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The availability, quality and processing aspects of gum arabic from Kenya.

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THE AVAILABILITY, QUALITY AND PROCESSING ASPECTS OF GUM ARABIC FROM KENYA

A Thesis submitted to the University of Wales for the degree of Doctor of Philosophy in Wood Science

By

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In memory of my daughter Sonia Akune who returned to the Lord in March 1992 while I was in the United Kingdom carrying out studies leading to this Degree.

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ABSTRACT

Four aspects on gum arabic from Kenya were examined in this Thesis; resource availability, physical and chemical properties, evaluation of methods for monitoring and quality control and processing.

Under resource availability, provisional *Acacia senegal* maps were produced showing that the resource occupies 14%, 20% and 37% of Isiolo District, south western Marsabit and Turkana Districts respectively. The main source of gum arabic was identified as *A. senegal* var. *kerensis* and was observed growing mostly in patches with high stocking on hills and occasionally along luggas (dry river streams). The relative amount of resource available for tapping varied between the three districts with Turkana District having the highest proportion and Isiolo District the least. The species grows in association with some of the other gum producing species. *Acacia seyal* and *A. tortilis* were recognised as the main species likely to produce gum in large quantities and thus possible contaminants.

Studies on characterisation of Kenyan gum (from var. *kerensis*) showed that it differs from the present commercial gum (from var. *senegal*) in specific rotation, nitrogen content and intrinsic viscosity showing that the taxonomic differences observed are well reflected in the chemical nature of the two varieties. Variations were also observed between samples and regions and were attributed to possible genetic effects and local adaptations respectively. These differences present future opportunities for improving gum production through tree improvement and gum handling practices. In terms of emulsification functionality,

gum arabic from var. *kerensis* was comparable and in most cases gave better results than gum from other commercial sources.

An evaluation of methods regarded as inexpensive for monitoring and quality control revealed that elution profiles produced by gel permeation chromatography (gpc) method provide a quick and consistent diagnosis of gum arabic of commerce. The use of physico-chemical and carbohydrate (analytical) method is also useful but tends to be influenced more by natural product variability and can sometimes result in rejection of otherwise authentic gum arabic. The Enzyme linked immunosorbent assay (Elisa) technique is useful but needs further refining to make it more specific to gum arabic from *A. senegal*.

Results on processing of gum arabic showed that heating gum solution at 100°C results in significant degradation of the gum molecule after 6 hours affecting emulsification functionality and that heating at 65°C seems to be a favourable alternative. Use of enzyme biotechnology has a promising future. All the three enzymes examined degraded the gum molecule but with different effects on the resulting gum properties. The protein degrading enzyme (pronase) has a fast rate of reaction causing reduction in viscosity and gelling but the gum also loses its emulsifying functionality. The two carbohydrate degrading enzymes (viscozyme and β-D-galactosidase) achieve the desired reduction in viscosity and gelling at about the same rate. However, the former slightly degrades the protein component resulting in unstable emulsions while β-D-galactosidase gave better results.

On the basis of these studies, areas for future attention are suggested.

CONTENTS

CHAPTER 1. GENERAL INTRODUCTION

Acacia senegal (L.) Willd. is the botanic source of gum arabic, an important article of commerce for thousands of years. The species is native to the hot and dry regions of Africa, parts of the middle East to as far as western India. However, it is within Africa that the species occurs most widely. It forms a belt of about 300 km along the southern frontier of the Sahara desert from Mauritania and Senegal in the west to Somalia in the Horn of Africa. From the Horn of Africa, it stretches southwards to Natal and Transvaal in South Africa. Pockets of the species have also been reported in Namibia and Angola (Giffard, 1975; Brenan, 1983).

Apart from gum arabic, the tree is also valuable in various ways. In Sudan and India it has proved useful as a windbreak by protecting sandy soil from being blown off and hence checks desert encroachment (NAS, 1979). The gum belt in Africa is said to act as buffer against desertification. Beside, the tree canopy intercepts rain drops while its taproot and extensive lateral roots make the species effective in reducing soil erosion and hence stabilises the soils (Muthana, 1988). It is a natural soil improver through its ability to fix nitrogen. The pods and foliage provide good fodder to the livestock and when in flower is a source of nectar for making honey. The tough wood of its stem and taproot are used for making tool handles whereas the strong fibres obtained from the long flexible surface roots are used in making ropes. Branches are normally cut for fencing livestock enclosures and when dry are used as a source of fuel wood. The wood yields excellent charcoal. These attributes make the species a suitable candidate for agroforestry development in the dry areas.

Acacia senegal is one of the species known to be quite variable. It sometimes grows as a bush or shrub of about 2 m high and sometimes as a well formed tree up to 15 m high with varying shades in between (Ross, 1979; Brenan,

1983). This variation has sometimes presented difficulties and uncertainties to taxonomists. Currently three varieties are generally recognised and sometimes a fourth. The three comprise of var. *senegal,* var. *kerensis and* var. *rostrata*. The fourth (var. *leiorachis*) is said to occur in two growth forms, a well formed tree with a rounded crown and a form with long straggling branches. However, recent studies have shown the former growth form to be a separate species as *Acacia circummarginata* (Hassan and styles, 1990; Coe and Beentje, 1991) while not much has been done about the later form. The distribution of the varieties within the species range reveal varietal preferences but with a higher representation in the Horn and East Africa. Two of the undisputed varieties (var. *senegal* and var. *kerensis*) and var. *leiorachis* are said to occur in Kenya but it is not clear whether one or all of the varieties produce gum arabic. One of the aims of the study was to identify the varieties growing in Kenya and specifically, the main source of gum arabic.

Sudan has through centuries been a leading producer of gum arabic and with it, a long history in the development and management of *A. senegal* resources. This has been possible because of the favourable climatic and soil factors which have resulted in extensive areas of pure stands, especially in the Kordofan and Darfur provinces giving the country comparative advantage of uniformity. In addition the country has a well developed gum management practice based on gum gardens and reforestation programmes that has resulted in successful dry land agroforestry. Production from both pure stands and cultivated plantations has given the country advantages of economics of scale (Awouda, 1990). Apart from Sudan, Nigeria and Senegal have improved their market share through improving methods of field production as well as incorporating practices in domestication and dry land agroforestry.

However, in most other countries *A. senegal* resources are still growing in the wild and completely unattended. Kenya falls in this category with her resources found growing in patches and in association with other species. The locations and extent of these patches are not clear. Most of the associates are other *Acacia* species and members of the *Commiphora* species forming *Acacia -Commiphora* bush land (Agnew and Waterman, 1989). *Boswellia* species (which together with *Commiphora* form the Burseracae family) and *Euporbia* species are some of the other common associates. Some of the *Acacia* species are known to produce gums which can be potential contaminants while members of the Buseracae family produce resins commercially known as myrrh from *Commiphora* species and frankincense from *Boswellia* species respectively. As a step towards improving gum arabic in the country, there is need to map out *A. senegal* resources to provide some idea on the commercial availability and identify likely contaminating species.

Gum arabic is produced by a tree following an injury as a physiological adaptation to prevent loss of water (Smith and Montgomery, 1959). Production is only in the dry season and yield varies between 0.15 kg to 2 kg per tree (Ballal Siddig, 1991). Visually it is produced in different sizes, colours and sometimes shapes depending on species, environmental conditions and mode of exudation. Nonetheless, typical samples vary in size between 2 - 10 cm, spheroidal in shape and with fissured surface markings (Obeid and Seif El Din, 1970). Colour varies from clear, amber to dark brown and is used commercially as a basis of assessing quality and hence grading. The three features are sometimes useful for initially distinguishing whether a nodule is of gum from *A. senegal*.

Chemically it consists of high molecular weight polysaccharides and their calcium, magnesium and potassium salts which on hydrolysis yield D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid. The latter and its 4-O-methyl

derivative are responsible for the acidity of the gum. The molar proportion of the sugars vary slightly but are usually characteristic of the species or variety. The amount of metals present vary with soils on which the tree is growing. However, excess amount beyond a specified limit can render the use of the gum in the food industry. In addition, gum arabic contains proteinaceous material covalently bonded to the polysaccharide. The total amount is less than 5%, the actual amount varying with species or variety and it is the one responsible for the functional properties of the gum as emulsifiers (Randall et al, 1988). On the basis of the above information, further chemical and molecular studies have shown that gum arabic is an arabinogalactan protein complex (Fincher et al, 1983; Akiyama et al, 1984; Vandevelde and Fenyo, 1985) and that it comprises of three fractions; a protein deficient arabinogalactan, a protein rich arabinogalactan complex and a glycoprotein (Randall et al, 1989a).

Gum arabic of commerce is defined as a dried exudation obtained from stems and branches of *A. senegal* Willdenow or related species of *Acacia*, Family leguminosae (FAO, 1986). This definition varies slightly in wording between various regulatory authorities but all acknowledge that the main component of gum arabic is from *A. senegal*. There is a growing debate as to whether the FAO definition of 1986 is satisfactory. One school of thought fears that the definition/specification is too lax to prevent gums from other than the specified source being sold and used. To overcome the fear FAO (1990) proposed more strict regulations with the aim of attempting to characterise more specifically gum arabic of commerce. However, the other school of thought believes that the parameters used in the proposed specification are too restrictive in characterising gum arabic of commerce and can result in rejecting otherwise authentic gum arabic if applied particularly, when only one method (analytical) is relied upon.

Most of the data used in the preparation of the specification of gum arabic of commerce is understandably from Sudan and to some extent Nigeria and possibly the French speaking west African states of Senegal and Chad. From the work of Brenan (1983), var. *senegal* appears to be the main source of gum arabic from these countries while in Kenya and may be East Africa in general, it is from var. *kerensis*. To date not much has been done to examine variation in gum arabic due to varieties. The present study was therefore devoted to characterising gum arabic from authentic sources of var. *kerensis* within the gum producing region in Kenya to establish mean values and hence typical properties. There was further need to examine suitable methods for characterising gum arabic that could be readily applied in monitoring and quality control in producer countries.

Gum arabic is one of the products that has been on the market since ancient times. The Egyptians used it in medicines and ceramic pottery over 4000 years ago (NAS, 1979). In modern times it is used in the manufacture of a great many products with the food industry being the principal consumer where it is used to give desired qualities through its influence on the viscosity, body and texture of food. However, before using, it has to be cleaned (processed) to remove foreign matter and ensure a uniform product. Current processing involves dissolution of the gum in water and heating the solution at boiling for a specified period to allow it flow readily and sterilise it. Whilst gum arabic is known to readily dissolve in water producing aqueous solution of over 50%, some gums behave like chewing gum at concentrations of 30 - 40% and need special processing (Woolen, 1982). The final aim of the study was to investigate ways of reducing viscosity and gelling observed in the Kenyan gum without drastically affecting its functional properties.

Studies presented in this thesis fall in two sections:

Section 1 covers an assessment of gum arabic resources. It is divided into two chapters. Chapter 2 presents a synthesis of existing information on the

taxonomy of *A. senegal* so as to understand the present state of knowledge on the varieties known. Distribution of *Acacia senegal* and associated species are briefly reviewed. Chapter 3 provides information on resource survey. Knowledge of these aspects are considered as important in planning and management for gum production.

Section 2 deals with studies on gum arabic. It is divided into four chapters. Chapter 4 presents background information on gum arabic by reviewing and harmonising current knowledge from different sources for consumption by Wood Scientists. Chapter 5 examines the chemical characteristics of gum arabic from Kenya. It describes experimental methods applied, presents and discusses the results. Chapter 6 evaluates the suitability of various methods developed for monitoring and quality control of gum arabic of commerce with an interest in those that can be readily adopted by producer countries. Chapter 7 examines some aspects of gum processing in general, but in particular, those aimed at reducing high viscosity and gelling behaviour observed in the Kenyan gum without drastic effect on its emulsification functionality. New opportunities in gum processing using enzyme biotechnology are considered.

The last chapter summarises and concludes work done in this thesis and offers suggestions for future work.

SECTION I: ASSESSMENT OF GUM ARABIC RESOURCES

CHAPTER 2: LITERATURE REVIEW ON *ACACIA SENEGAL*

2.1. Taxonomy of *Acacia senegal*

2.2.1. Classification

Acacia senegal (L.) Willdenow was first described by Linnaeus in 1753 under the name *Mimosa senegal* (Ross, 1973). A year later, Philip Miller adopted the generic name *Acacia* and described 24 species under the genus though he made no attempt to sub-divide the genus into groups. It was not until 1806 that Willdenow divided the species into seven groups on the basis of vegetative characters, but once again, the groups were not prefaced by any indication of rank. However, he was the first man to provide the name of *Acacia senegal* as it is known today. A more elaborate classification of the *Acacias* is credited to Bentham (1875). Bentham divided *Acacia* into six series, the series delimited primarily on foliage, on whether they were armed, and if armed upon whether or not spinescent were present. African *Acacias* fell into two series, namely gummiferae and vulgares with the former comprising all species with spinescent stipules and the latter those with non-spinescent stipules. *Acacia senegal* was placed in the series Vulgares. Bentham's classification was the most significant and important contribution because, for the first time, the generic limits of *Acacia* were clearly defined and those species which did not belong to the genus were excluded.

Since the work of Bentham, two methods have been employed to sub-divide African *Acacias* (Ross, 1973). Some authors have relied on whether or not stipules were spinescent while others have employed infloresence. Both methods have advantages. Guinet and Vassal (1978) proposed a classification that corresponds to Bentham's classification and includes additional valuable characters. They recognised three subgenera within the genus *Acacia*, namely; Acacia, Aculeiferum and Heterophyllum on the basis of characters of seed and seedlings,

on the occurrence of stipular spines and pollen characters. According to this classification, *A. senegal* belongs to the subgenus Aculeiferum which is characterised by non-endospermic seeds beside prickles or spines of non-stipular origin. It includes Bentham's series Vulgares and Filicinae. This subgeneric division has gained acceptance and is being widely used (Doran et al, 1983). On the basis of the above work, *A. senegal* can now be systematically classified as follows:

Phylum	Angiospermae
Sub-phylum	Dicotyledones
Order	Leguminales
Family	Leguminoseae
Sub-family	Mimosoideae
Genus	Acacia
Sub-genus	Aculeiferum

2.1.2. Variation in *Acacia senegal*

Although it has been easy to classify *A. senegal* to the rank of sub-genus, describing the species below this rank has not been satisfactory. Comprehensive taxonomic assessments have shown the species to be highly variable. It has been described as a shrub about 2 m or a tree up to 15 m high (Ross, 1975; Brenan, 1983). Its crown is slightly rounded or flattened and somewhat spreading, sometimes a slender tree with long whippy (straggling) branches. Bark on trunk is yellowish to greyish brown; rough and non-peeling or smooth, papery and peeling off in strips. Young branchlets are similar to the main trunk but glabrous to densely pubescent. Prickles 2-8 mm long, in threes, with central one curved downwards and the two laterals curved upwards, or singly with laterals absent. Leaves have petioles and rachis clothed with hairs; petioles usually glandular, pinnae 2-6 pairs with 7-25 leaflets per pinna and glabrous to somewhat pubescent. Flowers whitish

to yellowish white, more or less scented and in spikes 2-10 cm long. Pods dehiscent, grey to brown or yellowish brown, rounded to acuminate, pubescent or puberulous and 3-14 cm long. Seeds 4-8 per pod, pleurogram horse shoe shaped and lack endosperm (Guinet and Vassal , 1978; Ross, 1979 and Brenan, 1983).

The great variability in the species (habit and habitat) has presented difficulties and uncertainties to taxonomists. This is reflected in the numerous synonyms given to the species and varieties in particular (Brenan, 1983). Ross (1979) pointed out that the species is so widespread and variable that it can sometimes only be distinguished from related species with difficulty while Brenan (1983) mentioned seven species as those most similar to and likely to be confused with *A. senegal*. In Kenya, *A. circurmmarginata* and *A. thomasii* have sometimes been treated as separate species though related to *A. senegal* (Brenan, 1959; Dale and Greenway, 1961) and sometimes as varieties of the species (Brenan, 1970, 1983; Ross, 1979). Dale and Greenway (1961) made no distinction of the varieties in Kenya while Beentje (1990) acknowledged the difficulties of separating the three varieties supposed to occur in the country and preferred to see the species as single but quite variable. Part of the problem arises from continuous variation of characters in the species and inadequate information concerning exact nature of morphological variation in part of the species range and the fact that most of the information is based on herbarium specimens. Because of these difficulties, Ross (1975) considered all *Acacia* species with spicate inflorescence, and armed at or near the nodes with prickles in threes, the central one hooked downwards and the two laterals upwards or at times spreading laterally or else solitary as members of the *Acacia senegal* complex. Nevertheless, effort has been made to describe varieties of *A. senegal* within the complex. The varieties can be differentiated from the key as follows:

a. Tree with a distinct main stem;

i. tree with lax rounded or flattened and spreading crown; young branchlets sparsely to densely pubescent; inflorescence axis pubescent.

- bark on trunk yellow brown, non papery and non peeling.
- pod rounded to acute, seldom acuminate at apex ---------- var. *senegal*.

ii. tree with slender straggling branches or sometimes well formed tree with rounded crown;

- young branchlets glabrous or sub glabrous and rather smooth; inflorescence axis often purplish
- bark on trunk yellow, papery and peeling --- var. *leiorhachis*.

b. Shrub or bush, usually without a distinct trunk;

i. pod rounded to acute, seldom acuminate at apex,

- leaves with up to four pairs of pinnae --- var. *kerensis*.

ii. pod markedly acuminate or rostrate at apex,

- leaves with up to 12 pairs of pinnae ---- var. *rostrata*.

The following is a brief description of the four varieties in the light of present knowledge.

2.1.2.1. var. *senegal*: Brenan

A tree up to 15 m high with a variable crown, flat and spreading or lax and rounded (Ross, 1979; Brenan, 1983). Bark on trunk not papery and peeling but greyish to yellowish brown, rough, fissured and sometimes flaking. Young branchlets sparsely to densely pubescent. Inflorescence axis typically pubescent and rarely glabrous. Pods rounded to acute or pointed but rarely acuminate.

This is the typical variety described by Linnaeus in 1753. It is extremely variable with a wide range of variation in indumentum, leaflet size, flower size and general habit (Brenan, 1959, 1983; Ross, 1975,1979) as reflected in the ten synonyms given to the species (Brenan, 1983) though *A. verek* is the most commonly used. The major distinguishing morphological characters are its arborescent growth form, inflorescence axis, pods that are rounded to pointed apices and a characteristic none papery, none peeling, rough and (in mature trees) fissured bark (Ross, 1979). Recent studies (Hassan and Styles, 1990) confirm the high variation in its habit and habitat.

2.1.2.2. var. *leiorhachis*: Brenan

First described by Brenan (1953) as always a tree, it grows to about 12 m high either as a slender spindly tree with long straggling branches that form an open irregular crown or as a well grown tree with a rounded crown (Ross, 1979; Brenan, 1983). Bark on trunk is yellow, papery and peeling. Young branchlets and inflorescence axes are subglabrous to glabrous. Pods rounded to acute at apex and are several times longer than they are broad.

However, recent studies have shown that the growth form that grows to a well formed tree is of a specific rank as *A. cirmmarginata* Chiov. (Hassan and Styles, 1990; Coe and Beentje, 1991). It is found mostly in valleys or drainage sides on hills and is distinqished from *A. senegal* in habit, by having longer pods with some constrictions between seeds, more leaflet pairs per pinna and a longer

inflorescence-axis. The form with straggling branches and resembling *Acacia. thomasii* Harms has been reported to occur in Rift valley in Kenya (Coe and Beentje, 1991) and is also said to be endemic in eastern and southern districts. Because of lack of additional details apart from field observations, it is considered as *A. senegal* var. *leiorhachis*.

2.1.2.3. var. kerensis: Schweinf.

Is a shrub or bush 1-5 m high, branching from the base or with a short trunk . Bark on trunk greyish to dark brown and sometimes peeling. Young branchlets and inflorescence axes usually pubescent. Leaves have four pairs of pinnae. Pods rounded to acute at apex, rarely acuminate (Brenan, 1983).

The reliance on growth form in describing var. *kerensis* is considered as unsatisfactory since it is not known whether the variety represents a good taxon or merely embraces a heterogeneous assemblage of shrubby like growth forms (Ross, 1979). The variety is said to have smaller leaves than variety *senegal*. Hassan and Styles (1990) have shown that this variety is quite variable in Somalia with regard to the number of pinnae, the indementum and pod shape and regard it as a mere growth form of var. *senegal*.

2.1.2.4. var. *rostrata*: Brenan

Either a shrub branching at or near the base or a small tree up to 8 m high with a flattened and spreading or slightly rounded crown. Bark on trunk with a flaking papery peel, creamy yellow to grey brown. Young branchlets and inflorescence axes more or less pubescent. Leaves with 4-12 pairs of pinnae. Pods rostrate or acuminate at apex. Confined to southern Africa though specimens with rostrate pod apices are said to have been collected from Somalia, Kenya and Uganda (Ross, 1979; Brenan; 1983).

2.2. Distribution

2.2.1. Geographical distribution

A. senegal is native to the hot and dry regions of Africa and parts of the middle east. The distribution map (Fig. 2.1) indicates that distribution through its range shows varietal preferences. Var. *senegal* is the most widely distributed. It is present through a belt some 300 km wide immediately south of the Sahara desert from Mauritania and Senegal in the west to the Horn of Africa (Giffard, 1975). From the Horn of Africa, the range extents southwards to Tanzania. In Asia the variety has been reported in southern Sind and Baluchistan in Pakistan (Cheema and Qadir, 1973) as well as in Rajasthan, Punjab and Delhi in India. Var. *kerensis* is restricted to eastern and north eastern Africa in Ethiopia, Somalia, Kenya, Uganda and Tanzania (Ross, 1979; Brenan, 1983). Var. *rostrata* occurs across southern Africa, south of the Zambezi river. It is found in Angola, Namibia, Zimbabwe, Botswana and South Africa in Natal and Transvaal. Var. *leiorhachis* and *A. circummarginata* have discontinuous range being known in Ethiopia, Somalia, East Africa and south of the Zambezi river in Zambia, Zimbabwe and Transvaal in South Africa.

2.2.2. Factors influencing distribution

2.2.2.1. Climatic factors

Wide distribution of the species in the drier parts of tropical and subtropical Africa, Arabia, western Pakistan and north western India is evident. *Acacia senegal* is both drought resistant and frost hardy and grows in areas receiving from as little as 200 mm of rain and withstanding as many as 11 months of drought to well over 800 mm of rain (NAS, 1980; Ballal Siddig, 1991). Over most of the range however, the mean annual rainfall is 200-450 mm . Walter (1971) concludes the rainfall range of 250-400 mm as optimal but it is apparent that variation in the

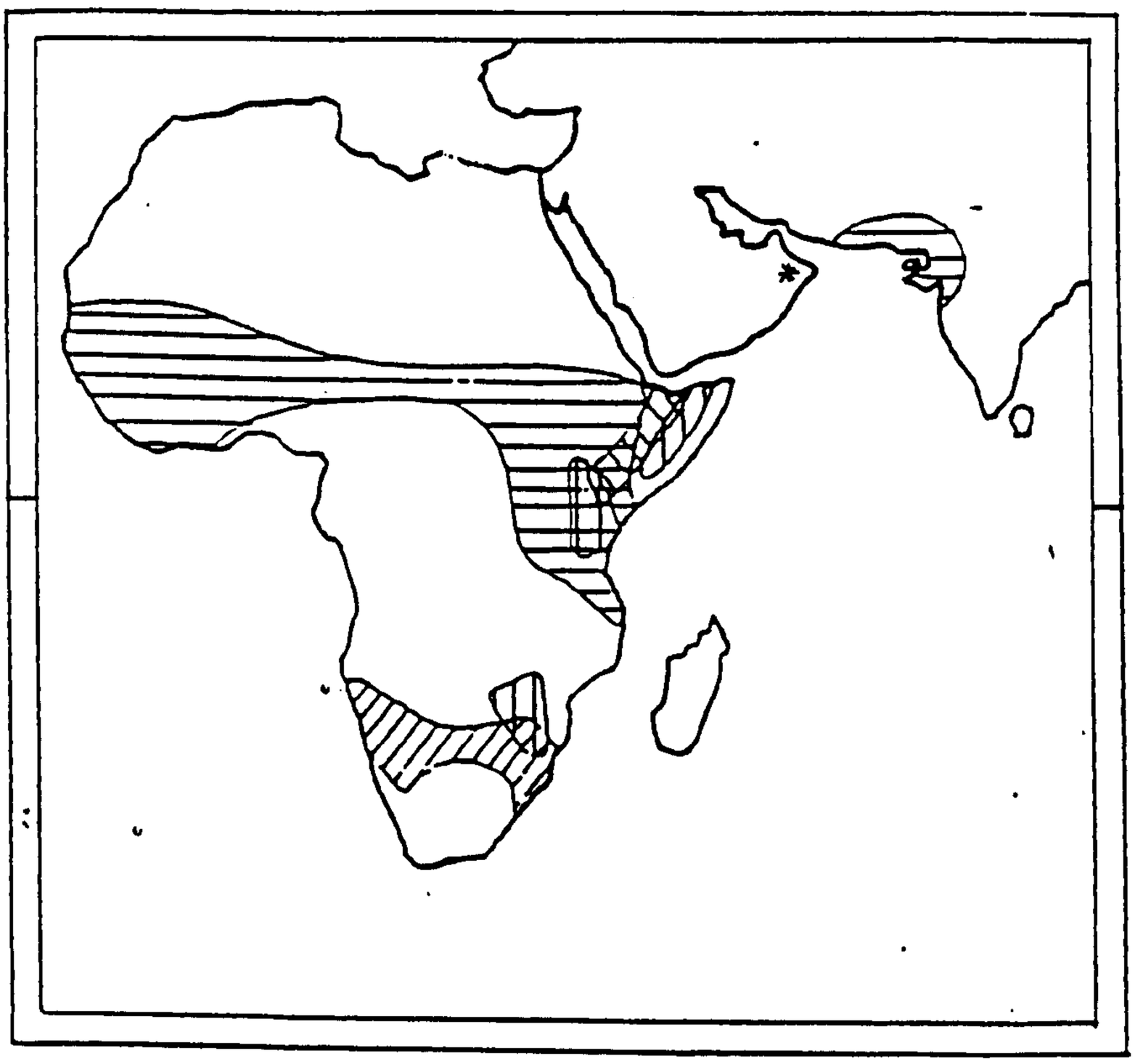


Figure 2.1: Map of *Acacia senegal* showing approximate distribution of the species and varieties. (Source: Brenan, 1983)

relationship with rainfall varies with soil type due to differences in texture. In Sudan the species occurs widely between the 200 mm and 450 mm isohyets on sands in the west but, mainly where mean annual rainfall is about 500 mm or more, on the dark cracking clay soils further east (Obeid and Seif El Din, 1970). In India the species grows successfully in Punjab (375-500 mm), Kutch (322 mm) and Jodhpour (391 mm). The species has been observed to tolerate extreme temperatures as low as -1.7°C and as high as 48.3°C (Ballal Siddig, 1991). The mean annual temperature over most of the species range is 25 - 27°C.

2.2.2.2. Edaphic factors

Edaphic factors have been described as important in influencing the growth and abundance of *A. senegal* (Cheema and Qadir, 1973). Irrespective of topographic conditions, occurrence was observed mostly on coarse textured soils. Pandya and Sidha (1985) noted the species on hills in India on soils that are mostly sandy intermixed with stones and pebbles. These are soils with low water holding capacity (optimum range 15-25 %), high permeability and rich in carbonates (Cheema and Qadir, 1973). Nevertheless, the species is also reported to thrive on other soils like clay and alluvial where rainfall is not too high to cause waterlogging and drainage is good.

2.2.2.3. Biotic factors.

Biotic factors have been covered in detail by Ballal Siddig (1991). Activities like overgrazing, over-cultivation, the use of fire in bush clearing adversely affects tree population (NAS, 1979, Obeid and Seif El Din,1970). Browsing by camels and goats results in stunted growth and is sometimes sufficiently severe to kill the trees (Ballal Siddig, 1991). Among insect pests, locusts are the most serious as they affect gum yield through defoliation.

2.3 Associated species

Acacia senegal has been reported to occur in a wide range of vegetation types. In Sudan, Smith (1949) used an ecological classification based on compositional criteria and identified three vegetation types; *Acacia*-desert scrub found on the low rocky hills of Nubian sandstones, *Acacia*-short grass within the main gum belt region and *Acacia*-tall grass in areas receiving between 450-800 mm of rain. In India Pandya and Sidha (1985) recognised two forest associations also on the basis of composition; *Euphorbia-Commiphora-Acacia senegal* association found on drier sites that have shallow sandy soils with exposed sandstones and *Acacia-prosopis* cineraria association common in the hilly central tract of the Kutch District. Three vegetation types have been recognised in Kenya: FAO (1971) described the vegetation types on the basis of geomorphological features and classified *A. senegal* under two of them in Isiolo District i.e. basement hills and pediments and basement ridges. Herlocker (1979) followed Pratt and Gwynne (1977) classification but modified by compositional criteria when classifying the vegetation of Marsabit District while Agnew and Waterman (1989) have described the vegetation type using both composition and physiognomy and identified *A. senegal* with the *Acacia-Commiphora* bushland.

Although no systematic analysis has been carried out on species association with *A. senegal*, an assessment reveals that it is associated mostly with species in the genus *Acacia*, *Commiphora*, *Boswellia*, *Euphorbia*, *Faidherbia*, *Balanites*, *cadaba* and *combretum*. An evaluation in the Sahelian region has shown that common associates are *Balanites aegyptiaca*, *Faidherbia albida*, *Commiphora africana* and *Zizyphus mauritania* (Giffard, 1975). In Sudan, *A. senegal* is associated with *Acacia radiana*, *Faidherbia albida*, *Maerua grassifolia* and *Leptadenia pyrotechnica* (Ballal Siddig, 1991) while in Somalia it has been shown to be associated with *Acacia tortilis*, *A. nilotica*, *A. edgeworth*, *A. bussei*,

Commiphora spp., *Euphorbia* spp. and *Grewia* spp (Hassan and styles, 1990). Within Kenya, an examination of available information shows that it is associated with *A. tortilis, A. horida, A.reficiens, Commiphora* spp. *Boswellia* spp. and *Eupohorbia* spp. (Agnew and Waterman, 1989; Herlocker, 1979; Olang, 1984)

2.4. Gum yield

Acacia senegal has a life span of 25-30 years, by which time it is fully mature (Awouda, 1973). For gum production, the tree starts at the age of 3-4, 5 or 6-7 years for coppices, plantations and natural stands respectively (Ballal Siddig, 1991). Maximum yield of gum is expected within a period of 20 - 25 years after which it declines appreciably.

Generally, information on yield is scant. Ballal Siddig (1991) made an attempt to establish quantitative data on yield based on different sources of information. Using individual trees and assuming an optimum density of 400 stems per hectare, he found that yield varies from 0.15 kg per tree (60 kg ha^{-1}) and 2 kg per tree (800 kg ha^{-1}). Studies carried out in Kenya show that mean yield in wild stands varies from 0.25 kg and 2 kg per tree (Mulinge and Abdille, 1988). The actual amount produced depends on various factors like heredity, environmental conditions and gum husbandry practices. Studies on effect and intensity of tapping in Sudan have shown that higher yields are realised shortly after the rains than later in the dry season. Frequent tapping also results in higher yields but is not recommended as it results in weakening the tree hence shortening its productive life span. Similar observartions on the time of tapping have been made in Kenya. Mulinge (1990) observed that gum production declines with increasing drought and infact trees stopped producing gum during the peak of the dry season.

CHAPTER 3. SURVEY OF *ACACIA SENEGAL* RESOURCES FOR GUM ARABIC IN NORTHERN KENYA

3.1 Introduction

Over 75% of Kenya is marginal, technically referred to as Arid and Semi Arid Lands (ASALs). It is characterised by low biological productivity resulting from scant and erratic rainfall, intense radiation, strong dry winds and poor soils. These conditions support drought deciduous bush and shrublands. The grass cover depends on the density of woody vegetation and is available mostly during the rain season. The harsh and uncertain environmental conditions have made nomadic pastoralism the most viable mode of life. Vegetation is thus an important component to these communities for the provision of grazing and browse resources. Additionally, it is a source of fuelwood, building, fencing material and traditional medicine. The government recognises this importance and has initiated various projects in such areas for the management of rangelands to stimulate livestock production.

