Chapter one Introduction and Literature Review

1. Introduction

Acacia gums are polysaccharides obtained from the stems and branches of plant species produces gums. It is mainly obtained from the genus *Acacia* as dried exudates (FAO, 1990). Gums of commercial importance have a significant ecological and socioeconomic impact on inhabitants of the semi-arid zones, especially, the African gum belt (fig 2).

These natural products enjoy remarkable diversity of applications which is mainly due to their ability to reduce surface tension, extremely high solubility in water and low viscosity (Osman, 1993). They are high molecular weight polymeric compounds, composed mainly of carbohydrate moieties capable of possessing colloidal properties in appropriate solvents.

They are either hydrophobic or hydrophilic in nature. Hydrophobic gums are insoluble in water and include resins such as Olibanum gum. Whereas hydrophilic gums are in water soluble and can be subdivided into natural, semi synthetic and synthetic gums (Glicksman, 1973).

Acacia seyal var. seyal Del. is a typical tree in the African semi arid zones. It is a small to mediumsized tree that reaches a height of 12-17 m National Academy of Sciences, NAS, 1980), von Maydell, 1990(Hall and McAllan, 1993; McAllan, 1993; has a stem diameter of 30 cm (Mustafa, 1997), or 60 cm under favorable conditions and develops a characteristic umbrella-shaped crown (von Maydell, 1990) plate (1 - 2).



Plate 1: Acacia seyal tree

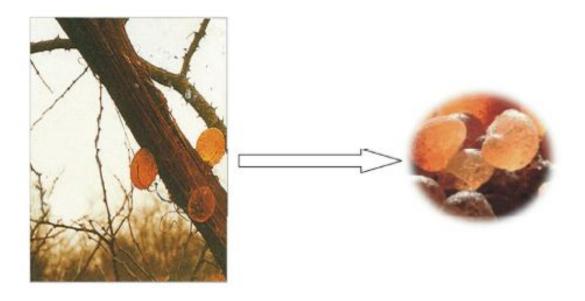
Several authors provided a valuable description of *Acacia* seyalvarseyal(NAS, 1980 ,von Maydell, 1990;Elamin, 1990; Hall and McAllan, 1993; McAllan, 1993; Mustafa, 1997;).

A. seyal var. seyal is widely, distributed in the African savannas (Booth and Wickens, 1988; McAllan, 1993), often dominates the vegetation community and in some areas forms pure stands (McAllan, 1993; Wickenset al., 1995).

It is considered one of the most common trees on clay plains that flood during the rainy season (McAllan, 1993).

The species is an important source of fuel wood, building poles, forage, commercial gums and tannins (; NAS, 1979,1980, von Maydell, 1990, ELamin, 1990; Wickens et al., 1995, Mustafa, 1997) and is a source of nectar for honeybees (Booth and Wickens, 1988). A. seyal var seyal produces gum was showed in plate(2) which though of inferior quality in Comparison to that of Acacia senegal var senegal, is traded in Sudan under the name "gum talha", recently the sudan A. Seyal var seyal represent nearly 70% of the value of gum export for sudan(sudan customs).

Plate 2. A. Seyal var. seyal gum(Abdullah2013)



Additionally, *A. seya l* var *seyal* serves valuable ecological functions such as reducing soil erosion and acting as a defence line for desert encroachment in many parts of the Sudan, as is the case for the selected location for the present study, the Umfakarin forest reserve. Like other Leguminous. *A. seyal* is a nitrogen fixing tree which can be integrated into an agro-forestry system to enhance the growth of agricultural crops.

1.2. Species and Distribution:

The species requires annual rainfalls of 250-1000 mm and it can withstand inundation better than other acacias (NAS, 1980,vonMaydell, 1990;). The species thrives in most soil types, even in heavy clay and stony soils found on the plains (NAS, 1980, McAllan, 1993;). It prefers temperatures between 15-35 °C (Vogt, 1995). It often grows with other tree species, such as *Acacia sieberana*, *Anogeissusleiocarpus*, *Balanitesaegyptiaca*, *Faidher -biaalbida* and *Ziziphusmauritiana* (McAllan, 1993).

In general, there are two main varieties of *A. seyal*; variety seyal and variety *fistula* figure (1). Variety *seyal* is found in both western and eastern Africa

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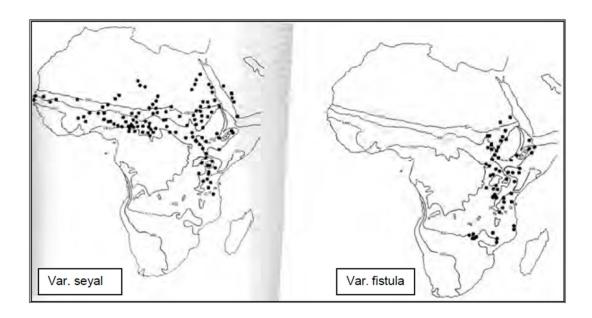


Figure 1. Distribution of Acacia seyal varieties in Africa,

(Source: Hall and McAllan, 1993; McAllan, 1993)

and also on the Arabian Peninsula, while variety *fistula* is found in the eastern parts of Africa (McAllan, 1993).

NAS (1980) and NFTA (1994) indicate that variety *seyal* is native to northern-tropical Africa and Egypt (figure 2). The two varieties can be easily distinguished; variety *seyal* has a reddish-brown bark, while variety *fistula* has white to greenish yellow bark (McAllan, 1993).

Besides clearance for mechanized farming and wood fuel, other factors such as grazing, deliberate and undeliberate fires also have a significant negative

impact, not only on natural stands of *A. seyal* but also on natural forests in Sudan.

In Sudan, the two varieties occur naturally in the low rainfall savannah zone and extend from Gadarif, Blue Nile, and White Nile to clay plains around Nuba Mountains and the Darfur Region (El Amin, 1990; Mustafa, 1997; Sahni, 1968). The species is distributed throughout its natural range, and is usually associated with *Balanitesaegyptiaca* in the *Acacia seyal*-Balanites woodland area (**fig 2**).



