stabilizers, emulsifiers, gelling, etc. Thickening and stabilizing agents such as gelatin, pectin, xanthan gum, starch and Carob bean gum etc. These bind free water in the milk, which helps to prevent whey separation ("syneresis") and can be used to obtain a variety of viscosity and texture modifications. (Ockerman , 1978).

5.2.1.1 Sample preparation

Yogurt containing *P. reticulatum* gum was used for this experimental.

Four samples were used for analysis A, B, C and D.

A: Yogurt control (0.0%) 1000ml (without addition gum)

B: Yogurt with 0.1 % g gum, 0.1/100 ×1000 = 1g

C: Yogurt with 0.3% g gum, 0.3 /100 × 1000 = 3g

D: Yogurt with 0.5% g gum, 0.5 /100 × 1000 = 5g

5.2.1.2 Results and discussion

Table (5.4) to Table (5.6) and Figure (5.5) to Figure (5.7) ,show chemical composition of samples after preparing and storage 6 and 12 days at 4° C. Result show decreased in moisture , lactose and pH four word from {A to D) and increased in protein, fat, ash , T.S , T.NF and acidity.

Table 5.4: Chemical composition of samples (A, B, C and D) after preparing

Parameters	A	B	C	D
Moisture	84.2	83.3	81.9	80.6
Protein	4.6	5.1	5.6	6.2
Fat	4.6	4.9	5.8	6.3
Ash	2	2.4	2.5	2.8
Lactose	4.6	4.3	4.2	4.1
T.S	15.8	16.7	18.1	19.4
T.N.F	11.2	11.8	12.3	13.1
pH	5.2	5	4.5	4
Acidity	1.4	1.7	2.1	2.4

T.S: total solid, T.N.F: total no fat

Table 5.5: Chemical composition of samples (A, B, C and D) after storage for 6 days at 4° C

Parameters	A	B	C	D
Moisture	84.9	84	82.5	81.3
Protein	4.5	5	5.5	6.1
Fat	4.4	4.7	5.6	6
Ash	1.8	2.2	2.3	2.6
Lactose	4.4	4.2	4.1	4
T.S	15.1	16	17.5	18.7
T.N.F	10.7	11.3	11.9	12.7
pH	5.1	4.7	4.1	3.8
Acidity	1.6	1.9	2.4	2.9

Table 5.6: Chemical composition of samples (A, B, C and D) after storage For 12 days at 4° C

Parameters	A	B	C	D
Moisture	85.7	84.9	83.2	82.1
Protein	4.3	4.8	5.2	5.8
Fat	4.3	4.6	5.5	5.9
Ash	1.6	2	2.1	2.4
Lactose	4.1	4	3.8	3.7
T.S	14.3	15.1	16.8	17.9
T.N.F	10	10.5	11.3	12
pH	5.1	4.7	4.1	3.8
Acidity	1.6	1.9	2.4	2.6

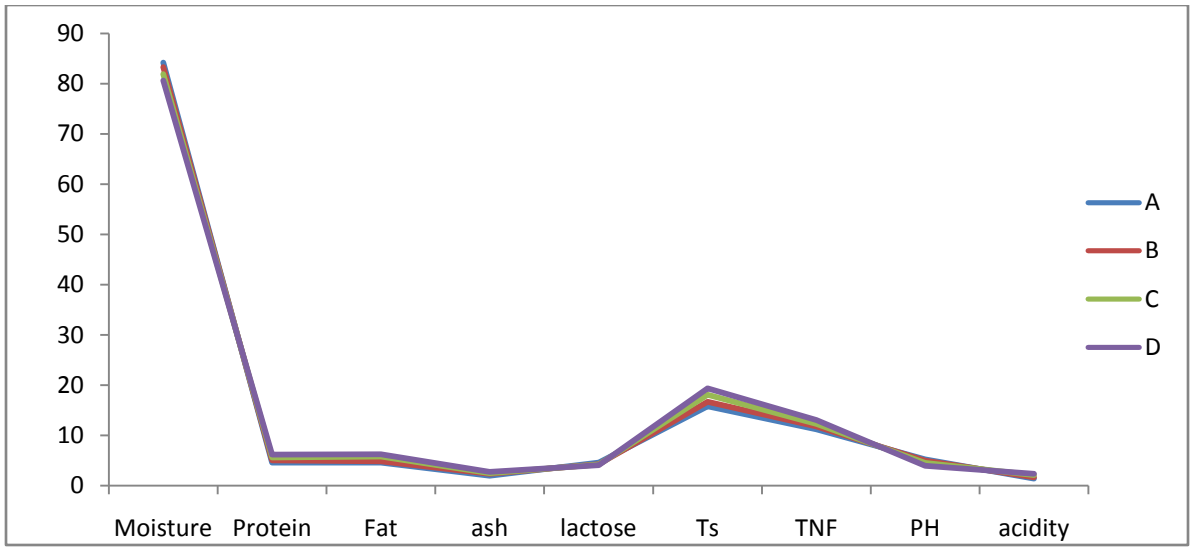


Figure (5.5): Chemical composition of samples (A, B, C and D) after preparation

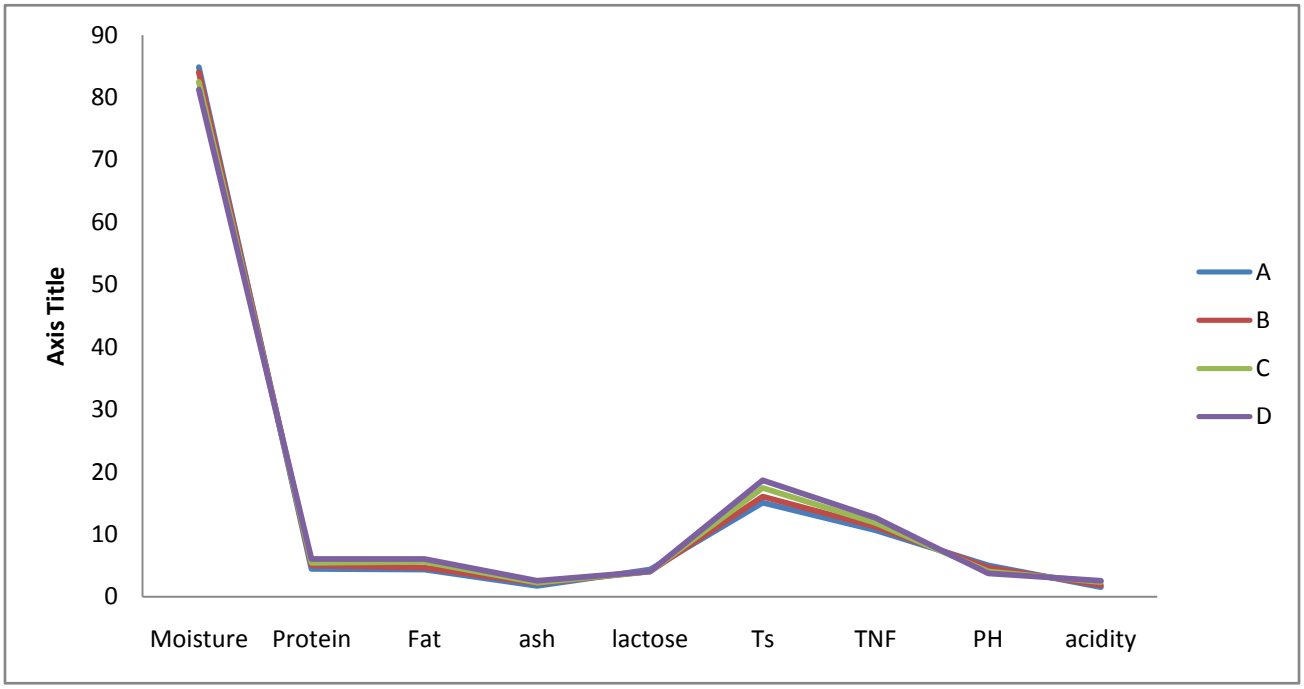


Figure (5.6): Chemical composition of samples (A, B, C and D) after storage for 6 days at 4°C

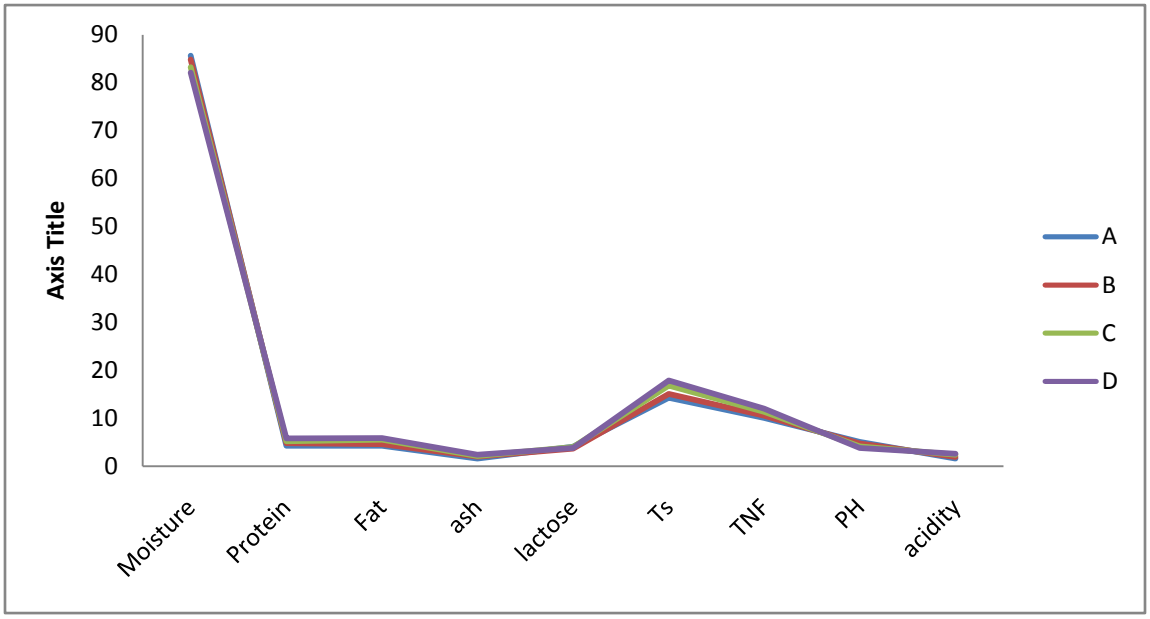


Figure (5.7): Chemical composition of samples (A, B, C and D) after storage for 12 days at 4°C

Table (5.7) to Table (5.9) and Figure (5.8) to Figure (5.10) show Sensory evaluation of samples (A, B, C and D) after preparing and after storage 6 and 12 days at 4° C . Result show increased in Texture four word from {A to D} and decreased in Color, Taste, Flavor, and overall from {A to D}.