Livestock production is but only one of the sources that the rangelands support. Most of the woody resources hold known or potential promise as producers of commercially viable products. For instance, gum arabic produced by *Acacia senegal* is an important article of commerce for thousands of years. Sudan is the leading producer that accounts for about 85% of world production (Awouda, 1990). Myrr and frankincense are important products from *Commiphora* and *Boswellia* species and until recently Somalia was the world's leading producer (Ali, 1986). One advantage about the resources is the ability to produce gums or resins only during the dry season when forage is scarse thereby allowing the communities to be occupied in meaningful economic activity. The indirect benefits from these resources are many (NAS, 1979; Stiles, 1988). Accelerated economic development of these areas therefore lies in recognising the

potential that exists in the vegetation resources and incorporate it in the development strategy.

There is now public awareness in the country about some of these resources following initiative from various groups working in the range lands in collaboration with research institutions. At present interest in the commercial production of gum arabic has grown and there is increasing activity in both production and marketing of the product within the country and for export. However, little is still understood about the status of the gum arabic resources in the country. This study was therefore undertaken as part of the initiative to provide initial data base on the availability of the resource and potential for comercial production. It was carried out as an exploratory survey to locate and map out gum arabic resources in three districts of northern Kenya. The primary aims were to produce *Acacia senegal* maps showing source locations and clarify varieties of *A. senegal* involved, gain some knowledge on stocking within the mapped units and proportion of resource available for tapping. Since the stands are still growing in the wild and thus mixed with other species, there was need to identify possible contaminanting species. As a secondary objective, initiative was undertaken to examine the relationship between stocking density and environment based on data collected.

3.2 Methods used in the resource survey

3.2.1 Selection of the study area

Preliminary work in 1989/90 showed *Acacia senegal* to be present in quantity in five districts of northern Kenya. These are Wajir, Isiolo, Samburu, Marsabit and Turkana. Beside, it was observed, though less abundantly in seven other districts: Mandera, Tana river, Taita Taveta, Kajiado, Laikipia, Baringo and West Pokot. However, only Isiolo, Marsabit and Turkana Districts offered favourable conditions for the study i.e they had suitable vegetation maps, good

infrastructure and relatively secure. In addition, local communities had become involved in the collection of gum arabic for sale to S.A.L.T.L.I.C.K (a non-governmental organisation) currently promoting the marketing of gum arabic in the country. These three districts were therefore selected the study (Fig. 3 1).

3.2.2. Field survey.

The vegetation map for each area formed the starting point for the survey and a basis for production of provisional *A. senegal* vegetation maps. Because original surveys were carried out independently, each vegetation map was first examined and a description of the various units understood. Following is a brief description of the maps: In Isiolo District EMI/ODA (1990) prepared a vegetation map for four of the five divisions. The map recognises nine physiognomic types superimposed on fourteen ecological land units (ELU) developed earlier by FAO (1971). For the present study, additional information on the remaining division was prepared from a combination of satellite, aerial photographs and topographical maps on a scale of 1:50,000 by a cartographer. For Marsabit District, the UNESCO/IPAL project produced a vegetation map of the south western sector (Lusigi et al, 1986). The map subdivides the area into 24 range types which are physiognomically distinct units delineated on the basis of aspect and composition. In Turkana District, the vegetation cover assessment map by Olang (1984) was used in the resource survey. He followed the classification of Pratt and Gwynne (1977) and subdivided the district into eight vegetation types.

For each area, vegetation units described as containing *Acacia* species and specifically *A. senegal* where applicable were identified and marked for field appraisal. In the case of Turkana District, initial screening of the main vegetation types was based on combined information from the map by Olang as well as data collected by Turkana Drought contigency planning unit (TDCPU) of the ministry

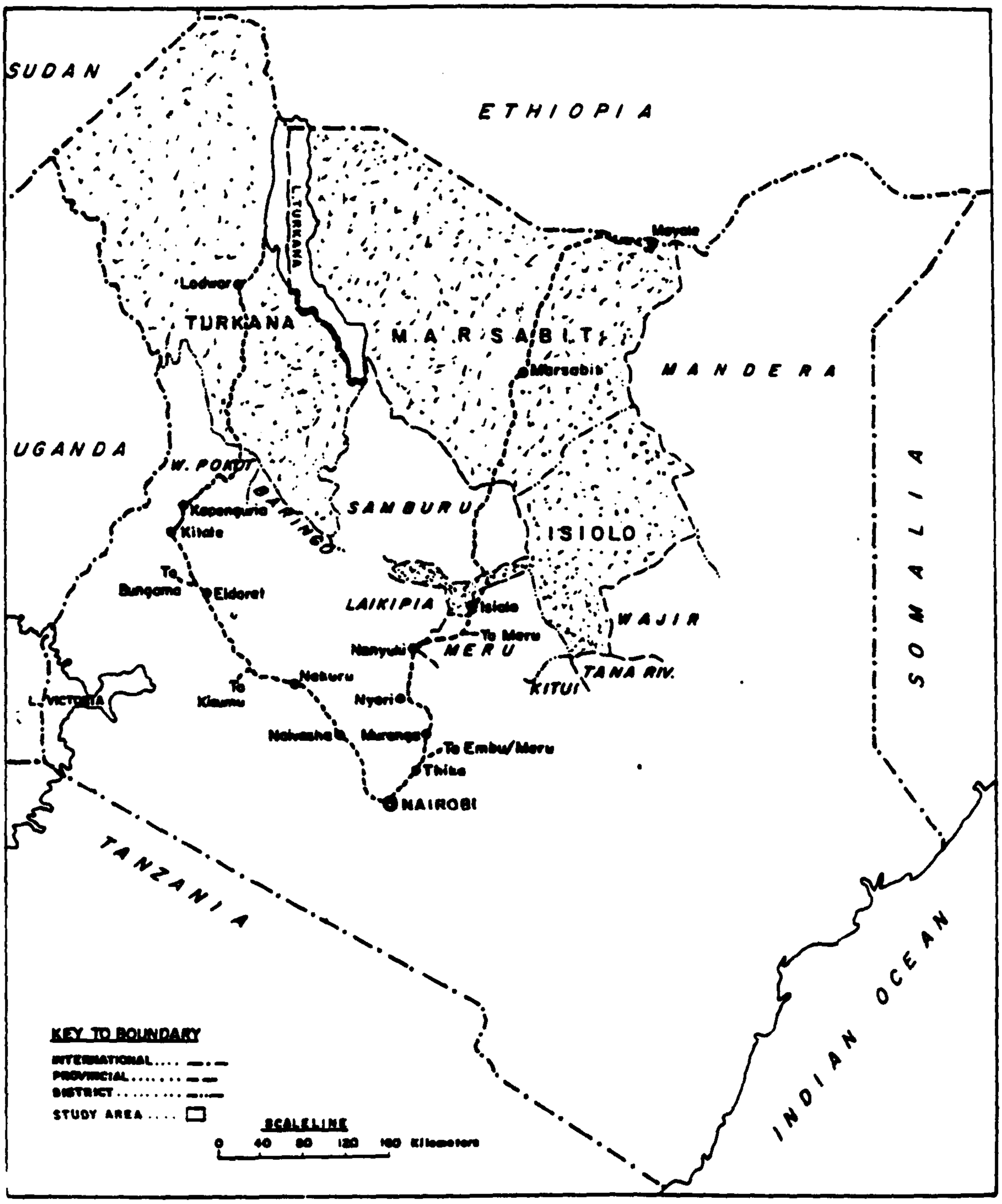


Figure 3.1: Location of the study area

of planning and unpublished work by Amuyunzu (1988b). Motorable routes and tracks were located on the maps and distances computed from predetermined points. This was followed by a reconnaissance to each unit in order to understand the general distribution of vegetation and select transect sites. Every reconnaissance was carried out in consultation with local residents familiar with the area or an officer who had worked on the project.

3.2.3 Sampling

Because of the expansive nature of most units and at times inaccessible conditions, transects were selected along motorable routes but in such a way that they were representative of the whole legend unit according to the method by Amuyunzu (1988). From the reconnaissance survey, two types of units were recognised: continuous and patchy. For each continuous unit, a transect on a set bearing was initiated at the unit boundary and the first sampling point positioned 500 m inside the unit and 500 m from the nearest road avoiding boundary and roadside effects. Three circular plots (each 0.1 ha) were established at every sampling location. Of these, a centre one was positioned on the transect and one other at 500 m away on a perpendicular bearing to each side. The objective of sampling three plots was to increase the sampling intensity at each point and thus reflect a fair representation of stocking density at a given site. Identical sets of three samples were enumerated at 3-4 km intervals depending on whether the terrain was a plain or hilly. For patchy units sampling was effected where *A. senegal* occurred. Attention was centred here on the character of patches of *A. senegal* and to collect these efficiently transects were aligned less formally and intervals depended on the distance separating the patches crossed by the transect. Again three (0.1 ha) plots were established per sample.

3.2.4 Recording of data

The following information was recorded from every sample plot:

- variety of *A. senegal* present.

- the number of trees of *A. senegal* by basal diameter. Previous experience had shown that most trees rarely exceeded 8 cm. Five diameter classes were therefore developed; < 2 cm, 2-3 cm, 4-5 cm, 6-7 cm and > 8 cm.

- density of other species of trees/shrubs present.

- soil type - soils were classified on the basis of texture and recorded as clay, loam, sandy or rocky. However, only two types of soils (sandy and rocky) on which *A. senegal* grows were encountered.

- terrain type - was recorded as hilly, plain or lugga (dry river stream)

Relative changes in the abundance of *A. senegal* between sample points along the transects were noted. Names of other species in the plots were recorded using their botanical names where positive identification was possible or by their known vernacular names which were later identified from relevant check-lists for the respective areas. Information was summarised in tables showing:

- terrain type, soils and density of *A. senegal* by basal diameter for each sample site and vegetation unit (Appendix I).

- summarised data on basal diameter to reflect age structure (Appendix II)

- names and density of other species associated with *A. senegal* by district (Appendix III)

3.2.5 Data processing and interpretation

. Preparation of *A. senegal* vegetation map

The vegetation maps from the three districts which had been used in the field survey were given to the survey branch of the Forestry Department for production of *A. senegal* maps. In each case, all units identified as containing

sufficient amounts of the species were marked. Area under each vegetation was computed in square kilometres.

. Stocking density of *Acacia senegal*

From appendix I data on mean density by vegetation unit per district were examined diagrammatically as histograms and differences in density between units subjected to analysis of variance. A one-way analysis of variance was found sufficient, providing a convenient summary of the data and an indication of the magnitude of variation between comparisons. Multiple comparison test using Fisher's Least Significance Difference (LSD) was however, used to asses significant differences. Meanwhile, standard deviation and coefficient of variation were computed from mean density per transect to examine variatation within a given vegetation unit. Quality of the resource was examined by considering stocking density by basal diameter for each district from data in appendix II (II. 2) and presented as histograms.

. Assessment of the relationship between stocking density and environment

To gain some understanding of the relationship between density and environment, data on density of *A. senegal* and associated species were subjected to multivariate analysis using Detrended Correspondence Analysis, DECORANA (Malloch, 1988). DECORANA is an ordination programme similar to reciprocal averaging technique but where the 'arch effect' found in many ordination techniques is said to have been removed by eliminating correlations between the first and higher axes. It provides both sample and species ordinations giving scores on the first four axes of the ordinations. A standard default run was used for both sample and species data four axes being extracted in each case. Scores generated

for samples with respect to these axes were plotted for exploratory analysis of the relationships of *A. senegal* with districts, terrain types and soils by superimposition of external data on the ordination scatters. Discernible patterns were statistically evaluated by analysis of variance. Relationship of other species with sample scores on the axes extracted were examined to check if patterns or correlations were present in the data.

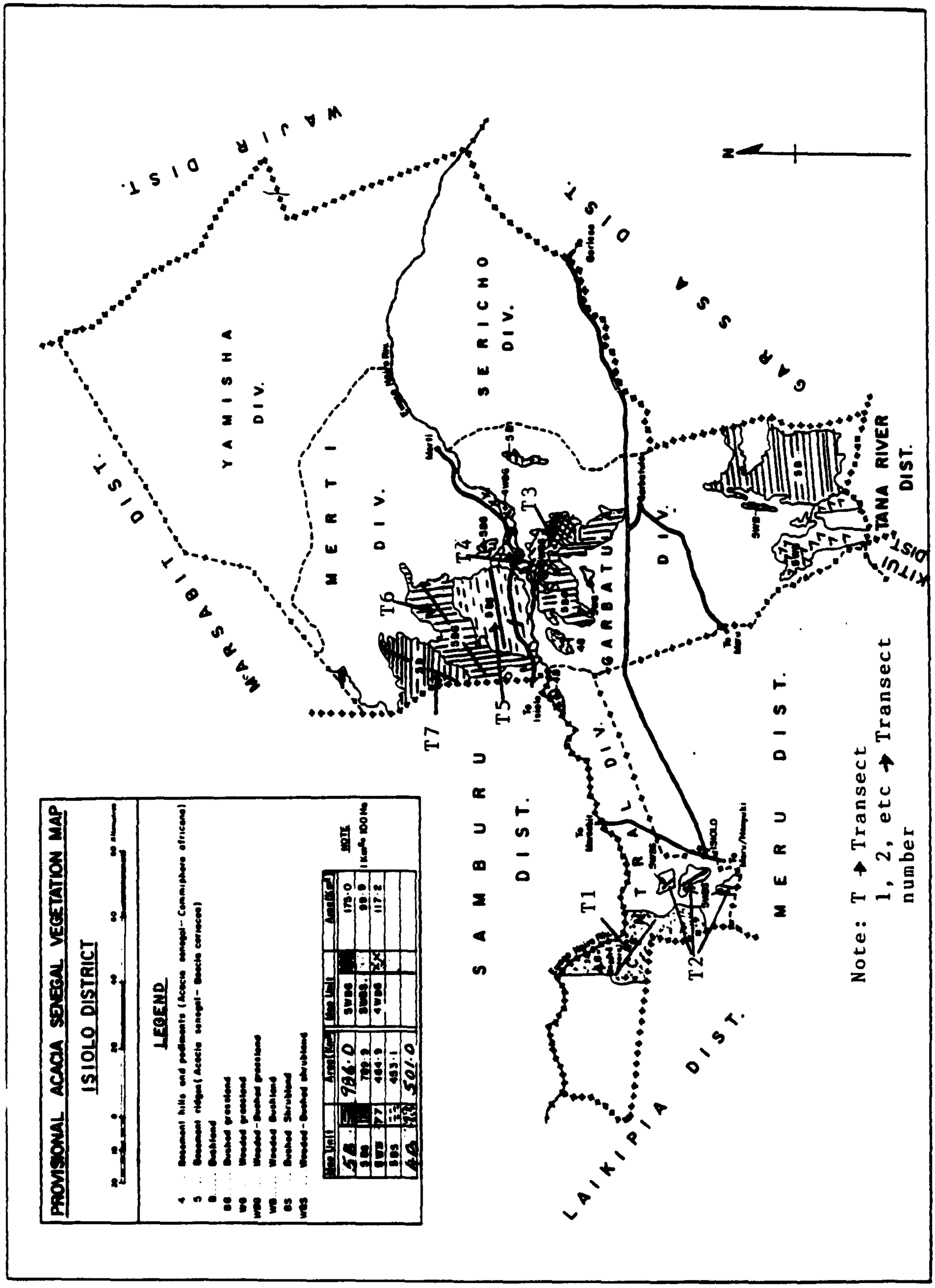
3.3 Results

3.3.1. Resource mapping and stocking density by district

3.3.1.1. Isiolo District

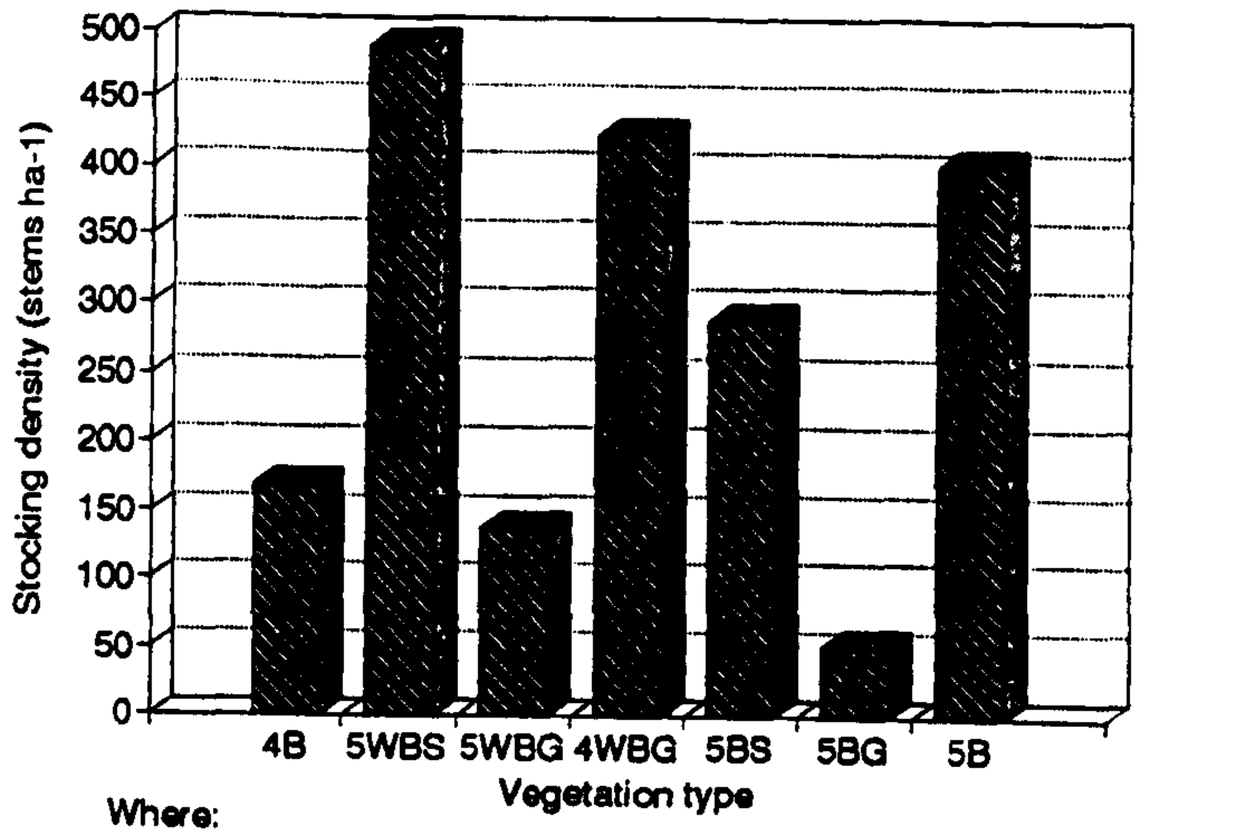
Figure 3.2 shows source locations of *A. senegal* in Isiolo District. The species was restricted to central, Garba Tula and Merti divisions and in two eclogical land units (ELU). These are the basement hills and pediments (ELU 4) and basement ridges (ELU 5). Ecological land unit 4 was represented by two vegetation types; bushland (4B) and wooded bushed grassland (4WBG) while ecological land unit 5 was represented by bushland (5B), bushed grassland (5BG), bushed shrubland (5BS) wooded bushland (5WB) and wooded bush shrubland (5WBS). The total area covered by vegetation types with sufficient amount of *Acacia senegal* was 3587 km^2 or 14% of the district.

Transects were run in each vegetation type to obtain information on stocking. Each vegetation type was identified by a local name beside the physiognomic classification used for ease of planning for gum production. Results are presented in Figure 3.3 and data in appenidx I. Highest density (491 stems ha^{-1}) was observed in Ranges (5WBS) that comprise of several ridges within central division while lowest density (52 stems ha^{-1}) was observed in Timut (5BS) that has relatively high human settlement. Analysis of variance (Table 3.1) revealed significant differences in stocking between the various vegetation types. Ranges



Note: T → Transect
1, 2, etc → Transect number

Figure 3.2: Provisional Acacia senegal map for Isiolo District



Where:

4B (Engare Ntare) 5WBS (Ranges) 5WBG (Malkadaka)

4WBG (Bisani Biliku) 5BS (Hot Spring) 5BG (Timut) 5B (Kom)

Figure 3.3. Stocking density of *Acacia senegal* in Isiolo District

Table 3.1. Analysis of variance for difference in density of *Acacia senegal* in Isiolo District

Local name	Vegetation type	Transect number	Mean density (sph)
Engare Ntare	4B	1	169ab
Ranges	5WBS	2	491d
Malkadaka	5WBG	3	138ab
Bisani Biliku	4WBG	4	425cd
Hot spring	5BS	5	290bc
Timut	5BG	6	52a
Kom	5B	7	399cd

Means with the same letter are not significantly different at p = 0.05

(5WBS), Kom (5B) and Bisani Biliku (4WBG) supported significantly higher densities than Engare Ntare (4B), Malkadaka (5WBG) and Timut (5BG). Ranges also had significantly higher density than Hot Spring (5BS). It is however, important to note that these densities do not necessarily reflect total amount in a given vegetation type as those types with bigger areas are likely to support more resource than those with less. Stocking within the units was also variable. To examine magnitude of variation therefore, standard deviation and coefficient of variation were computed from mean density per heactare. Coefficient of variation was used to examine homogeneity between samples where a value of 35% means that there is less variation between samples in a vegetation type or unit. Ranges, Bisani Biliku, Hot spring, Timut and Kom had values below 35% indicating low variation between samples while Engare Ntare and Malkadaka showed generally high variation in the distribution of the resource. The species was observed on all the three terrain types with generally higher densities on the hills and luggas but low densities on plains

Quality of the resource was assessed from data on basal diameter by considering all trees up to 3 cm as regenerates, trees 4 cm - 7 cm as intermediate and those above 8 cm as mature. Results are presented in Figure 3.4 and are expressed as a percentage of the resource by diameter class. Isiolo District has a higher proportion of younger stands with poor representation in the mature classes. An examination by type showed higher proportion of regenerates in Engare Ntare, Ranges and Hot spring. Var. *kerensis* (Figure 3.5a) was observed in all the units and is the main source of gum arabic. However, *A. circummarginata* (thought earlier to be a growth form of var. *leiorhachis*) was observed in Malkadaka, Hot spring and Bisani Biliku and was restricted to luggas (Fig. 3.5b). Experiments to collect gum from the tree were not successful confirming experience of the local communities.

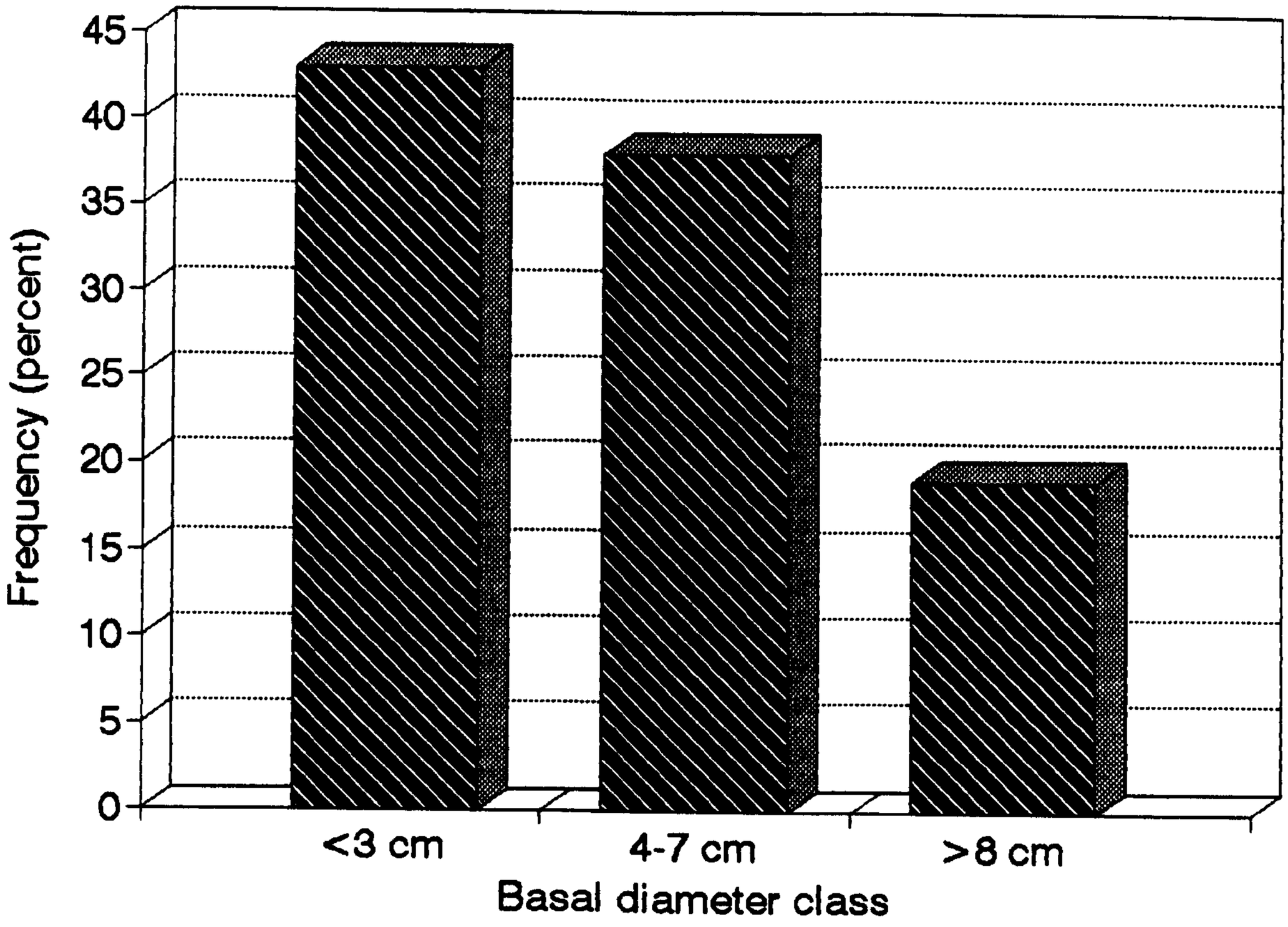


Figure 3.4: Basal diameter class in Isiolo District

Figure 3.5a. *Acacia senegal* var. *kerensis* . Photo. taken at Engare Ntare, Isiolo District, Kenya; November 1990.

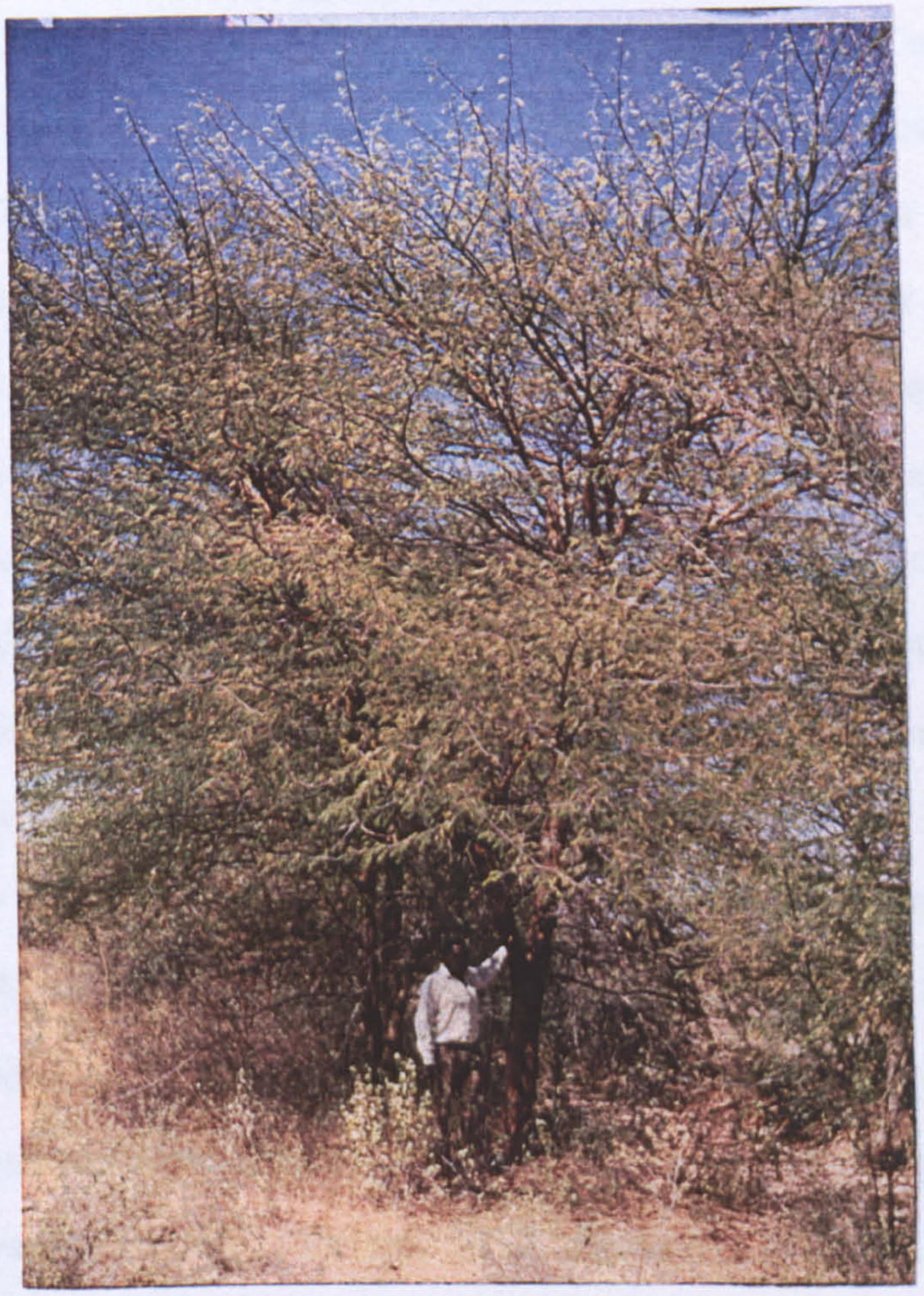


Figure 3.5b. *Acacia circummarginata*. Photo. taken at Bisani Biliku, Isiolo District, Kenya; November, 1990.

3.3.1.2. Marsabit District

A. senegal was well represented in seven range types of south western Marsabit defined by Lusigi et al (1986). These were *Acacia* with *Duosperma/Lippia* and *Brachieria* bush land (designated as 8E), *Acacia* with *Duosperma* and *Blepharis* bush land (9K), *Acacia* with *Duosperma* bush land (10F), *Acacia with Duosperma* shrub land (18G), *Acacia* wood land (20C) and *Acacia* scattered bushes in lava rocks (23X). The first four range types are found in the northern part while the last two in the southern part of the study area (Fig. 3.6). Total area under *Acacia senegal* in the region was estimated at 4578 km^2 or 20% of the area. Each range type was surveyed and results are presented in Fig 3.7 while data in appendix I. Density varied appreciably between range types with Kurkum (10F) and Olturot (18G) supporting significantly higher densities than the others (Table 3.2). Density in the two range types was 556 and 431 stems ha^{-1} while for other types it varied between 122 stems ha^{-1} (Falama) and 272 stems ha^{-1} (Ilaut). Ilaut also supported significantly higher density than Ngurunit. Assessement of stocking within a range type revealed again high variation. Within Kurkum, Olturot and Ilaut the species was mostly observed on hills and in luggas and had more or less uniform distribution between samples (coefficient of variation varied between 13% and 28%). However, in Hedad, Falama and Ngurunit the species was observed on plains and in luggas with low stocking in the former but high stocking in luggas resulting in generally high variation with coefficient of variation varying between 47% and 60%.