Figure 2: shows the Acacia seyal plant distribution in Africa

In such formation, *A. seyal* is the dominant species, forming pure dense stands in many areas. According to Mustafa (1997), this formation begins to emerge with an increase in the annual rainfall to accumulations of more than 500 mm. In the savanna region of Sudan, A. seyal has been subjected to large-scale clearing for mechanized agriculture (Vink, 1990; Wickens*et al.*, 1995Mustafa, 1997;) associated with firewood and charcoal production to meet energy requirements.

1.3. Sudan gum production

Sudan is the world's biggest producer and exporter of gum Arabic. Sudan production data are given in Tables 1a and 1b: 5year annual averages since

1960 are given in Table 1a and yearly figures for the crop years 1988 - 94 are shown in Table 1b. The data in Table 1a shows a drop in production by more than half in the last decade compared to that in the 1960s (when it averaged about 4,500 tonnes /year). Before that year between 1950 - 59 (not shown) productions averaged was just under 41,000 tonnes/year. The relation between the production of gum Talha to gum Arabic in Sudan is shown in Tables (1a and 1b), which is found to be proportion with a ratio of around 5 - 15%. However, in recent years (Table 1b) has varied from less than 200 tonnes (3%) in 1992 to 11,000 tonnes (33%) in 1994. However, the

comparison with the production data is difficult because of the uncertainty in the level of carry-over of stocks from one year to the next. Sudan export both *A. Senegal* (Hashab) and *A. seyal* var *seyal* (Talha gum) table (2).

Table 1a. Production of Talha and Hashab gum in Sudan during 1960-94 (tons):

	1960- 64	65-69	70-74	75-79	80-84	85-89	90-94
Annual average	46550	50576	35073	37408	31079	23721	18358
Of which:							
Hashab gum	44299	47434	30910	36026	26721	19777	15038
Talha gum	2251	3142	4163	1382	4358	3944	3320

Source: Gum Arabic Company, Sudan

Table 1b. Production of Talha and Hashab gum in Sudan during 1988-1994 (tons):

	1988	1989	1990	1991	1992	1993	1994
Total	26000	28948	25733	12351	7616	12865	33227
Of which:							
Hashab gum	20000	24256	22408	11756	7439	11410	22178
Talha gum	6000	4692	3325	595	177	1455	11049

Source: Gum Arabic Company, Sudan

Table 2.Exporation of Sudan Gum Arabic (1988-94) in tons

	1988	1989	1990	1991	1992	1993	1994
Total	18603	19352	26912	24978	14068	15730	22735
Of which:							
Hashab gum	16672	17385	22960	21543	8198	9925	18339
Talha gum	1931	1967	3952	3435	5870	5805	4396

Source: Gum Arabic Company, Sudan

1.4. Chemical composition of (A. seyal var seyal)

1.4.1. Molecular weight of A. seyal var. seyal

Anderson *et al.* (1969) estimated the weight average molecular weight for *A. seyal* var. *seyal* and the value was 8.5×10^5 . The value of Mw of *A. seyal* var. *seyal* was found to be 15.5×10^5 . For *A. senegal* var. *senegal* were found to be 8.64×10^5 and 2.86×10^5 , Younes (2009) obtained the weight average molecular weights of *A. Senegal* var. *senegal* and *A. seyal* var. *seyal* samples; it was ranged from 8.08×10^5 to 1.34×10^6 for *A. senegal* var. *senegal* and ranged from 6.40×10^5 to 1.90×10^6 for *A. seyal* var. *seyal*.

1.4.2. Proximate analysis

Approximate analysis of gum especially moisture, ash and nitrogen are important for quality specifications.

- (1) Moisture content.
- (2) Ash content.
- (3) Nitrogen content.
- (4) Specific optical rotation.

1.4.2.1. Moisture content

Moisture content of the gum determines the hardness of the gum and hence the variability of densities and the amount of air entrapped during nodule formation. It can be determined by measuring the weight loss after water evaporation. Reducing the moisture content of the natural gum can be readily used as a tenable method of reducing the microbial counts (Karamallah, 1999), Anderson *et al.* (1963) reported the moisture content of *A. seyal* var. *seyal* gum in the range from 11% to 16.1%.

Karamallah (1999) measured the moisture content in *A. senegal* var. *senegal* and *A. seyal* var. *seyal* gum collected between 1960 and 1999 in Sudan, it was found equal to 10.75% and 9.4% respectively, Hassan (2000) in the study of *A. seyal* var. *seyal* gum from different locations of Sudan reported an average of 8.5% moisture content, Hassan *et al.* (2005) reported that the moisture content of *A. seyal* var. *seyal* gum in the range from 7.4% to 8.3%. Siddiget al. (2005) reported the value of 12.6% for the moisture content of *A. seyal* var. *seyal* gum. Omer (2006) found that the moisture content of *A. seyal* var. *seyal* gum. Seyal gum were in the range of 11.76% to 14.8% and 5.66% to 11.11% respectively, moisture content in *A. seyal* var. seyal gum was determined by Abdelrahman (2008) found that moisture to be 11.07%.

1.4.2.2. Ash content:

The ash content indicates the presence of inorganic elements existing in saltform. Anderson *et al.* (1968) and Karamallah (1999) showed that the type

of soil(clay or sand) affected the ash content significantly; Anderson et al. (1963) reported that ash content of A. seyal var. seyalgum in the range from 1.94% to 3.55%. Anderson (1977) reported that ash content of A. senegal var. senegal and A. seyal var. seyal gum in the value of 2.87% and 3.93% respectively. Again Karamallah (1999) reported a value of 3.7 and 2.3 Ash content for A. senegal var. Senegal, and A. seyal var. seyal gum respectively collected between 1960 and 1999 in Sudan. Hassan et al. (2005) in the study of A. seyal var. seyal gum from different locations of Sudan reported an average of 0.21% ash content, Omer (2006)reported the ash content of A. senegal var. senegal in the average of 3.27% and in the average of 2.61% for A.seyal var. seyal gum, The mean value of ash content reported by Abdelrahman (2008) in A. senegal var. Senegal and A. seyal var. seyal gum in the average of 3.32% and 2.43% respectively