Table 5.7: Sensory evaluation of samples (A, B, C and D) after preparing

Parameters	A	B	C	D
Color	6.46	5.32	4.22	4.21
Taste	6.57	5.88	4.95	3.25
Flavor	6.84	5.75	4.95	4.16
Texture	4.57	5.35	6.24	6.68
Overall Acceptability	5.37	5.67	4.46	3.30

Table 5.8: Sensory evaluation of samples (A, B, C and D) after storage for 6 days at 4° C

Parameters	A	B	C	D
Color	6.20	5.26	4.27	4.18
Taste	6.47	5.80	4.75	2.25
Flavor	6.58	5.65	4.81	3.14
Texture	4.51	5.28	6.13	6.56
Overall Acceptability	5.35	5.50	4.28	3.15

Table 5.9: Sensory evaluation of samples (A, B, C and D) after storage for 12 days at 4° C

Parameters	A	B	C	D
Color	5.26	5.20	4.19	4.12
Taste	5.46	5.63	4.75	2.11
Flavor	5.58	5.52	4.78	3.12
Texture	4.34	5.22	6.10	6.45
Overall Acceptability	5.23	5.30	4.14	3.10

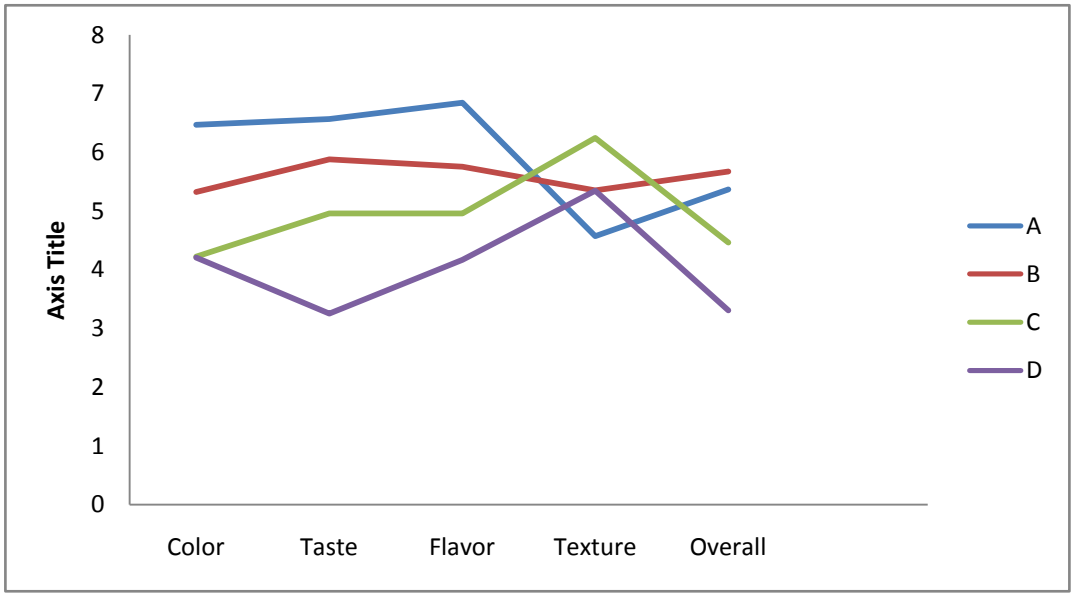


Figure (5.8):Sensory evaluation of samples (A, B, C and D) after preparing

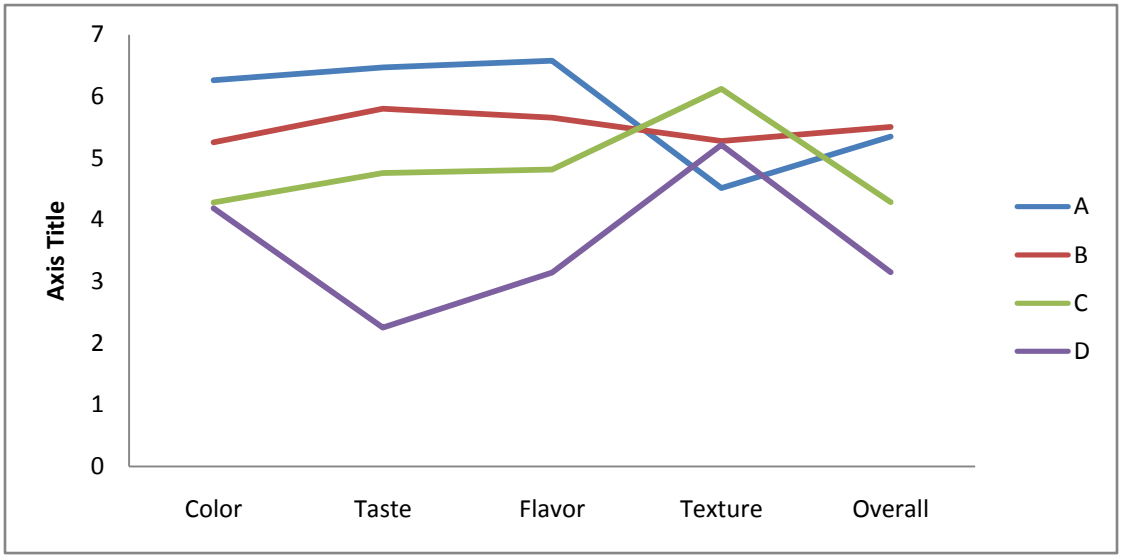


Figure (5.9):Sensory evaluation of samples (A, B, C and D) after storage for 6days at 4°C

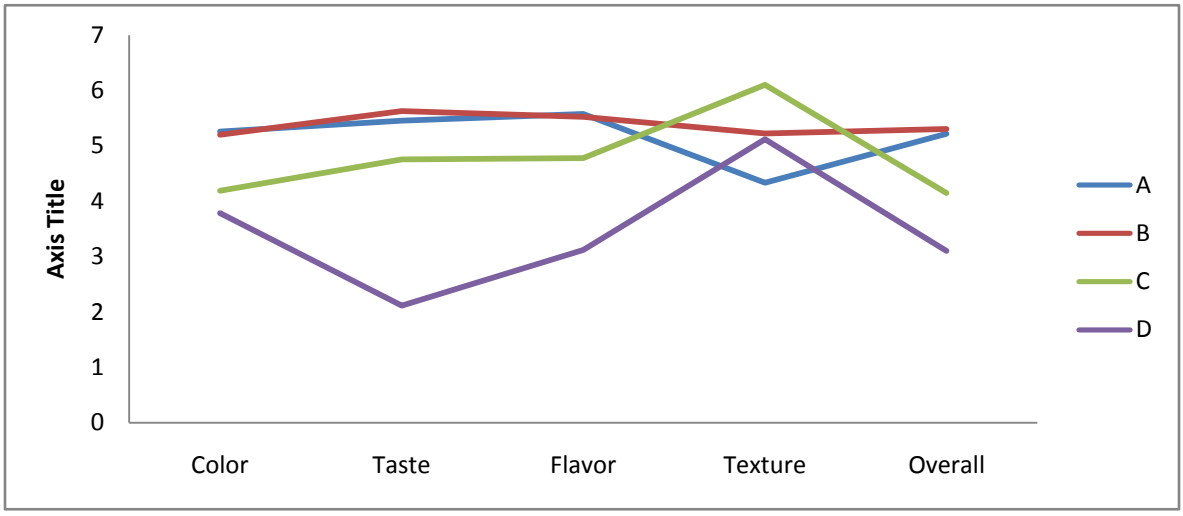


Figure (5.10): Sensory evaluation of samples (A, B, C and D) after storage for 12 days at 4°C

But to comparison between Preparing and different storage of Sensory evaluation shows increased in Texture four word and decreased in Color, Taste , Flavor and overall acceptability from preparing to storage 6,12 days.

Table (5. 10) to Table (5.12) and Figure (5.11) to Figure (5.13) show Bacteria count analysis of samples after preparation and after storage 6 and 12 days at 4 ° C . Result show decreased in TVC and *Staphloyccus* from {A toD} for all condition.