Size class distribution for variety *kerensis* showed good representaion in all classes with adequate recruitment from lower ones (Fig. 3.8). There were however, differences between range types. Falama and Kurkum had higher proportion of regenerates while Hedad, Olturot, Ngurunit and Ilaut had higher proportion of trees in the intermediate and mature classes. Var. *kerensis* was

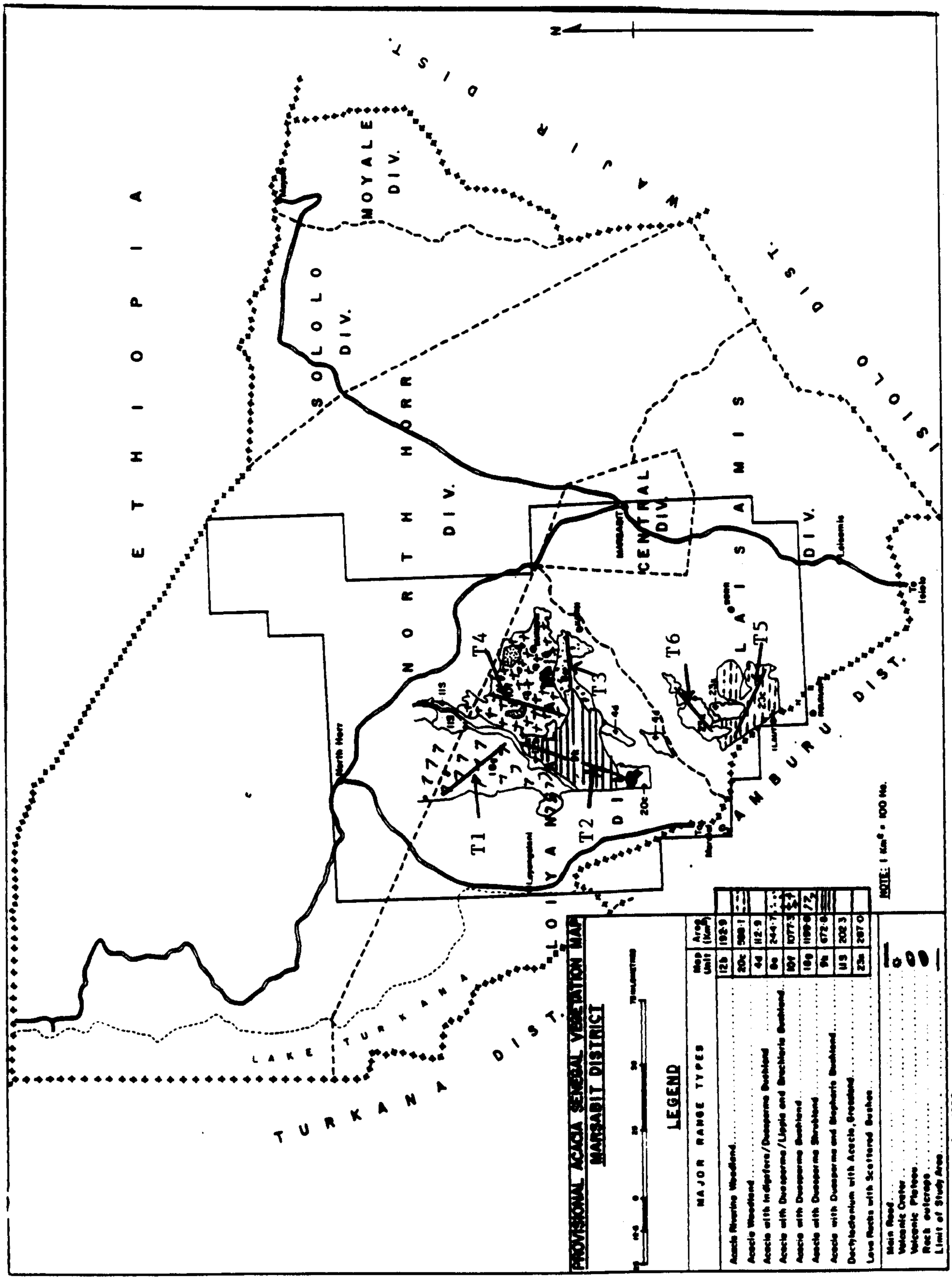
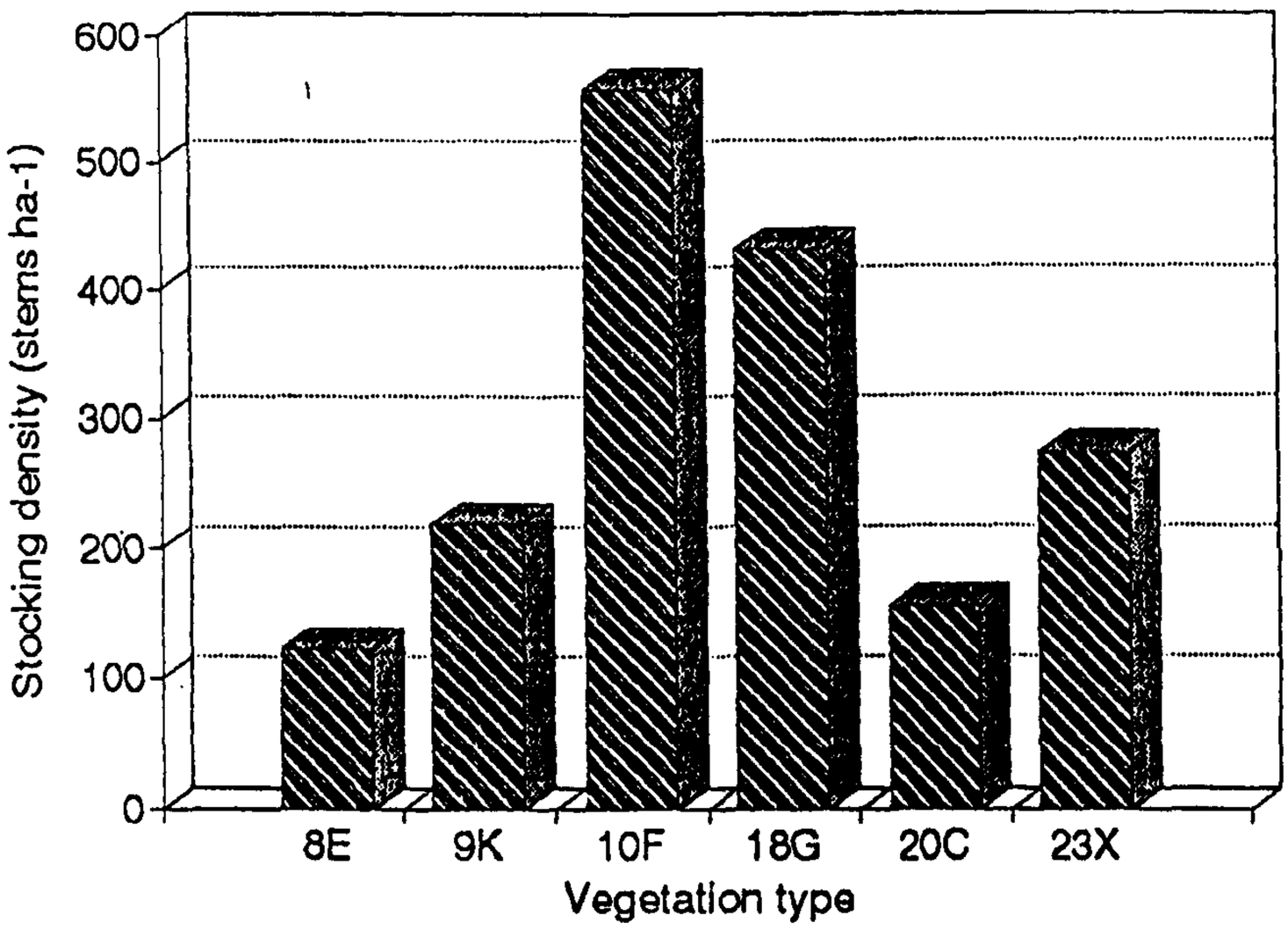


Figure 3.6: Provisional Acacia senegal map for Marsabit District

observed in all the range types while *A. circummarginata* was observed in Ngurunit and Ilaut along luggas.



Where: 8E (Falama) 9K (Hedad) 18G (Olturot)
10F (Kurkumu) 20C (Ngurunit) 23X (Ilaut)

Figure 3.7: Stocking density of *Acacia senegal* in Marsabit District

Table 3.3. Analysis of variance results to examine difference in the density of *Acacia senegal* in six range types.

Local name	Vegetation type	Transect number	Mean density (sph)
Olturot	18G	1	431c
Hedad	9K	2	214ab
Falama	8E	3	122a
Kurkum	10F	4	556c
Ngurunit	20C	5	154a
Ilaut	23X	6	272b

Means with the same letter are not significantly different at $\rho = 0.05$

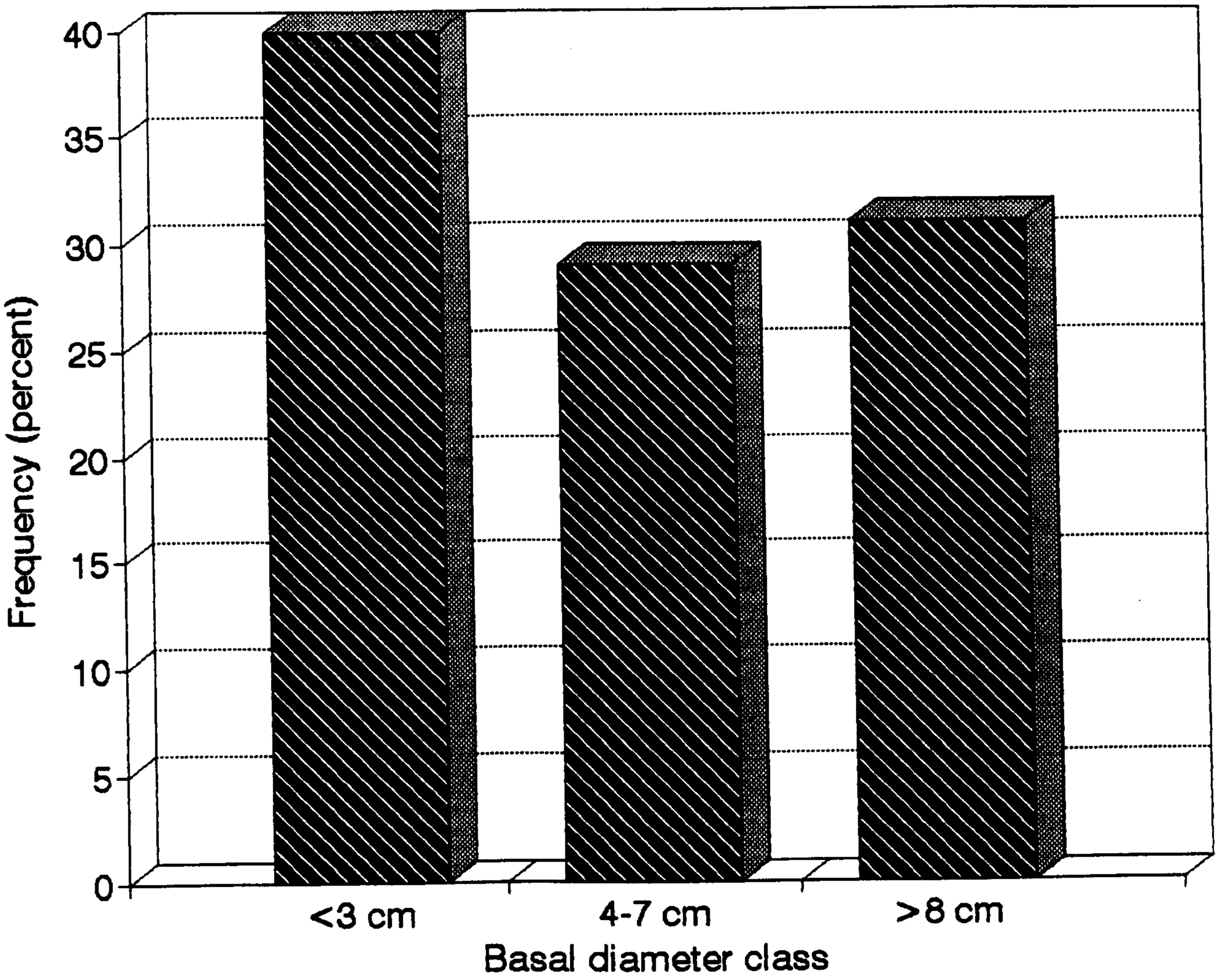
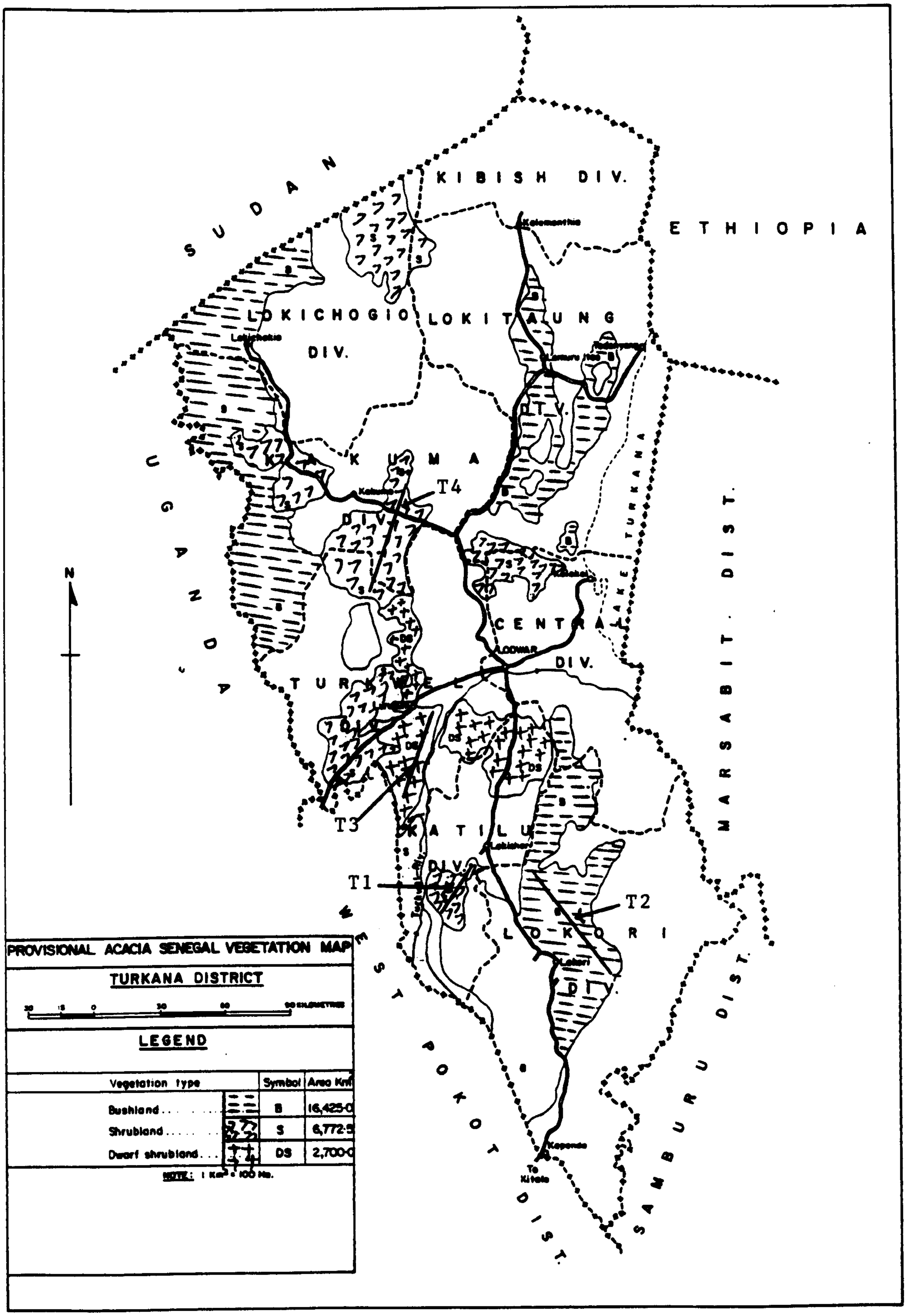


Figure 3.8: Basal diameter class in Marsabit District

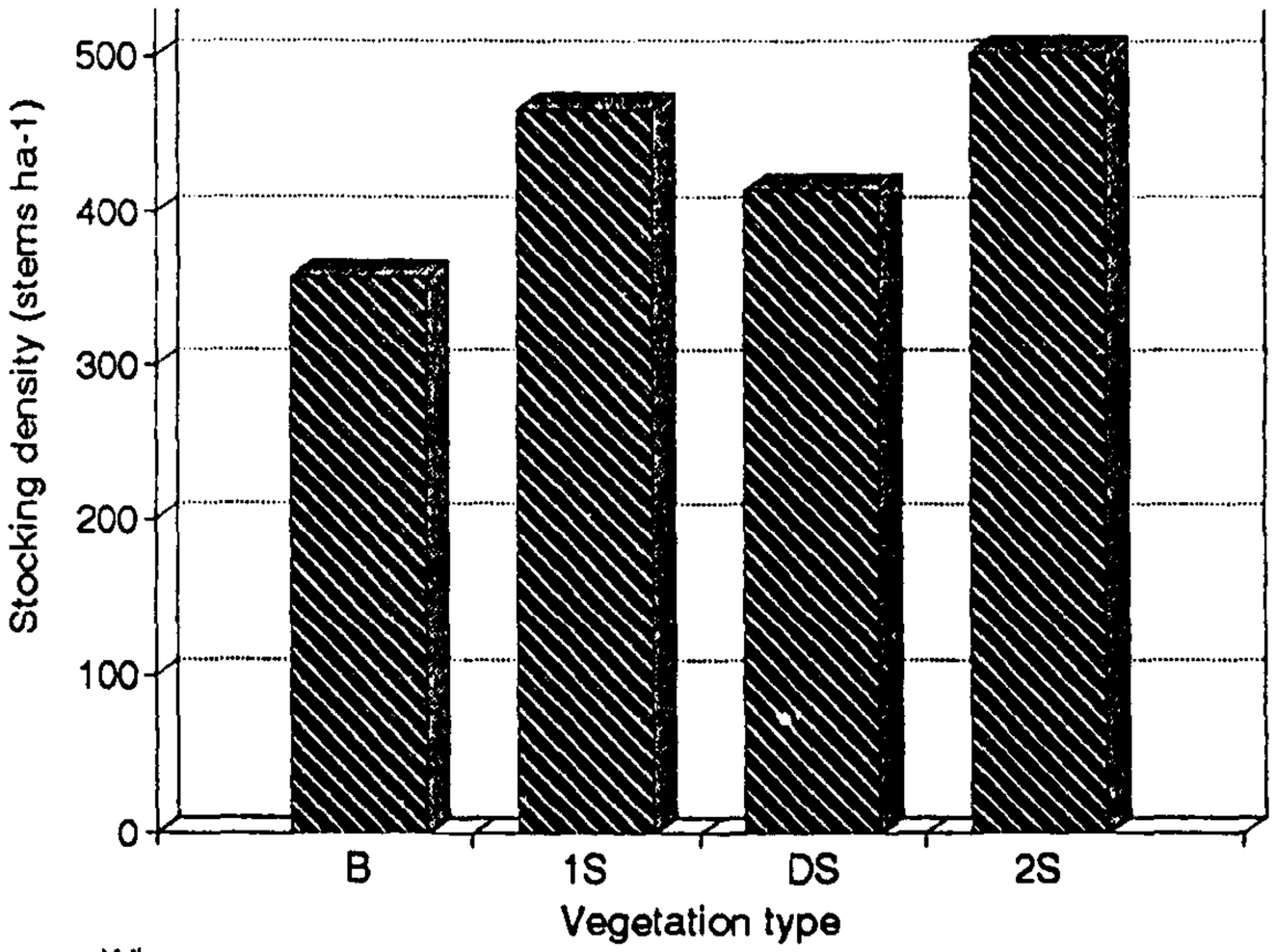
3.3.1.3. Turkana District

Out of the eight vegetation types recognised in the district, (Olang, 1984) *A. senegal* was observed in three of them: bushland (B), shrubland (S), and dwarf shrubland (DS) which occupy 25898 km^2 or 37 percent of the district (Fig. 3.9). Four areas representative of these vegetation types were surveyed to examine stocking and quality of the resource (Fig. 3.10). The areas had average to high stocking with density varying between 359 stems ha^{-1} (Lokori) and 502 stems ha^{-1} (Kakuma). The species was found mostly on hills and in luggas. Variation between the vegetation types was low and analysis of variance did not show significant difference between them. Difference between samples in a vegetation type was also low with a coefficinet of variation between 9% (Lokori) and 31% (Lokichar). An examination of basal diameter distribution revealed also fairly uniform distribution in all the three classes with slightly higher representation in the younger ones (Fig. 3.11). Only var. ***kerensis*** was observed in the different vegetation types.



Drawn by S M Servo - Sept 1992

Figure 3.9: Provisional Acacia senegal map for Turkana District



Where:

B (Lokori) 1S (Lokichar) DS (Lorugum) 2S (Kakuma)

and B -> Bushland, S -> shrubland, DS -> dwarf shrubland

Figure 3.10. Stocking density of *Acacia senegal* in Turkana District

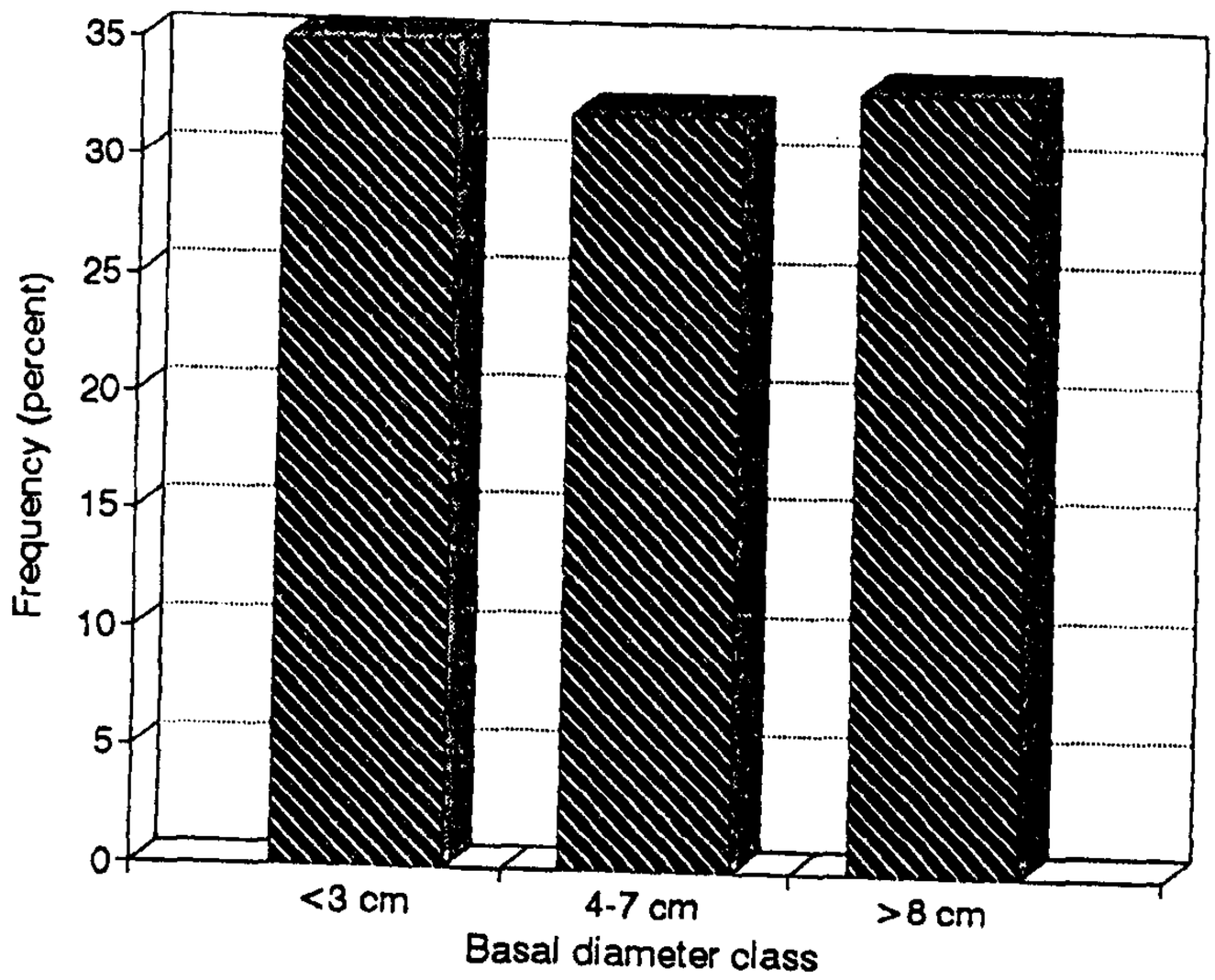
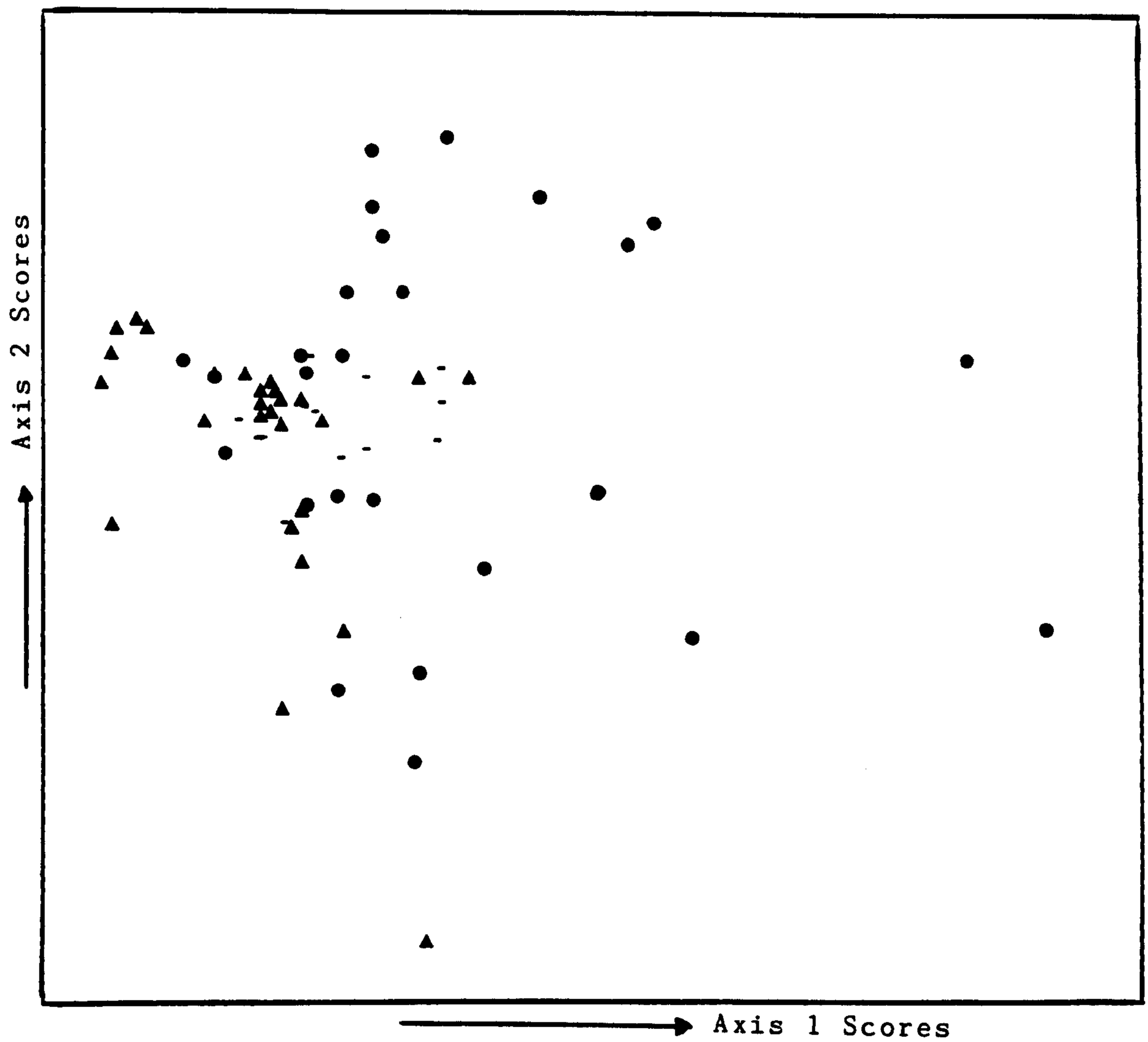


Figure 3.11. Basal diameter of *Acacia senegal* in Turkana District

3.3.2 Relationship between stocking density and environment and associated species

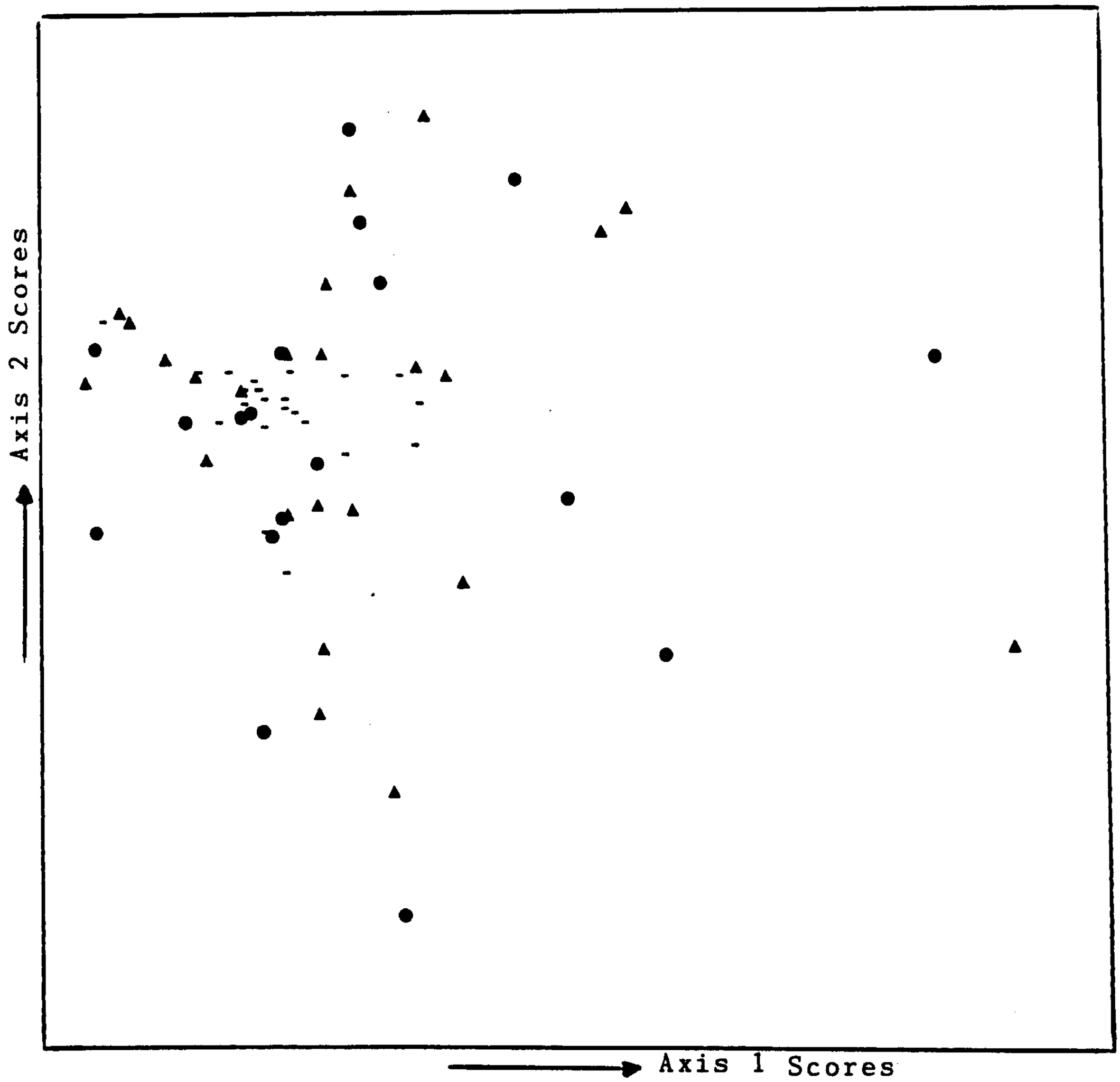
This study was carried out to examine if a trend existed in the general distribution of the resource with respect to region, topography or soils. Data on 17 transects comprising 65 samples from the three districts were used in the analysis. The four Axes extracted from scores generated by DECORANA were associated with 36%, 26.0%, 13% and 10% of the variation in the data respectively. Three scatter plots were compared for observable patterns. The scatter plot for axis 1 and 3 was omitted as it resulted in a curvelinear spread of scores indicating that the two axes were not independent of each other. Interpretation was therefore carried out for either a combination of Axes 1 and 2 or 2 and 3.

A regional trend in the samples with respect to districts was established. Within the ordination scatters, samples from Turkana and Isiolo Districts received low loadings on Axis 1 while those from Marsabit received more variable loadings. The Marsabit and Isiolo samples received more variable loadings on Axis 2 but on this axis samples from Turkana were limited to a narrow range of scores (Fig. 3.12). A trend was also established with respect to terrain types (Fig. 3.13). Superimposition of terrain categories on the scatter indicates rather low Axis 1 scores for samples originating from hilly terrain but higher loadings on Axis 2. Samples from plains and luggas were scattered with respect to both axes. No grouping of any soil type within the scatter was noted.