1.4.2.3. Nitrogen and protein content

The role of nitrogen and nitrogenous component in the structure, Physicochemical properties and functionality of gum was recently subjected to intensive investigations (Dickinson *et al.*, 1988, Randall *et al.*, 1989). Dickinson(1991) studied the emulsifying behavior of gum and concluded that there was a strong correlation between the proportion of protein in the gum and emulsifying stability. Anderson *et al.* (1963) reported that nitrogen

content of A. seyal var.seyal gum ranged from 0.09 – 0.19% w/w. Nitrogen content of A. senegal var. senegal gum had been determined by Anderson (1977) and was found to be 0.29% and for A. seyal var. seyal 0.14%. Juraseket al. (1993) reported 0.28 to 0.35% nitrogen content for A. senegal var. senegal samples and 0.14% for A. seyal var seyal. Karamallah (1999) reported nitrogen content in comparative analytical data for A. senegal var. senegaland A. seyal var. seyal gums collected between 1960–1999 in Sudan to be 0.33% for A. senegal var.senegal gum, and 0.11% for A. seyal var. seyal gum. Hassan et al. (2005) reported protein content of A. seyal var. seyal had a mean value of 0.96%. The nitrogen content of A. seyal var. seyal gum had been determined by Siddiget al. (2005), it was found to be 0.15% and hence protein content found to be 1.0%. Omer (2006) determined the nitrogen content for samples of A. senegal var.senegal and A. seyal var. seyal from different locations, the values were 0.35% and 0.14% for A. senegal var. senegal and A. seyal var. seyal respectively, whereas protein content had a value of 2.3% and 0.93 respectively. Abdelrahman (2008) reported the average value of nitrogen content of A. senegal var. senegal gum 0.37% whereas equal to 0.14% for A. seyal var. seyal gum.

1.4.2.4. Sugar composition

Monosaccharide composition of gum is determined by acid hydrolysis of the gum, complete hydrolysis yields four basic sugar constituents, D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. Anderson (1977) reported that sugar content of A. senegal var. senegal was 41% galactose, 27% arabinose, 14% rhamnose and 14.5% glucuronic acid. Jurasek*et al.* (1993) reported sugar composition as 34 - 46% galactose, 23 - 35% arabinose and 9-16% rhamnose for A. senegal var. senegal, and 38% galactose, 46% arabinose and 4% rhamnose for A .seyal var. seyal Karamallah (1999) reported comparative analytical data for A. senegal var. senegal and A. seyal var. seyal gums collected between 1960 and 1999 in Sudan, he reported sugar content had a value of 36 - 42% galactose, 24-29% arabinose, 12-14% rhamnose and 16-17% glucuronic acid for A. senegal var. senegal, whereas had a value of 37-38% galactose, 41 - 45% arabinose, 3 - 4%rhamnose and 11-12% glucuronic acid for A. seyal var. seyal.

Islam *et al.* (1997) and Williams *et al.* (2000) reported the sugar content of *A. seyal* var. *seyal* as 38% galactose, 46% arabinose, 4% rhamnose and 6.5% glucuronic acid. Flindt*et al.* (2005) reported the sugar content of *A. seyal* var. seyal 34.9% galactose, 26.5% arabinose, 11.5% rhamnose and 11.6% glucuronic acid. Siddig*et al.* (2005) reported the sugar content of *A. seyal*

var. seyal 36% galactose, 44% arabinose, 3% rhamnose and 16% glucuronicacid. The average values of sugar content determined by Abdelrahman (2008) of A. seyal var. seyal as 28.8% galactose, 34% arabinose and 1.6% rhamnose.

1.4.5. Cationic composition

The most four abundant cationic elements present in gum are calcium, potassium, magnesium, and sodium. It had been cited in the final report of the safety assessment of different Acacia gum that Anderson *et al.* (1990) reported the cation composition of Sudanese *A. senegal* var. *senegal* samples between 1904 and 1989. In the same report United States Pharmacopoeia reported the specifications grade of Acacia gum as arsenic (3ppm), lead (0.001%) and heavy metals (0.004%). The specifications for food grade Acacia gum include arsenic (3mg/kg maximum), heavy metals (0.002% maximum) and lead (5mg/kg maximum) had been cited in the same report.

1.4.6. Physical properties of gum:

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

40% and become pseudo plastic at higher concentrations (Verbeken*et al.*, 2003). The pH of the solutions is normally around 4.5-5.5, but maximal viscosity is found at pH 6.0.

1.4.6.1. PH value

The hydrogen ion concentration plays great importance in the chemistry and industry of the gums. The change in the concentration of hydrogen ion may determine the solubility of gum and the precipitation of protein, therefore 14 functional properties of a gum may be affected by change in pH for example viscosity and emulsifying power. Crude gum is slightly acidic because of the presence of few free carboxyl groups of its constituent acidic residues, D-glucuronicacid and its 4-O-methyl derivative.

Karamallah*et al.* (1998) reported the pH mean value of 4.66 for the 755 authentic. *A. senegal* var. *senegal* gum samples, collected in season 1994/1995. The same author in the same study reported the mean value of 4.54 for commercial samples collected between 1992 and 1996, also they reported an average value of 4.4 for *A. senegal* var. *senegal* gum samples, collected between 1960 and 1995. Karamallah (1999) reported 4.66 pH values for *A. senegal* var. *senegal* and 4.2 for *A. seyal* var. *seyal* gum. The pH value had been determined by Younes (2009), he reported a value of 4.78 for *A. senegal*var. *senegal* and 5.16 for *A. seyal* var. *seyal* gum. Satti (2012)

reported the meanvalue of PH value for A. nilotica var. nilotica the range was 5.15 - 5.24.

1.4.6.2. Specific optical rotation

The optical activity of organic molecules (saccharides and carbohydrates) is related to their structure and is a characteristics property of the substance, and thus the specific rotation is considered as the most important criterion of purity and identity of any type of gum. Anderson et al. (1963) reported the specific optical rotation of A. seyal var. seyal gum in the range from +44° to +56°. Anderson (1977) reported a value of -30° specific optical rotation for A. senegal var. senegal and +51° for A. seyal var. seyalgum, Karamallah (1999) reported -30.3° specific rotation for A. senegal var. senegal and +50.6° for A. seyal var. seyal gum. Hassan (2000) reported that A. seyal var. seyal gum exhibit dextrorotatory optical rotation ranging from +40° to +62°. Hassan (2005) reported $+53^{\circ}$ mean value of specific optical rotation of A. seyalvar. seyal gum. Optical rotation of A. seyal var. seyal gum had been determined by Siddiget al. (2005) and found to be +450. Omer (2006) reported that an average values of specific optical rotation equal to -32°, and +49.4° for A. senegal var. senegal and A. seyal var. seyal respectively. Abdelrahman (2008) reported the average value of optical rotation of A.