Table 5. 10: Bacteria count of samples (A, B, C and D) after preparation

Parameters	A	B	C	D
TVC	$3.92x10^3$	$3.69x10^3$	$3.48\ x10^3$	$3.38\ x10^3$
E.coli	-//-	-//-	-//-	-//-
Staphyloccus	$1.29\ x10^3$	$1.24\ x10^3$	$1.21x10^3$	$1.16\ x10^3$

Table 5. 11: Bacteria count of samples (A, B, C and D) after storage for 6 days at 4 ° C

Parameters	A	B	C	D
TVC	$3.90\ x10^3$	$3.67\ x10^3$	$3.46\ x10^3$	$3.36\ x10^3$
E.coli	-//-	-//-	-//-	-//-
Staphyloccus	$1.27\ x10^3$	$1.23\ x10^3$	$1.18\ x10^3$	$1.16\ x10^3$

Table 5.12: Bacteria count of samples (A, B, C and D) after storage for12 days at 4 ° C

Parameters	A	B	C	D
TVC	3.88 x10^3	3.59 x10^3	3.42 x10^3	3.32 x10^3
E.coli	-//-	-//-	-//-	-//-
Staphyloccus	1.23 x10^3	1.22 x10^3	1.18 x10^3	1.12 x10^3

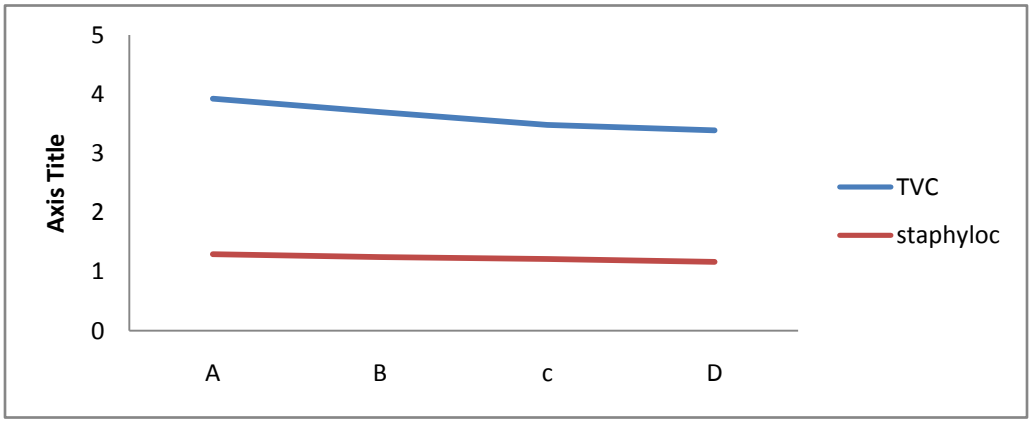


Figure (5.11):Bacteria count of samples (A,B ,C and D)after preparation

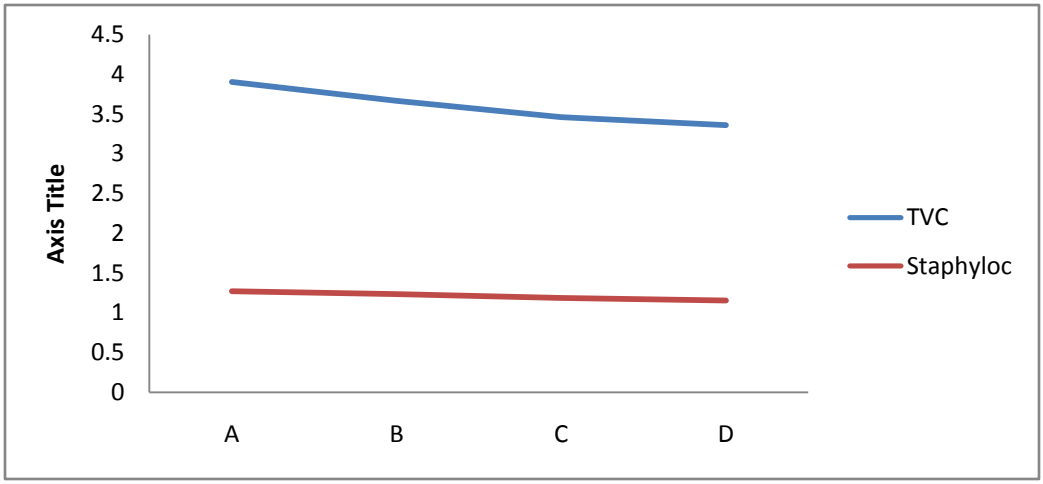


Figure (5.12): Bacteria count of samples (A, B, C and D) after storage for 6days at 4 ° C

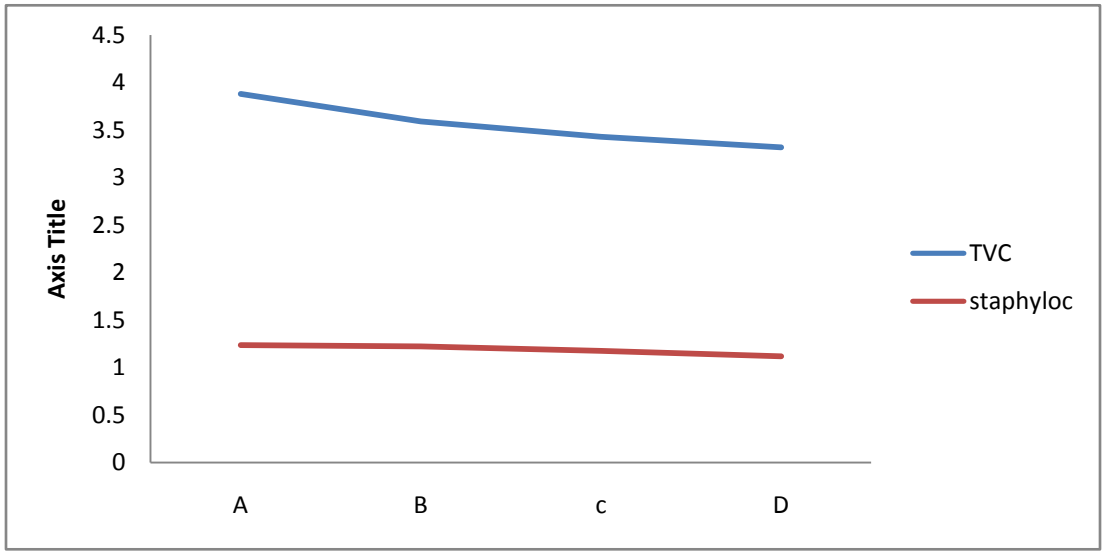


Figure (5.13): Bacteria count of samples (A, B, C and D) after storage for 12days at 4 ° C

From the presented result, it is clear that when addition *Piliostigma reticulatum* gum to the Yogurt it is good thickening and stabilizing agents because prevent whey separation (“syneresis”) and can be used to obtain a variety of viscosity and texture modifications.

5.2.2 Baking test

5.2.2.1 Preparation of bred samples

Wheat flour containing *Piliostigma reticulatum* gum was used for making bred according to (Badi *et al*., 1978). A 250 g flour, water (according to farinograph water absorption), salt (1%) , yeast (2%) , sugar (2%) and oil (2%) were weighted and mixed to form a dough in mono – universal laboratory dough mixer for 5 min, at medium speed . The dough was allowed to rest for 10 min, at room temperature , then there pieces of 120g dough were weighed , made in to pan and placed in the fermentation cabinet for fine proof for 1 hr. Baking was done in an oven at 220° C with saturation of steam for 10 – 15 min . After the loaves were cooled, they were weighted then the volumes measured in ml using the millet seed displacement method (volumeter) and their specific volumes (v/w) were calculated. The loaves were sliced with an electrical knife, and then each loaf with its slice was photographed.

5.2.2.2 Bread weight

The weighed of the loaf bred was taken in gram.

5.2.2.3 Bread volume

The loaf volume was determined by the seed displacement method according to (pyler , 1973) . The loaf was placed in container of known volumeter which small seed (millet seeds) were run until the container is full. The volume of seeds displaced by the loaf displaced by the loaf was considered as the loaf volume.

5.2.2.4 Bread specific volume

The specific volume of the loaf was calculated according to the AOAC method (2000) by dividing volume by weight (g).

5.2.2.5 Results and discussion

Table (5.13) and Figure (5.14) show the effect of addition of *Piliostigma reticulatum* gum powder at ratios 0.5, 1.0, 1.5 and 2.0 % on the baking of wheat flour. Result show that the control loaf bread volume of wheat flour is 366.6 cm; addition of gum sample gave difference in bread volume. The loaf bread of 2. 0 % *Piliostigma reticulatum* gum gave the highest volume 376.7 cm, while the ratios 0.5, 1.0 and 1.5% *Piliostigma reticulatum* gum gave the result of 363.3, 346.7 and331.7 cm respectively. Specific volume of control bread of wheat flour was 3.36 cm/g , the highest value of specific volume gave at ratio 2.0 % *Piliostigma reticulatum* gum as 3.45 cm/g.

Table 5.13: Effect of *Piliostigma reticulatum* gum on bread properties

Level of *Piliostigma reticulatum* gum in wheat flour	Bred volume (cm)	Bread weight (gm)	Bread specific volume (cm/g)
Control (0.0)%	366.6	109.1	3.36
0.5 %	363.3	109.06	3.33
1.0 %	346.7	110	3.15
1.5 %	331.7	110.5	3.0009
2 %	376.7	109.3	3.45

Figure (5.14): Bread prepared from wheat flour containing different levels of *Piliostigma reticulatum* gum

A: bread of wheat flour

B: bread of wheat flour + 0.5% *Piliostigma reticulatum* gum

C: bread of wheat flour + 1%% *Piliostigma reticulatum* gum

D: bread of wheat flour+ 1.5% *Piliostigma reticulatum* gum

E: bread of wheat flour+ 2% *Piliostigma reticulatum* gum

From the present result, it is clear that the specific volume of the bread was affected by the addition of *Piliostigma reticulatum* gum and improver the quality of bread at two (2%) levels of the gum.