▲ Isiolo ● Marsabit - Turkana

Figure 3.12: DECORANA ordination of samples of Acacia senegal woodland in Isiolo, Marsabit and Turkana Districts, based on 65 samples for regions



- Hilly ● plain ▲ lugga

Figure 3.13: DECORANA ordination of samples of Acacia senegal woodland in Isiolo, Marsabit and Turkana Districts, Kenya based on 65 sample for terrain types.

Analysis of variance revealed that the observed trends were associated with differences in stocking density between districts and terrain types (Table 3.3). In Turkana District, mean density of *Acacia senegal* (438 stems ha^{-1}) was significantly higher than either Marsabit (292 stems ha^{-1}) or Isiolo (281 stems ha^{-1}). The analysis also revealed that on hilly terrain there were significantly higher densities (442 stems ha^{-1}) than either along luggas (294 stems ha^{-1}) or on the plains (275 stems ha^{-1}). No difference was observed with soil types.

Table 3.3. Analysis of variance results to examine difference in the distribution of *Acacia senegal* with districts and topography.

a. Districts

District name	Number of sample sites	Mean density	Std. dev.	Coef. of var. (%)
Isiolo	28	281a	135	48
Marsabit	25	292a	120	41
Turkana	12	438	66	15

b. Topography

Topographic type	Number of sample sites	Mean density	Std. dev.	Coef. of var. (%)
Hilly	17	442b	130	29
Plain	18	275a	149	54
Lugga	30	294	96	33

Means with the same letter are not significantly different at $P = 0.05$

3.3.3. Association with other species

Twenty four species were recorded as associates of *A. senegal*. Table 3.4 gives an arrangement of the sample analysis in increasing Axis 1 scores. The table shows samples from Isiolo predominantly on the left side and those from Marsabit to the right with samples from Turkana interspersed. The groups appear to be influenced by species with restricted distribution as will be observed from the ordination data below. Three species (*Acacia tortilis*, *Acacia reficiens* and *Commiphora africana*) were most common associates represented in more than 50% of the samples. Superimposition of tallies of stems on ordination scatters revealed some relationship between stocking and either region or topography. Samples relatively rich in *A. tortilis* received high loadings on Axis 3 and an examination revealed that they comprised of samples originating from Isiolo and Marsabit (Fig. 3.14a). The species was mostly observed on plains and in luggas. For *A. reficiens*, samples rich in the species received relatively high loadings on Axis 1 (Fig. 3.14b). An examination of the samples revealed average to high density in Marsabit and Turkana with the species being common on plains and in luggas. Samples rich in *Commiphora africana* received relatively high loading on Axis 2 and relatively low loading on Axis 3, higher abundance being typical for samples from Isiolo District while it occured at low to average density in Marsabit and Turkana Districts (Fig. 3.14c). For this species, there was no marked preference for any of the terrain types. For the other species repeatedly recorded, three revealed relationships. Samples rich in *Commiphora confusa* received high loading on Axis 2 and were restricted to Isiolo (Fig. 3.14d). Samples rich in *C. holtiziana*, abundant in Isiolo and Marsabit also received high loading on Axis 2 (Fgi. 3.14e) while those for *C. pseudopaolii*, mostly from Marsabit and Turkana received high loading on Axis 3. All the three species were characteristic of hilly terrain.

Table 3.4: Species that are noteworthy associates of Acacia senegal in Kenya arranged in a squence of increasing Axis 1 scores

District	1	1	1	1	1	1	2	1	2	1	2	3	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1	3	1	2	2	2	3	3	1
Species																																			
Acacia mellifera																							*										*		*
Acacia nubica											*																								
Acacia reficiens					*				*							*			*			*					*		*			*	*	*	*	*
Acacia senegal	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Acacia tortilis	*	*	*	*	*			*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*
Balanites aegyptiaca									*												*															
Balanites rotundifolia																						*														
Boscia coriacea			*	*									*																							
Boswellia hildebrandtii							*		*		*	*																			*					
Boswellia neglecta	*			*				*							*																				
Cadaba farinosa																																	*		
Cadaba rotundifolia																																	*	*	
Commiphora africana	*	*	*	*	*			*		*		*	*	*	*	*	*	*	*	*	*	*		*	*		*		*				*	*	
Commiphora campestris																																			
Commiphora confusa	*	*				*							*	*	*		*	*	*	*			*	*					*		*				*
Commiphora holtziana	*					*							*	*	*	*	*	*	*	*			*	*	*		*		*						*
Commiphora kua							*		*		*																				*		*			
q																																			
Commiphora pseudopaolii												*										*						*				*	*		
Cordia sinensis				*																															
Euphobia cuneata									*		*											*								*			*		
Grewia tenax							*																							*		*			
Lannea elata	*	*																																	
Maerua crassifolia		*							*					*	*	*		*	*	*	*								*	*		*			
Salvadora persica				*								*										*												*		

Legend:

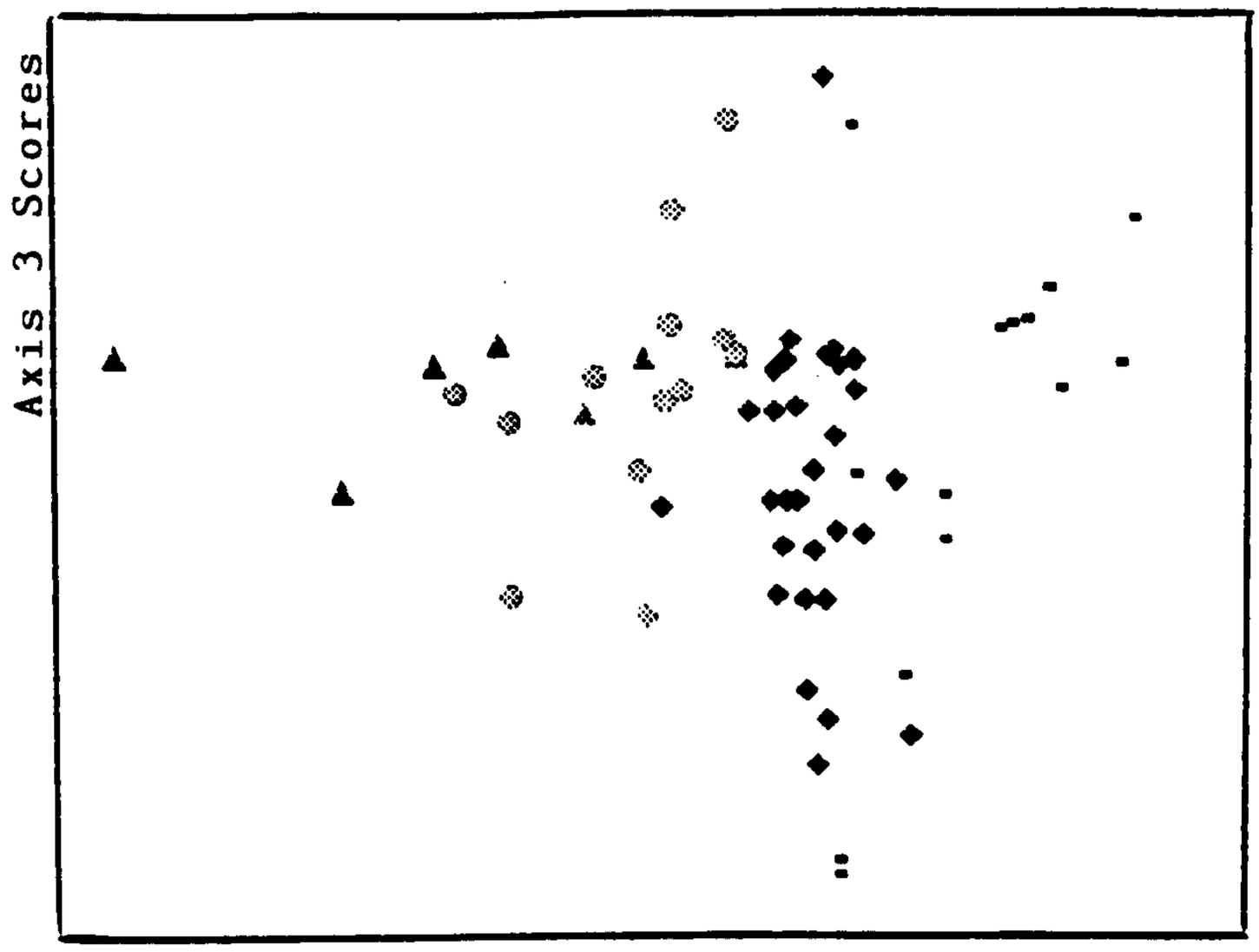
1 -> Isiolo District

2 -> Marsabit District

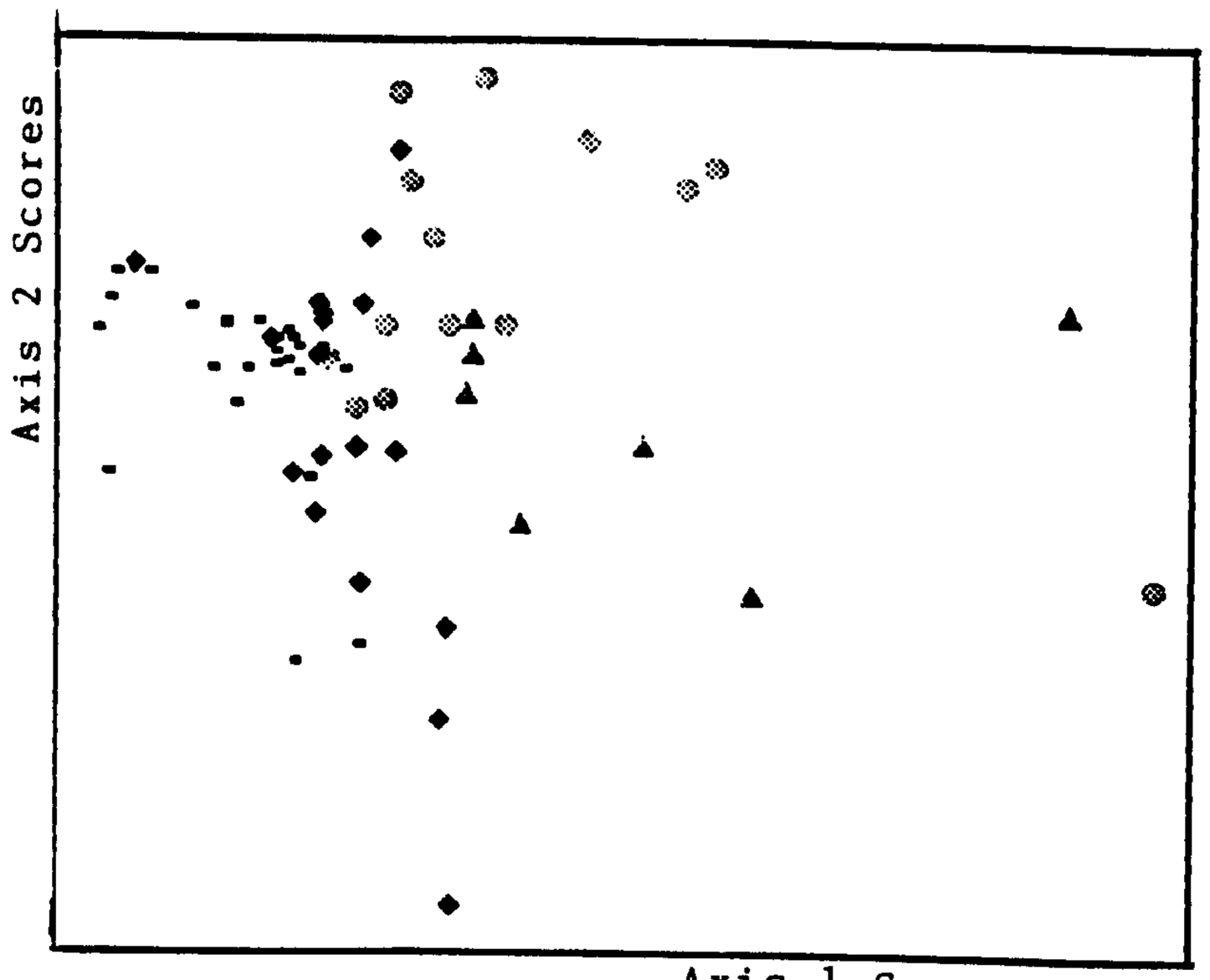
3 -> Turkana

Table 3.4: continued

	2	2	3	1	2	2	3	3	2	2	2	2	2	2	2	1	1	3	3	3	2	1	2	2	3	2	2	2	2	2	Frequency
Species																															
Acacia mellifera								*			*	*								*	*			*		*	*	*	*		13
Acacia nubica						*									*																3
Acacia reficiens			*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*	39
Acacia senegal	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	65
Acacia tortilis	*	*	*	*			*	*	*					*	*		*	*	*	*	*		*		*	*	*	*		*	51
Balanites aegyptiaca																	*											*			4
Balanites rotundifolia			*				*																								3
Boscia coriacea							*																								4
Boswellia hildebrandtii	*	*							*									*							*						10
Boswellia neglecta																															4
Cadaba farinosa						*					*	*	*											*			*		*		7
Cadaba rotundifolia								*											*	*											4
Commiphora africana			*	*		*		*			*	*	*			*		*	*	*		*		*	*				*		40
Commiphora campestris																														*	1
Commiphora confusa																*						*									17
Commiphora holtziana	*												*		*															*	20
Commiphora kua	*				*	*				*		*	*								*			*		*	*	*	*		17
Commiphora incisa									*				*																		3
Commiphora pseudopaolii		*	*					*	*				*					*	*	*	*				*	*				*	18
Cordia sinensis						*				*	*	*									*			*							7
Euphobia cuneata		*	*					*			*									*								*			13
Grewia tenax					*					*											*		*				*		*		9
Lannea elata																															2
Maerua crassifolia	*	*			*				*	*	*	*		*		*						*	*			*	*		*	*	28
Salvadora persica			*				*						*	*				*													9

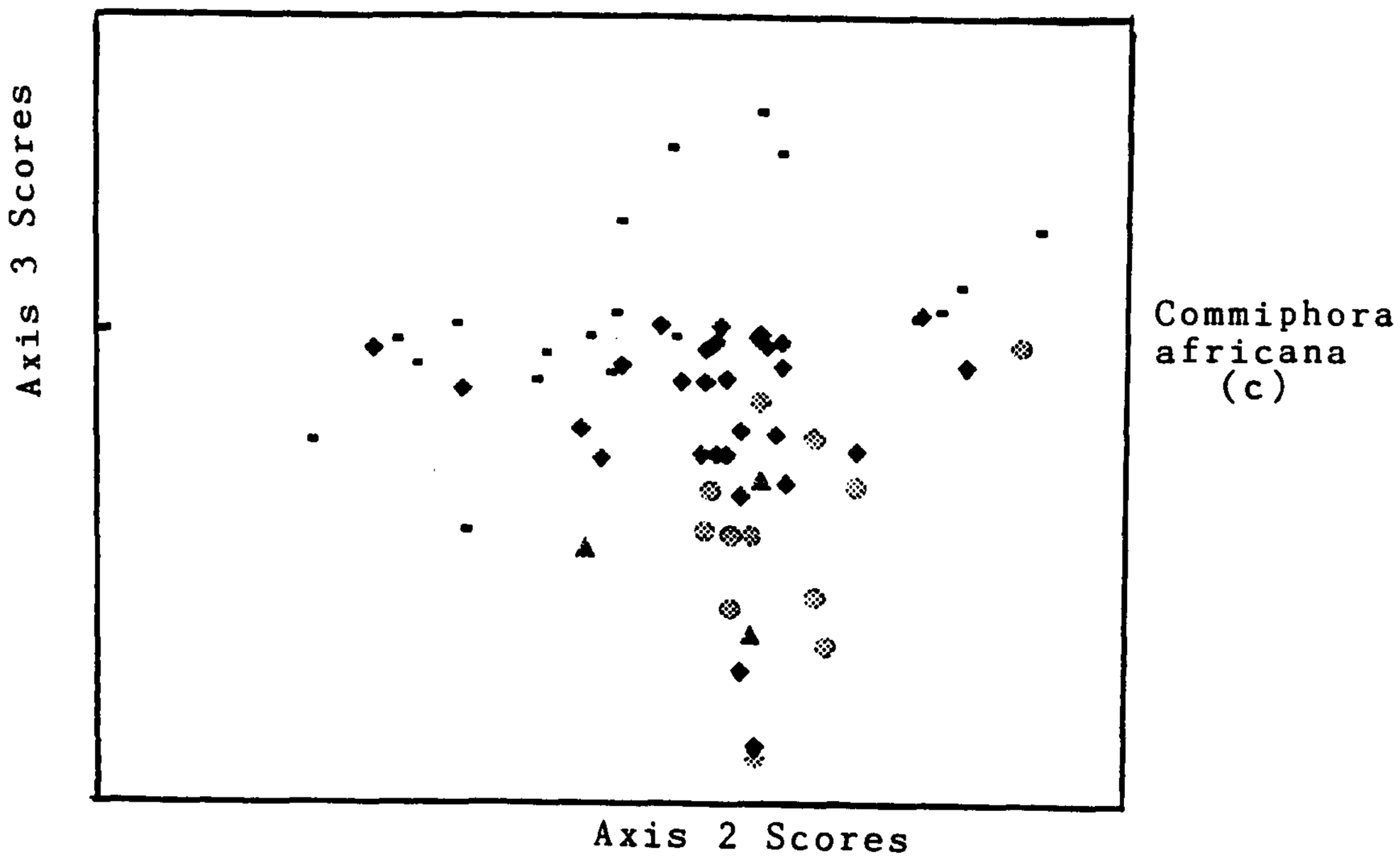


▲ ≥100 stems ❁ 50-99 stems ◆ 1-49 stems - tortilis absent



▲ ≥ 100 stems ● 50-99 stems ◆ 1-49 stems - reficiens absent

Figure 3.14: DECORANA ordination of samples of Acacia senegal woodland in Isiolo, Marsabit and Turkana showing distribution of Acacia



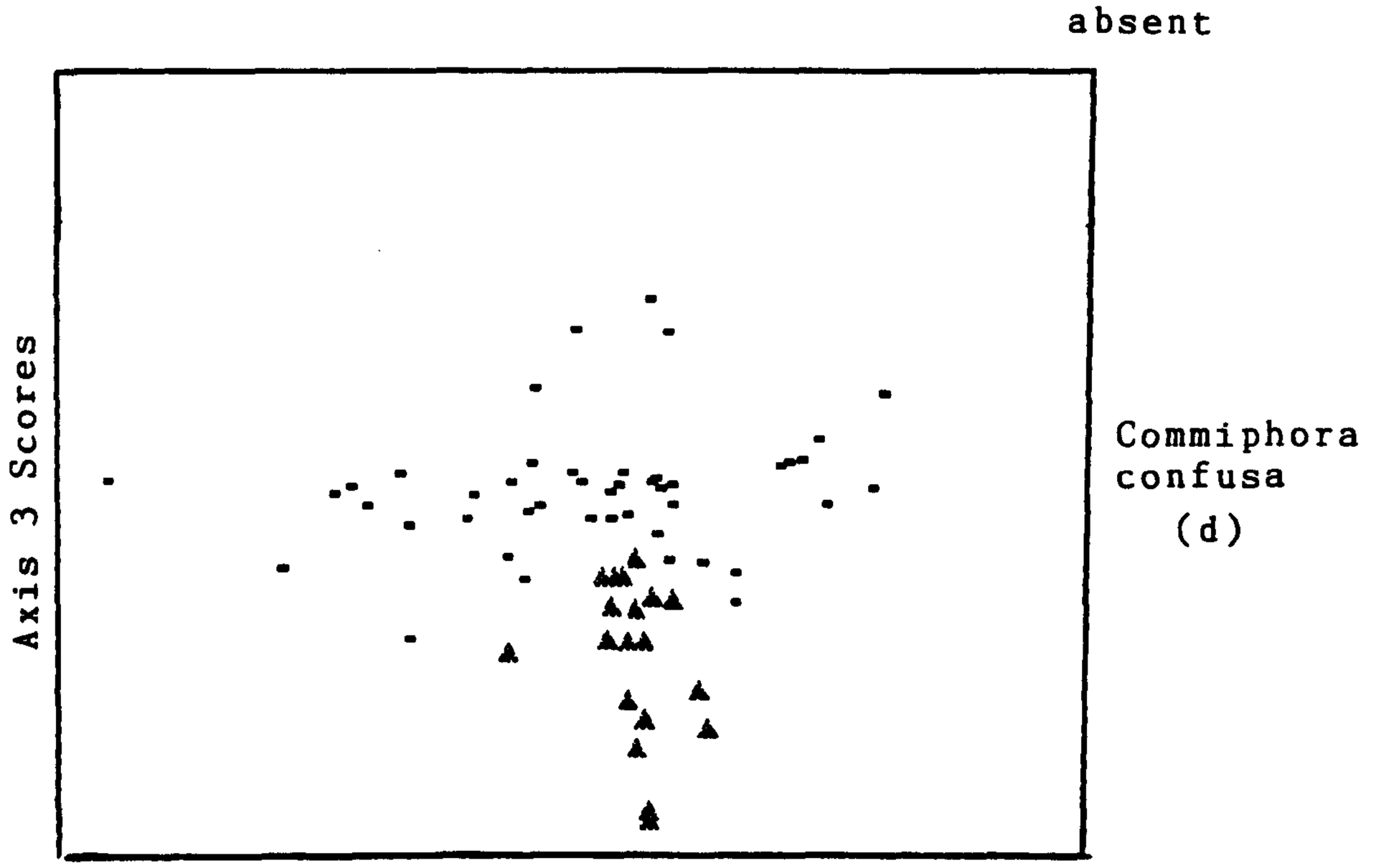


Figure 3.14: DECORANA ordination of samples of Acacia senegal woodland in Isiolo, Marsabit and Turkana Districts showing distribution of Commiphora africana (c) and C. confusa (d).

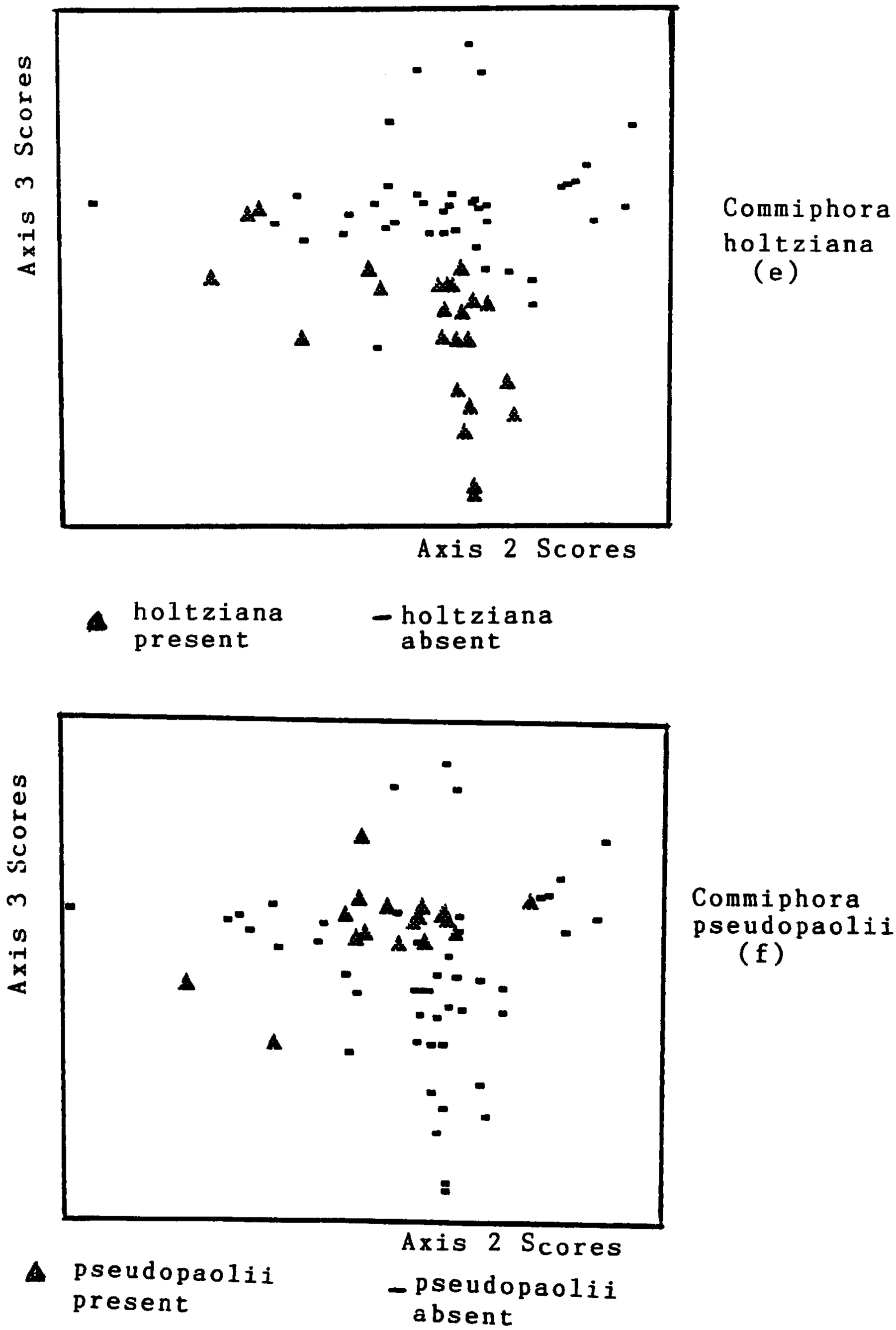


Figure 3.14: DECORANA ordination of samples of Acacia senegal woodland in Isiolo, Marsabit and Turkana Districts showing distribution of Commiphora holtiziana (e) and C. pseudopaolii (f)

3.4. Discussion

It is evident from results of ordination and analysis of variance that irrespective of district, stocking of *A. senegal* across the study region is influenced by terrain type. Most samples with stocking exceeding 200 stems ha^{-1} received high loadings on Axis 2 and a further examination revealed that they comprised of samples predominantly from hilly terrain and occasionally luggas. Invariably, all samples from Turkana (except 1) were from either of the terrain types. Even within Isiolo District where there was overall low stocking, samples from hilly terrain and luggas closely associated with hills had high stocking and received high loadings on Axis 2. This preference for hilly terrain may relate to topographic and soil factors. Lind and Morrison (1974) in a study of the vegetation of East Africa indicated that while large plant formations are influenced by climate, differences in plant communities are attributed mainly to topographic and soil conditions. Abundance of the species on hilly terrain had been noted previousily. FAO (1971) in the classification of vegetation based on geomorphological features found that *A. senegal* was an indicator species for basement hills and ridges in Isiolo District. Most of the hills are derived from tertiary-recent volcanic activity that have given rise to rocky pebbles and sandy volcanic soils (Saggerson, 1972). In Marsabit District the species was found to be characteristic of the precambrian rocky gneissic soils and sandstones of the Ndotos, Ol Doinyo Mara ranges and Mt. Nyiru that have undergone metamorphic processes (Herlocker, 1979) while in Turkana it was found to be abundant on slopes of high hills that are somewhat excessively drained (Amuyunzu, 1988a). The hills generally support soils that are coarse in texture and well drained and thus suitable for the growth of the species. Luggas occasionally support high stocking and more so in the relatively arid environments due to the availability of soil moisture that result from runoff and subsurface

drainage. The species was reported to grow extensively in shallow drainage lines in the Koroli desert in Marsabit District (Herlocker, 1979).

Climatic conditions also play a part in the overall distribution and hence stocking density. The influence of rainfall was not examined in the present study but it has been shown that *A. senegal* thrives best in regions with mean rainfall between 200 - 450 mm (Obeid and Seif El Din, 1970; NAS, 1979). Within the study region, Turkana District is the driest with a mean annual rainfall of 250 mm over most of the area (Amuyunzu and Oba, 1991) while Isiolo District on the other hand receives relatively high rainfall. The mean annual rainfall in Isiolo District for the period between 1978 and 1987 was 592 mm with a range from 443 mm and 787 mm (Ministry of planning 1989). Beside, it experiences long periods of cool dry months which limit growth of the species over most places and could partly explain the overall low stocking density observed. More importantly however, is the fact that this conditions tend to restrict the productive gum season.

An assessment of *A. senegal* resources by district revealed that within Isiolo District the resource covers approximately 14 percent of the area. This small proportion is understandable when one recognises that it occurs in only two ecological land units found in the District (FAO, 1971). Highest disparity in distribution was observed and was thought to be influenced by topography and soils. However, the influence of man was identified also as major factor. For example the unit refered to as Ranges(5WBS) with exceptionally high stocking comprises of several ridges that are usually reserved by local communities for dry season grazing though they also fall within the holding ground of Livestock Marketing Division (LMD) and have extra restriction from widespread grazing. The other two types (Bisani Biliku and Kom) with high stocking represent areas bordering Uaso Ngiro river and luggas respectively. In the former, the area is also reserved for dry season grazing while preference of the species for luggas has been

discussed. On the other hand units with low densities (Engare Ntare, Malkadaka and Timut) are mainly in the bushland or bushed grasslands with high human settlement. It seems therefore that besides natural factors (climate and soils), human activity plays a greater part in the distribution of the species. The human factor was identified by Pratt and Gwynne (1977) as one of the main factors affecting vegetation types in East Africa. Earlier studies had shown that in settled areas woody resources are cut down for making homesteads, livestock enclosures and for fuelwood (Lusigi et al, 1986). Indeed during the survey of Isiolo District, there was evidence of removal of *A. senegal* and *A. tortilis* by local communities for fencing enclosures. The poor representation in mature diameter class is therefore attributed to removal through harvesting. Young trees are also good fodder for camels and goats and studies carried out elsewhere (Obeid and Seif El Din, 1970) have shown that regeneration of *A. senegal* can be greatly affected by browsing and especially by goats.

In south western Marsabit, *A. senegal* resources covered 20 percent of the area. The species occurs mostly in patches with high variation in density between units reflecting differences in soil and climatic conditions. These observations support earlier studies which had shown that growth and distribution of *A. senegal* in Marsabit is influenced by parent rock and soil moisture (Herlocker, 1979). It is within Marsabit District that it exhibits wide differences in habbit probably reflecting extreme environments. For example, var. *kerensis* grows to its extremes within this region. On the deeper white sands of the Hedad plains in range type 9K it grows to its upper limit of 5 m and sometimes as a spreading multi stem bush having up to seven stems. On the drier parts in Falama and Olturot it is a shrub of about 2 m and mainly confined along luggas or drainage areas while in Kurkum the two limits are represented. Within Ngurunit and Ilaut in the south and on deeper red sands it again grows to its upper limit with a short and sometimes distinct stem

while on rocky and drier sites it grows as a dwarf bush or shrub. It was this variation in height that supported our use of basal diameter in describing age either as regenerates, intermediate or mature. In the latter range types, *A. circummarginata* was observed. It occurs as a well formed tree up to 14 m high and is confined along luggas and other drainage areas. Tapping trials to collect gum from this variety were unsuccessful as it only produced limited quantities of gum like exudate that did not justify collecting. Kurkum and Hedad areas to the north and Ngurunit/Ilaut areas to the south are suitable areas for production of gum arabic as they had good representation of trees in intermediate and mature classes that permits tapping. In Turkana, the species occupies an area estimated at 37 percent of the total area. The species has high stocking density probably reflecting ideal climatic and soil conditions for the species growth as discussed earlier. Variety *kerensis* was the only one observed as a bush/shrub and is well represented in all the age classes. It is mostly confined on hills and luggas. The district has sufficient resources that can support tapping operations for production of gum arabic.

Analysis of association with other species revealed that *Acacia tortilis*, *Acacia reficiens* and *Commiphora africana* are the most common associates. *A. tortilis* had high abundance in Isiolo and Marsabit while *A. reficiens* was more abundant in Marsabit and Turkana Districts. Both species were mostly associated with *A. senegal* on the plains and in luggas. *C. africana* had high abundance in Isiolo decreasing towards Turkana and did not show association with respect to terrain type. It is known to be a dominant species in the southern part of the country (Agnew and Waterman, 1989). The other species of *Commiphora* revealed some relationship between stocking and topography or region. Apart from *C. pseudopaolii* all the *commiphora* species encountered in the survey were restricted either to Isiolo or Isiolo and Marsabit and were abundant on hills.

Most associates of *Acacia senegal* are either other species of *Acacia* or *Commiphora* species and therefore of interest as capable of producing gums or resins. Among the *Acacia* species, *A. tortilis* produces gum which is distinctly dark, of smooth texture and shiny. *A. mellifera* has also been reported to produce some gum with properties comparable to that of gum arabic from *A. senegal* (Anderson and Farquhar, 1979) but quantities are generally too small for meaningful commercial production. *Commiphora holtiziana* and *C. confusa* produce resins, commercially referred to as myrrh (Waterman et al., 1987), distinctly different from the water soluble gums. It may however be noted that *Acacia seyal* whose gum often enters the market in admixture with gum arabic from *A. senegal* was not encountered in any samples though it is known to occur in Isiolo and Marsabit Districts and represents a major threat to adulteration.