Senegal var. senegal gum -31.50 whereas equal to +61° for A. seyal var. seyal gum.

1.4.6.3. Viscosity

The viscosity of a liquid is its resistance to shear, to stirring or to flow through a capillary tube, Viscosity was considered as one of the most important analytical and commercial parameters, since it is a factor involving the size and the shape of the macro - molecule (Anderson et al., 1969), Viscosity can be presented in many terms such as relative viscosity, specific viscosity, reduced viscosity ,inherent viscosity and intrinsic viscosity, It is also presented as kinematic or dynamic viscosity. The intrinsic viscosity has great practical value in molecular weight determinations of high polymers, This concept is based on the Mark-Houwinkrelation suggesting that the intrinsic viscosity of a dilute polymer solution is proportional to the average molecular weight of the solute raised to a power in the range of 0.5 to 0.9 Values of the proportionality constant and the exponent are well known for many polymer-solvent combinations. Solutions viscosities are useful in understanding the visco-clastic behavior some polymers.

The stiffness of the polymer can be known from the relationship between intrinsic viscosity and changing ionic strengths of gum solutions. Anderson (1977) reported a value of 13.4 cm³g⁻¹ intrinsic viscosity for *A. Senegal* var.

senegal and 12.4 cm³g⁻¹ for *A. seyal* var. seyal gum. Duvalletet al.(1993) found that the intrinsic viscosity ranged between 13.4-23.1 cm³ g⁻¹ for *A. seyal* var. senegal and equal to 12.4 cm³g⁻¹ for *A. seyal* var. seyal.

Karamallh*et al.* (1998) reported that the mean value of intrinsic viscosity of 1500 samples of *A. senegal* var. *senegal* was 16.44cm³g⁻¹. Also Karamallh, (1999) reported the intrinsic viscosity was equal to 16.6 cm³g⁻¹ for *A. senegal* var. *senegal* and 11.0 cm³g⁻¹ for *A. seyal* var. seyal. Hassan *et al.* (2005) reported that the intrinsic viscosity of *A. seyal* var. *seyal* in the ranges between 11.9–17.6cm³g⁻¹. The intrinsic viscosity had been determined by Flindt*et al.* (2005), they reported that the intrinsic viscosity of *A. seyal* var. *seyal* fall in the range from 11.6 to 17.7cm³g⁻¹.

The intrinsic viscosity of *A. seyal* var. *seyal* gum had been determined by Siddig*et al.* (2005), it was found to be 14cm³g⁻¹. Omer (2006) found that an average values of intrinsic viscosity equal to 14.6cm³g⁻¹, 11.4cm³g⁻¹ for *A. senegal* var. *seyal* respectively. Abdelrahman (2008) reported the average value of intrinsic viscosity of *A. senegal* var. *senegal* gum 15.4cm³g⁻¹ whereas equal to 11.6cm³g⁻¹ for *A. seyal* var. *seyal* gum. The intrinsic viscosity had been determined by Elmanan et al., (2008), they reported that the intrinsic viscosity ranged between 14.7 to 17.3cm³g⁻¹

for A. senegal var.senegal and between 14.6 to 14.9cm³g⁻¹ for A. seyal var.seyal.

1.5. Application of Gums

Exudate gums are used in an overwhelming number of applications, mainly situated in the food area. However, there are also considerable non-food applications. Its uses fall into three main areas: food, pharmaceutical and technical.

1.5.1. Food Applications

Gum Arabic(GA) is mainly used in the confectionery industry, where it is incorporated in a wide range of products. Gum Arabic is being widely used for industrial purposes such as a stabilizer, a thickener, an emulsifier and an encapsulating in the food industry and to a lesser extent in textiles, ceramics, lithography, cosmetic and pharmaceutical industry. In the food industry,(GA) is primarily used in confectionery, bakery, dairy, and beverage and as a microencapsulating agent (Mariana *et al.*, 2012). They are find in a wide applications in the Food and Beverages Industries as a natural emulsifier, particularly, for citrus oils (Egadu*et al.*, 2007).

As noted earlier, the FAO specification for gum arabic intended for food use stipulates that it should come from *A. Senegal varsenegal*or closely related species. Even apart from legislative requirements, the quality and technical performance of gum from this source makes it the material of choice in most cases. In Europe, the food additive number of gum arabic is E414.

Confectionery remains a major use for gums, although supply and price pressures have led to a marked reduction in the amount of gum used in some traditional items such as "fruit gums" and pastilles. The role of gum in confectionery products is usually either to prevent crystallization of sugar or to act as an emulsifier. In candy products it is also used as a glaze. It finds wide application as a means of encapsulating flavours (for example, spraydried flavours and citrus oils) and is also used in a range of dairy and bakery products (especially as a glaze or topping in the latter). It is used in soft and alcoholic drinks, either as a vehicle for flavouring or as a stabilizer or clouding agent. It has a long tradition of use in wine gums, where it produces a clarity that is higher than can be obtained with other hydrocolloids. Furthermore, it prevents sucrose crystallization, provides a controlled flavor release and slows down melting in the mouth, making the wine gum long lasting. It also provides the appropriate texture to for candies, which are easily deformed in the mouth but do not adhere to the teeth (Arjaet al., 2011).

In low-calorie candy, gum Arabic is used to compensate for the loss of texture, mouth feel and body, resulting from the replacement of sugars by artificial sweeteners. It is also used in chewing gum as a coating agent and as a pigment stabilizer. In aerated confectionery products, such marshmallows, nougats and meringues, gums acts as a whipping and stabilizing agent. It is also used in toffees and caramels as an emulsifier, to maintain a uniform distribution of fat across the product. In jelly products, it is used to provide a fibrous, fruit-like texture (Tadesse*et al.*, 2007). Gums, are, widely, used as an emulsifier in the manufacture of soft drinks. Due to its stability in acid conditions and its high solubility, gum s arewell suited for use in citrus and cola flavor oil emulsions. High levels of gum are used to ensure a complete coverage of the interface and to prevent flocculation and coalescence of oil droplets. Normally, a weighting agent is added oil-phase density, inhibiting to increase the to creamingdestabilization (Wyasu and Okereke, 2012). Gums can be also form a stable cloud in the drink, imitating the effect of added fruit pulps and juices. Gum Arabic is used increasingly as a source of soluble fiber in lowcalorie and dietetic beverages. In powdered beverage mixes, gums are added

to produce the same opacity, appearance, mouth feel and palatability of natural fruit juices (Wyasu and Okereke, 2012).