Chapter Six

6 Toxicological Study of *Piliostigma reticulum* gum and germ

6.1Safety and toxicity of Carob (Locust) bean gum

Carob bean gum is used as a food thicker and stabilizer, in the Europe it is labeled as E410. Carob gum may be used in coffee, fish products, dried pasta, fermented milk, cream and Infant formula (www.NUTRIENTSREVIEW .com) Home (Carbohydrates / Carob or locust bean gum).Carob gum is approved for use as food additives by the US Food and Drug Administration and is on the list of substances that is a generally recognized as safe (GRAS). And has the Acceptable Daily Intake (ADI) not specified status (the highest safety category by.(JECFA, 1982). . During a two year animal study, rats were given locust bean gum as 5 % of their diets, no carcinogenic or other toxic effects were observed. Locust bean gum has also been studied in humans as a potential cholesterol lowering compound. The potential toxicity of gum of Carob (Locust) bean gum has been studied.

6.1.1**Biochmical Aspects**

- in a bioavaialable calorie assay, groups of 10 male weanling rats (Sprague – Dawley) were given 5g basal diet plus 0.5, 1,2g sucrose or o.5, 1 ,2 g gum for 10 days. Comparison of the carcass weight gain showed that carob bean gum was not a source of bioavilable calories (Robaislek , 1974).Carob bean gum has been noted to contain tannins, which depress appetite and growth, and trypsin inhibitors , which are also growth inhibitory (LSRO/ FASEB , SCOGS -3,.1972)

6. 2Toxicological Studies of Carob bean gum

6.2.1Special studies on mutagenicity

Carob (locust) bean gum was evaluated for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations. Indicator organisms used were Saccharomyces cervisiae and Salmonella typhimurium, strains TA- 1535, TA- 1537 and TA-1538. Mammalian metabolic activation preparations were from mouse (ICR adult), rat (Sprague – Daeley adult) and monkey (Macaca mulatta adult). Carob bean gum did not exhibit genetic activity in any 0f the assays employed (Maxwell. W.A and Newell, G.W, 1972 and Litton Bionetics , Inc, 1975) .

6.2.2Special studies on reproduction

A three- generation reproduction study was carried out in CD strain Charles River albino rats. Groups of 10 male and 20 female animals were fed a rat chow diet containing 2 0r 5% locust bean gum (LBG) or 5% alpha cellouse (control). The same doeses and animal numbers were used throughout the study. In each generation the parental animals received the test diet for 11 weeks prior to mating and through mating gestation and weaning. Two or three litters were raised per generation and the second litter was used to produce the following generation. Ten males and 10 females from each treatment group of the F_{3b} generation were selected for histopathological examination of 12 major oranges' and stissues and organ weight analysis. All others animals were subject to gross necropsy.

There were statistically significant decreases in premating body weight gain in the F_0 female fed 2% LBG and in final body weight in the females fed 5% LBG.

There were the following significant differences in oragn weight ratio in the F_{3b} 5% LBG group as compared to the controls : smaller spleen to brain weight , absolute liver weight , liver to brain weight the highly variable values for these parameters in young rats and the fact that all the animals may not have been at the

same age at sacrifice . The factor could have had an effect on organ weight ratios in young a nimals.There was no significant treatment – related effects on reproductive indices or gross microscopic pathology (Domanski *et al*., 1980)

6.2.3Special studies on teratogenicity

Teratogenice experiments with four species of animals (rats, mice, hamsters and rabbits) did not indicate that the test material was a teratogen to mice at 280 m/kg and 1300 mg/kg, although 5/21 dams diet at the latter dose. Up to 1300 mg/kg in rats, up to 1000 mg/kg in hamsters and at 196 mg/kg in rabbits no teratological effects were seen. At 910 mg/kg in rabbits, most of the pregnant dams diet (Morgareidge, 1972).

6 .2.4 Observation in Man

A clinical study of commercial preparation of carob bean gum grain as laxative in doses of " two heaping teaspoonfuls " in 56 patients , some of whom took the preparation regularly for two years, resulted in no untoward effects related to the gastrointestinal tract , and no allergenic reaction (Holbrook ,1951).

Eight infants between the ages of two – and - a half to five months were fed meals of sugared milk plus a 1% powder extract from carob bean .Addition of the carob supplement did not alter the duration of the gastrointestinal transit time of the meal. Physiological aerophagy was markedly suppressed by the supplement (Rivier, 1952).

6 .2.5 Comments

In vitro tests with human enzyme preparations show little utilization by gut. Carob bean gum was not teratogenic in several mammalian species although it did produce terata in the chick embryo assay. The effects noted in feeding trials are those expected of anon- metabolized polymeric substance acting as a bulking agent. In the previous evaluation, it was noted that the following studies were not available for evaluation: reproduction, teratogenicity and mutagencity. The new

data submitted showed that Carob bean gum did not cause any significant compound – related effects in a three – generation reproduction study. It was not mutagenic in microbial systems, with and without a activation. In lifetime feeding studies in the rat and mouse, Carob bean gum not carcinogenic.

The aim of this chapter is to study the cytotoxic effects of *Piliostigma reticulatum* gum and germ solution to assess the safety of *Piliostigma reticulatum* gum and germ. The effect of *Piliostigma reticulatum* gum and germ solutions on two cell lines human cancer cell line: MCF.7 (breast cancer) and HepG.2 (liver cancer) were obtained frozen in liquid nitrogen from the American Type Culture Collection (ATCC, Manassas, USA). The results were represented as half maximal inhibitory concentration IC_{50} of the gum and germ.

6.3 Toxicological Types

A number of common toxicity terms can be defined as follows

6.3.1 Toxicity

The degree to which something is poisonous

6.3.2Cytotoxicity

The degree to which something is toxic to living cells, denoting certain drugs used in the treatment of cancers. The prefix cyto- denotes a cell. It comes from the Greek kytos meaning hollow, as a cell or container. Toxic is from the Greek toxicant meaning arrow poison

6.3.3 *In vitro*

In vitro(Latin: *within glass*) refers to studies in experimental biology that are conducted using components of an organism that have been isolated from their usual biological context in order to permit a more detailed or more convenient

analysis than can be done with whole organisms. In contrast, the term *in vivo* refers to work that is conducted with living organisms in their normal, intact state. Common examples of *in vitro* experiments include (a) cells derived from multicellular organisms (cell culture or tissue culture), (b) sub cellular components (e.g. mitochondria or ribosome's), (c) Cellular or sub cellular extracts (e.g. wheat germ or reticulocyte extracts), or (d) purified molecules in the test tube (often proteins, DNA, or RNA, either individually or in combination).

6.3.3.1 Methods of use

According to Christopher Lipinski and Andrew Hopkins, "Whether the aim is to discover drugs or to gain knowledge of biological systems, the nature and properties of a chemical tool cannot be considered independently of the system it is to be tested in". Compounds that bind to isolated recombinant proteins are one thing, chemical tools that can perturb cell function another, and pharmacological agents that can be tolerated by a live organism and perturb its systems are yet another. If it were simple to ascertain the properties required to develop a lead discovered *in vitro* to one that is active *in vivo*, drug discovery would be as reliable as drug manufacturing (Lipinski *et al*., 2004)

6.3. 3.2Tissue culture

Is the growth of tissues or cells separate from the organism this is typically facilitated via use of a liquid, semi-solid, or solid growth medium, such as broth or a gar. Tissue culture commonly refers to the culture of animal cells and tissues, while the more specific term plant tissue culture is being named for the plants.

6.3.3.3 Modern usage

In modern usage, tissue culture generally refers to the growth of cells from a tissue from a multicellular organism *in vitro*. These cells may be cells isolated from a donor organism, primary cells, or an immortalized cell line. The term tissue culture

is often used interchangeably with cell culture. The literal meaning of tissue culture refers to the culturing of tissue pieces, i.e. explants culture. Tissue culture is an important tool for the study of the biology of cells from multicellular organisms. It provides an *in vitro* model of the tissue in a well defined environment which can be easily manipulated and analyzed.

6.3.3.4 Cell line

Cells of a single type taken from an animal or human and grown in the laboratory, in other words, cell line is a product of 99mmortal cells that are used for biological research. Cells used for cell lines are immortal, that happens if a cell is cancerous. The cells can perpetuate division indefinitely which is unlike regular cells which can only divide approximately 50 times. These cells are useful for experimentation in labs as they are always available to researchers as a product and do not require what is known as 'harvesting' (the acquiring of tissue from a host) every time cells are needed in the lab.

6.3.3.5 The median lethal dose (LD_{50})

The dose required to kill half the members of a tested population after specified test duration. Table (6.1) shows the median lethal dose (LD_{50}) relative toxicity of some agents. Table (6.2) shows toxicity classification and (LD_{50}) of oral, inhalation and skin respectively.

6.3.3.6 The half maximal inhibitory concentration (IC_{50})

Measure of the effectiveness of a compound in inhibiting biological or biochemical function .This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. In other words, it is the half maximal (50%) inhibitory concentration (IC_{50}) of

a substance (50% IC, or IC_{50}).It is commonly used as a measure of antagonist drug potency in pharmacological research .Table (6.3) shows the classification of the cytotoxicity for natural ingredients (Shirazi *et al*., 2004).

Table 6.1: The median lethal dose (LD_{50}) relative toxicity

Agent	LD_{50} (mg/kg)
Ethyl Alcohol	10.000
Sodium chloride	4.000
Morphine sulphate	900
Strychnine sulphate	2
Nicotine	1
Hemicholinium-3	0.2
Dioxin	0.001
Botulinum toxin	0.00001

Table 6.2: Example of toxicity classification

toxicity classification	LD_{50} Oral (mg/kg)	LD_{50} Inhalation (mg/kg)	LD_{50} Skin (mg/kg)
Supertoxic	<5	<250	<250
Extremely toxic	5-50	250-1000	250-1000
Very toxic	50-500	1000-10000	1000-3000
Moderately toxic	500-5000	10000-30000	3000-10000
Slightly toxic	>5000	>30.000	>10000

Table 6.3: Classification of the cytotoxicity for natural ingredients (Balantyne, 1999).