SECTION II: STUDIES ON GUM ARABIC

CHAPTER 4: LITERATURE REVIEW ON GUM ARABIC

4.1. General description

4.1.1. Definition and nomenclature

Although different regulatory organisations have defined gum arabic of commerce somewhat differently, they all acknowledge it as a dried gummy exudate obtained from the stems and branches of *A. senegal* (L.) Willd. or closely related species (US pharmacopoeia 1985; FAO, 1990). The name gum arabic derives from the fact that it was formerly shipped to Europe from Arabian ports (Obeid and Seif El Din, 1970). There are also different local names depending on the area of origin. Ballal Siddig (1991) gave a comprehensive list of local names from different countries. In Kenya, it is known by the following names: Ekunoit (Turkana), Babito or Bura dima (Boran), Iderikes (Samburu), Idado (Somali), Mongoli (Kamba), Ol munshuin (Maasai), Chepkomon (Kipsigis), Matengewa (Bajuni) and Kikwata (Swahili) (Coe and Bentjee, 1991).

4.1.2 Formation and function

According to a hypothesis on the mechanism of formation proposed for gum arabic, the gum acid has as its precursor, some highly branched arabinogalactan of a hemicellulosic type, to which is added rhamnose, glucuronic acid and 4-O-methyl glucuronic acid (arising from oxidation related mechanisms) terminated side chains in the final stages of gum production (Anderson and Dea, 1968). It is envisaged that enzyme systems probably differ at different parts of the tree and the dark brown (Hennawi) gum formed on the main trunk is thought to be manufactured by a different enzyme.

The site of formation has been comprehensively studied (Ghosh and Purkayastha, 1962). In a study of the anatomy of wood and bark of *A. senegal* Ghosh and co-worker observed that gum comes from gum cysts which develop in the inner bark of some trees that naturally exude it. The cysts are developed in the tangential rows of the axial parenchyma strands of the phloem adjacent

to the cambial zone. They are first developed schizogenously but later on enlarge considerably (lysigenously) due to the breakdown of the surrounding cells. The cysts do not have a particular shape or size but appear as vertically aligned, sinuous and sometimes interconnected passages ending abruptly. The development is preceded by certain widespread changes like profuse development of parenchymatous tissues, disappearance of starch etc in both xylem and phloem. This observation is supported by work of Anderson and Dea (1968) who also reported lack of starch in the wood tissues of excised *A. senegal*. A recent study by Joseleau and Ullman (1990) provides further biochemical evidence for the site of formation of gum arabic in *A. senegal*. By comparison of the carbohydrate analysis of the tissues from the inner bark, cambial zone and xylem of the gum producing branch with corresponding tissues of a none producing branch, they found comparable molar proportions of the sugars (galactose, arabinose, rhamnose and glucuronic acid) in the inner bark of the former branch as in gum arabic.

Gum is believed to be formed by a tree in order to seal off the injured parts, not so much to prevent infection, but to prevent loss of water (Smith and Montgomery, 1959). This suggestion is supported by Obeid and Seif El Din (1970) who noted that gum is exuded naturally from lesions caused by drought, sun scorch and fire or from wounds caused by animals as a defence mechanism to avoid dehydration. That it is produced under conditions of stress or disturbance when in stress is further reported by Awouda (1973) though his suggestion of defence against infection has been rejected.

4.1.3. Production

Gum is produced either naturally (spontaneous exudation) or after an injury. In countries like Sudan where gum production is an established activity, tapping is the common source of the commercial product. The process is carried out by cutting and peeling off pieces of bark, 10 - 20 cm long by 2 - 4 cm wide from branches of the tree using either a traditional tapping axe or a

sonke (modified spear like equipment designed for tapping). Gum exudes from the wounds as droplets of clear viscid fluid which increase in viscosity as they loose water by evaporation and harden from the outside. Continuous exudation forces the outer skin to break repeatedly allowing the droplet (nodule) to increase in size until the flow rate declines and the outer skin becomes too thick and hard. The size of the nodule is variable and ranges from 2 - 10 cm in diameter (Obeid and Seif El Din, 1970). The first crop of nodules takes 3 - 6 weeks to harvest, the exact period depending on climatic conditions. Subsequent crops are harvested at shorter intervals of 1 - 2 weeks.

4.1.4. Grading

Is done to improve quality of the gum coming into the market. The practice, in principle, involves sorting gum nodules by hand according to their size and colour. The method of grading varies among the major producing countries (Adamson and Bell, 1974). In Sudan for example, the main grades are:

- "natural" grade - consists of gum arabic as it is picked from the tree with all associated impurities
- "cleaned" grade - one where impurities like bark, twigs and other varieties of gums together with smaller fragments of dust have been removed.
- "cleaned and sifted" - as for cleaned grade but where smaller pieces of gum have also been removed
- hand picked selected grade - a special grade that consists of only better pieces of gum, essentially the larger pieces of uniform pale colour.
- "siftings and dust" - the waste from other grades, particularly the cleaned and sifted grades.

At present, the Gum Arabic Company of Sudan has adopted only the cleaned, cleaned and sifted and hand picked selected grades for export. These grades are regarded internationally as model grades for both quality and price.

In Nigeria, the main grade of gum arabic is called 'Falli' or 'kolkol'. It is of good appearance and quality comparable to the kordofan gum though inferior as it tends to produce a slightly dark colour in solution. French speaking countries in West Africa appear to export their gum under more or less same conditions. Principal producers are Senegal and Chad and smaller quantities also come from Mali, Niger and Mauritania. About six grades are recognised (Admson and Bell, 1974).

- gomme blanche - colourless and comparable to kordofan hand picked selected.
- gomme petit blanche - small pieces of the same
- gomme blonde - a darker colour
- gomme petit blonde - small pieces of the same.
- gomme vermicelle - a whitish to pale yellow gum
- gomme fabrique - rejected pieces of gum (because of their dark colour).

4.2. Properties of gum arabic

4.2.1 Physical and chemical properties

Gum arabic readily dissolves in water forming a slightly acidic solution with a pH range between 4.2 and 4.6. The acidity is due to glucuronic acid and its 4-O-methyl ether (Smith, 1939). Some of the free carboxyl groups are partly neutralised by calcium, magnesium, sodium, potassium and other cations in smaller amounts notably, iron, copper, zinc and manganese (Adamson and Bell, 1974; McDougal, 1987). It can thus be also referred to as the part neutralized salt of an acidic polysaccharide. Good quality gum arabic dissolves in water to give colourless or pale yellow solutions with a sweet smell. Gums that give coloured solutions, are less soluble or have a distinct rotten or irritating smell

are considered to be of poor quality. In solution, gum arabic gives a negative optical rotation. Typical values for Sudanese and Nigerian samples have specific rotation values between -27° to -33° (Anderson et al, 1990). It is long known that optical rotation is influenced by the composition and nature of the sugars present (Stoddart,1971).

One of the important physical properties of gum arabic is its ability to dissolve in water to yield solutions of very high concentrations (up to 55%). At 5% (w/v) it forms solutions of low viscosity in comparison to other naturally occurring hydrocolloids. This property makes the gum very useful commercially (Whistler , 1959). Analytical studies on a wide range of samples of gum arabic gave mean intrinsic viscosity values of 16 ml/g for Sudan and 18 ml/g for Nigeria with a range from 13-22 ml/g for a 1% concentration (Anderson et al, 1990). The viscosity of gum arabic has been shown to be closely related to molecular weight (both being dependent on molecular size distribution and shape). Measurement of intrinsic viscosity allows the estimation of a molecular weight value from an expression (Mark Houwink) of the form $(\eta) = K.Mw^a$ (Anderson and Rahman, 1967) where:

(η) = intrinsic viscosity (ml/g)
K = a constant characteristic of the polymer and solvent at a specified temperature.
Mw = weight average molecular weight.

a = a constant related to the shape of the polymer.

Anderson and Rahman (1967) deduced the values as $K= 1.3\times10^{-2}$ and a=0.54 for gum arabic. It has long been established that the molecular weight distribution is broad and skewed and has values ranging from 0.1×10^6 to 1.18×10^6. A value of 0.58×10^6 has been considered as most representative. Vandevelde and Fenyo (1985) found values from 0.44×10^6 to 2.2×10^6 for gum arabic based on laser light scattering technique. Because of the broad distribution exhibited, the term heteropolymolecular is also applied to such a gum i.e. a polymer system having either a variation in monomer composition

and/or a variation in the mode of linking and branching of monomer units in addition to a distribution in the molecular weight (Anderson and Stoddart, 1966).

Complete hydrolysis of gum arabic with dilute acid yields D-galactose, L-arabinose, L-rhamnose and D-glucuronic acid and its 4-O-methyl ether (Cree, 1966). Cree gave a detailed review of the properties of gum arabic which revealed that the molar proportions of sugar residues in the gum are of the order of 3:3:1:1 for galactose, arabinose, rhamnose and glucuronic acid respectively. However, values seem to vary from 3:2:1:1 to 3:3:1:1 to 4:2:1:1 (Anderson et al, 1990, 1991) which appear to reflect variation in regions and variety. The acidic component in the gum is usually expressed as uronic acid anhydro sugar. Analysis shows that it consists of glucuronic acid and its 4-O-methyl ether with values ranging from 13-25% and 0.24-1.5% respectively (Cree, 1966; Anderson et al, 1990).

Gum arabic also contains proteinaceous material covalently bonded to the polysaccharide. Values of protein content vary from 1.9-2.3% though higher values of 4.7% have been observed in some gums (Anderson et al, 1990; 1991). The peptide/protein part contains eighteen amino acids of which hydroxyproline, proline, serine, threonine and leucine account for 82% (Anderson and McDougal, 1987). Most of the amino acids are contained in the internal structure of the gum (i.e. in the branched galactan core) with only a smaller part associated with the periphery. This partly explains why the amino acid content of the gum cannot be readily reduced by mild chemical treatments or by action of enzymes. Further, gum cannot be completely deprotenised without gross degradation of the gum molecules and destruction of its functionality (e.g. emulsification) and surface activity.

As earlier mentioned, gum arabic contains cations which exist as part neutralised salts of acidic polysaccharides. A total of fourteen cations have been detected in the gum though calcium, potassium, sodium and magnesium

are considered as most abundant (Douglas, 1989). The actual amount depends on the relative abundance of the elements in the soils at different locations. Higher occurrence of some elements, particularly the heavy metals for which upper limits are specified by regulatory authorities can lead to rejection of such gums for food uses (Anderson and Weiping, 1991).

4.2.2 Molecular structure and properties

The first elaborate description of the structure of gum from *Acacia senegal* was by Anderson and Stoddart (1966) based on the results of sequential Smith degradation (Goldstein et al, 1965). They showed that the gum molecule consists of β-D-(1-3) galactan core and β-D-(1-6) linked galactan branches ramified with side chains of arabinose, rhamnose and glucuronic acid as terminal groups. Subsequent work on whole and partially hydrolysed gum subjected to second Smith degradation followed by gel permeation chromatography showed that the gum consist of uniform subunits of β-D-(1-3) galactopyranose residues of about 8000 molecular mass (Churms et al; 1983) while further work by Street and Anderson (1983) revealed that the lowest Smith degradation product has about 116 β-D-(1-3) linked galactopyranose blocks with β-D-(1-6) linked branch units. Re-interpretation of the revised Street and Anderson structure (1983) using computer modelling and stepwise reconstruction of structures of precursors has led to a possible structure shown in Figure 4.1 (Osman, 1993). In the structure arabinose occurs partly as short arabinofuranosyl side chains and partly as arabinopyranosyl end groups of β-D-(1-3) linkage. Rhamnose is present entirely as end group and is said to be attached to carbon 4 of glucuronic acid. The linkage is thought to be either α-L-rhamnopyranosyl (1-4) β-D-glucuronic acid or its β-D glucuronic acid (4-ome).

The above structure is based entirely on the carbohydrate component of the molecule. However, as previously stated, gum arabic is also known to contain small amount of protein (Anderson and Stoddart, 1966) and hence

belongs to a group of proteoglycans known as Arabinogalactan Proteins (Fincher et al, 1983; Akiyama et al, 1984). Confirmation that it is indeed an arabinogalactan protein complex came from the work of Vandevelde and Fenyo (1985) who used size exlusion chromatography to separate the gum into two components; a major component comprising 70% of the total gum but deficient in protein and a minor protein rich component consisting of 30% of the gum. On the basis of this information, two models describing the structure of gum arabic as an Arabinogalactan Protein (AGP) have been proposed: the "wattle blossom" and the "twisted hairy rope".

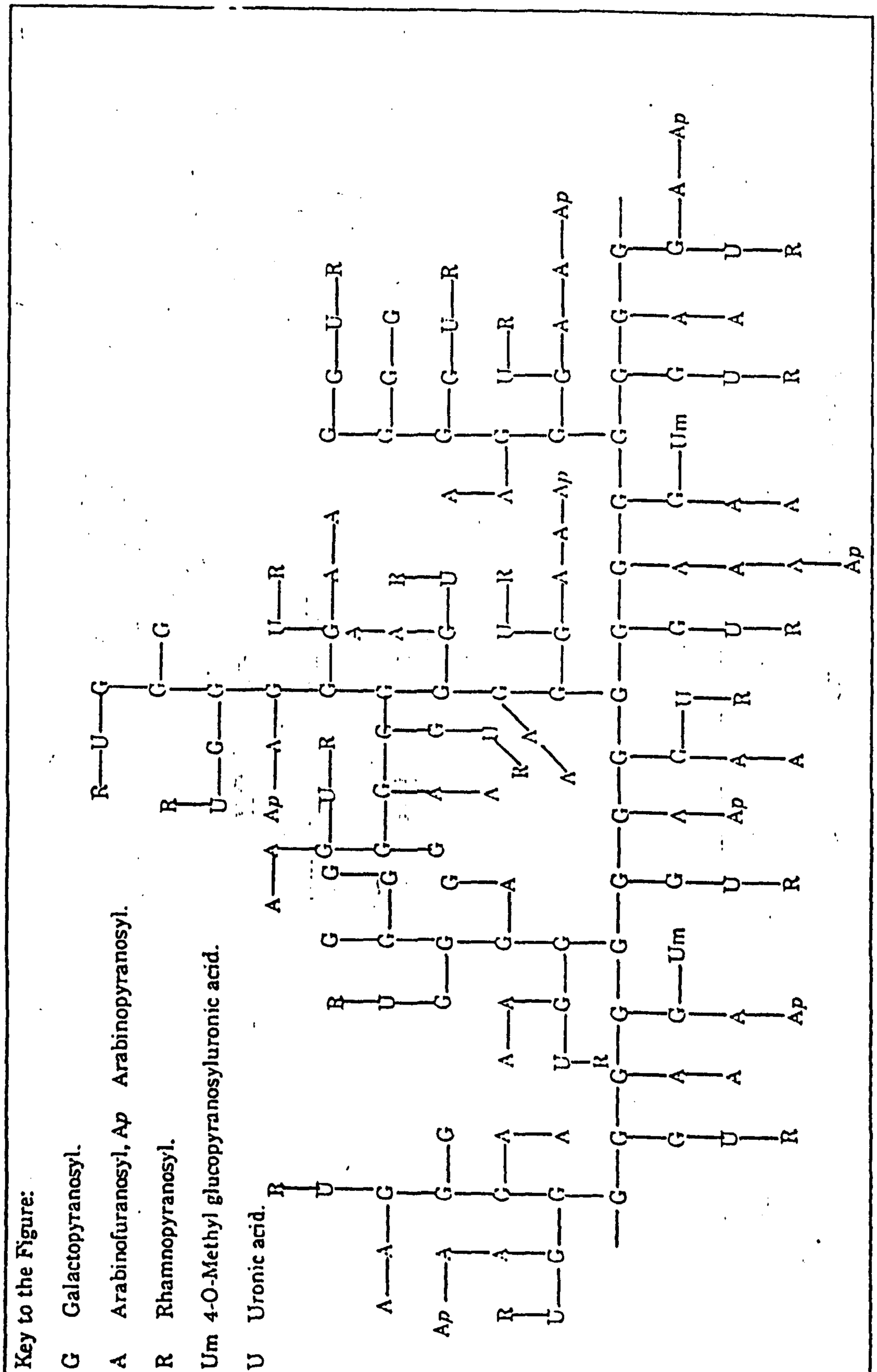
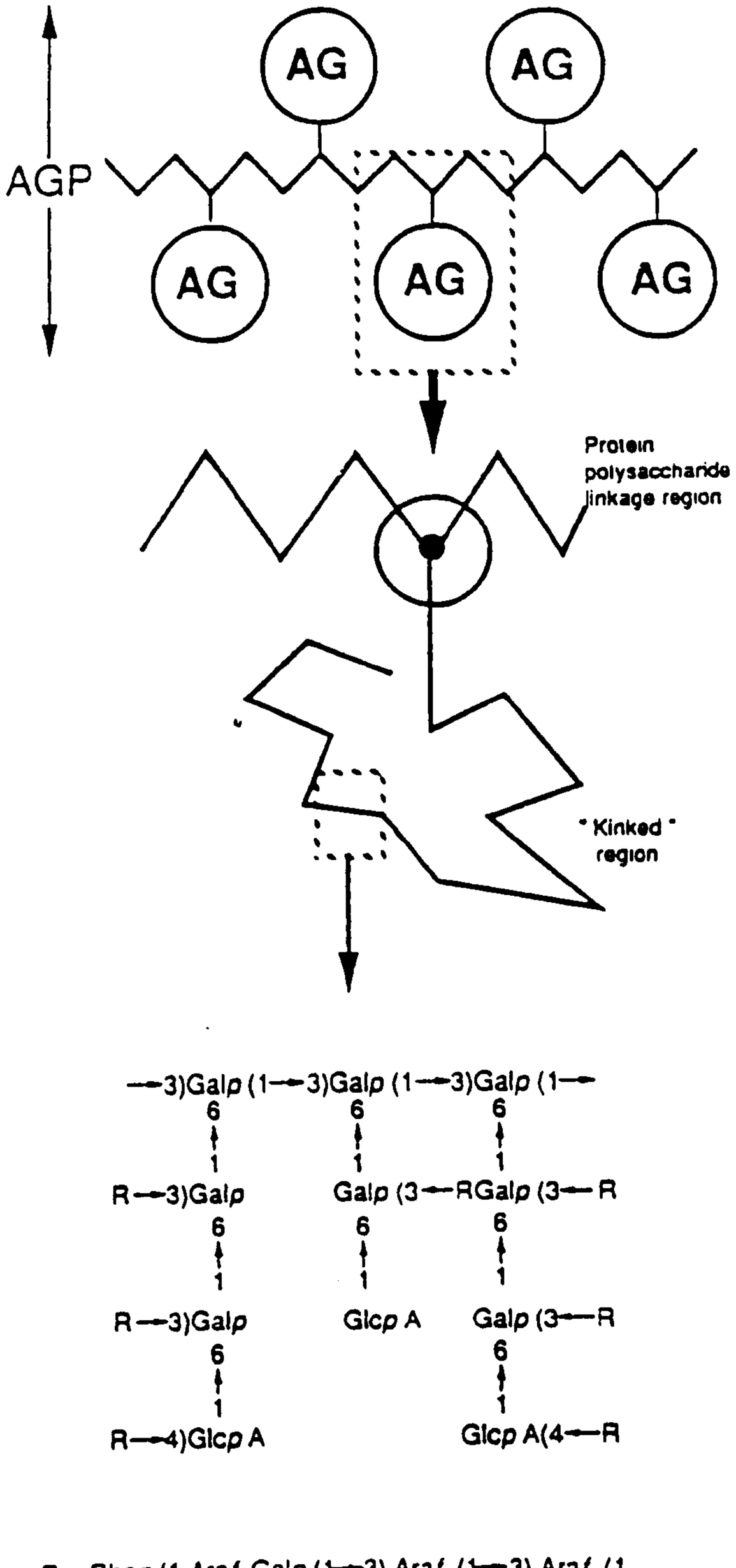


Figure 5.1: The structure of the polysaccharide from Acacia senegal (Street and Anderson, 1983)

The "wattle blossom" model was first proposed by Fincher et al (1983) in which the AGP is envisaged to have a wattle blossom appearance with clusters of ovoid or spheroidal Arabinogalactans (AGs) covalently linked to a polypeptide core (Fig. 4.2). In the light of this model, subsequent work on the AGP fraction of gum arabic using gel permeation chromatography (gpc) and hydrophobic interaction chromatography (hic) techniques led to the conclusion that each AGP fraction consists of five carbohydrate blocks of molecular mass of 2.8×10^5 covalently linked to a polypetide chain of approximately 1600 amino acid residues (Connolly et al, 1988; Randall et al, 1989a).

The "twisted hairy rope" model was proposed by Qi et al (1991). It is similar to the "wattle blossom" model with respect to the gum having a polypeptide backbone (hydroxyproline rich) to which are attached polysaccharide subsitituents. However, it differs with respect to the number of carbohydrate substituents attached to the backbone and shape of the molecule. The model envisages a hyroxyproline rich polypeptide backbone comprising of 10 to 12 repetitive amino acid units. Three of the hyroxyproline residues are linked to a tri-arabinoside chain and the remaining residues are linked through galactose to a branched "glucuronorhamnoarabinogalactan" polysaccharide substituent comprising about 30 sugar residues (Fig. 4.3). The overall peptide-saccharide arrangement represented in a statistical model would organise the polysaccharide side chains in an orderly fashion aligned along the long axis in the form of a twisted hairy rope. Using additional information from electron microscopy, they concluded that the AGPs contain a semi flexible polypetide backbone (hyp/pro. rich, ratio of 30:1) of 400 residues that make the molecule rodlike rather than spheroidal.



"Wattle blossom" - type structure of AGP

= polypeptide backbone

= AG substituent.

In an AGP there may be 25 Hyp residues each of which may bear an AG substituent, the molecule as a whole ia spheroidal.
Each AG has M.W ~ $2x10^5$

AG substituent showing blocks of 1,3 linked galactan backbone interrupted by periodate.
(Ara or 6 substituted Gal).
There may be 12 residues in each stretch of the backbone of the galactan framework between periodate suscepible linkages and perhaps 10 or more galactan stretches per substituent.

⊙ O- galactosyl-hydroxyproline linkage

Galactan backbone

Portion of AG substituent showing 1,3 linked Gal backbone and galactosyl side branches with saccharide substituents. (Data from *Acacia senegal gum* analysis). The galactan backbone may assume a helical conformation.

R = Rha*p* (1,Ara*f*, Gal*p* (1—3) Ara*f* (1—3) Ara*f* (1.

Figure 4.2: The 'Wattle blossom' model of the arabinogalactans proteins after Fincher et al (1983) and Randall et al (1989).

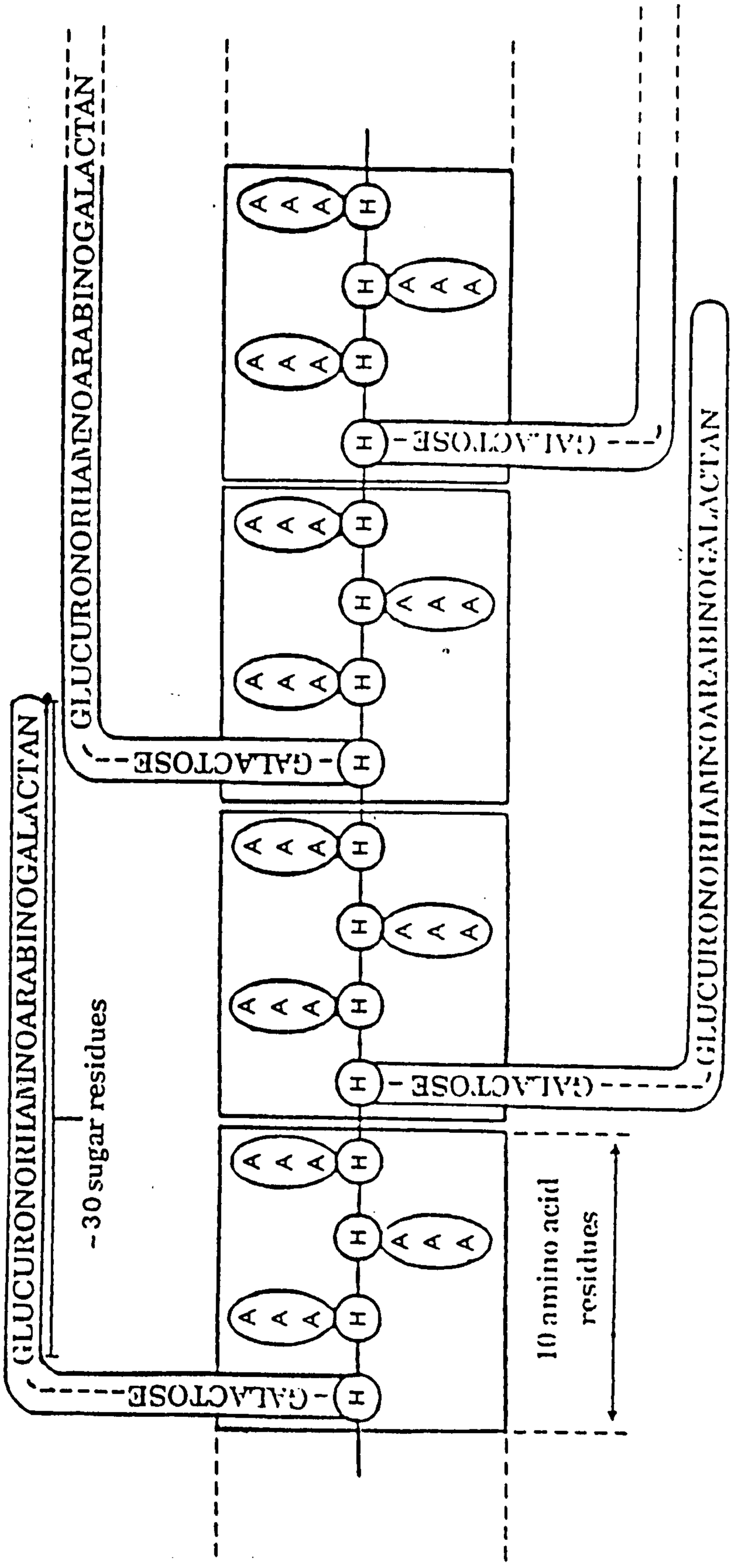


Figure 5.3: The "Twisted hairy rope" model of gum arabic glycoprotein (Qi et al,1991)

4.3. Uses

Gum arabic is used in food and non food industrial applications (Sandford and Baird, 1983).

4.3.1. Food and allied applications

The food industry is the main consumer of world production of gum arabic. The main areas are bakery, noncarbonated beverages, confectionery, soft drinks and brewing. Its major function in the bakery industry is to act as an adhesive (bakery glaze) while in confectionery it is used as a crystallization inhibitor (retard crystallization of sugar), emulsifier (assists in attaining a fine dispersion) and stabilizer i.e maintains the dispersion (Adamson and Bell, 1974). Emulsifying property is believed to be due to the gum containing hydrophobic protein moieties and hydrophilic carbohydrate residues (Randal et al, 1988). Good emulsifiers have high content of hydrophobic amino acids. The properties of emulsification and stabilization enables the gum to be used in other areas like foam stabilization. Its high solubility in water, non toxic, colourless, odourless and tasteless nature makes it a suitable additive to various formulations to provide "body", "mouthfeel" and texture to foods. It has been awarded the status "Acceptable Daily Intake (ADI) Not Specified" by FAO (1982) following extensive toxicological studies.

To prevent gum from botanical sources other than that from *A. senegal* being used in the food industry without having been subjected to positive toxicological evidence of safety, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recently published a revised specification for gum arabic (FAO, 1990). According to the document, gum arabic must satisfy all of the following criteria:

- solubility - gum arabic dissolves overnight in twice its volume of cold water. The solution flows readily and is acid to litmus; it is insoluble in ethanol.
- hydrolysis products - on hydrolysis it yields galactose, arabinose,

rhamnose and glucuronic acid

The following specifications must be met:

- loss on drying - not more than 15% (105°,5h)
- specific rotation - between -26° to -34° (calculated on dry weight basis).
- total ash - not more than 4%.
- acid insoluble ash - not more than 0.5 %
- acid insoluble matter - not more than 1%
- starch and dextrin - absent
- tannin bearing gums - absent
- nitrogen - 0.27% to 0.39%
- arsenic - not more than 3 mg/kg
- lead - not more than 10 mg/kg
- heavy metals - not more than 40
- microbial criteria - salmonella sp. negative in 1 gm; E. coli negative in 1 gm.

The above criteria are now the subject of further review

4.3.2. Industrial applications

4.3.2.1. Pharmaceutical and cosmetic industry

The properties of gum arabic as an emulsifier/stabilizer and binding agent lead to its use in the above industry. It is used in coating sugar coated pills and drug encapsulation. It has been used in the preservation of vitamin A in margerine and stabilisation of vitamin C in aqueous solutions. Moreover, it produces a smooth viscous syrup and prevents crystallization of sugar in the preparation of cough syrup (Adamson and Bell, 1974). Its use in the cosmetic industry arises from the non toxic nature and low tendency to produce allergic reactions. It is thus used in lotions and protective creams where it stabilises the emulsion and forms a protective coating.

4.3.2.2. Other uses

The other industries in which gum arabic has found application include textiles, films, printing inks and adhesives. In the textile industry, the gum is used in finishing silk and rayon, as a sizing agent and in imparting patterns to the fabrics. The principal function of the size is one of film forming, film elongation and film tensile strength. Because of the cost however, starches are most commonly used on cotton and rayon and gum is only limited to specialised application. For economic reasons therefore less expensive non food grades are more preferred. Gum arabic has many functions in the film industry (or lithography). It is an important ingredient of the foundation solution and a protector during storage of the plate. Advantage is taken of its ability to add good wettability and provide viscosity control over the solutions used as a base for light-sensitive chemicals.

It is used in printing inks as a thickener to provide proper viscosity for application and to increase the stability of some products. It is thus an important ingredient in the manufacture of lithographic, letter press and screen printing inks, particularly the water and water-alcohol based types because of its suspending, emulsifying and protective colloid properties. Inorganic thickeners like fumed silica and modified bentonites are the most common in oil and alcohol based inks. The binding properties of the gum and ability to be made into a mucilage makes it suitable for use as an adhesive for general domestic and office use, on envelopes and stamps. It has also found uses in other adhesives as a thickener to control viscosity of the product, improve the handling (flow) during application and improve the finished product (film thickness). In the latter, it has been used either alone or in combination with poly(vinyl acetate) emulsions.

CHAPTER 5: CHARACTERISATION OF GUM ARABIC FROM *ACACIA SENEGAL* VAR. *KERENSIS* FROM KENYA

5.1: Introduction

Sudan is the principal producer of gum arabic of commerce which, until recently, accounted for about 85% of the world production. The remaining amount comes from Nigeria, Senegal and Chad with minor quantities from Mauritania, Mali and Niger. However, vast resources of *A. senegal* are also found in some countries of the Sahel notably the Horn of Africa including East Africa and Southern Africa. In Kenya, *A. senegal* grows extensively in the Arid and Semi Arid Lands found to the northern, eastern and southern parts of the country in what is known as the *Acacia-Commiphora* bushlands.

Production and trade in gum arabic started in Kenya in 1990 and the activity is expanding rapidly. However, knowledge on the quality of gum arabic produced is still scant and hence confidence in the product among established international merchants is low. Available information seemed to indicate that gum arabic from Kenya and Tanzania was dark or brown thereby excluding it from use in food applications (Robbins, 1987). It is now known that such gum was either a mixture of gums from other sources or had a high content of iron, reflecting abundance of the cation in the soils. The main problem however, arose from uncertainty as to the precise identity of *A. senegal* in East Africa. The main varieties of *A. senegal* found in the sub region have been covered in chapter 2 while chapter 3 has identified *A. senegal* var. *kerensis* as the main source of gum arabic in Kenya. The later variety is different from the one in Sudan based on the work of Brenan (1983). Meanwhile apart from the work of Anderson and Weiping (1990) who suggested that the difference observed between a gum sample from Marsabit, Kenya and those from Sudan and Nigeria could be due to different varieties, no work has been reported which acknowledges variation in *A. senegal* as varietial.

The present study was carried out in order to establish mean (hence typical) properties of gum arabic from variety *kerensis* based on an extensive

sampling programme. Comparison was then made with gums from known sources of Sudan, Nigeria and Uganda as well as any differences between regions and samples.