In microencapsulation, liquid, solid or gaseous substances are coated with a protective layer to prevent chemical deterioration and the loss of volatile compounds. It is a useful technique to convert liquid food flavors to flow able powders that can be used in dry food products. Some Gumsare effective encapsulation agents because of their high water solubility, low viscosity and emulsification properties and is used in soups and dessert mixes. Gums are also, used, to prevent gelation in canned gravy based pet foods, they inhibit proteinsextraction from meat into the gravy (Verbeken*et al.*, 2003). applications food of gums, such as: Meat products, dairy products, bakery products, beverage, confectionery and flavors. Gum Arabic has excellent emulsifying properties. The hydrophobic polypeptide back bone strongly adsorbs at the oil-water interface, while the attached carbohydrate units stabilize the emulsion by steric and electrostatic repulsion. Fractionation studies show that, although emulsifying properties, generally improve with increasing molecular weight and protein content, the best results are obtained with mixtures of different fractions. Seemingly, the heterogeneous nature of the gum makes it an excellent emulsifier (Verbekenet al., 2). They are

finding in wide applications in the Food and Beverages Industries as a natural emulsifier, particularly, for citrus oils (Egadu*et al.*, 2007)

1.5.2. Pharmaceutical use

Gum arabic's use in pharmaceuticals is much less than it once was, and it has been displaced in many of its applications by modified starches and celluloses. However, it still finds some use in tablet manufacture, where it functions as a binding agent or as a coating prior to sugar coating, and it is also used as a suspending and emulsifying agent, sometimes in blends with other gums. They are also used in the pharmaceutical industry as a suspending agent and stabilizer (Fennema, 1996).

1.5.3. Non-Food Applications; technical and miscellaneous uses:

In cosmetics, gums function as stabilizers in lotions and protective creams, where they increase viscosity, imparts spreading properties and provides a protective coating and a smooth feel. It is used as an adhesive agent in blusher and as a foam stabilizer in liquid soaps (Arjaet al., 2011). Gum Arabic is also used in the preparation of etching and plating solutions in the lithography industry. They are used as a dispersant in paints and insecticidal/acaricidal emulsions, respectively keeping the pigments and

active components uniformly distributed throughout the product (Verbeken*et al.*, 2003).

In the textile industry, they are used as a thickening agent in printing pastes for the coloration of knitted cellulose fabrics. Other applications are ink and pigment manufacture, ceramics and polishes (Verbeken*et al.*, 2003).

An important non-food/pharmaceutical application of gum arabic is in the printing industry, where it is used to treat offset lithographic plates: as a protective coating to prevent oxidation; as a component of solutions to increase hydrophilicity and impart ink repellency to the plates; and as a base for photosensitive chemicals.

1.5.4. Animal feed:

The bark is a valuable part of *Acacia seyal*. It is extensively used for feeding cattle, goats and sheep during the dry season. The bark is thick, smooth and relatively soft when fresh. In the dry months of February and March in Kenya, animals browse the bark and eat the leaves, which, however, are relatively few at that time. Often the whole tree is cut to a height of 1.5 to 2 m and new growth arises from the previous cut. As animals often browse the bark of standing trees, deformation is common. Round service-wood, poles, posts, building material, fuel wood, fuel is subject to wood borers and readily

reduced to dust when stored, "Zeribas" (= "Bomas") building and fencing, browsing of leaves, twigs and pods, branches are often pollarded by shepherds.

Nutritional Quality and Animal Production

Animals will eat up to 5.5 kg. of bark a day, sufficient for maintenance and the production of 4.5 litres of milk. The crude protein content of the bark is between (10.6%-4.1). The Calcium values are very high, with over 4 %, but the phosphorus content of under 0.1 % was low (Dougall and Bogdan, 1958).

Objectives:

1/ to fractionate Acacia seyal var. seyal gum in to two fractions (hydrophobic and hydrophilic fractions).

 $2/\mbox{\ study}$ the $\mbox{\ physicochemical properties}$ of the fractions .

Chapter Two

Materials and methods

2.1. Materials:

Authenticated gum samples from *A. caciaseyalvarseyal were*obtained from Gum Arabic Company Ltd., Khartoum, for season2014/2015.

2. 1.1 Sample cleaning and preparations:

One kg of crude gum nodule and powder were cleaned from bark, Soil, dust particles and other impurities prior to use. The nodules were ground to form gum powder.

2.2. Methods:

Physicochemicals methods used include

2.2.1. Moisture content of gums:

Moisture content was determined according to Mantell (1965). One g of sample was weighed, and then heated in a porcelain crucible in an oven at 105°C for 5 hrs. It was then cooled in a desiccator and weighted. The percentages of the moisture contents were calculated as follow:

% Moisture content=Sample weight before drying-sample after drying X100. Sample weight before drying

2.2.2. Fractionation of the gums:

2.2.2.1. Foam fractionation:

Fivety grams of the gum sample were weighted on dry weight bags and dissolved in 100ml of distilled water, then transferred toavolumetric flask (1000ml)and completed with water up to the mark of the flask. A beaker of 1000 ml was place in bowl to trap any lost drops as a result of air pumping. A pipe fromosmometerwas inserted into the beaker, and the foams formed were counted . The experiment continued till all foams were trapped. The foams collected in separate dish and allowed to dry at room temperature overnight. The residue was collected in a bottle and subjected to freeze drying (Edwards 2507 phase 1, volts 220 / 240Hz. 50 made in Britain by Edwards high vacum). The powder from both foams and residue solution were collected and kept for further analysis. Foaming efficiency was recorded according to the following formula:

% Foaming efficiency = Weight of foamsample X 100
Weight of original sample

2.3.properties of gum fractions:

2.3.1. Moisture content for the fractions:

The moisture contents for foaming powderand residue fractions were determined as described in 2.2.1. and data were recorded.