Category	IC_{50}
Potentially very toxic	IC_{50}<10μg mL^{-1}(million cells mL^{-1})
Potentially toxic	10μg mL^{-1}< IC_{50}<100μg mL^{-1}
Potentially harmful	100μg mL^{-1}< IC_{50}<1000μg mL^{-1}
Potentially non toxic	IC_{50}>1000μg mL^{-1}

Sometimes, it is also converted to the pIC_{50} scale (-log IC_{50}), in which higher values indicate exponentially greater potency.

According to the FDA, IC_{50} represents the concentration of a drug that is required for 50% inhibition *in vitro*. It is comparable to an EC_{50} for agonist drugs. EC_{50} also represents the plasma concentration required for obtaining 50% of a maximum effect *in vivo*.

6.3.3.7 Determination of the IC_{50} of a drug

The IC_{50} of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC_{50} values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC_{50} values are very dependent on conditions under which they are measured. In general, the higher the concentration of inhibitor, the more agonist activity will be lowered.

This study was carried out to investigate the cytotoxicity of the *Piliostigma reticulatum* gum and germ in two types of cancer human cell lines to assess the safety of *Piliostigma reticulatum* gum and germ.

6.4 Procedure

Cells were grown under aseptic conditions with complete medium in a25 cm^3 cell culture flask with humidified atmosphere and 5%CO_2 at 37° C, Cultured monolayer at 80 % confluence was washed with PBS then trypsinized by 2ml trypsin – EDTA solution, incubated for 2 min. Then the flask was tapped to detach the cells, complete culture medium was added to stop the reaction. The cell suspension was counted using hemocytometer (R . Guillermo *et al*., 2008) and cell viability checked by trypan blue (99% viability). The cells were diluted with medium to have approximately concentration of 10 0,000cells /ml ;the cell suspension was agitated gently and placed it in a sterile reservoir.200 ml of the cell suspension (containing ~ 20,000 cell per well) was dispensed by multichannel pipette into the inner 60 wells of the 96 well plate. The peripherals wells were filled with PBS; and the plate was incubated for 24 hours. After cell seeding and attachment the media was discarded gently and different concentrations of the compounds prepared. Gum and germ Samples were dissolved in H_2O and heated at 65° C for 15 min in(a water bath) , then sterilized by 0.22µm syringe filter and different concentrations were prepared (1000 , 500 , 100, 10, 1 and 0.1 µg / ml) by diluted in DMEM media. 200µl of treatment media was dispensed into 4 replicates for each concentration, 16 wells were` filled with media only (as negative control) and two filled with media containing Doxorubicin HCL (3µg /ml) as a positive control. After that the 96 wells plate covered with lid, was incubated at 37° C for 24 h. After the incubation period, the cultures were examined, under inverted microscope, recording changes in morphology of cells

due to cytotoxic effects of the test chemical and photos were taken, the medium was decanted gently .100 µl of Neutral red medium (after centrifugation at 1800 rpm for 10 min, to remove any precipitated dye crystals) are added into each well and incubated again for 3h at 37°C.After incubation, the dye containing medium was decanted and each well was rinsed gently twice with 0.2 ml PBS solution to remove the unabsorbed neutral red dye contained in the wells. 150µl of destain solution were added and incubated for 10 min with shaking(R . Guillermo *et al*., 2008) . The absorbance of acidified ethanol solution containing extracted neutral red dye was measured at 540 nm (BioTek, ELX808).Viability % was calculated dose – response curves were drawn to determine a 50% inhibition of the uptake (IC_{50}).

6.5 Results and discussion

The cytotoxicity test with cancer human cells was conducted to identify the cytotoxic potential of *Piliostigma reticulatum* gum and germ.

The IC_{50} values (the concentrations of drug required to produce 50 % inhibition of cell growth) were calculated from the viability %. The experiment was repeated 3 times for each cell line.

Table (6.4) and Figure (6.1) , show the effects of *Piliostigma reticulatum* gum on Liver cancer cell line (HepG-2). The results show that the IC_{50} of *Piliostigma reticulatum* gum was 11184.4µg mL^{-1} which is categorized in the range potentially non toxic substance for (HepG-2) cell line .Table (6.5) and Figure (6.2), show the effects of *Piliostigma reticulatum* germ on Liver cancer cell line (HepG-2). The results show that the IC_{50} of *Piliostigma reticulatum* germ was 848 µg mL^{-1} which is categorized in the range potentially harmful substance for(HepG-2) cell line.

Table 6.4: Cytotoxicity of *P. reticulatum* gum on Liver cancer cell line (HepG-2)

Concentration By µg/ml	Viability %
1000	87.6
500	89.8
100	91
10	91
1	91
0.1	92

IC_{50}=11184.8µg/ml

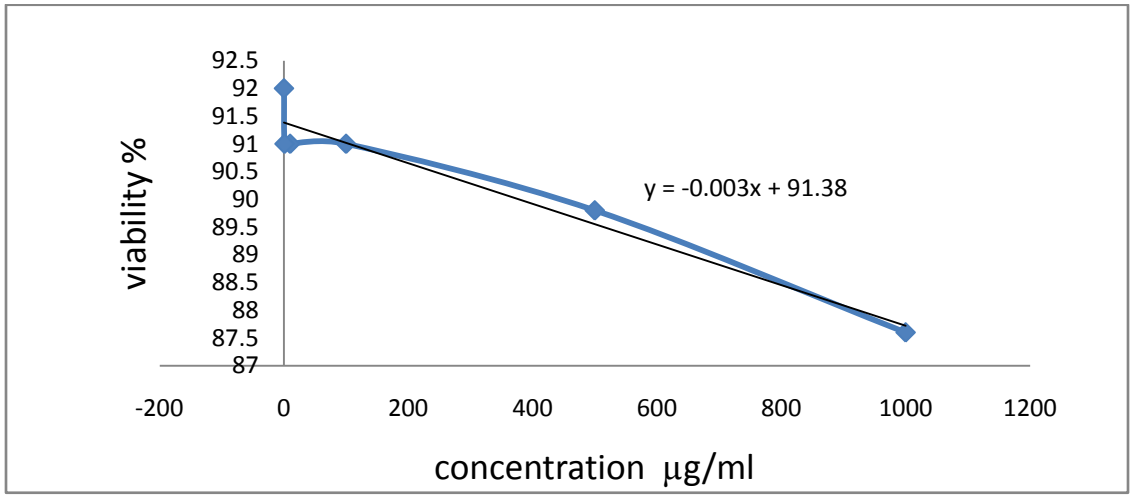


Figure 6.1: **Cytotoxicity of *P .reticulatum* gum on Liver cancer cell line (HepG-2).**

Table 6.5: Cytotoxicity of *P. reticulatum* germ on Liver cancer cell line (HepG-2)

Concentration By µg/ml	Viability %
1000	42.6
500	69.6
100	71.9
10	83
1	96.6
0.1	97.7

IC_{50}=848 µg/ml

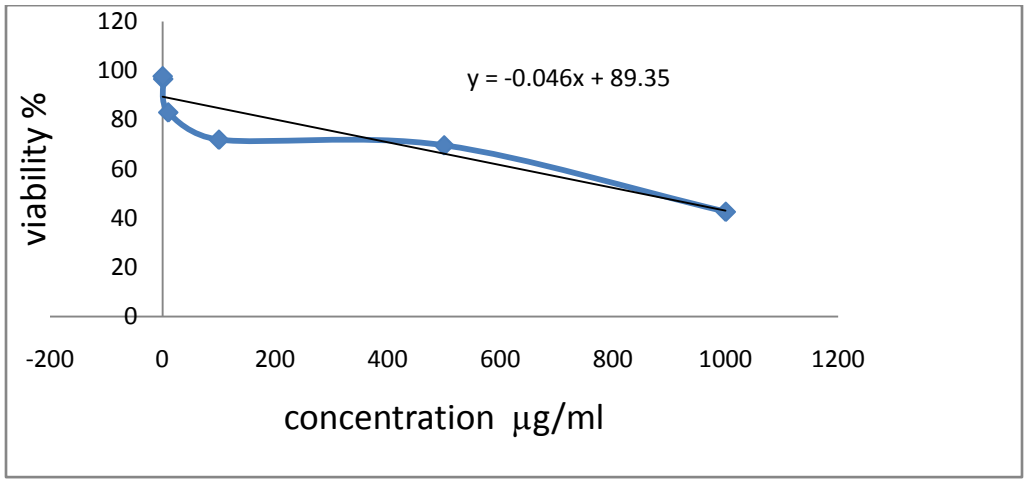


Figure 6.2: Cytotoxicity of *P. reticulatum* germ on Liver cancer cell line (HepG-2)

Table (6.6) and Figure (6.3) , show the effects of *Piliostigma reticulatum* gum on breast cancer cell line (MCF-7). The results show that the IC_{50} of *Piliostigma reticulatum* gum was 7865.4 μg mL^{-1} which is categorized in the range potentially non toxic substance for (MCF-7) cell line. Table (6.7) and Figure (6.4) , show the effects of *Piliostigma reticulatum* germ on breast cancer (MCF-7) cell line. The results show that the IC_{50} of *Piliostigma reticulatum* germ was 462 μg mL^{-1} which is categorized in the range potentially harmful substance for (MCF-7) cell line.