5.2. Materials and methods

5.2.1 Origin of gum samples

Fifty seven samples of good quality gum (i.e clear or amber) were collected during the survey of gum arabic resources from single trees of *A. senegal* var. *kerensis* in three districts as follows:

a) Sixteen samples from Isiolo District coded as I.

b) Thirty one samples from Marsabit (15 from Ngurunit and 16 from Kargi) coded as NM and KM.

c) Ten samples from Turkana District (5 from Lokichar and 5 from Kakuma) coded as LT or KT.

Each sample was packed separately and properly labelled to indicate tree number, origin, mode of exudation and date of collection. Details of the samples are given in appendix IV.

In the laboratory, each sample was examined visually and graded as clear or amber on the basis of colour. The samples were cleaned by hand to remove bark and other foreign matter where possible though most of them were of relatively pure form. Representative specimens were ground to powder in a Kika Werk model grinder.

5.2.2. Analytical methods

The analytical methods used in this study can be summarised into two groups:

. physical methods

- moisture content
- ash content
- pH and gel
- specific rotation

- intrisic viscosity

- emulsification properties

. chemical methods

- nitrogen content

- glucuronic acid content

- neutral sugars determination

- amino acid content

- tannin content

- metal content

Most of the above methods are those used or developed by Dr. Anderson and his group in the gum research laboratory, University of Edinburgh, unless otherwise stated.

5.2.2.1. Moisture content.

150 mg gum was heated at 105°C in an oven overnight. The samples were allowed to cool in a dessicator before reweighing. Moisture content was expressed as a percentage ratio of lost to original weight.

5.2.2.2. Ash content

Gum from the above experiment was heated in a muffle furnace at 550° C until completely ashed. Ash content was expressed as a percentage of ash to dry gum weight.

5.2.2.3. pH and gel determination

A 25% gum solution (w/v) was prepared by dissolving the gum in water at room temperature overnight. pH was determined using a pH meter (alpha 200 model) at room temperature after calibration with standard buffer solutions at pH 7.0 and 4.0. Gelling properties were determined from the same solution qualitatively. A four point scale was established on the basis of whether there was no gel (-), light gel (+), moderate gel (++) or heavy gel (+++) (Anderson ,1992).

5.2.2.4. Specific rotation

100 mg gum was dissolved in 10 ml water (1% w/v) overnight. The solution was filtered, placed in a glass cell of path length 10 cm care being taken to avoid air bubbles getting trapped in the solution and measured in a Perkin Elmer Model 141 polarimeter set at 589 nm wavelength and 20° ±2°C. The degree of rotation was displayed and read from the instrument panel and corresponding specific rotation was calculated from the relationship:

$$[\alpha]_D = 100\alpha/L.C$$

Where:

α = the measured rotation

D = wavelength of light (Sodium line, 589 nm)

L = path length in decimeters

C = concentration of solution in gm/100 ml

The results were corrected for loss on drying.

5.2.2.5. Intrinsic viscosity

200 mg gum was dissolved in 20 ml of molar sodium chloride overnight. The gum was filtered and 12 ml of clear solution measured by flow time in an Ubbelohde suspended level dilution viscometer at 25 ±0.1°C. The times were measured to within 0.1 second by means of a stop watch. Flow times were also obtained for successive dilutions with M-Sodium chloride solution (four additions of 2 ml each).

Assuming the densities of M-Sodium chloride and gum solutions to be equal for low concentrations of gum, the intrinsic viscosity number (η) is given by:

$$(\eta) = \lim_{C \to 0} \frac{\eta sp}{C} = \lim_{c \to 0} \frac{t - t_o}{Ct_o}$$

Where C is the concentration of gum solution (g/ml), ηsp is the specific viscosity and t_o and t are the flow times (seconds) for solvent and solution respectively. Linear extrapolation of the plot $\frac{t - t_o}{Ct_o}$ against concentration C (when C→0) gives (η), (Cowie, 1973). The value (η) in ml/g is obtained as the Y-intercept.

5.2.2.6. Nitrogen content

Two procedures based on the kjeldahl method were used: back titration and the semi kjeltic auto analyser method

. using back titration method

150 mg gum sample was heated under reflux with 3 ml of conc. sulphuric acid using copper and potassium sulphate (ratio 1:20) as catalyst until all the nitrogen was converted to ammonium sulphate giving a clear blue solution. The solution was made alkaline with 15 ml NaOH (35% w/v) and total gaseous ammonia liberated estimated by bubbling the vapours through 5 ml of 0.014 M hydrochloric acid. Back titration of the excess acid with 0.014 m NaOH gave ammonia content from which the nitrogen content was calculated as:

$$N\% = \frac{V(HCl)\times M(HCl) - V(NaOH)\times M(NaOH)\times 14\times 100}{\text{weight of the sample}}$$

Where:

V = volume in ml

M = molarity

The above method was used in Edinburgh

. Direct titration with boric acid using kjeltec auto analyser

500 mg of gum samples were transferred into digestion tubes. 15 ml conc. sulphuric acid plus sufficient amount of catalyst (sodium sulphate, copper sulphate and Selenium as catalyst, ratio 20:2:1) were added to the tubes and

then placed in the "Tecator" digestion heating system (model 1015) preheated to 240°C. Complete digestion is attained when the heated solutions turn to a pale blue colour indicating that gum nitrogen has been converted to ammonium sulphate. The tubes were allowed to cool for 10 minutes and 70 ml of distilled water added. Two tubes containing 15 ml sulphuric acid plus catalyst (as blanks) were included in the digestion. The solutions were made alkaline with 50 ml of 10M (40% w/v) sodium hydroxide in a kjeltec distillation "Tecator" unit (model 1002) followed by steam for five minutes.

The total gaseous ammonia liberated was estimated by bubbling through 50 ml of 2% boric acid with bromophenol blue as indicator. The resulting solution was titrated against 0.025M sulphuric acid. Nitrogen content was calculated from the relationship:

$$1 \text{ ml } 0.5M\ H_2SO_4 \equiv 14 \text{ mg N};$$

$$\therefore 20 \text{ ml } 0.025M\ H_2SO_4 \equiv 14 \text{ mg N}$$

$$\text{Hence } N\% = (Y \text{ ml} \times \frac{14}{20X}) \times 100$$

where:

Y ml = volume of $0.025M\ H_2SO_4$ used
X mg = weight of gum sample used (gm).

5.2.2.7. Uronic acid anhydro and hence glucuronic acid

50 mg gum was dissolved in 20 ml of water (in a 250 ml conical flask) overnight. Gum solution was filtered and uronic acid anhydride determined by method of cation exchange resin. Amberlite cation exchange resin, IR-120 (H^+ form) was packed half full in a glass column. The column was washed with 0.1M sulphuric acid and excess acid removed by washing down the column with deionised water until neutral. A sample of gum solution was then passed down the column slowly, the eluent being collected in a 250 ml conical flask. Three bed volumes of deionised water was washed down the column and

collected in the conical flask. The combined volume was titrated against 0.01M sodium hydroxide using phenolphthalein as indicator.

Thus the acidity of the gum solution was measured and equivalent weight (in grams) of dry salt free gum required to neutralize one mole of sodium hydroxide estimated as:

$$\text{Eq. wt. (gm)} = \frac{\text{sample wt. g} \times 1000}{\text{Vol. of titre} \times \text{Molarity of NaOH}}$$

Hence uronic acid content is calculated from equivalent weight as:

$$\text{UAA} = \frac{\text{molar mass of UAA} \times 100}{\text{acid eq. wt.}}$$

Where:

UAA = uronic acid anhydro

Glucuronic acid is expressed as a percentage of uronic acid anhydro because that best quantifies the amount of glucuronic acid present in a polymerised form (Anderson, 1993).

5.2.2.8. Emulsification properties

5% gum solution (w/v) was left overnight to dissolve. The solution was clarified by centrifugation and 2 ml of the clear gum solution were transferred to two test tubes and covered with 0.5ml limonene. The resulting mixture was treated in an Ultra Turrax homogeniser, model T25 set at 15,000 revolutions per minute (speed preset using a strobe light) for 60 seconds.

0.25ml of the treated mixture was removed by a syringe and placed in a test tube containing 25 ml of distilled water (a 1-in-100 dilution). The mixture was shaken well and a sample removed and its absorbance determined in a UV spectrophotometer (Hewlett Packard 8452A Diode array) set at 500 nm wavelength. The absorption given by the resulting emulsion is quoted as emulsion activity (EA) i.e. ability to form emulsion (James and Patel, 1988; Anderson et al, 1991). The mixture was left for 30 minutes and a sample taken from the lower half. The absorbance obtained after storage for 30 minutes

expressed as a percentage of emulsion activity is quoted as the emulsion stability from the relationship:

$$ES\% = \frac{\text{Absorbance at 500 nm of emulsion stored for 30 minutes}}{\text{Emulsion activity at 500 nm of fresh emulsion}} \times 100$$

Where:

ES = emulsion stability

5.2.2.9. Sugar content

500 mg gum was hydrolysed with 0.5M sulphuric acid at 100°C for 14 hours. Hydrolysates were neutralised with barium carbonate, filtered, deionised with Amberlite cation exchange resin (IR-120, H^+ form), filtered and concentrated to a syrup in a rotary evaporator at 40°C.

Sugars were separated by applying a band of hydrolysate syrup on Whatman 3MM papers using ethyl acetate: pyridine: water (10:4:3, v/v/v) as solvent in a descending column for 14 hours. Neutral sugars were located by staining side strips with diphenylamine/aniline/phosphoric acid reagent (Chaplin and Kennedy, 1986), bands were cut and corresponding sugars eluted from the paper with 20 ml boiling water in boiling tubes for 30 minutes in an oil bath. Eluted sugar solutions were filtered and volume adjusted to 25 ml. 2 ml in duplicate for each sugar were placed in test tubes and reacted with 1 ml phenol (5%) and 3 ml conc. sulphuric acid for 10 minutes to allow colour to develop. The absorbance of the resulting solution was determined on a UV spectrophotometer at 430 nm. The sugar content (as a ratio) was calculated from the corresponding sugar factors (from calibration curve) after correction for glucuronic acid (Anderson and Stoddart, 1966; Anderson, 1992).

5.2.2.10. Amino acid content

The analysis was carried out at the Department of Chemistry, University of Edinburgh. 50 mg gum was weighed and transferred to a 100 ml round bottomed two necked flask. Anti-bumping granules and 20 ml of 6M hydrochloric acid were added and the flask connected to an 800 mm air cooled

condenser. The apparatus was purged with oxygen free nitrogen and contents heated at 180°C under reflux for 18 hours. The solution was filtered through Whatman No. 42 filter papers and evaporated to dryness at 40°C. The residue was dissolved in 4 ml of 0.2M sodium citrate buffer (pH 2.2), filtered through 0.22 micro meter Millipore filter and stored frozen for analysis.

Analysis was effected with a Rank Hilger chromaspec analyser. About 60 micro litre was applied to a 350 × 3 micro metre stainless steel column of cation exchange resin and constituent amino acids separated by high pressure chromatography by elution with lithium citrate buffers of increasing ionic strength and pH. The eluted amino acids were detected by reaction with ninhydrin in a continous flow analytical system and quantified by reference to standard solutions at 570 nm (and 440 nm for proline and hydroxyproline). The amino acid composition is reported as residues of amino acid per 1000 total amino acid residues.

5.2.2.11 Tannin content

Tannin was determined according to the procedure outlined by FAO (1990). A 2% solution was dissolved overnight. The solution was centrifuged and 0.1ml ferric chloride added. A blackish colouration or precipitate was indicative of the presence of tannin.

Samples which showed a positive test for tannin were examined further to determine the amount of tannin. A 0.5% solution was left overnight to dissolve, filtered and 0.1ml of 9% ferric chloride solution added. The absorbance was determined on a UV spectrophotometer set at 312 nm since earlier wavelength scan for gum ferric chloride complex had shown peak absorbence at 312 nm. Calibrations were effected with tannic acid solutions. Absorption of ferric chloride solution was used as blank.

5.2.2.12. Metal content

200 mg ash was dissolved in 10 ml conc. nitric acid and the solution made up to 25 ml in a volumetric flask. About 10 ml was carefully removed and used in the determination of trace elements by Atomic Absorption Spetroscopy. Aluminium and Chromium were analysed using an

acetylene/nitrous oxide flame while the other trace elements using an acetylene flame. Meanwhile, 1 ml was removed from the stock solution (original 25 ml) and 100 ml water added. The resulting solution was used for analysis of potassium, sodium, calcium and magnesium also by Atomic Absorption Spectroscopy. For each metal, standard solutions were prepared with pure metal salts and used to calibrate the spectrometer.

5.3 Results.

5.3.1 Properties of gum arabic from Kenya and comparison with data from main commercial sources

Analytical data relating to the physico-chemical and carbohydrate parameters are presented in Table 5.1. The Table gives mean values together with standard deviation (std. dev.) and coefficient of variation (coef. of var.) for each property based on the total of 57 samples. In addition, representative data of gum arabic from Sudan, Nigeria and Uganda as well as proposed FAO (1990) specification for food grade gum arabic are included for comparison.

Moisture and ash contents compare favourably with gum arabic from the three countries and fall within the proposed FAO specification for food grade. Three properties i.e. specific rotation, nitrogen content (hence protein) and intrinsic viscosity differ appreciably. Specific rotation had a higher value in comparison with gum from the other three countries though it was within the proposed specification. The greatest difference was observed in the nitrogen content which was higher than gum from the other countries and exceeded the upper limit set by FAO by about 13%. The gum from Kenya was also more viscous. Intrinsic viscosity was higher by between 22 - 45%.

An examination of the emulsification properties showed that gum from Kenya has better emulsification activity and comparable emulsification stability though it had a tendency to form gel that became more evident at a solution concentration of 25%. Gum from Uganda shows relatively lower emulsification functionality. The Kenyan gum did not contain tannin and pH was similar to

that of gum from other sources. Compared to the mean values of gum from the three countries galactose content was relatively lower by 15% while arabinose and rhamnose were higher by 17 and 23 percent respectively.

Table 5.1. Comparison of physico-chemical and carbohydrate data for gum arabic from Kenya and other commercial sources

	Kenya n=57	std. dev.	coef. of var.	Sudan *	Nigeria *	Uganda **	FAO 1990
Loss on drying (%)	14.7	0.4	2.7	13.0	13.0	14.3	<15
Total ash (550°C,%)	3.0	0.2	6.7	3.6	3.7	3.9	<4
Specific rotation (deg)	-34	2	5.9	-30	-30	-31	$-26°$-34^0
Nitrogen content (%)	0.44	0.04	9.1	0.34	0.35	0.27	0.27 to 039
Hence protein (NCF×6.63)	2.9	0.2	6.9	2.3	2.3	1.8	1.8 to 2.6
Intrinsic viscosity (ml/g)	21.9	3.4	15.5	16.0	18.0	15.1	
Mw $\times 10^6$	0.94	-	-	0.53	0.66	0.47	
Neutralization equivalent. weight	995	40	4.0	1050	980	1071	
Hence Uronic acid anhydro (%)	18	1	5.6	17	18	17	
Emulsification activity	1.66	0.04	2.4	1.60	nd	1.51	
Emulsification stability (%)	93	1	1.1	95	nd	60	
Tannin (2% sol.)	0	0	0	0	0	0	
Gel (25% sol.)	+			nd	nd	nd	
pH (25% sol.)	4.4			4.3	4.3	nd	
Sugar composition after hydrolysis							
Glucuronic acid	18	1	5.6	17	18	17	
Galactose	39	4	10.3	44	47	47	
Arabinose	28	2	7.1	25	23	25	
Rhamnose	16	2	12.5	14	12	12	

Note:

*→Anderson et al, 1990

**→Anderson and Weiping, 1991

NCF→ Nitrogen conversion factor

+ → gel present

nd → not determined

Mw → calculated from the Mark Houwink equation

Results of amino acid composition based on 30 samples including std. dev. and coef. of var. representative of the study area together with data for Sudan, Nigeria and Uganda are presented in Table 5.2. Kenyan gum showed higher amount of threonine but lower arginine, aspartic acid, lysine, isoleucine and phenylaline in comparison with the other countries. It is worth noting that coef. of var. for cystine was exceptionally high due to the high variation between samples. The content of cystine varied between 0 and 12 ppm. Gum from Uganda shows relatively higher alanine, glutamic acid, lysine, tyrosine and valine but lower hydroxyproline and serine. The other differences are minor.

Table 5.2. Comparison of the amino acid composition (residues per 1000 residues) for gum arabic from Kenya and gum from other commercial sources

	Kenya n=30	std. dev.	coef. of var.	Sudan *	Nigeria *	Uganda **
Nitrogen (%)	0.45	0.07	15.6	0.34	0.35	0.27
Alanine	25	3	12.0	27	24	38
Arginine	8	1	12.5	13	12	14
Aspartic acid	45	4	8.9	68	61	65
Cystine	3	2	66.7	2	0	6
Glutamic acid	38	3	7.9	42	42	54
Glycine	53	4	8.0	50	50	52
Histidine	53	3	5.7	44	48	52
Hydroxypro.	322	15	4.7	304	335	235
Isoleucine	10	1	10.0	12	13	15
Leucine	71	3	4.2	66	69	67
Lysine	19	2	10.5	25	24	44
Methionine	1	0	0	2	1	1
Phenylalanine	26	2	7.7	33	29	48
Proline	66	8	12.3	63	55	66
Serine	138	3	2.2	129	129	116
Threonine	87	4	4.6	68	67	67
Tyrosine	14	1	7.1	14	14	20
Valine	32	4	12.5	35	32	43
Hence NCF	6.63	0.09	1	6.62	6.65	6.61

Where:

*→Anderson et al, 1990

**→Anderson and Weiping, 1991

Metal content was also analysed and mean values together with std. dev. and coef. of var. compared with gum from the three countries. Results are presented in Table 5.3. Gum from Kenya had on average lower total metal content as reflected in the lower ash. An examination of the elements present revealed comparable values for calcium, cobalt, copper and magnesium with gum from Sudan and Nigeria. It had lower values for chromium, lead, manganese, nickel, potassium, sodium and zinc but higher values for aluminium and iron. Two samples had higher content of lead and chromium arising from appreciable differences between samples that did not seem to reflect regional differences. Differences were observed between gum from Kenya and Uganda. The latter showed higher levels of aluminium, chromium, cobolt, copper, iron, lead, mangenese, nickel, potassium, sodium and zinc. Several of these elements are heavy metals whose limit has been specified by regulatory organisations.

Table 5.3. The cation composition (µg/g ash)

Sample code	I2	I7	I10	KM2	KM3	NM11	NM6	LT1	LT4	KT3	Mean	SD	CV	Ex-Uganda	Ex-Sudan	Ex-Nigeria
Ash (%)	3.0	3.5	3.5	1.9	2.3	3.3	2.3	3.0	2.9	2.9	2.9	0.5	17.2	3.9	3.7	3.7
Aluminium	307	539	502	266	358	428	341	289	205	245	348	105	30.2	1746	190	311
Calcium	173729	356201	366098	203390	255009	317202	187713	276436	208545	280585	262491	69090	26.3	150841	256000	316000
Chromium	9	23	9	38	17	7	7	13	25	4	15	10	66.7	994	47	34
Cobalt	0	0	0	0	0	0	0	0	0	0	0	0	0	8	<1	<1
copper	47	44	68	63	69	73	71	72	53	79	64	11	17.2	1248	52	66
Iron	1078	946	1304	1004	1083	1328	1316	691	513	682	995	274	27.5	1331	128	110
Lead	<1	8	2	0	5	5	4	0	0	0	3	3	100	34	6	11
Magnesium	44746	34969	42718	50848	50091	40667	39005	38610	38149	45148	42495	4958	11.7	37041	38000	39000
Manganese	24	49	38	14	22	38	40	50	36	44	36	11	30.6	74	100	57
Nickel	4	6	7	3	6	5	6	5	5	6	5	1	20	16	10	12
Potassium	165763	172603	175527	278450	203780	161651	226719	97960	161495	128241	177219	47675	26.9	277069	237000	221000
Sodium	2913	2440	2232	5448	3677	3050	3084	3758	3726	3013	3334	858	25.7	6847	9400	10200
Zinc	6	9	3	9	7	7	6	5	3	4	6	2	33.3	71	24	40

5.3.2. Variation in the properties

Though the mean values provide an overall picture and an indication of the chemical nature of gum arabic from Kenya, distinct differences were observed among various samples. To understand the nature of variation therefore, analytical data was compared for regions and samples within a region.

5.3.2.1. Variation between regions

Data for Isiolo, Marsabit and Turkana are presented in Table 5.4. Two regions were recognised in Marsabit (Ngurunit and Kargi) which showed some differences in the properties and are therefore presented separately. Moisture and ash contents show slight differences between the four regions. Samples from Ngurunit show slightly higher moisture content than average. Discernible differences were observed in the specific rotation, nitrogen content and intrinsic viscosity. Isiolo and Ngurunit regions have values within the FAO specification for specific rotation but values for the other two regions are higher with Turkana exhibiting greater difference. Nitrogen content was higher for all regions but also variable. Values varied between 0.40% and 0.50% with Marsabit District providing gum with the highest nitrogen content. Viscosity was also variable (16.6 to 25.9 ml/g) and Marsabit gum was the more viscous. Gum from Turkana is comparable to commercial gums from Sudan and Nigeria.

To examine if the differences observed were significant, analysis of variance was performed on the three properties and multiple comparison test carried out (using Least Significant Difference) to show regions that differed significantly. The results are presented in Table 5.5.

Table 5.4. Physico-chemical and carbohydrate data for gum arabic from four regions

	Isiolo	Marsabit		Turkana
		Ngurunit	Kargi	
	n=16	n=15	n=16	n=10
Loss on drying (%)	14.5	15.3	14.2	14.6
Total ash (%)	3.2	3.0	2.7	2.9
Specific rotation (degrees)	-33	-32	-35	-37
Nitrogen content (%)	0.40	0.43	0.50	0.42
Hence protein (NCF*6.63)	2.5	2.7	3.1	2.7
Intrinsic viscosity (ml/g)	23.5	25.9	21.4	16.6
$Mw \times 10^6$	1.08	1.29	0.91	0.57
Neutralization equiv. wt.	1003	927	1029	1021
Hence Uronic acid anhy. (%)	18	19	17	17
Emulsification activity	1.60	1.66	1.68	1.69
Emulsification stability (%)	92	92	93	95
Tannin (2% sol.)	0	0	0	0
Gel (25% sol.)	(+)	(+)	(+)	(-)
pH (25% sol.)	4.4	4.3	4.4	4.4
Sugar composition after hydrolysis				
Glucuronic acid	18	19	17	17
Galactose	39	43	41	33
Arabinose	27	25	28	31
Rhamnose	17	13	14	19

Note:

(+)→gel present

(-)→gel absent

Table 5.5. Results of analysis of variance between regions

		Isiolo	Marsabit		Turkana
			Nguurunit	Kargi	
	df				
Specific rotation	3	-33a	-32a	-35b	-37c
Nitrogen content	3	0.40a	0.43a	0.50b	0.42a
Intrinsic viscosity	3	23.5bc	25.9c	21.4b	16.6a

Regions with same letter are not significantly different at p = 0.05

Gum samples from Kargi and Turkana had significantly higher specific rotation in comparison with those from Ngurunit and Isiolo. The difference between the two regions was also significant but no difference was observed between Ngurunit and Isiolo. A comparison of the nitrogen content showed gum from Kargi to contain significantly higher nitrogen than the other three regions though no difference was observed between Ngurunit, Isiolo and Turkana. In the case of intrinsic viscosity, Turkana had siginificantly lower viscosity than the other regions. However, gum from Kargi was siginificantly less viscous than Ngurunit but no difference was observed between the latter and Isiolo or between Isiolo and Kargi.

There were slight differences in the emulsion properties between regions. Isiolo gave gum with emulsion activity similar to Sudan while values for Marsabit and Turkana were higher. No tannin was observed in the gum from any of the regions and pH values did not differ from any other. However, the results reveal that there was a tendency for the gums from all the regions to form gels. An examination of the sugar content reveal differences among the regions, particularly with regard to the molar ratios of galactose and arabinose. The former varied between 33 and 43 while the latter between 25 and 31 percent. Ngurunit in Marsabit gave gum that was more acidic while the highest value for rhamnose was recorded for Turkana.

In analysing results of amino acid composition, two aspects are of interest, (1) whether differences observed in the nitrogen content among regions could be reflected in the amino acid content and (2) whether there was a trend in the content of certain amino acids with increase in the nitrogen content. The results are presented in Table 5.6.

Table 5.6. Amino acid acid composition by region and nitrogen content

	By region				By nitrogen content		
	Isio. n=8	Ngur. n=7	Karg. n=7	Turk. n=8	<0.40 n=10	0.40-0.49 n=10	>0.50 n=10
Nitrogen (%)	0.41	0.43	0.49	0.42	0.37	0.44	0.56
Alanine	28	25	20	26	26	24	22
Arginine	8	7	7	8	8	7	7
Aspartic acid	48	45	38	47	48	43	43
Cystine	3	2	2	6	1	2	5
Glutamic acid	42	35	34	40	42	35	37
Glycine	50	51	55	58	49	56	59
Histidine	49	52	56	54	48	53	53
Hydroxypro.	298	337	324	328	308	335	301
Isoleucine	11	9	9	10	10	9	10
Leucine	76	71	71	67	73	67	75
Lysine	22	19	16	20	21	18	19
Methionine	1	1	1	1	2	1	1
Phenylalanine	29	24	24	27	28	24	26
Proline	68	52	73	68	72	58	68
Serine	134	139	143	136	130	139	143
Threonine	82	86	90	91	85	85	91
Tyrosine	15	12	12	14	17	12	12
Valine	38	32	26	31	34	30	30

There were no distinct regional differences in the amino acid composition. The only differences observed related to about one or two amino acids per region; for example Turkana showed exceptionally higher value for cystine, Ngurunit higher value for hydroxyproline but lower proline, Isiolo lower hydroxyproline and Kargi higher serine. The significantly higher nitrogen content observed for Kargi was not reflected in abundance of any amino acid or group of amino acids. Considering varying amount of nitrogen content, a trend could be seen in the content of some amino acids. The amount of alanine, aspartic acid, tyrosine and valine were lower for higher levels of nitrogen while cystine, glycine and serine increased.

Cation composition revealed comparable results for calcium, chromium, copper and magnesium (see Table 5.3). The levels of aluminium decrease from Isiolo to Turkana. The latter has also low iron and zinc. However, the

interesting phenomenon is the close similarity in the cation content among samples from same region. Samples 1-3 were from Isiolo, 4-7 from Marsabit and 8-10 from Turkana. Although there were slight differences in some cation contents, the overall picture was that samples from a particular area had similar cation composition to one another.

5.3.2.2 Variation between samples in a region

Results of the preceding section have shown that there exist regional differences in three main properties; specific rotation, nitrogen content and intrinsic viscosity. There was need to carry out further examination on samples within a region for any discernible differences. In all cases, coef. of var. was used to examine homogeneity in the data. It is long known that for biological data a figure below 35% reflects less variation or homogenous data and was adopted in this study. Results for Isiolo District are shown in Figure 5.6a. Coef. of var. for all the properties was below 35%. However, when data of the three main properties are examined further, specific rotation was seen to vary between -28° and -36°, one sample (I3) had exceptionally high nitrogen while one (I12) had a low value than the mean resulting in a higher range. Similar observation was noted for intrinsic viscosity where some samples had higher than average values while others lower than average values.

Data for Ngurunit, Marsabit are shown in Figure 5.6b. Again coef. of var. for all the properties fall within acceptable limits. Sample NM6 gave gum with exceptionally higher nitrogen content while samples NM6, NM11 and NM14 had exceptionally high viscosity. Overall, Ngurunit region gave gums with the highest viscosity. General variation in samples from Kargi Marsabit was also low though five samples had specific rotation greater than the mean of -34° and half of the samples had nitrogen content in excess of 0.50%. Viscosity was variable with some samples having values comparable to gum from Sudan while other samples had higher values.