2.3.2 Ash content:

Ash content was measured according to Anderson (1959). One gram of each fraction, samples was weighed in a dry porcelain crucible. The crucible was ignited in a furnace at 550°C for 6 hrs.; until became free from carbon. It was then cooled in a desiccator and weighed. The percentages of total ash of dry sample were calculated as follows:

% Ash content = Weight of crucible after ignition X 100

Weight of crucible before ignition

2.3.3. pH of the solutions:

0.1 gram of each sample were weighed and dissolved in 100 ml of distilled water.

The pH was determined using 350 pH meter 20200, Jun 2010, Made in P.R.C. and data were recorded.

2.3.4. Specific rotation:

0.1 gram samples were weighed and dissolved in 100 ml of distilled water.

Optical rotation of gum fraction was measured using ADP 220 36 – 601 Version16; (Bellingham + Stanley Limited); using 2 dm Polari meter tube, according to the International Commission for Uniform Methods of Sugar Analysis (ICUMSA). Blank reading was determined by filling the tube with distilled water and zero reading was obtained. Then the tube filled with the gum fraction and oil rotation was computed and recorded.

The specific angular rotation for a samples can be determined by using the

$$[\alpha]_{\mathbf{D}}^{\mathbf{T}^{\mathbf{O}}\mathbf{C}} = \frac{\alpha_{\mathbf{D}}^{\mathbf{T}}}{\alpha_{\mathbf{N}}^{\mathbf{I}}}$$

equation:

Where:

 $[\alpha]_{\Sigma}^{T^{\circ}C}$ = The specific rotation in angular degrees per dm and per g / cm³, at $T^{\circ}C$ and measured using a sodium 'D' light source.

 α_D^T = The optical rotation in angular degrees at T^OC and measured using Sodium 'D' light source.

T°C = the room temperature in degrees.

c = the concentration of the solution in g / cm^3 .

1 = the length of the polarimeter tube in dm.

2.3.5 Nitrogen (Protein) content:

Protein content was determined 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.
 - Sample + H₂SO₄ (conc.) catalyst +Heat → (NH₄)₂SO₄
- (2) Distillation of the ammonia into a trapping solution.
 - $(NH_4)_2SO_4 + 2 Na OH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$
 - NH₃ + H₃BO₃ → NH₄⁺ + H₂BO₃⁻
- (3) Quantification of the ammonia by titration with a standard solution. The reactions involved in these steps can be shown as follows:

2.3.5.1 Method

In Khaldal digestion flask 0.2 g of each fraction samples was taken. 0.8 g of catalyst mixture (96% anhydrous sodium sulphate and 4% cupricSulphate) was added.3.5 cm³ of concentrated sulphuric acid were added to the flask, and the contents were digested for 2 hrs till a colorless liquid was obtained. The digested material was cooled, diluted and then transferred to the Distillation unit using minimum volume of distilled water. It was then

madealkaline with 20 cm³of 40% aqueous sodium hydroxide solution. The ammoniawas distilled into 10 cm³of 2% boric acid solution for 5-10 minutes. Three drops ofbromocresol green-methyl red mixed indicator were added. The apparatus was steamed out for 5 minutes and the distillate was titrated against 0.02 M HCl.

The nitrogen and protein percentages were calculated according to the following equations:

Nitrogen% =
$$V \times M_A \times 0.014 \times 100$$

W

Where:

V = the volume of the titrant.

 M_A = the molarity of the acid.

W = weight of the sample.

Protein% = Nitrogen% x 6.6 (Anderson, 1986).

2.3.6. Determination of Uronic acid Methods:

The methods used for the determination of uronic acid in *Acacia seyal* in this work were the acid – alkali analysis (Osman, 1993)

Reagents:

- Sulphuric acid (2.0 mol. Dm⁻³)
- Sodium hydroxide (0.1 mol. Dm⁻³)

Method:

The acation exchange column packed with Amberlite (IR $-120~H^+$) resin was thoroughly washed with 2.0 mol dm⁻³ H₂SO₄, followed by distilled water washing until the column was sulphate free.

Three grams of both gum fractions were dissolved in 100 ml distilled water.

Gum Arabic samples (50 cm³of 3 % w/v) were slowly passed down the column and washed by distilled water. The eluent and washing (300 cm³) were collected and titrated against standard sodium hydroxide 0.1 M. using methyl red as an indicator. The experiment repeated three times and data were recorded.

Calculation:

The equivalent weight of poly saccharides containing uronic weight of the polysaccharide (in the acid form) against standard alkali. The acid equivalent weight of poly saccharine and uronic acid are calculated as follows:

Acid equivalent weight = weight of sample x 100

Volume of titer x molarity of alkali

Total uronic acid = Molar mass of uronic acid (194) x100

Acid equivalent weight

2.3.6 Viscosity

Viscosity was determined according to Diamante and Lanrefractometer. The absolute viscosities of the different gum fractions were determined using a Lamy Viscometer RM100 (Lamy, France), a rotating viscometer with coaxial cylinder. Five grams from both gum fractions were weighed and dissolved in 100 ml distilled water. Approximately 25 mL of gum solution was placed in the tube DIN 1 outer cylinder, and then the bob MK Din-9 was inserted. The radius of the tube (R_a) is 16.25 mm and the radius of the bob is (R_i) 15.5 mm. The length of the bob is 54 mm. The correct mode was set for the appropriate measuring system (MS 19) and the measurement time was fixed at 60 seconds. All viscometric measurements of the samples were conducted in triplicate.

2.3.7 Determination of cationic composition:

The cationic composition was determined according to Maurer (1977) and Motweli (2001).

2.3.7.1 Potassium and Sodium:

Flame Photometer was used to determine Potassium and Sodium. The flame photometer used was PF P7- JENWAY system, with filter to select Na, K, Ca, Ba and Li; Sensitivity: fine to course. Before analysis, fraction samples were prepared by ashing and extraction.

The content of each element in the sample was recorded as percentage.

2.3.7.2 Calcium, Magnesium, zinc, chromium and lead:

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 monochromator is used in conjunction with the hollow cathode lamp to isolate the desired spectrum. The radiation that finally reaches the detector system is amplified. Flame Atomic Absorption spectrophotometer was used to determineCalcium, Magnesium, zinc, chromium and lead. The spectrophotometer used has the model AA - 6800, SHIMADZU, Kyoto, Japan. Before analysis fraction samples, were prepared by ashing and extraction. The content of each element in the sample was recorded as concentration percentage(Anonymous, 1976; Hanlon et. al., 1989: SHIMADZU, 2010).