Table 6.6: Cytotoxicity of *P. reticulatum* gum on breast cancer cell line (MCF-7)

Concentration By μg/ml	Viability %
1000	86.3
500	87.67
100	90.4
10	90.4
1	90.4
0.1	93

IC_{50}=7865.4μg/ml

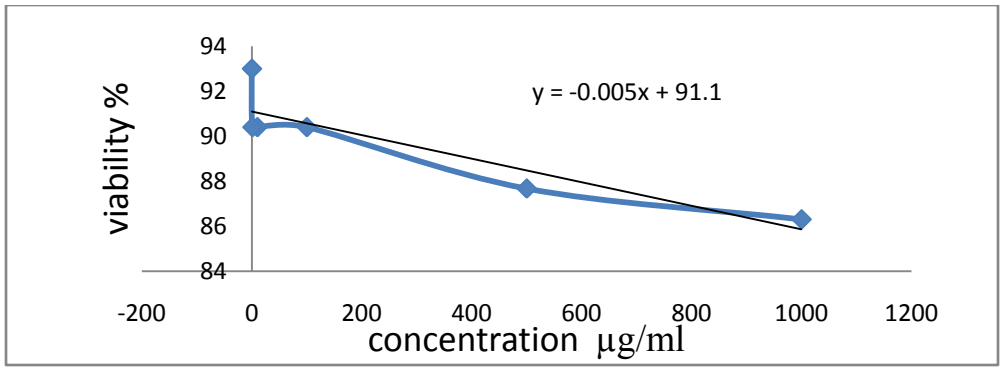


Figure 6.3: Cytotoxicity of *Piliostigma reticulatum* gum on breast cancer cell line (MCF-7)

Table 6.7: Cytotoxicity of *P. reticulatum* germ on breast cancer cell line (MCF-7)

Concentration By µg/ml	Viability %
1000	6.8
500	57.5
100	76.7
10	79.45
1	79.45
0.1	82.2

IC_{50}=462 µg/ml

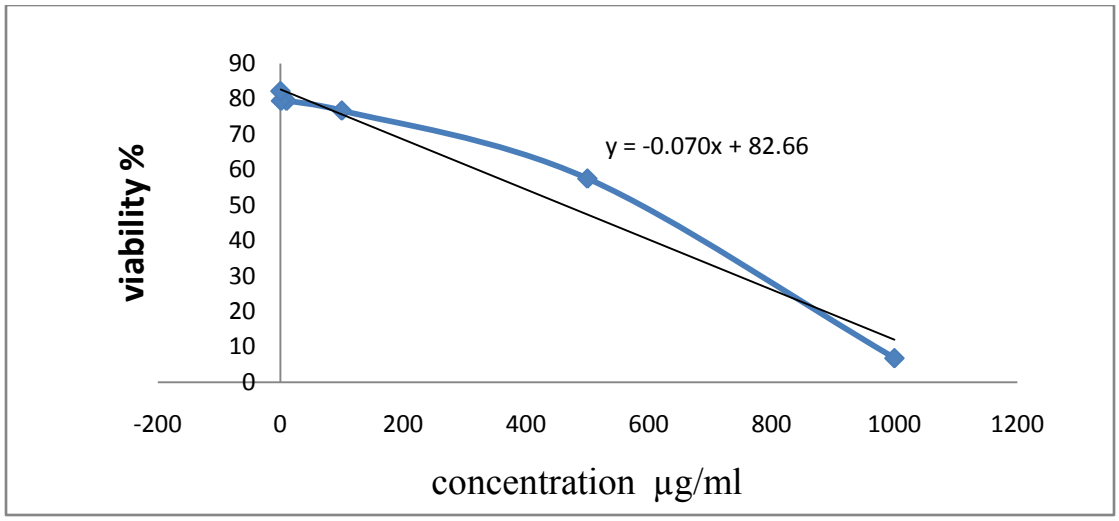


Figure 6.4: Cytotoxicity of *P. reticulatum* germ on breast cancer cell line (MCF-7)

In conclusion, *Piliostigma reticulatum* gum is a safe food additive, since it has a similar safety of Carob bean gum which is used as a safe food additives (US Food and Drug Administration)and is on the list of substances that is, generally recognized as safe (GRAS). And has the (Acceptable Daily Intake (ADI) not specified status (the highest safety category (JECFA, 1982).

Conclusions

Piliostigma reticulatum gum has:

• Low ash.

•High protein composition.

• a galactomannan 1:2 ratio.

• Rich in Ca and K.

•The gum has a non Newtonian behavior.

• Safe to use as a food additive, can be use as a thicken for yogurt and improve the quality of bread at two levels of the gum.

• The germ has a high protein composition can be use as animal feed.

• The germ has antic cancer effect for liver and Breast cancer both.

Suggestions for Further work

• Further studies may be needed for determination of molecular weight of gum and germ .

• More investigation may be needed for Studying toxicity of *Piliostigma reticulatum* gum and germ on normal human (cell line).

References

Akin – Osanaiye , B.C., Agbaji , E . B and Abdulkadir , O . M. (2009). Proximate Composition and the Functional Properties of defatted seed and Protein Isolates of Kargo (*Piliostigma reticulatum*) Seed .*African Journal of Food, Agriculture , Nutrition and Development* **6**: 1365- 1377.

Amira , A. E. Satti. (2011). Ph.D. Thesis, department of Chemistry, Faculty of Science, Sudan University of Science and Technology, Khartoum, Sudan.

Anderson, D. M. W. (1977). *Proc. Biochem*. **12**.24.

AOAC. (1998). Official Methods of Analysis. *Association of Official Analytical Chemists, Washington, DC.*

AOAC. (2000). Official Methods of Analysis of the *Association of Official Analytical Chemists, 17 th ed. Washington, P.C.*

AOAC. W.H., (1990) . Official Methods of Analysis of *Association of Official Analytical Chemists. Arlington .VAUSA.*

A. Ragheb, K. Haggag and I.Abd El-thalouth.(1989).Preparation and characterization of carbooxymethyl carboin .*Acta polymeric* **40**. Nr-11.

Aspinal, E. M. (1970). "*Polysaccharides*" Pergamon Press , Oxford, New York. pp. 1.

Awouda, H. M. (1974). Production and Supply of Gum Arabic. *Khartoum Sudan*.

Bastista MT, Amaral MT,Proenca Da Cunha . A. (1996) . Carob fruit as a source of natural antioxidants, In proceedings of the III International Carob Symposium. Cabanas – Tavira , Portugal.

Batlle , I., Tous , J .(1997). Carob Tree: *Ceratonia Siliqua* L.Promoting the conservation and use IF underutilized and neglected crops. . *Institute IF plant Genetics and crop plant Research,* Gatersleben / International plant Genetic Resources Institute, Rome. Italy.

Baumer, M.(1983). Notes on *Trees and Shrubs in arid and Semi- arid regions.* Ecological management, of arid and semi – arid rangeland in Africa and the Near and Middle East (EMASAR) – Phase2. FAO, Bome , Italy. 270pp.

Bengoechea , C., Romero, A., Villanueva, A., Moreno, G., Alaiz, M., Millan, F., Guerro, A., and Puppo, M.C. (2008) . Composition and structure of Carob (*Ceratonia siliqua*.) germ proteins . *Food chemistry*, **107**, 675-683.

Bentham, G. (1875). *Tran. Linn.Soc. London*, **30**,355-66.

Berhaut , J.(1975).Flore illus tree due Senegal .Dicotyledonous .Volume 4, Ficoidees legumineuses.

Blunt, H. S.(1926). gum Arabic with special reference to its production in Sudan. Oxford University Press.; pp. 11 – 43.

Bourbon, A. L., Pinheiro , A.C.,Riberio,C., Miranda ,C.,Maia ,J.M., Texixeria,J.A.,and Vicente ,A.A., (2010) .Characterization of galoctomannans extracted from seeds Gleditia triacanthos and Sorphora japonica through shear and extensional rheology: Comparison with guar gum and locust bean gum .*Food Hydrocol*.**24,**184- 192.

Braun, D.B. and Rosen, M.R. (2000). Rheology Modifiers Handbook-Practical Use and Application. William Andrew Publishing , Norwich, New York, USA. P. 14-27.

Burkill . H .M. (1995) . "*The useful plants of west tropical Africa*" . *2nd Edition* .Volume 3 , Families J- L. Royal Botanic Gardens , kew , Richmond , United Kingdom .857pp.

Carlson, W and Domanski , J. (1980). Two year chronic oral toxicity study with locust bean gum in albino rats . Unpublished study. Industrial Bio-Test Laboratories, Inc.

Casimir, C. and Akoh, D. B., Min. (2008): Chemistry, Nutrition and Biotechnology, *Food lipids*, Marcel Dekker, New York, pp 77-80.

Chau ,C., Cheung, K. and Wong , Y. (1997). Functional properties of protein concentrate from three Chinese in digenouse legume seeds. *Journal of Agricultural and food chemistry*, **45**, 2500- 2503.

Cox, G. E., Baily, D. E. and Morgareidge, K. (1974) .Subacute feeding in

dogs with a pre-cooked gum blend. Unpublished report from the Food and Drugs Laboratories, Inc., submitted to the World Health Organization by Hercules BV.

Crawford L., McDonald G. M., and Friedman M . (1990) .Composition of sickle pod *(Cassia obtusifolia)* toxic weed seeds. *J. Agric. Food Chem.* **38** (12):2169-2175.

Dakia,P.A.,Blecker , Ch.,Wathelet , B., and Paquot ,M.(2008).Composition and physicochemical properties of Locust bean gum extracted from whole seeds by acid or water dehulling pre-treatment .*Food Hydrocol*.**22**,807- 818.

Dakia , P,A., Blecker , C.,Robert , C., Wathelet ,B. and Paquot ,M . (2008) .Composition and Physicochemical Properties of Locust Bean Gum Extracted from whole seeds by Acid or Water Dehulling pre - treatment .*Food*

Hydrocolloids , **22**, 807 – 818. http: //dx.doi.org/10.10161j. *Food hyd* . 2007 .03.007.

Davis, H. T. (1994): Physicochemical and Engineering Aspects, *Colloids and Surfaces*, **91**, 9–24.