Table 5.6a: Physico-chemical and carbohydrate data for gum samples from Isiolo District

Sample number	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11	I12	I13	I14	I15	I16	Mean	SD	CV
Loss on drying (%)	15.2	14.4	13.6	13.7	13.6	13.9	15.7	14.5	13.3	14.9	14	17.5	16.2	14	13.7	14.5	14.5	1.1	7.6
Total ash, 550 C(%)	2.7	3.0	3.1	3.3	3.0	2.9	3.5	3.8	3.5	3.1	3.2	2.9	3.1	3.3	3.5	3.3	3.2	0.3	9.4
Specific rotation (degree	-34	-32	-35	-36	-32	-36	-32	-28	-30	-34	-33	-35	-33	-31	-31	-32	-33	2	6.1
Nitrogen (%)	0.42	0.46	0.53	0.40	0.41	0.47	0.45	0.38	0.41	0.33	0.39	0.30	0.34	0.42	0.35	0.39	0.40	0.06	15.0
Hence protein	2.8	3.1	3.5	2.7	2.7	3.1	3.0	2.5	2.7	2.2	2.6	2.0	2.3	2.8	2.3	2.6	2.7	0.4	14.8
Intrinsic viscosity mol/dm Nacl (ml/g)	19.4	29.2	29.6	18.2	22	18.6	25.8	23.0	27.4	26.8	23	18.2	29.5	22.4	20.8	22.8	23.5	3.9	16.6
Neutral. eq. wt.	964	987	1081	1009	1162	1001	970	870	1156	996	1022	963	881	1093	1030	870	1003	86	8.6
Hence UAA (%)	18	18	16	17	15	18	18	20	15	18	17	18	20	16	17	20	18	2	11.1
Emulsification activity (limonine)	1.65	1.61	1.70	1.60	1.59	1.60	1.67	1.64	1.70	1.60	1.62	1.58	1.59	1.61	1.62	1.6	1.6	0.04	2.5
Emulsion stability (%)	93	92	95	88	90	92	94	92	95	93	94	90	88	95	91	90	92	2	2.2
Tanin (2% sol.)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Sugar compostion after hydrolysis																			
Glucuronic acid	18	18	16	17	15	18	18	20	15	18	17	18	20	16	17	20	18	2	11.1
Galactose	40	39	32	31	36	35	39	40	39	49	43	32	49	42	40	37	39	5	12.8
Arabinose	26	23	28	36	33	32	25	23	26	20	26	26	21	28	28	27	27	4	14.8
Rhamnose	16	20	24	17	15	15	18	17	20	13	14	23	10	14	15	16	17	4	23.5

Table 5.6b: Physico-chemical and carbohydrate data for gum samples from Ngurunit, Marsabit

Sample number	NM1	NM2	NM3	NM4	NM5	NM6	NM7	NM8	NM9	NM10	NM11	NM12	NM13	NM14	NM15	Mean	SD	CV
Loss on drying (	15.4	15.1	15.3	14.5	15	14	14.2	14	15.9	16.8	15	15.8	15.3	14.9	18.8	15.3	1.2	7.8
Total ash, 550 C(	3.0	3.7	3.3	3.2	3.4	2.3	2.8	2.9	3.3	3.1	2.9	2.5	2.7	3.4	3.1	3.0	0.4	13.3
Specific rotation	-32	-30	-34	-28	-31	-35	-32	-30	-34	-33	-31	-34	-33	-33	-34	-32	2	6.3
Nitrogen (%)	0.46	0.42	0.44	0.43	0.37	0.61	0.44	0.33	0.43	0.46	0.37	0.47	0.33	0.48	0.35	0.43	0.1	23.3
Hence protein	3.1	2.8	2.9	2.9	2.5	4.0	2.9	2.2	2.9	3.1	2.5	3.1	2.2	3.2	2.3	2.8	0.5	17.9
Intrinsic viscosity mol/dm Nacl (ml/g)	20.6	21	24.4	26	20.8	31	26	27.1	26.6	28.6	31.5	26.6	22	30.4	25.2	25.9	3.5	13.5
Neutral. eq. wt.	875	902	862	850	982	937	932	886	1008	876	980	837	977	982	1022	927	59	6.4
Hence UAA (%)	20	20	20	21	18	19	19	20	18	20	18	21	18	18	17	19	1	5.3
Emulsification activity (limonine)	1.70	1.64	1.67	1.62	1.69	1.70	1.67	1.75	1.70	1.62	1.60	1.63	1.64	1.61	1.65	1.66	0.04	2.4
Emulsion stablity	96	92	90	94	92	94	89	95	93	94	89	90	94	93	95	93	2	2.2
Tanin (2% sol.)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gel (25			++			+	+											
PH (25% sol.)			4.3			4.4	4.3									4.3	0.1	2.3
Sugar compostion after hydrolysis																		
Glucuronic acid	20	20	20	21	18	19	19	20	18	20	18	21	18	18	17	19	1	5.3
Galactose	46	50	42	45	44	39	40	39	42	44	41	42	39	44	43	43	3	7.0
Arabinose	23	20	28	24	25	30	26	25	24	25	28	23	25	26	24	25	2	8.0
Rhamnose	11	10	10	10	13	12	15	16	16	11	13	14	18	12	16	13	3	23.0

Table 5.6c: Physico-chemical and carbohydrate data for gum samples from Kargi, Marsabit

Sample code	KM1	KM2	KM3	KM4	KM5	KM6	KM7	KM8	KM9	KM10	KM11	KM12	KM13	KM14	KM15	KM16	Mean	SD	CV
Loss on drying (%)	15.3	15.1	14.6	13	14.3	14.7	13.2	13.6	13.4	13.3	14	15.1	16.2	14.8	13.4	14.1	14.2	0.9	6.3
Total ash, 550 C(%)	1.8	1.9	2.3	3.0	3.6	2.8	3.0	2.9	3.1	3.2	3.3	3.0	1.9	2.7	2.8	3.1	2.7	0.5	18.5
Specific rotation (degrees	-34	-32	-32	-36	-34	-35	-37	-35	-33	-34	-34	-36	-34	-34	-33	-39	-35	2	5.7
Nitrogen (%)	0.47	0.41	0.38	0.43	0.36	0.6	0.49	0.5	0.44	0.61	0.42	0.51	0.7	0.57	0.5	0.56	0.5	0.09	18.0
Hence protein	3.1	2.7	2.5	2.9	2.4	4.0	3.2	3.3	2.9	4.0	2.8	3.4	4.6	3.8	3.3	3.7	3.3	0.6	18.0
Intrinsic viscosity mol/dm Nacl (ml/g)	25.8	23	26.3	16.6	19.6	18.4	17.6	26	22.8	26	21.2	18.6	25.8	22.4	15	16.6	21.4	3.8	17.8
Neutral. eq. wt.	972	1135	1012	1165	930	984	1092	993	946	1004	996	1064	988	859	1164	1152	1029	88	8.6
Hence UAA (%)	18	16	17	15	19	18	16	18	19	18	18	17	18	18	15	15	17	1	5.9
Emulsification activity (limonine)	1.65	1.63	1.63	1.6	1.64	1.71	1.66	1.64	1.7	1.69	1.66	1.7	1.76	1.74	1.69	1.7	1.68	0.04	2.4
Emulsion stablity (%)	90	91	96	85	94	96	91	90	96	95	94	94	96	94	95	94	93	4	4.3
Tanin (2% sol.)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Gel (25% sol.)	(-)	(-)	(-)	(-)	(-)		(-)				(-)	+ +	+ +						
PH (25% sol.)	4.4	4.5	4.4	4.5	4.3		4.4				4.4	4.5	4.3				4.4	0.1	2.3
Sugar compostion after hydrolysis																			
Glucuronic acid	18	16	17	15	19	18	16	18	19	18	18	17	18	18	15	15	17	1	5.9
Galactose	38	46	45	39	44	37	35	40	50	41	38	33	48	50	39	34	41	5	12.2
Arabinose	26	26	24	26	25	35	39	24	22	25	28	33	21	22	30	46	28	5	17.9
Rhamnose	18	12	14	20	12	10	10	18	10	16	16	17	13	10	16	15	14	3	21.4

Table 5.6d: Physico-chemical and carbohydrate data for gum samples from Turkana District

Sample number	LT1	LT2	LT3	LT4	LT5	KT1	KT2	KT3	KT4	KT5	Mean	SD	CV
Loss on drying (%)	13.6	13.9	14	14.1	13.8	14.8	14.5	15.7	15.1	16	14.6	0.8	5.4
Total ash, 550 C(%)	3.0	3.3	3.0	2.9	3.1	3.0	3.0	2.9	2.4	2.5	2.9	0.3	10.3
Specific rotation (degrees)	-38	-39	-40	-35	-36	-39	-36	-34	-39	-36	-37	2	5.4
Nitrogen (%)	0.50	0.45	0.51	0.46	0.44	0.36	0.38	0.40	0.38	0.34	0.42	0.06	14.3
Hence protein	3.3	3.0	3.4	3.1	2.9	2.4	2.5	2.7	2.5	2.3	2.8	0.4	14.3
Intrinsic viscosity mol/dm Nacl (ml/g)	15.2	15.5	17	15	17.4	18	19.1	19	16.5	13.7	16.6	1.7	10.2
Neutral. eq. wt.	1060	873	977	1053	1074	1059	1076	984	1013	1040	1021	60	5.9
Hence UAA (%)	17	20	18	17	16	17	16	18	17	17	17	1	5.9
Emulsification activity (limonine)	1.72	1.69	1.70	1.68	1.67	1.66	1.70	1.71	1.69	1.70	1.69	0.02	1.2
Emulsion stablity (%)	96	94	95	96	95	94	96	95	94	95	95	1	1.1
Tanin (2% sol.)	0	0	0	0	0	0	0	0	0	0	0	0	0
Sugar compostion after hydrolysis													
Glucuronic acid	17	20	18	17	16	17	16	18	17	17	17	1	5.9
Galactose	31	27	25	38	35	30	40	42	29	34	33	5	15.2
Arabinose	35	33	35	28	30	30	31	21	31	32	31	4	12.9
Rhamnose	17	20	22	17	19	23	14	18	24	17	19	3	15.8

Table 5.7: Amino acid composition (residues per 1000 residues) for selected gum samples

Amino acid	NK5 1	SSM2 2	HK2 3	NK2 4	NK8 5	KT2 6	KT3 7	HK8 8	NK1 9	HK6 10	MI6 11	HK4 12	LT1 13	HK9 14	MI3 15	HK3 16	NK3 17	HK7 18
Nitrogen (%)	0.33	0.35	0.36	0.37	0.37	0.38	0.40	0.42	0.43	0.44	0.47	0.49	0.50	0.51	0.53	0.6	0.61	0.61
Alanine	26	34	16	28	20	24	24	21	27	21	24	20	28	20	18	27	25	17
Arginine	7	8	6	7	7	9	6	7	8	7	8	6	9	7	7	7	6	6
Aspartic acid	45	64	32	49	34	47	37	40	48	40	45	31	54	44	43	41	38	35
Cystine	9	0	0	0	8	4	7	0	0	0	0	2	7	3	4	12	10	0
Glutamic acid	37	57	30	40	29	42	29	36	38	36	37	28	45	38	38	35	32	33
Glycine	52	38	53	46	52	57	56	58	53	58	57	52	59	59	71	51	49	56
Histidine	52	40	54	44	56	54	57	53	49	53	51	56	50	57	57	58	54	59
Hydroxyproline	324	250	366	335	353	280	354	329	320	330	335	354	290	271	261	309	344	307
Isoleucine	11	12	9	9	8	10	7	10	11	10	9	9	11	10	10	8	8	10
Leucine	74	83	69	73	67	68	65	66	75	66	62	69	68	82	89	71	70	71
Lysine	20	27	14	21	16	22	17	15	20	15	19	13	22	19	19	17	17	16
Methionine	1	2	2	1	1	1	1	1	1	1	0	1	1	0	0	0	1	1
Phenylalanine	26	39	20	27	19	26	19	26	28	23	26	21	31	29	28	24	22	22
Proline	52	77	76	50	58	83	52	69	53	69	56	67	59	75	78	70	51	83
Serine	138	124	134	130	143	133	143	144	137	145	131	146	132	146	143	133	144	151
Threonine	83	74	88	84	93	93	89	85	82	86	83	89	85	94	91	91	86	100
Tyrosine	12	24	10	18	9	15	11	12	13	12	12	16	13	14	14	14	11	9
Valine	33	47	22	37	23	31	25	26	35	26	33	22	36	31	29	31	30	23
Hence NCF	6.64	6.83	6.65	6.77	6.58	6.55	6.57	6.61	6.65	6.6	6.62	6.64	6.60	6.55	6.5	6.76	6.63	6.53

Gum samples from Turkana District also revealed overall low variation. A characteristic feature of these samples is the generally higher negative specific rotation with all the samples (except KT8) having values that exceeded the mean recorded for *Acacia senegal* var. *kerensis* . These data reveal also the apparent relationship between specific rotation and sugar composition. Samples with higher specific rotation had lower proportion of galactose to arabinose compared to sample 8 that has a value within the normal range. The same phenomena was observed in similar samples from the three areas mentioned above. Two samples gave nitrogen content higher than the average value recorded for *A. senegal* var. *kerensis*. A close examination at the nitrogen values from the two areas in Turkana revealed some differences with samples from Lokichar giving higher levels.

Data on the amino acid composition representative of 18 samples and arranged in increasing order of nitrogen content are presented in Table 5.7. There were observable differences between samples though no pattern could be attributed to regional differences. Taking the two major amino acids for illustration the amount of hydroxyproline varied between 261 and 354 units and serine from 124 to 151. Additionally, no distinct trend was observed with increasing nitrogen content between samples as was observed for the mean values and the overall distribution was fundamentally similar.

5.4 Discussion

A total of fifty seven samples collected from single trees within the gum producing region in Kenya provide sufficient data from which acceptable average values and ranges can be obtained to establish typical properties of gum arabic from Kenya. The results reveal that Kenyan gum differs from Sudanese, Nigerian or Ugandan gum by having higher specific rotation, nitrogen content and viscosity. The values for specific rotation and intrinsic viscosity fall outside the limits currently specified (FAO, 1990). These differences may be a reflection of the type of resource producing the gum.

Studies in chapter 4 have shown that var. *kerensis* is the main source of gum arabic in Kenya. Earlier studies (Anderson and Weiping, 1990) have shown some analytical and structural differences between samples from Marsabit and classical Sudanese gum arabic and suggested that these could be due to a different variety or from a member of the *A. senegal* complex. The similarities observed between a sample coded Marsabit (Anderson and Weiping, 1990) and samples coded KM13 (Table 5.6b) indicate that it was from var. *kerensis.* As for Sudan and Nigeria, an *A. senegal* distribution map (Brenan, 1983) shows that var. *senegal* is the only one growing there and hence principal source of gum arabic. Results from Uganda indicate that though the two countries are neighbours, gum from the two areas differ in total ash, specific rotation, nitrogen content, emulsification properties and sugar composition. Since gum from Uganda is analytically similar to gum from Sudan and in the absence of any further details as to the variety, it is logical to conclude that it could have been collected from var. *senegal.* Additional analytical differences are seen in the tendency of Kenyan gum to form gel and slightly different sugar composition, particularly the lower galactose content. Overall however, gum arabic from Kenya is comparable in functional properties to commercial gum arabic as seen from the emulsion properties. The slight differences in the cation content might be due to abundance of the cations in the soils at different locations rather than genetic factors.

It is appropriate at this stage to critically examine the analytical parameters in terms of their influence on the functional and other gum properties. Good quality gum arabic in a dry state is required to have a moisture content of up to 15%. In this state the gum is generally safe from attack by degrading organisms. Higher moisture content results in increased microbial activity causing degradation and it is generally an indication of poor storage condition. The higher moisture content observed among some samples from single trees in the present study is because they were picked fresh from

the trees and packed immediately unlike commercial consignments that are normally dried before shipping.

The ash content is a measure of the quantity of cations present in the gum which normally exist as part neutralised salts of acidic polysaccharide (McDougal, 1987). The actual amount depends on relative abundance in the soil at a particular location. Gum arabic from Kenya shows lower overall cation content and in particular, the cation composition for heavy metals falls within the limits specified by FAO (1990).

The high optical rotation of Kenyan gum has been noted. Average values varied between -34° and -37° between the regions and even higher values of -40° were recorded for some individual samples. Data from FAO (1990) cite a range of -26° and -34° as acceptable for gum arabic for food grade. The main reason for providing the above range was to offer greater food safety assurance by excluding majority of the untested and therefore non-permitted gums from other sources. This assumption was based on the available data then which suggested that values for gum arabic fall within the range given (Anderson et al, 1990). Values outside the range were thought not to be for gum arabic or were blends of gum carefully formulated to meet the requirement. Whereas the above criteria provide a useful guide for gums from some sources, it is clear from the present study that gum arabic from *A. senegal* variety *kerensis* is more optically negative and for some regions in Kenya falls outside the range currently specified. It should be emphasised that sugar blend leading to this higher regular specific rotation may not lead necessarily to undesirability in food uses. Objection regarding to toxicity or allergies is needed.

Interest was then centred on the possible reason for the more negative specific rotation of the Kenyan gum. It is known that specific rotation is influenced by the nature and composition of the sugars present (Stoddart, 1971). A close examination of the Sudanese and Nigerian gums reveals that

they tend to have overall mean values of around 45% galactose, 24% arabinose, 13% rhamnose and 17% glucuronic acid or a ratio of 4:2:1:1 (Anderson et al, 1990). Earlier studies showed the ratio to be in the order of 3:3:1:1 (Cree, 1966). These studies suggest that gum arabic is comprised of a higher proportion of galactose than arabinose or sometimes in the same molar ratios with rhamnose and glucuronic acid present in similar but lower amounts. An evaluation of the optical properties of the sugars show that galactose and glucuronic acid are of the D-series while arabinose and rhamnose of the L-series. It is thought that arabinose and rhamnose for gum arabic have a negative specific rotation (Anderson, 1994).

Based on these studies an evaluation was made on the Kenyan gum. It was noted that most samples whose specific rotation fall within the normal range had molar proportion (gal:arab:rham:glucuA) ranging from 4:2:1:1 to 2:2:1:1 indicating higher or same molar ratio of galactose to arabinose. In the latter case, the amount of galactose was still greater than arabinose. However, in samples where the specific rotation was more negative (most samples from Turkana and various samples from Kargi), the molar ratio was about 2:2:1:1 and in most cases the proportion of arabinose was slightly greater than that of galactose. While many sugars contribute to the specific rotation value, it is logical to conclude that the higher specific rotation in the Kenyan gum could be due to the higher proportion of arabinose. Additionally, the relatively higher rhamnose observed among some samples could equally contribute along with arabinose in influencing the overall specific rotation. Randall et al, (1989) also associated variations in the sugar composition of gum arabic results to a difference in the specific rotation.

The nitrogen and hence protein content of Kenyan gum arabic is relatively high. Values varied between 0.40% and 0.50% between the four regions, all exceeding the upper limit of 0.39% in the proposed FAO specification. Again the specification assumes that higher values greater than

0.39% are from sources other than *A. senegal*. Experience from this study has shown that values from individual samples can be as high as 0.70%. The significance of nitrogen or protein in the functional properties is now becoming clear. It has been established that the special film forming properties of the gum arise from its protein fraction (Dickinson et al, 1991). This fraction is associated with a high molecular weight portion of the gum representing about 30 percent of the total (Vandevelde and Fenyo, 1985) and is the one that adsorbs more strongly at the oil water interface and probably responsible for the emulsifying and stabilising properties (Randall et al, 1988). Dickinson et al (1988) working on Acacia gums with nitrogen content ranging from 0.1 - 7.5 % (protein content 0.5 - 47 %) found a strong correlation between the protein in the gum and its surface properties at the oil water interface. It appears therefore that the high nitrogen (or protein) content observed in the Kenyan gum might be of advantage in its use as emulsifier and stabiliser. This is further confirmed from the overall high emulsion properties recorded for the Kenyan gum which varied between 1.60 to 1.69 compared to 1.60 for Sudanese gum. However, it should be emphasised that it is not just the overall amount of the proteinaceous component which is important but rather its nature and distribution (Dickinson et al, 1991).

Although not directly emphasised in the FAO specification, gum viscosity is an important parameter that influences its functional nature and hence use. The high solubility and yet viscous nature allows its use in the food industry to impart body and texture to a variety of foods. This would make Kenyan gum arabic with high viscosity more desirable in some user requirements. However, high viscosity can also be undesirable, especially during processing. Experience shows that it can result in a condition known as stringiness i.e it behaves as a chewing gum at concentrations of 30 - 40 % (Woolen, 1982). This condition has been observed more in the west African

gums that tend to have higher viscosities. In addition, there was the tendency of the gum to form gel which can also result in difficulties in processing.

Beside understanding the general nature of Kenyan gum and its significance to the functional properties, it is equally important to understand the differences that exist. Variation between regions and samples were noted. The regional differences may reflect climatic and soil factors resulting in adaptation by the trees in the specific areas. Advantage might be taken of these differences and production areas selected to meet specified user requirements. For example, gum from Turkana has low viscosity. On the other hand, gum from Marsabit is more viscous and though it has good emulsion properties would require special processing. The tree to tree differences might reflect genetic differences and present greatest opportunity for tree selection for agroforestry development with the aim of improving quality and yield. It is worth noting that the extent of possible genetic influence was not examined since it was not part of the present study and, in any case requires a different approach to sampling.

CHAPTER 6: AN EVALUATION OF METHODS FOR CHARACTERISING AND MONITORING GUM ARABIC OF COMMERCE AND RELATED *ACACIA* GUMS

6.1. Introduction

The joint FAO/WHO expert committee on Food Additives (JECFA) defines gum arabic of commerce as 'a dried exudation obtained from the stems and branches of *Acacia senegal* Willdenow or related species of *Acacia*, Family leguminosae' (FAO, 1986). However, complaints were raised to the effect that the specification was too lax to prevent gums from other than the specified source being sold and used. As a result, JECFA announced a revised specification which includes three additional criteria (FAO, 1990): a) the source of gum arabic is now defined as from *A. senegal* and closely related species, b) nitrogen content must be between 0.27% - 0.39% and c) specific rotation must lie between -26° and -34°. The objective of these amendments was to characterise more specifically gum arabic of commerce.

While the need to minimise adulteration has been accepted, introduction of the new criteria has raised questions as to whether they adequately address fully the issue of the specification for gum arabic of commerce. Recent findings have found that this may not be so. In a study comparing the physical and chemical properties of gums derived from related vulgares and gummiferae series in the genus *Acacia*, it was found that there are no readily identifiable parameters which distinguish between the two series (Phillips and Williams, 1994). On the other hand, the same study showed that there was wide variation between nodules within a species arising from either age of the tree, season or region that sometimes result in wide variability in a single parameter. Studies in chapter 5 have shown that gum arabic from *A. senegal* var. *kerensis* shows analytical differences from var. *senegal*

and also confirms differences due to regions. Relying on the proposed specification would therefore result in rejection of otherwise authentic gum arabic.

There is need to establish suitable methods that can adequately characterise gum arabic of commerce. Various methods have been developed. The methods for physico-chemical, carbohydrate and amino acid composition (i.e. analytical) are well known (Anderson et al, 1990). Techniques of molecular characterisation (Osman, 1993) and use of an immunoassay specific to gum arabic (Menzies, 1992) have recently been developed. ^{13}C-NMR spectra to examine the structural characteristics of gum arabic and other *Acacia* gums (Weiping, 1993) and use of a chemometric method based on the Principal Component Approach (PCA) and Discriminant Component Approach to examine a chemotaxonomic link between *Acacia* gums from the closely related vulgares and gummiferae series as well as for non-*Acacia* gums (Jurasek et al, 1993a) are also reccent developments. The purpose of this study was to evaluate the first three methods (analytical, gpc and Elisa) in characterising gum arabic of commerce and some of the related gums of *Acacia* species. The three methods were selected because they have been suggested as inexpensive for monitoring and quality control and therefore consistent with the aim of the present study. ^{13}C-NMR which has also been recommended as unique and unambiguous in checking the identity of commercial samples is quite expensive and interpretation of data generally sophisticated; it cannot be afforded in producer countries and for general routine testing. It has thus been applied in this study only to confirm the structural nature of brown samples of gum arabic. The chemometric method is a statistical rather than analytical technique and is only considered here on account of classifying gum arabic.

6.2. Materials

Four types of samples were collected for the study:

6.2.1. Authentic commercial samples of gum arabic.

Four samples (one from each of the four areas covered in the survey in chapter 3) were collected from farmers at the local collecting centres. The samples were identified as DI (Daaba, Isiolo), NM (Ngurunit, Marsabit), KM (Kargi, Marsabit) and KT (Kakuma, Turkana). The fifth sample (GA) was obtained by mixing representative pieces from each of the above samples.

6.2.2. Brown samples of gum arabic

Ten brown samples of gum arabic were collected from single trees of *A. senegal.* var. *kerensis*. The samples comprised of three from Isiolo (I17, I18 and I19), six from Marsabit (NM16, NM17, NM18, NM19, NM20 and KM17) and one from Turkana (LT6).

6.2.3. Commercial samples suspected as contaminants

Four samples comprising of unusual nodules were provided by two gum traders. They were identified as:

- MK1 and MK2 sold at Kargi, Marsabit. Both samples comprised of broken pieces of medium size, brown in colour and of brittle character. The samples were believed to be *A. seyal*.
- X was sold in Isiolo and believed to be *A. seyal.* It comprised medium size nodules, clear to light brown and sticky.
- Y was sold in Isiolo and believed to be *A. paoli*. It comprised medium size nodules, amber to light brown with coarse appearance.

6.2.4. Authentic samples from other *Acacia* species

These comprised:

- A sample of *A. reficiens* collected at Ngurunit, Marsabit in July,92. It consisted of small nodules and fragments with a smooth glossy appearance, brown colour.
- A sample of *A. tortilis* collected at Ngurunit, Marsabit in July,92. It consisted of medium size nodules, smooth to rough fissures and brown in colour.
- A sample of *A. mubica* collected at Ngurunit, Marsabit in July,92. It consisted of medium size nodules, oily in appearance and light brown in colour.

6.3. Methods

6.3.1. The analytical methods

The physico-chemical and carbohydrate methods used are described in section 5.2.2.

6.3.2. Determination of molecular mass distribution by gel permeation chromatography (gpc)

6.3.2.1. Theoretical considerations

Gum arabic belongs to a group of proteoglycans known as Arabinogalactan Proteins (Fincher et al, 1983). Molecular analysis has shown that it consists of three distinct components similar in the proportion of various sugars but differing in their molecular masses and nitrogen contents (Randall et al, 1989). The main component (88.4% of the total) is an arabinogalactan (AG) with a molecular mass of 2.79×10^5 and deficient in protein. The second component (10.4% of the total) is an arabinogalactan protein complex (AGP) with a molecular mass of 1.45×10^6 containing about 50% of the protein. The third component (1.2% of the total) is a glycoprotein (GP) with a molecular mass of 2.5×10^5 containing about 25% of

protein. The components have been well characterised by the gpc technique (Osman, 1993).

The technique of gpc was introduced by Moore and Hendrickson in the 1960s. It is a liquid chromatographic method where the polymer molecules are separated by their molecular size in solution. As the solvent elutes through a column packed with porous material (stationary phase), smaller molecules diffuse into the pores whereas the larger ones are excluded. Therefore molecules of larger size elute first. In principle, the volume of liquid at which the polymer elutes from the column (Ve) is related to the physical parameters of the column by the relationship:

$$V_e = V_o + K_d V_i$$

where:

V_e is the elution volume

V_o is the void (dead) volume of the column

Vi is the internal volume of the column

K_d is the distribution coefficient

The total volume of the column is given by the relation

$$V_t = V_o + V_i$$

The distribution coefficient (K_d) depends on the size and shape of the polymer molecules and on the size and shape of the pores of the packing material. Though it is ideally expected to be temperature independent, it varies slightly beacause the pore structure and size of the gel are affected by temperature.

The dependence of molecular size on elution volume is illustrated in Figure 6.1a. The void volume (V_0) corresponds to the total exclusion of the polymer molecules from the pores of the gel. The exluded molecules are said to be significantly larger than the largest available pore size. Selective separation of the polymer molecules takes place between V_0 and V_t. Beyond V_t separation of solute

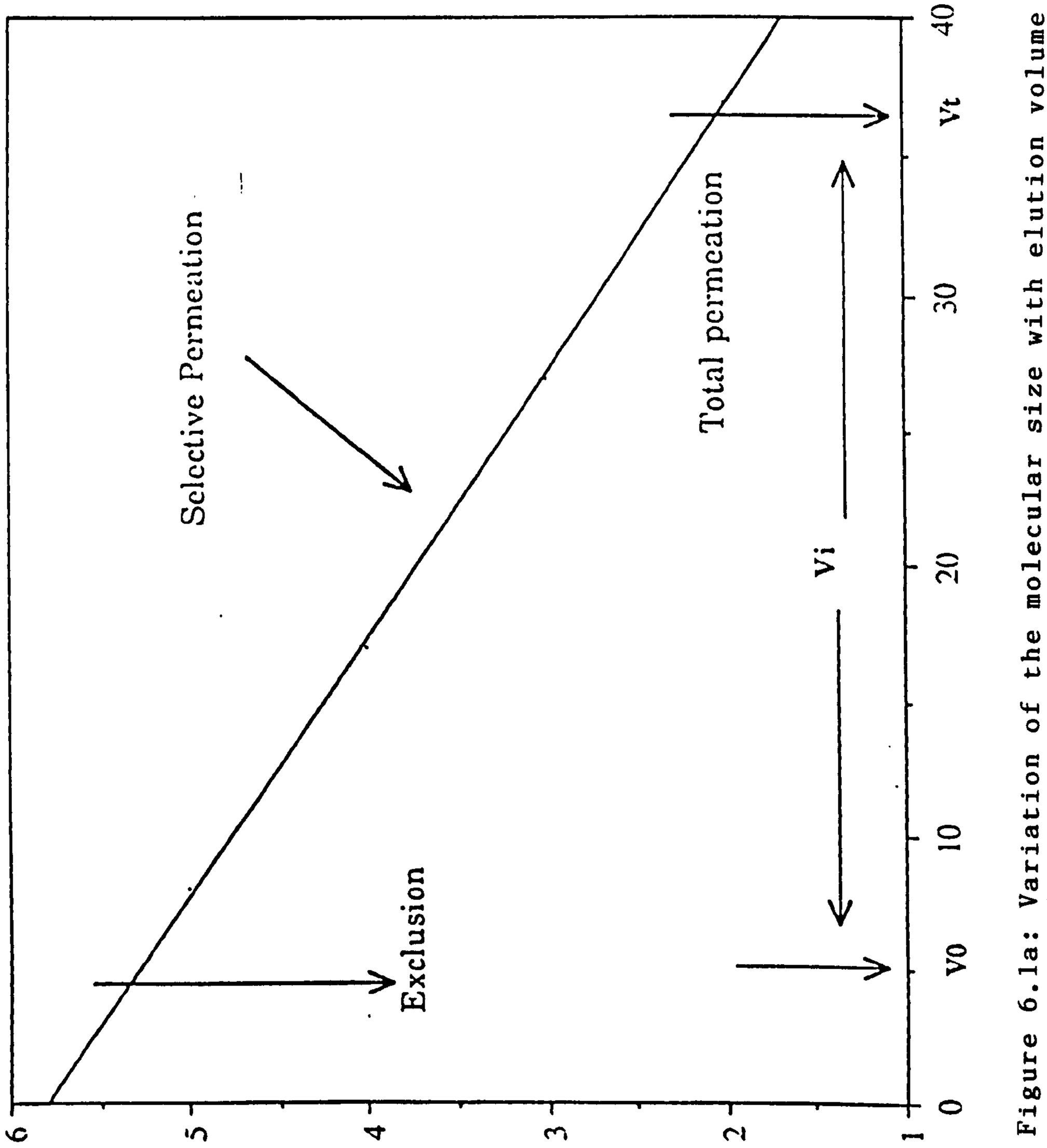


Figure 6.1a: Variation of the molecular size with elution volume (Osman, 1993)

molecules is not achieved solely due to size exclusion mechanism and the solute molecules are retained on the column (stationary phase), possibly by an affinity mechanism (Osman, 1993). In this case K_d is greater than unity.

6.3.2.2. Experimental

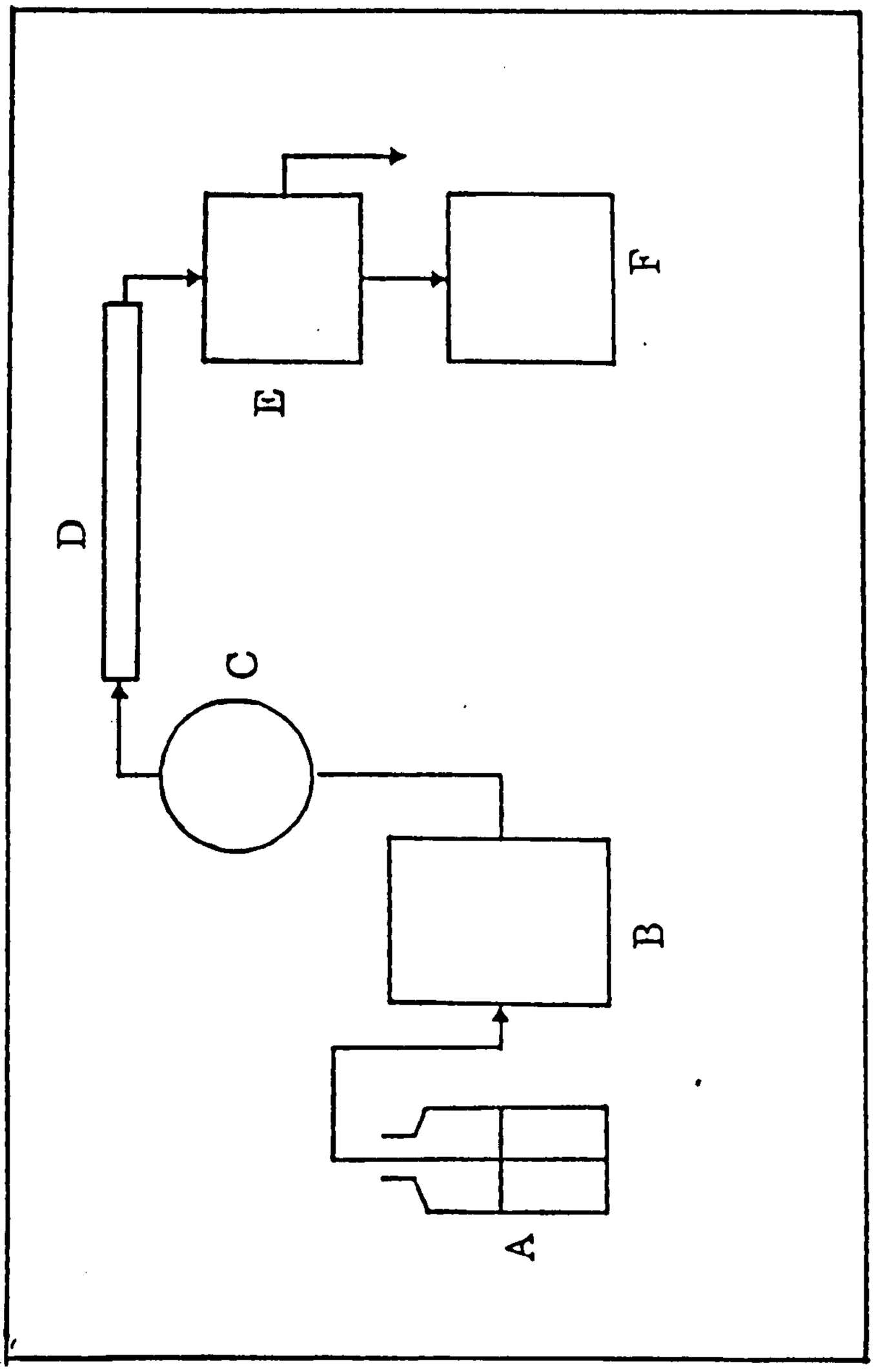
The analyses were carried out at the North East Wales Institute (NEWI), Deeside using a Pharmacia fast protein liquid chromatography (FPLC) system (Osman, 1993). The system comprises of a column packed with Superose 6, a gradient programmer (GP-250), a high precision pump (P-500) and a two channel recorder (REC-428). A diagram of the instrumentation is shown in Figure 6.1b.

Gum samples were prepared to 1% solution (w/v) in 0.5 mol dm^{-3} NaCl. First, elution buffer (0.5 mol. dm^{-3} NaCl) was filtered, degassed and passed through the column to provide a suitable baseline. Gum samples were then filtered and sequentially injected via a v7 valve (injection port) into the column. The flow rate was maintained at 0.5 cm^{-3} min^{-1} and eluent monitored by UV detector (LKB 2238 unicord SII) and refractive index (RI) detector (RI, waters associates inc., Mass. USA) linked to a two channel recorder (REC-428, Pharmacia). The elution profiles from UV and RI provide the required qualitative information in the form of comparative chromatograms.