2.5.Determination of sugar composition

2.5.1. Hydrolysis:

Solutions of 77.0 \pm 0.1 and 25.0 \pm 0.1% sulfuric acid were prepared from concentrated sulfuric acid (Merck, sp gr 1.84). Both acids were put in the refrigerator in the 0 °C compartment 1 h before they were used. About 100 mg samples were weighed into 50 ml round bottom flasks with grounded necks (NS 29/32). Glass rods with a diameter of 5 mm and of a suitable length were put in the round bottom flasks so that the top of the rod emerged about 2 mm. Hollowed ground stoppers were then used to ensure tight closure. The stoppered flasks were kept for about 15 min in the refrigerator at 5 °C before the acid was added. Draining along the glass rod, 1.00 ml of cold 77% acid was slowly added to each flask with a volumetric pipette and mixed thoroughly with the material for about 1 min. The stoppered flasks were then put in a small refrigerator, where the temperature was kept constant at -5.0 ± 0.5 °C. The samples swollen in cold acid were kept for 12-14 h (overnight) and the next day 1.00 ml of cold 25% acid was transferred into each flask and stirred well with rods. Closed tightly with stoppers, the samples were allowed to warm up to ambient temperature before they went into an oven where a constant temperature of 55 \pm 0.5 °C was maintained. The treatment in the warm oven lasted 2 h and, about 10-15 min after the

start, each flask was opened once, stirred briefly, closed and put back in the oven. At the end of the period, the flasks were taken out, left to cool down to room temperature and then 10.00 ml of cold, distilled water was slowly drained along the glass rod into the flasks. The flasks containing the hydrolyzates (about 12% sulfuric acid solutions) were attached to reflux condensers standing over a water bath with a constant temperature of 95 \pm 0.5 °C. This last stage of hydrolysis took 1 h. After cooling to ambient temperature, the hydrolyzates were filtered off through fritted glass crucibles of medium porosity. Round bottom flasks and the crucibles were rinsed several times and filtrates and washings were transferred into a 100 ml volumetric flask, which was then filled to the mark with distilled water. By using a 50 ml volumetric pipette, half of the acidic hydrolyzates were transferred into a 250 ml beaker, the empty weight of which was noted to the nearest 10 mg. The amount of barium hydroxide, which is about 20-30 mg less than that required to neutralize 50 ml of acidic solution, was weighed in another 150 ml beaker and dissolved with 100 ml of distilled water (approximately 2.56 g of Ba(OH)₂ 8 H₂O is needed to neutralize sulfuric acid in 50 ml of dilute hydrolyzate). Both beakers, 250 ml with 50 ml of hydrolyzate and 150 ml with barium hydroxide solution, were put in a water bath set at 80-85 °C for about 5 min before the neutralization began. One

drop of methyl red indicator was added to the hydrolyzate and by stirring with a glass rod the first 25 ml portion of 100 ml base was slowly poured. After 2-3 min of stirring the second 25 ml base was added and the other third and fourth portions were added to the hydrolyzate in the same way. Although most of the acid was neutralized, the end point was reached by adding 0.05 M Ba(OH)₂ dropwise (light pink color of the BaSO4 suspension disappears). This last step should be carried out slowly and carefully. It is also important to stir well. The entire neutralization step in the water bath takes about 20 min. The beakers were taken out of the bath, and the glass rods were rinsed with 1-2 ml of distilled water and allowed to cool. Digestion results in wellseparated barium sulfate precipitates within 5-10 min. However at least 1 h is necessary to cool the supernatant to room temperature. Before each beaker with neutral solution and precipitate was weighed, some drops of water condensed on the inside wall of the beaker over the liquid should be removed with a clean paper tissue. To determine the exact weight of neutralizate, the weight of BaSO4 formed should also be considered. This calculation is performed by subtracting the empty weight of each beaker and 1.9 g (the weight of precipitate) from those with solution and precipitate. Since the exact weight of the neutralizate was known, about half of the supernatant (75-80 ml) was decanted in a 250 ml round bottom evaporator

flask and weighed to the nearest 10 mg. In this way, the exact ratio of the amount of sample, which will be analyzed by HPLC, to the hydrolyzed sample, was determined.

The neutralizate was then evaporated to dryness in a rotary evaporator with water bath at 40 °C, dissolved in 10 or 20 ml of ultra pure water, and filtered through a $0.45~\mu$ membrane.

Column insert Sustain NH2 5v m 250 x 4.6 id mm. Mobile phase: Acetonitrile: Distilled water 75.25. Detector: RI detector. Conditions:Flow rate: 1.0 ml/min, Injector volume: 20 l, Column temperature Ambient, RI detector temperature 27.1 C- 27.8 C.

Then 10 µl were injected in the HPLC chromatograph Instrument (Syknm) according to the above conditions.

The sugar composition of each fraction was assessed with 2 replicates and by a minimum of 2 but often 3 injections from each replicate. Two standard injections were performed before and after each injection of specimen. The reproducibility was between 0.5 and 1.0% for higher amounts of sugars (>10%), glucose, xylose and mannose for instance. In the case of sugar yields less than 2-3%, the reproducibility was adversely affected and

increased to 3-5% and in cases where the yield of an individual sugar was around 0.5 to 1%, the reproducibility increased up to 10%.

Chapter three

Results and Discussions

Table (3.1). Physicochemical properties of Foam and Residual fraction of $A.seyal\ var\ seyal\ gum$:

Gum	Moisture	Ash(w/w)%	Protein%	Nitrogen%	Uronic
fractions	(w/w)%				acid%
Foam	7.65	2.64	1.75	0.28	1.73
fraction					
Residual	6.23	2.89	1.925	0.308	3.23
fraction					
Minerals		Gum fractions(w/w)%			
		Foam fr	raction	Residual	fraction
K		0.068		0.090	
Na		0.008		0.008	
Ca		0.849		0.978	
Mg		0.312		0.352	
Cr		2.29*10 ⁻⁴		5.74*10 ⁻⁵	
Zn		nd		nd	
Pb		nd		nd	

	nd = not detected			
Sugars compositions	Gum fractions(w/w)%			
	Foam fraction	Residual fraction		
Rhamnose%	0.2186	0.1464		
Arabinose%	2.2440	1.8664		
Galactose%	1.90245	1.4715		
	Gum fractions (w/w)%			
_	Foam fraction	Residual fraction		
Optical rotation	+47	+51		
Viscosity	2.94	3.611		
pH	5.04	4.33		

3.1. Moisture content of gum:

Moisture content in crude original talha gum was 11.22% w/w. these results agree with Anderson etal.(1963) repoted the moisture content of *A. seyalvarseya*l gum in the range from 11% to 16.1%.