Dea , E. M., DA Rees, J Welsh, HA Barnes, J Price. (1977). "Associations of like and unlike polysaccharides: mechanism and specificity in galactomannans, interacting bacterial polysaccharides and related systems." *Carbohydrate Research***57**: 249-272.

Dea , I. C. M. (1979). Interactions of Ordered Polysaccharide Structures - Synergism and Freeze -Thaw Phenomena. Polysaccharides in Food . J. M. V. Blanshard, JR Mitchell. London, Butterworths**:** 229-247.

Dea , I.C.M., and Morrison ,A.(1975).Chemistry and interactions of seed galactomannans . Ad. *Carobhydrate Chem, and Biochem*., **31**,241-312.

Deis, R. (2001) . Salad Dressings and Sauces: Through Thick and Thin. *Food Product Design:* **1**-9.

Denkler M. (1997) . Based on U.S. Pat . No. 4 743 659; Derivates of Cassia . Freedom ChemicalDiamalt GmbH. Private Communication , January 3 1.

Diack ,M, Sene ,M ., Badiane ,A.N ., Diatta, M. and Dick, R.P .,(2000).Decomposition of native shrub , *Piliostigma reticulatum* ,Litter in soils of semiarid Senegal . Arid Land Research and Management **14** (3) : 205- 218.

Dickinson, E. (1995): Emulsion stabilization by polysaccharides and Protein–polysaccharide complexes, edited by A. M. Stephen, *Food Polysaccharides and their applications*, (pp. 501–515), New York, Dekker.

Dickinson .E., and Stainsby . G. (1988).Emulsion stability .In E. Dickinson and Stainsby . Advance in *Food Emulsion and Foams* (pp.**1**-44). London

: Elsevier Applied Science.

Djuma . (2003) . Djuma Game Reserve copyright © 1998 – 2003.

Domanski, J., Carlson, W and Frawley, J. (1980) .Three generation reproduction study on locust bean gum in albino rats. Unpublished study .

Doublier , J. L.(1981) . Rheological studies on starch-Flow behavior of wheat starch pastes. Starch/ Staerke **33**:415-420.

Doublier, J. L., Llamas , G., and Lemeur, M. (1987) . A rheological
Investigation of cereal starch pastes and gels . Effect of pasting procedures . *Carbohydr. Polym*. **7**:251-275.

Dow chemical company. (1999). Method Food grade gum .

El-Amin, H. M. (1990): Trees and shrubs of the Sudan. Ithaca press Exeter. 160-163.

Elegbede J .A. (1998).Legumes .In: *Nutritional quality of plant Foods*.

Ershoff, B. H and Wells , A. G. (1962) . Effects of gum guar, locust bean gum and carrageenan on liver cholesterol of cholesterol-fed rats, Proc. Soc. Exp. *Biol. Med.*,**110** (3), 580-582.

Evans, I. D., and Haisman , D. R. (1979). Rheology of gelatinized starch
suspensions . J.*Texture Stud*. **10**:347-370.

Fahren bach , M. J., Riccardi, B. A and Grant, W. E. (1966). Hypocholesterolemic activity of mucilaginous polysaccharides in White Leghorn cockerels, Proc. Soc. Exp. *Biol. Med*., **13** , 321-326.

Farahnaky,A.,Darabzadeh ,N.,Majzoobi,M., Mesbahi,Gh,Rezvani, E.,Schleining, G.(2013) . Production and Rheological properties of Locust Bean Gum from Iranian Carob Seeds . In side *Food Symposium*, **9**-12 April,Leuven, Belogium .

FAD Research Laboratories, Inc. (1972) .Teratologic evaluation of FDA 71-14. Teratologic evaluation of carob bean (locust) gum.Submitted under Contract No. FDA **71**-260. Maspeth, N.Y.

FAO of the United Nation. (2010) . Ecocrop (online resource Eco crop).

Food Chemicals Codex. (1996): Fourth Edition. *National Academy Press , Washington, D.C.*

Galla, N.R.; Dubasi, G.R . (2010)*: Chemical* and functional characterization of gum karaya (*Sterculia urens* L.) seed meal. *Food Hydrocol., **24***, 479–485.

Gaonkar , A.G.(1991) . Interfacial activates and emulsions characteristic of some Food hydrocolloids. *Food Hydrocolloids*, **5**(4**)**, 329 – 337.

Garti, N. (1999). What Can Nature Offer From an Emulsifier Point of View: Trends and Progress*Colloids and Surfaces A: Physicochemical and Engineering Aspects, **152***(1), 125-146.

Garti .N., Madar ,Z., Aserin .A., and Sternheim .B.(1997).Fenugreek galactomannan as food emulsions .*Food Science and Technology* .**30**.305-337.

Geerling , C. (1982).Guide de terrain des ligneous Saheliens et Soudano Gunineens.

Glicksman , M. (1973): *Industrial gums Polysaccharide and their derivates*, 2nd Ed, Academic press, New York, San Francisco, London, pp. 173-363.

Glickisman, M., and Sand. R. E., (1993). In Whistler, R. L. (ed.) *" Industerial Gums*" 2nd ed. Academic Press, New York p. 197.

Griffiths, C. (1952). Food **21**: 58.

Goodwin, J. W ., and Roy, W.H. (2008). Rheology for Chemists: An Introduction, 2nd Edition. RSC Publishing. P. 5- 30.

Grover .J.A . (1993) . Methylcellose and its derivatives. In R.L., Whistler and J.Bemiller . *Industrial Gums* .3rd Ed, (pp475-504) . London: Academic Press

Guillermo Repetto, Anadel Peso andJorge L Zurita . (2008): Neutral red uptake assay for the estimation of cell viability cytotoxicity, Nature Protocols.

Hallagan J.B., La Du B.N., Pariza M.W., Putnam J.M., and Borzelleca J.F. (1997) .Assessment of *Cassia* gum. *Food Chem* . *Toxicol.* **35**:625-632.

Han, C.D. (2007): Rheology and Processing of Polymeric Materials- Polymer Rheology. Oxford University press. P.1- 51.

Henson J.B., Dollahite, J.W., Brigdes, C.H. and Rao .(1965) . Myodegeration in cattle grazing *Cassia* species . *J. Am. Vet. Med. Ass'n* **147**:142-147.

Hoefler, A. C.(2004).: *Hydrocolloids.* Eagan Press Handbook Series . Eagan Press . St. Paul, Minnesota,.

Holbrook, A. A. (1951): The behavior of Carob bean in the gastrointestinal tract of man, Amer. J. Dig. Dis., **18**, 24-28.

Holmberg, K. (2006): Organic Reactions in Emulsions and Micro emulsions, In Emulsions and emulsion stability ,2nd Edition, *Surfactant Science series*, edited by Sjoblom, J. , Bergen, Norway.

Hoyt, J. (1966): "Friction reduction as an estimation of molecular weight." J Polymer Sci **4**: 713.

Irwin Hs , Barnaby. R.C. (1981).Adv in Legume Systematic vol 1, Royal Botanical Gardens , Kew , England , pp 97- 106.

JECFA, (1981) .WHORAO Reviews of Carob (locust) bean gum and Guar gum Listed in Summary of Evaluations Performed by the Joint FAOIWHO Expert Committee on Food Additives ,**INS** 410, 1981 and **INS** 412, 1975.

JECFA, (1982).: Toxicological evaluation of certain food additives, 526. Arabic gum (gum a rabic). In: Twenty-sixth Report of the Joint FAO/ WHO Expert Committee on Food Additives. WHO Food Additive Series 17 .

JECFA, (1993): *Toxicological evaluation of certain food additive sand contaminants*, 789: Carotenes from natural sources (algal and vegetable). World Health Organization, Geneva.

Kamal, M. S. (2011). Physico-chemical Study of *Acacia sieberana*. var.*sieberana* Gum. Ph. D. thesis, Sudan University of Science and Technology.

Khachatryan, A. (2003): Randam centroid optimization of lutein-enriched oil water emulsion at acidic pH, A Thesis for the degree of Master of Science, Louisiana State University, pp 11.

Klose R.E. and Glicksman , M. (1968) . Gums: In Handbook of Food Additives. Thomas E. Furia, editor. pp. 335-337. The Chemical Rubber Co., Ohio.

Koshioka M. and Takino Y. (1978) . Studies on the evaluation of crude drug I . Quantitative estimation of anthraquinones in *Cassia* seeds . *Chem. Pharm. Bull.* ***25***:(5)1345-1 349.

Krantz, J. C., jr, Carr, C. J. and De Farson , C. B. (1948). Guar poly- saccharide as a precursor of glycogen, J. Amer. Diet As 24, 212.

Kratzer, F. H ., Rajaguru , R.W.A.S.B and Vohra, P. (1967).The effect of polysaccharides on energy utilization, nitrogen retention fat absorption in chickens, *Poultry Sci*., **48**, 1489 -1493.

Krog, J. N. (1990) : In Food Emulsifiers and their chemical and physical properties, Grindstet Products, Brabrand, Denmark, pp 128.

Kubal, N. G. (1948):"Physiochemical properties of Karaya gum and locust bean mucilage." *Food Colloid* Science **3**: 457-460.

Laughlin, R., (1994) : In the Aqueous Phase behavior of Surfactants, *Academic Press*, New York.

Launay , J. D., G Cuvelier .(1986). Flow properties of aqueous solutions and dispersions of polysaccharides: *Functional Properties of Food Macromolecules*. D. L. JR Mitchell. London, *Elsevier Applied Science***: 1**-78.

Lee, W. J., and J.A.Lucey . (2004) .Structure and physical properties of yogurt gels: Effect of inoculation rate and incubation temperature. J.Dairy Sci.**87**:3153-3164.

Leimgruber R. and Marbot H. (1991) : Application *for the GRAS evaluation of Cassia gum*.: Registration and Consulting Company, Ltd., Basel, Switzerland. RCC Project 250424, September 2.

Leung A. Y**.** and Foster S. (1 996) . Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. John Wiley and Sons, Inc., New York.

Lin MJY, Humbert ES and FW Sosulski . (1974) : Certain functional properties of sunflower meal products. *Journal of Food Science;* 39: 368.

Lipinski, C. and Hopkins, A. (2004): *Nature*, **432**, 855.

Litton Bionetics, Inc. (1975) .FDA Project No. 2468, *Food and Drug Administration Contract No*. **223**-74-2104: Evaluation of test compound locust bean gum, FDA **71**-14.

Lutchmedial , M.,Ramalal , R., Badrie , N.and Changyen , I.,(2004): Nutritional and Sensory quality of stirred soursop(Annonamuricatal)yoghurt. International of Food Sciences and Nutrition, **55**(5):4o7 -414.

LRSO/ FASEB. (1972). Evaluation of the health aspects of Carob bean gum As a food - SCOGS-3 . Prepared for Bureau of Foods, US Food and Drug Administration Contract No. FDA **72**-85.

Maier, M. A., C Karl, K Magnuson, RL Whistler. (1992). Guar, locust bean, tara, and fenugreek gums . Industrial Gums . J. B. RL Whistler. San Diego, Academic Press**:** 205-215.

Maier, M., Anderson ,M., Karl ,C., and Magnuson ,K. (1993). Guar, Locust bean , tara , and fenugreek gums InWhistly ,L.R., and BeMiller ,J.N.(Eds).Industrial gums : polysaccharides and Their Derivatives . **3** rd ed.Academic press. New York.

Mason, T. G., Wilking, J. N., Meleson, K., Chang, C. B., and Graves, S. M. (2006). Nanoemulsions: Formation, Structure, and Physical Properties. *Journal of Physics: Condensed Matter*, ***18***(41), R635.

Maxwell, W. A and Newell, G. W. (1972) . Study of the mutagenic effects of FDA-71-14 (locust bean gum). Unpublished report from the Stanford Research Institute, submitted to the World Health Organization by Hercules BV.

Mc Cleary BV, Nurthen E, Taravel FR, Joseleau JP. (1983) .*characterization of the oligosaccharides* produced on hydrolysis of galactomannan with beta – D – mannanose carbohydrate Res 118 pp91 – 109.

McClements, D. J. (2004). Protein-stabilized Emulsions. *Current Opinion in Colloid and Interface Science, **9***(5), 305-313.

Meer, W. (1977): *Plant Hydrocolloids*. Food Colloids . H. Graham. Westport, AVI Publishing**:** 522-539.

Mezger, T. G. (2002): In the Rheology Handbook: For users of rotational and oscillation rheometers, Vincentz Verlag.

Morgareidge, K. (1972) .Teratological evaluation of FDA-71-14 (locust

bean gum). Unpublished report from the *Food and Drug Research Laboratories, Inc.*, submitted to the World Health Organization by Hercules BV.

Naber, E. C. and Smothers, S. E. (1975) .Patterns of toxicity a teratogenicity in the chick embryo resulting from administration of certain nutrients and food additives, *PoultrySci*., **54** (5), 1806.

National Toxicology Program . (1980) : Carcinogenesis bioassay of locust bean gum. DHHS publication No . (NIH) **81**-71 - 77.

Neukom , H. (1988). Carob Bean Gum . Properties *and* Applications. Proceeding of II International Carob symposium , 551 – 555.

Neuwinger , H.D ., (2000) .African traditional medicine : a dictionary of plant use and application s . Medpharm Scientific , Stuttgart, Germany .589 pp.

NTP (1982b) Carcinogenesis bioassay of tara gum. NTP-TR-224 .

Ockerman, H.(1978) : Source Book for Food Scientsist. Westport, AVI publishing Company, Inc.

Paraskevopoulou, A., Kiosseoglou, V., Alevisopoulos, S., and Kasapis,

S. (1997): Small deformation properties of model salad dressings

prepared with reduced cholesterol egg yolk, *Journal of Texture*

Studies, **28**(2), 221–237.

Pazur , J.H., Chaplin ,N.F., and Kennedy , J.F., (1986) .Carbohydrates Analysis A practical Approach Oxford press .London.

Pellet, P.L and Yong, V.R (1980): "Nutritional evaluation of protein foods." Published by the United Nation University .

Phillips, G.O., Wedlock, D. J., and Williams, P. A., (1995): In "Gums and Stabilisers in the Food Industry", Vol. **8**. INEC communication to the *JECFA* Secretariat .

Phillips G.O., Williams P.A. (2001): Tree exudate gums: natural and versatile food additives and ingredients, *Food Ingred Anal Int* , **23**,26, 28.

Poethke W., Rao **D.A.,** and Loscher K.D . (1968) .*Pharm. Zenthralh.* 107571.

Puhan Z, Wielinga MW , Products derived from carob pods with particular emphasis on carob bean gum (un published report of INEC).

Pyler,E.J.(1973).Baking sciences and technology .Vol. Siebel publishing *Company. Chicago,* I IL.

Randell B.R. (1995) : Taxonomy and evolution of *Senna obtusfolia* and *S. tora. J. Adelaide Bot.Gard.* 1655-1658 **.**

Randall, R. C., Phillips, G. O., and Williams, P. A., (1989). *Food Hydrocolliods* . **3**, 65.

Richardson, J. W., TJ Foster. (1998). "Dilute solution properties of guar and locust Bean gum in sucrose solutions." *Food Hydrocolloids***12**: 339-348.

Rivier , C. (1952). Recherché sure le mode d'action du Nestargel, Schweiz. Mediz., **82**, 256.

Ross, A. H., Eastwood, M. A., Brydon, W. G., Anderson, J. R and Anderson, D. M., (1983). Astudy of the effectsof dietary gum Arabic in humans. *American Journal of Clinical Nutrition* **37**(3): 368- 375.

Salage r, J. L. (2006): Emulsion Phase Inversion Phenomena, In Emulsions and emulsion stability , 2nd Edition, *Surfactant Science series,* edited by *Sjöblom,* J., Bergen, Norway.

Schramm, G. (2000): A practical Approach to Rheology and Rheometry. Gebrueder HAAKE GmbH, Karlsruhe, Germany.

Shirazi, F.H., Ahmadi, N. and Kamalinejad , M. (2004). *DARU*, **12**, 3.

Silva, D. A. and de Paula. R. (2007). Graft copolymerisation of acrylamide onto cashew gum. *European polymer journal***43**(6): 2620-2629.

Srivastava .M., and Kpoor , V.P., 2005. Seed galactomnnans: An overview.*hem* . *and Biodiver*.**2**,295-317.

Stittikijyothin, W., Torres, D., and Goncalves, M.P., (2005).*Modeling the* rheological behavior galactomnnan aqueous solutions.: *Carbohydrate poly*.**59**, 339-350.

Survey.(1977) : Industry on the Use of Food Additives . Committee on GRAS List Survey - Phase III,Food and Nutrition Board, National Research Council, *National Academy* of Sciences, Washington DC, pp.64-66.

Torio, M.A.O .; Saez, J.; Merc, F.E. *(*2006*)*: Physicochemical characterization of galactomannan from sugar palm *(Arenga saccharifera Labill.)* endosperm at different stages of nutmaturit. *Philippine J. Sci., **135**, 19–30.*

Upadhyaya S.K. and Singh V. (1986): Phytochemical evaluation of *Cassia obtusifolia* and *Cassia tora. Proc. Indian Acad. Sei., Plant Sei.* 96 :2 1-26.

Vassal, J. (1972). *Bull. Soc., Hist. Nat. Toulouse*. **108**,i.

Verbeken, D., and Dierckx. S., (2003) . Exudate gums: occurrence, production, and applications." *Applied Microbiology and Biotechnology* **63**(1): 10-21.

Vohra, P. and Kratzer, F. H. (1964) . Growth inhibitory effects of certain Polysaccharides for chickens , *Poultry Sci*., **43** , 1164-1170.

Vohra, P., Shariff, G and Kratzer, F. H . (1979) . Growth inhibitory effect of some gums and pectin for Tribolium castaneum larvae,chickens and Japanese quail, Nutr. Rep. Internal's, **19** (4) 463-469.

Von Maydell, H. (1986).Trees and shrubs of the Sahel: their characteristics and uses .Schriftenreihe der GTZ No 196. Deutsche Gesellschaft fur Technische Zusammenarbeit , Eschborn , Germany .**525** pp.

Walstra, P. (2003) : Physical chemistry of foods. New York: Marcel Dekker, Inc. France.

Wielinga, A. M. (2000): Galactomannans. Hand book of Hydrocolloids. P. W. GO. Wielinga MW . Phillips . Boca Raton. (1990) : Production and application of seed `gums. Gums and stabilizers for the food industry **5**, CRC Press**:** 137-154.

Wong, R. B. K., and Leliever , J. (1982). *Rheological characteristics of wheat starch pastes* measured under steady shear conditions. J. Appl. Polym. Sci**. 27:**1433-1440.

Wu.Y., Cui.W., Eskin ,N.A.M., and Goff ,H.D., (2009) .An investigation of four commercial Galactomannan on their emulsion and rheological properties .*Food Ress* . Inter .**42**, 1141- 1146.

X.Hang , Y.Kakuda and W.chi.(2001) . *Hydrocolloids in emulsions* : Particle Size distribution and interfacial activity.

Yelemou, B.Bationo , B.A, Yameogo, G. and Millogo- Rasolodimby ,J. (2007) .Gestion traditionnelle et usages de *Piliostigma reticulatum* .

Youmbie , D.D.B. (2008).Effects a nalgesiques et ant- in flammatoires del extra it aqueux de *Piliostigma reticulatum*.