3.2. Physicochemical properties of gum fractions:

The results of analysis of gum fractions were illustrated in Table (3.1). The results showed that foam fractions gain the highest moisture compare Residual one. While Residual fraction contains high As, Protein and

Nitrogen content than foam one. While the other compositions were high due to low moisture content, due to that residues contain more precipitation of minerals and other particles than foam. Moreover, moisture content obtained less than what found by Anderson *et al.* (1963) reported the moisture content of *A. seyal*var. *seyal* gum in the range from 11% to 16.1%, and also less than Karamallah (1999) measured the moisture content in *A. seyal* var. *seyal* gum collected between 1960 and 1999 in Sudan, it was found equal 9.4%

. Siddiget al. (2005) reported the value of 12.6% for the moisture content of A. seyal var.seyal gum. Omer (2006) found that the moisture content of A. senegal var.senegal and A. seyal var.seyal gum were in the range of 11.76% to 14.8% and 5.66% to 11.11% respectively, moisture content in A. seyal var.seyal gum was determined by Abdelrahman (2008) found to be 11.07%.

But it was agree with Hassan *et al.* (2005) range who reported that the moisture content of *A. seyal*var.*seyal*gum in the range from 7.4% to 8.3%.

For Ash content compare to crude gum the results agree with the following; Anderson *et al.* (1963) reported that ash content of A. *seyal* var. *seyal* gum in therange from 1.94% to 3.55%. Anderson (1977) reported that ash content of A. *seyal* var. *seyal* gum in the value of 3.93%. Again Karamallah (1999) reported a value of 2.3 Ash content for A. *seyal* var. *seyal* gum collected

between 1960 and 1999 in Sudan.Omer (2006)reported the ash content of inthe average of 2.61% *for A.seyal*var. *seyal* gum, The mean value of ash contentreported by Abdelrahman (2008) in *A. seyalvar.seyal* gum in the average of 2.43%. While it is higher than what was found by Hassan *et al.* (2005) in the study of *A. seyal* var.*seyal* gum from differentlocations of Sudan reported an average of 0.21% ash content.

Nitrogen is greater than what was found by Anderson et al. (1963) reported that nitrogen content of A. seyal var.seyal gum ranged from 0.09 – 0.19% w/w. and Anderson et al. (1978, 1979) and Karamalla (1965) they stated thatNitrogen% 0.09-0.14. Abdelrahman (2008) reported the average value of nitrogen content for A. seyal var.seyal gumof equal to 0.14%, and to Juraseket al. (1993) reported nitrogen content 0.14% for A. seyavarseyall. And also with Omer (2006) determined the nitrogen content for samples of A. seyalvar. seyal from different locations, the value of 0.14% for A. seyal var.seyal, whereas protein content had a value of 0.93 %. The high protein in the residues are agree with that a smaller fraction (1% of total) (residues fraction) composed by a glycoprotein (GP) consisting of the highest protein content (50 wt%) with an amino acid composition different from the complex AGP (Williams et al., 1990). And there is increasing experimental evidence that associate the antioxidant function with its protein fraction,

mainly by amino acid residues such as histidine, tyrosine and lysine, which are generally considered as antioxidants molecules (Marcuse, 1960, 1962; Park *et al.*, 2005).

The results showed that Uronic acid in was lower in foam fraction sample was 1.73 % and in residual fraction sample was 3.23%. These results may be due to that uronic acid have a protein nature, and according to the above results protein tend to increase in residual than foam.

K, Ca and Mg are high in residue fraction than foam, while Cr mineral in the table (2) were highest in foams. The rest namely; Zn and Pb were not available in the two fractions. The results as we mentioned inTable 3.1above are due to that minerals and particles were tend to be precipitated. Zn not available not detected while Osmanet al.(1993) and Williamset al.(1990) found 11- 45 (ppm) in seyal, this due to variation of soils and environmental conditions, which agree with Al-Assaf, et al.(2005 (a,b)),Flindtet al. (2005), Hassan et al.(2005) and Siddiget al. (2005) they stated that chemical composition of Gum Arabic may vary slightly depending on its origin, climate, harvest season, tree age and processing conditions, such as spray dying.

The results showed low viscosity than Menzies*et al.*(1996) stated that specific optical rotation of seyalvarseyal gum is +41° to +61°. The results

showed that optical rotation was highest in residual than foam, this results may due to that residues contains high amount of minerals and precipitated particles the for it affected the rotation angle. Specific viscosity was high in residual gum than foam. This may be due to that the residue contains metals and other heavy materials than foam. And also the concentration in

this samples is (5%) showed low viscosity. This agree with Glicksman (1969) stated that higher viscosity is not obtained with gum until the concentration of about 40-50%. pH highest in foam fraction than residences this may be due to that as in the above 3.3. the residue contains high minerals and consequently tend to shift the pH towards alkalinity.

The results of sugar composition of the two fractions of A. seyalvarseyal gums were shown in Table (3.1). The results showed that the three sugars are lowr than what foundby Anderson et al. (1978, 1979) and Karamalla (1965) they stated that Galactose (46%) Arabinose (4%) Rhamnose (4%). Also the results revealed that Rhamnose, Arabinose, and Galactose were higher in foam than residual. The presence of more sugars in the foam fractions agree with that the main fraction (88-90%) (foam fraction) of a polysaccharide of β -(1 \rightarrow 3) galactose, highly branchedwith units of rhamnose, arabinose and glucuronic acid (which is found in nature likesalts of magnesium, potassium

and calcium). This fraction is called Arabinogalactan (AG) and contains a low protein content (\sim 0.35%) and MW \approx 300 kDa (Renard*et al.*, 2006, Sanchez *et al.*, 2008).

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