6.3.3. An Enzyme Linked Immunosorbent Assay (ELISA) for gum arabic

6.3.3.1. Theoretical considerations

Williams et al (1992) and Menzies et al (1992) have developed an ELISA technique which can detect gum arabic from *Acacia senegal* and gums related to it. The technique is based on obtaining antibodies raised in rabbits against gum arabic which recognise and interact with a specific binding site in the molecule of the test sample. The degree of interaction is known as cross reaction which refers the ratio of the weight of a specific antigen required to reduce binding of the



Key to the figure

A Mobilephase resevoir
B Pump
C Injection port
D Column
E Detector
F Recorder

Figure 6.1: A schematic illustration of gpc instrumentation (Osman, 1993)

specific antibody by 50% to the weight of the cross reactant (i.e. test substance) required to reduce the binding of the antibody by the same amount expressed as a percentage (Morris, 1985). Percent cross reaction is expressed by the relationship (α) as follows:

$$\% (\alpha) = \frac{\text{conc. of A. senegal at 50\% max. absorbance}}{\text{conc. of a test sample at 50\% max. absorbance}} \times 100$$

Cross reactivity value (α) is regarded as a useful diagnostic parameter in characterising gums that are related. Closely related samples cross react more to a specifically produced antibody than substances which are unrelated. By this method *A. senegal* gum can be readily differentiated from other *Acacias* as well as non-*Acacia* gums.

6.3.3.2. Immunoassay procedure

The assay was carried out according to the method described by Menzies (1992). The procedure followed is presented in the scheme outlined in Figure 6.2. Using plates pre-coated with gum arabic (as antigen), solutions of test gum (concentrations of 10 - 100 ug cm^{-3} w/v, in wash buffer, pH 7.8) were added to separate wells of the micro-elisa plates. Anti-gum arabic antibody was then added (step 1). The plates were left at room temperature for 60 minutes, emptied, thoroughly washed with wash buffer and blot dried. At this stage, if the gum is not from *A. senegal* the antibody recognises and interacts with only the gum already adsorbed on the plates. However, if the test gum has features similar to *A. senegal* competition occurs and the antibody can interact either with the molecules in solution or those adsorbed onto the plate.

A second antibody (Anti rabbit IgG) conjugated to an enzyme (Peroxidase) in citrate buffer was added to each well (step 2) and the plate left for another hour, washed with wash buffer and blot dried. The anti rabbit IgG is said to recognise

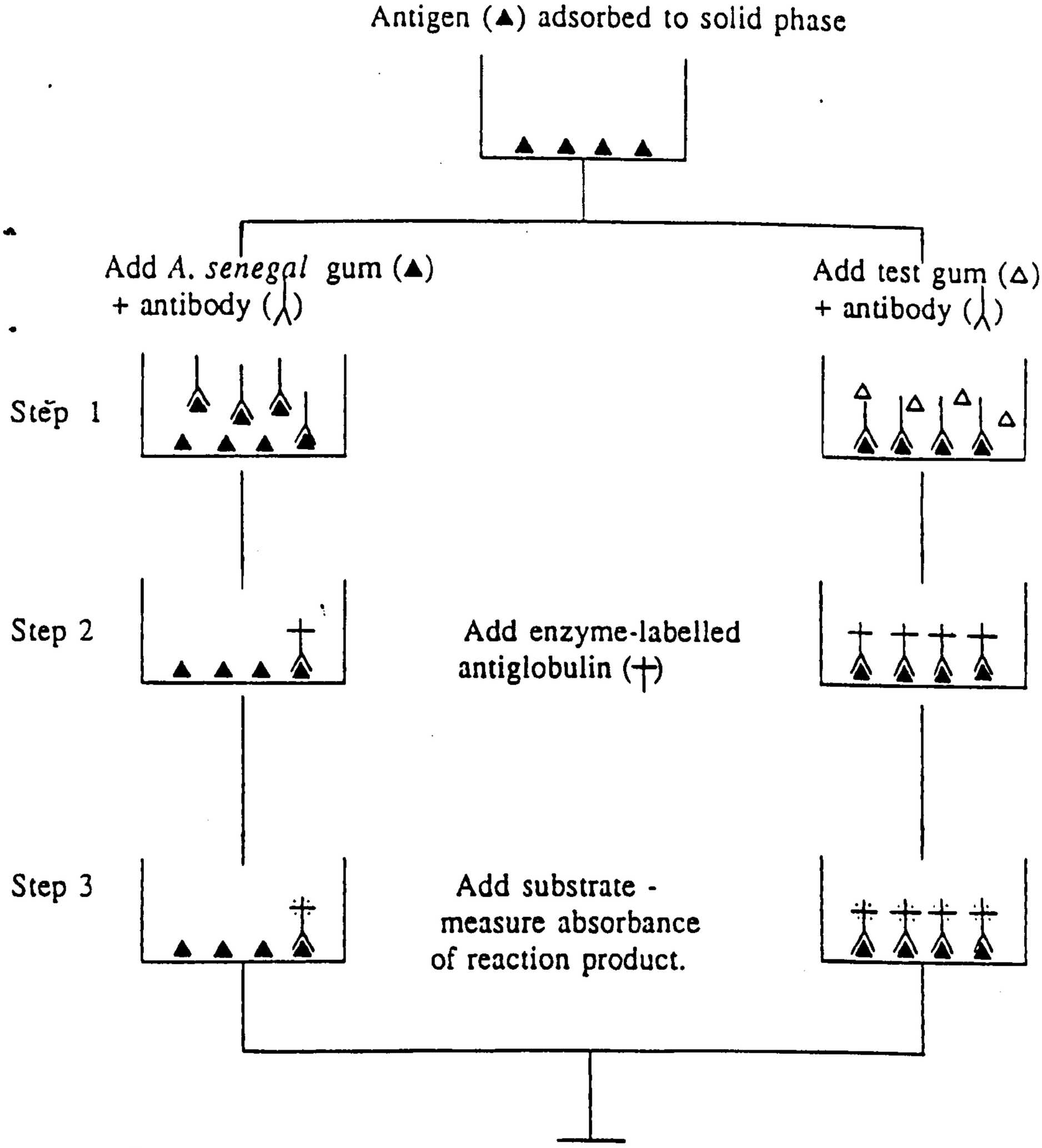


Figure 6.2: Schematic illustration of the ELISA procedure (Menzies, 1993)

and interact with the gum arabic anti-body on the plate. Finally, a chromogenic substrate was added to each well (step 3) and the change in absorbance (A405 nm) was recorded at 10 to 20 minute intervals. The chromogenic reagent normally interacts with a second antibody producing a blue green colouration. The intensity of the colour is proportional to the amount of the second antibody attached to the plate. Little or no colour indicates , therefore, that the test gum is from *A. senegal* while an intense colour indicates that it is not.

6.3.4. ^{13}C-NMR spectroscopic method·

6.3.4.1. Theoretical considerations·

^{13}C-NMR has proved to be an efficient spectroscopic method for structural determination of polysaccharides (Jennings and Smith, 1980). It has been used to obtain information on anomeric configurations, linkages and branching arrangements. The most informative method is based on correlation of the chemical shifts of the carbon atoms of the polysaccharide units using related monosaccharides as novel compounds. It has been shown that chemical shifts of standard monosaccharides are similar to those of the monosaccharides within the gum molecules except for substituent effects (Weiping, 1993). Additional information on overall structural arrangement can be obtained by examination of ^{13}C line width. Narrow peaks are given by sugar units which are terminal groups in side chains while broader peaks indicate more restricted segmental motions or a greater variability of chemical environments such as found in the backbone of a polysaccharide (Selvendran and Ryden, 1990). Besides the quantitative approach, ^{13}C-NMR spectra finger prints can be used to compare similarities between samples.

6.3.4.2. Procedure

Fourier transform ^{13}C-NMR spectra were obtained for 10% gum solutions in D_2O at room temperature at 62.90 MHz with a Brucker AC 250 spectrometer. The spectra were recorded overnight.

6.4. Results and discussion

6.4.1. Evaluation of gum arabic of commerce

6.4.1.1. Use of analytical methods

Analytical data for various samples are given in Table 6.1. Samples number 1-8 provide data for gum arabic from *A. senegal* var. *kerensis* (Kenya) with the first five representing commercial samples described in section 6.2.1. Samples number 6 and 7 are good quality gum from single trees (included for comparison) while sample number 8 is the mean value for fifty seven samples from Table 5.1. The last four samples are for *A. senegal* var. *senegal* (Sudan). Samples number 9 and 10 is data by Osman (1993) while number 11 and 12 is data by Anderson et al (1990).

A number of observations can be made from the results. Gum arabic of commerce from Kenya (samples number 1-5) shows analytical difference compared to that from Sudan (samples number 9-12). Kenyan gum has higher specific rotation, nitrogen content and intrinsic viscosity. This observation is consistent with earlier data (chapter 5) where the observed differences are reported to be associated with different varieties. On the basis of analytical data therefore it seems possible to distinguish between the gums of the two varieties of *A. senegal*. It was observed further that when compared to data of authentic samples, the four commercial samples show data that is representative of the areas in which they grow. For example sample KT is comparable to KT5 in the three properties (specific rotation, nitrogen content and intrinsic viscosity) while KM compares with KM11 and more so with sample KM13 (in Table 5.6c). Sample GA appears

Table 6.1: Analytical data for gum arabic of commerce

Sample code	DI	NM	KM	KT	GA	KM11	KT5	EX-KENY	SA1	SA2	SA3	EX-SUDA
and number	1	2	3	4	5	6	7	8	9*	10*	11**	12**
Moisture,%	17.2	13.7	16.4	13.4	14.4	14.0	16.0	14.7	14.0	14.5	13.0	13.0
Ash content, %	1.4	3.1	1.2	3.2	3.0	3.3	2.5	3.0	3.5	nd	3.4	3.6
Spec. rotation (deg.)	-34	-34	-34	-38	-34	-34	-36	-34	-30	-29	-31	-30
Nitrogen, %	0.44	0.46	0.67	0.38	0.44	0.42	0.34	0.44	0.37	0.35	0.31	0.34
Hence protein (n*NCF	2.9	3.1	4.4	2.5	2.9	2.8	2.3	2.9	2.4	2.3	2.1	2.3
Intr. viscosity (ml g-1)	26.5	23.0	29.1	17.4	23.8	21.2	13.7	21.9	16.5	nd	16.0	16.0
Emulsifcation activity	1.72	1.67	1.73	1.64	1.65	1.66	1.70	1.66	1.62	nd	nd	nd
Emulsification stability	95	95	95	92	93	94	95	93	94	nd	nd	nd
Equivalent weight	965	963	888	1070	887	996	1040	995	1068	1040	1120	1050
Hence UAA	18	18	20	16	20	18	17	18	16	17	16	17
Tannin (2% solution)	0	0	0	0	0	0	0	0	0	nd	nd	nd
Gel (25% solution)	++	++	++	+	++	-	nd	+	-	nd	nd	nd
pH (25% solution)	4.4	4.4	4.3	4.4	4.3	4.4	nd	4.4	4.4	nd	4.5	nd
Sugar composition after hydrolysis												
Glucuronic acid	18	18	20	16	20	18	17	18	16	17	16	17
Galactose	39	45	40	30	38	38	34	39	42	43	45	44
Arabinose	29	24	26	33	26	28	32	28	28	26	23	25
Rhamnose	14	13	14	21	16	16	17	16	14	14	16	14

where:

nd -> not determined

* -> Osman, 1993

** -> Anderson et al, 1990

'- -> no gel

'+ -> moderate gel

'++ -> heavy gel

to representative of the four samples and is comparable to sample number 8 which is also the mean for fifty seven samples. Two samples (numbers 1 and 3) show higher moisture content which indicates lack of proper drying that can easily result in deterioration as discussed earlier. In the light of the proposed JECFA specification, gum arabic of commerce from Kenya can be regarded as different from authenticated gum from *A. senegal*. Specific rotation for one sample (KT) falls outside the limit specified as does the nitrogen content for three samples (DI, NM and KM). The analytical approach thus has some limitation in defining gum arabic of commerce. Furthermore there are pronounced differences between samples rendering the proposed limits unable to fully characterise gum from even a single source. These observations are consistent with earlier studies (Phillips and Willams, 1994) and the results of chapter 5. It can be concluded that while the method can distinguish between gum from different varieties it cannot be entirely relied upon as a method of specification where the proposed specification is applied and because of the natural variability in the product. Full knowledge of the properties of varieties of *A. senegal* producing gum arabic is needed and acceptable limits set before it can be used singly.

6.4.1.2. The use of gpc method

Five samples from those listed in Table 6.1 were analysed by gpc. They were samples KM11, KT5 and GA1 from var. *kerensis* and SA1 and SA2 from var. *senegal*. In addition one sample (GA2) supplied as a commercial sample from Kenya was included.

The elution profiles of the above samples as monitored by UV and RI are shown in Figures 6.3a and 6.3b respectively. The UV profile is said to be more sensitive to the chemical composition of the various molecular mass fractions of the gum molecule and detects the π-π^* transition of the carbonyl and carboxylic groups associated with the protein/polysaccharide components while the RI

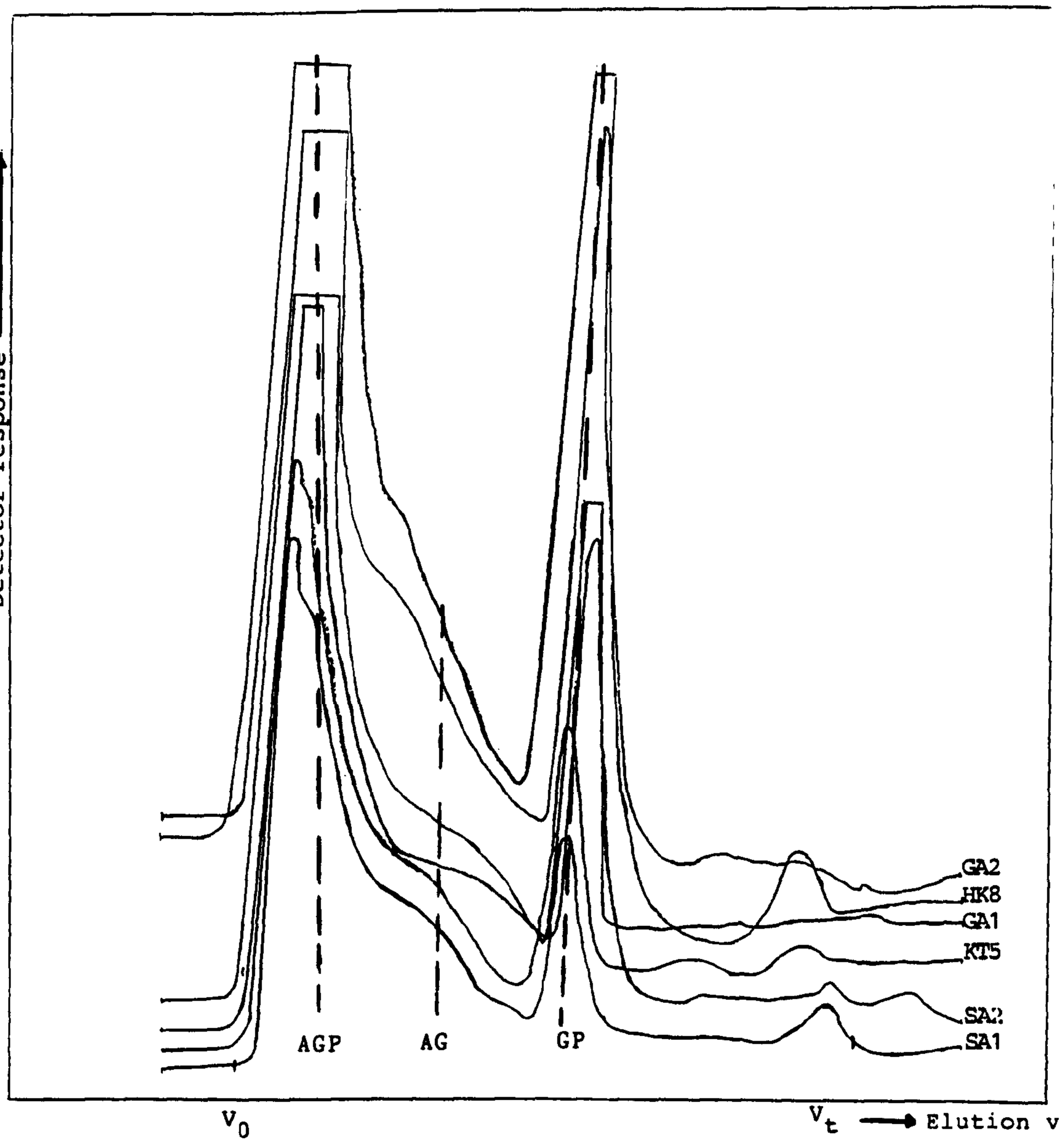


Figure 6.3a: GPC elution profiles of gum arabic as monitored by UV

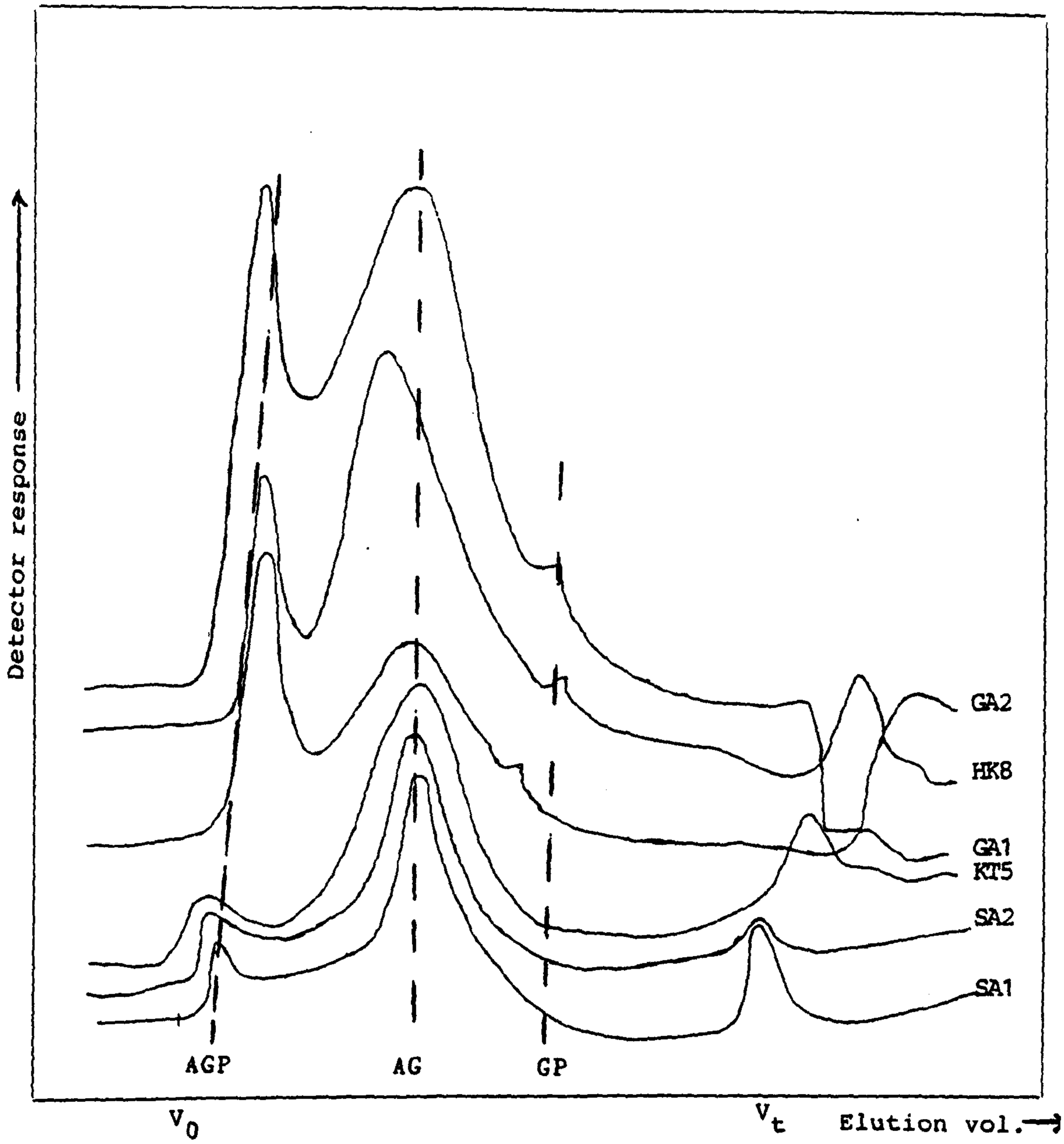


Figure 6.3b: GPC elution profiles of gum arabic as monitored by RI

reflects the concentration of the gum and hence indicates the true molecular mass distribution (Osman, 1993). The enhanced peaks of the UV profiles are said to be due to the very high molecular extinction coefficient of the protein that results in intense absorption (Randall et al, 1989a).

An examination of Fig. 6.3a reveals some characteristic features of the samples. All the samples show three distinct components designated as AGP, AG and GP. The presence of these fractions with identical profiles irrespective of the variety of *A. senegal* reflects the close similarity in the molecular composition of the samples. Osman et al (1993) have established that these fractions are characteristic of gum from *A. senegal* while Phillips and Williams (1994) believe that the presence of the AGP component can be regarded as a marker for gum arabic. A commercial sample from Kenya labelled as GA2 and which was analysed earlier was examined and showed a profile typical of the two samples (KM11 and GA1) which is characteristic of *A. senegal* var. *kerensis*. The RI profile has two main peaks corresponding to AGP and AG fraction of the gum with the latter being the larger clearly showing that it is the main component. One more observation can be made from the profiles. Besides the three components typical of *A. senegal*, samples KM, GA1 and GA2 show more enhanced UV profile due to the AGP and GP compared to typical gum from Sudan. The RI profile also shows a minor peak corresponding to the GP fraction. The enhanced UV peaks and presence of the minor RI peak may be associated with higher overall protein component in these samples. Indeed an examination of the analytical data for samples KM11 and GA reveal overall higher nitrogen contents compared to gum from Sudan. KT5 with a nitrogen content of 0.34% has a profile similar to that obtained for Sudanese gum. Hence it can be seen that the method is able to show differences arising from the protein component and can thus distinguish between the two varieties of *A. senegal* that differ appreciably in their content of nitrogen.

From these results, the gpc is a powerful technique in the characterisation of gum arabic of commerce. The UV profile is able to detect the three chemical species which have now been confirmed as diagnostic of normal gum from *A. senegal* irrespective of variety. It can also distinguish between gum from var. *senegal* and var. *kerensis.* More complete interpretation of the profiles is however, offered when information of the gpc is combined with analytical data (section 6.4.1.1).

6.4.1.3. The use of Elisa method

Gum arabic samples used for the gpc studies except (GA2) were tested in the Elisa for cross reaction. The results are presented in Table 6.2.

Table 6.2. Cross reaction data of gum arabic

Sample	Specific rotation(%)	Nitrogen content(%)	Cross reaction
KM11	-34	0.42	1.74
KT5	-36	0.34	0.90
GA1	-34	0.43	1.52
SA1	-30	0.37	1.00
SA2	-29	0.35	0.98

All the samples cross reacted in the Elisa indicating that they have close molecular characteristics. The extent of cross reaction however varied between the samples with KM11 and GA1 having higher values than SA1 (control). An examination of the nitrogen content reveals that samples with higher cross reaction values contain also higher levels of nitrogen hence protein. Earlier studies had shown that the antibody has strong affinity for the GP fraction, a lesser affinity for the AGP and little or no affinity for the AG fraction (Williams et al, 1992) while Menzies (1992) has suggested that the cross reaction with *A. senegal* gum is due to the presence of the GP fraction. This study did not go that far but when UV

profiles for two samples (KM11 and GA1) with higher cross reaction are examined they were also shown to have enhanced AGP and GP peaks reflecting that increased affinity of the antibody is due to the higher proportion of the groups recognised by the antibodies in the protein component of the gum. The method has scope in characterising gum arabic of commerce but because it seems to be influenced more by the amount of protein present, it needs refining to make it specific to *A. senegal*. It is worth noting that the variation observed above reflect the between sample variation either to variety or location as well as probably age and tend to follow the same pattern as observed for the analytical data.

6.4.2 Evaluation of related *Acacia* gums and suspected contaminants

An assessment of the methods was then extended to gums of other *Acacias*. Two types of samples were examined; three authentic *Acacia* species (section 6.2.4) and four suspected contaminants (section 6.2.3). Results of the physico-chemical and carbohydrate parameters are shown in Table 6.3.

Gums from the three species of *Acacia* (*A. nubica, A reficiens* and *A. tortilis*) have positive specific rotation and are thus distinct from *A. senegal* gum which has a negative specific rotation. They also differ from *A. senegal* with respect to the nitrogen content, intrinsic viscosity and sugar composition, in particular, they generally have lower glucuronic acid (except *A. reficiens*) and rhamnose. From the taxonomic point, the three species belong to the gummiferrae series in Bentham's classification which in general terms tend to posses the characteristics given above Anderson (1987). Recent studies (Phillips and Williams, 1994) have shown that there is some overlap in the properties between gummifferae and vulgares series. This does not accord with the earlier view that there is a clear distinction between the two groups. Of all the analytical parameters, specific rotation remains the most diagnostic in distinguishing members from the two series. When data for the three species are compared with

Table 6.3: Analytical data for gum from related Acacia gums and suspected contaminants

Species name and number	Acacia seyal 1a	* MK1 2	* MK2 3	Acacia drepan 4b	Acacia nilot. 5b	Acacia nubica 6	* X 7	Acacia tort. 8	Acacia refi. 9	Acacia paoli 10c	Acacia poly. 11	Acacia mell. 12d	* Y 13	Acacia sen 14
Loss on drying (%)	13.4	13.4	13.3	nd	nd	11.5	13.6	12.1	14.8	13.8	nd	8.7	13.7	14.7
Total ash, 550 C(%)	2.8	1.9	1.8	2.5	2.5	2.0	0.4	1.4	2.4	1.2	2.9	2.9	2.6	3.0
Specific rotation (degrees)	51	58	56	78	108	84	88	88	76	90	-12	-56	-36	-34
Nitrogen (%)	0.14	0.15	0.17	1.11	0.02	0.20	0.18	2.47	4.10	0.05	0.37	1.45	0.80	0.44
Hence protein	0.9	1.32	1.12	7.3	0.1	1.3	1.2	16.3	27.1	0.3	2.4	9.1	5.28	2.9
Intrinsic viscosity mol/dm Nacl (ml/g)	12.0	13.2	14.1	18.0	10.0	7.9	10.8	11.6	10.4	4.0	16.0	23.5	16.7	21.9
Neutral. eq. wt.	1470	1244	1246	1980	1890	2010	1869	1800	1050	2300	1900	832	843	995
Hence UAA (%)	12	14	14	9	9	9	9	10	17	8	9	21	21	18
Sugar compostion after hydrolysis														
Glucuronic acid	12	14	14	9	9	9	9	10	17	8	9	21	21	18
Galactose	38	34	38	38	44	37	40	34	40	33	54	43	36	39
Arabinose	46	47	44	52	46	53	47	49	35	55	29	27	35	28
Rhamnose	4	5	4	1	1	1	3	7	8	4	8	9	8	16

Note:

a -> Anderson et al, 1984
b -> Anderson, 1978
'c -> Anderson and Weiping, 1990
d -> Anderson and Fraquhar, 1979
* refers to suspected contaminants

published literature there are slight analytical differences. For example *A. tortilis* and *A. reficiens* gave highest nitrogen content recorded for the species. These differences may be due to different subspecies, varieties or regions of the samples analysed. For example, four subspecies of *Acacia tortilis* are recognised (Brenan, 1983); subsp. *tortilis*, subsp. *raddiana*, subsp. *spirocarpa* and subsp. *heteracantha*. Furthermore two of the subspecies i.e. subsp. *raddiana* and subsp. *spirocarpa* are known to have varieties: var. *radiana* and var. *pubscens* are found within subsp. *raddiana* while var. *spirocarpa* and var. *crinita* are found under subsp. *spirocarpa*. Two of the subspecies; subsp. *spirocarpa* and subsp. *raddiana* are reported to occur in Kenya (Brenan, 1983; Hassan and Styles, 1990). *Acacia reficiens* is also known to have subspecies. Two subspecies are recognised with subsp. *misera* reported to occur in Kenya while subsp. *reficiens* is found in Angola and Namibia (Hassan and Styles, 1990).

The elution profiles of the three *Acacia* species are shown in Figures 6.4a and 6.4b. They all differ from the typical profile for *A. senegal* and there are differences between the individual gum samples. The UV profile for *A. nubica* indicates two molecular mass fractions probably corresponding to the AG and GP, the former showing a broad but low peak suggesting low protein content as discussed in the analytical data. The RI profile shows one peak probably reflecting the main AG fraction. The UV profile for *A. tortilis* shows three components but in different proportion from that in *A. senegal*. The AGP fraction gives a minor peak probably deficient in the proteinaceous part. The next fraction is a very broad and enhanced profile reflecting an AG/GP complex. There is also a third peak eluted after the AG/GP complex. Analytical data reveal that *A. tortilis* has a high protein content which appears in this case to be associated more with the

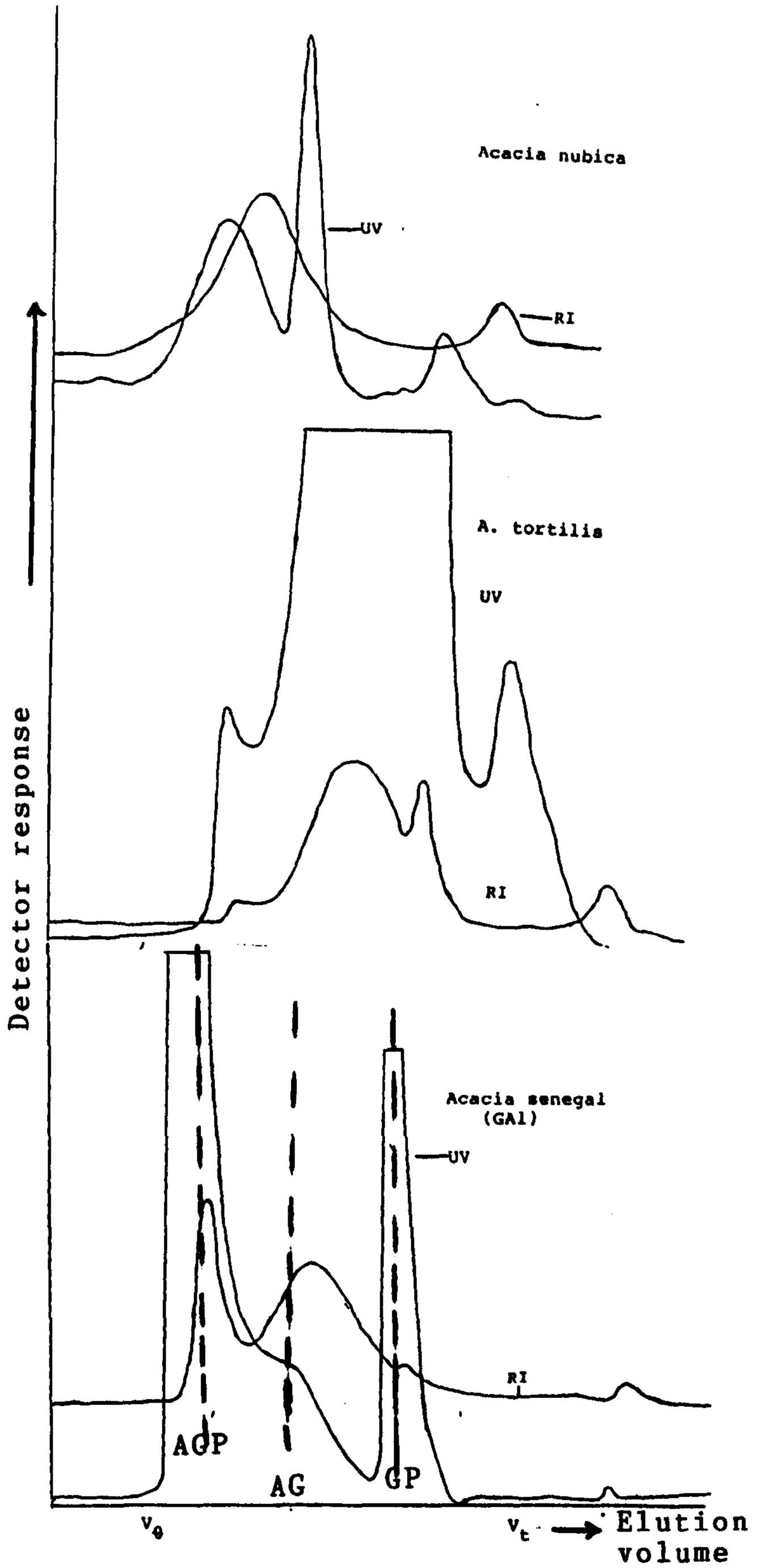


Figure 6.4a: GPC elution profiles of Acacia nubica, A. tortilis and Acacia senegal.

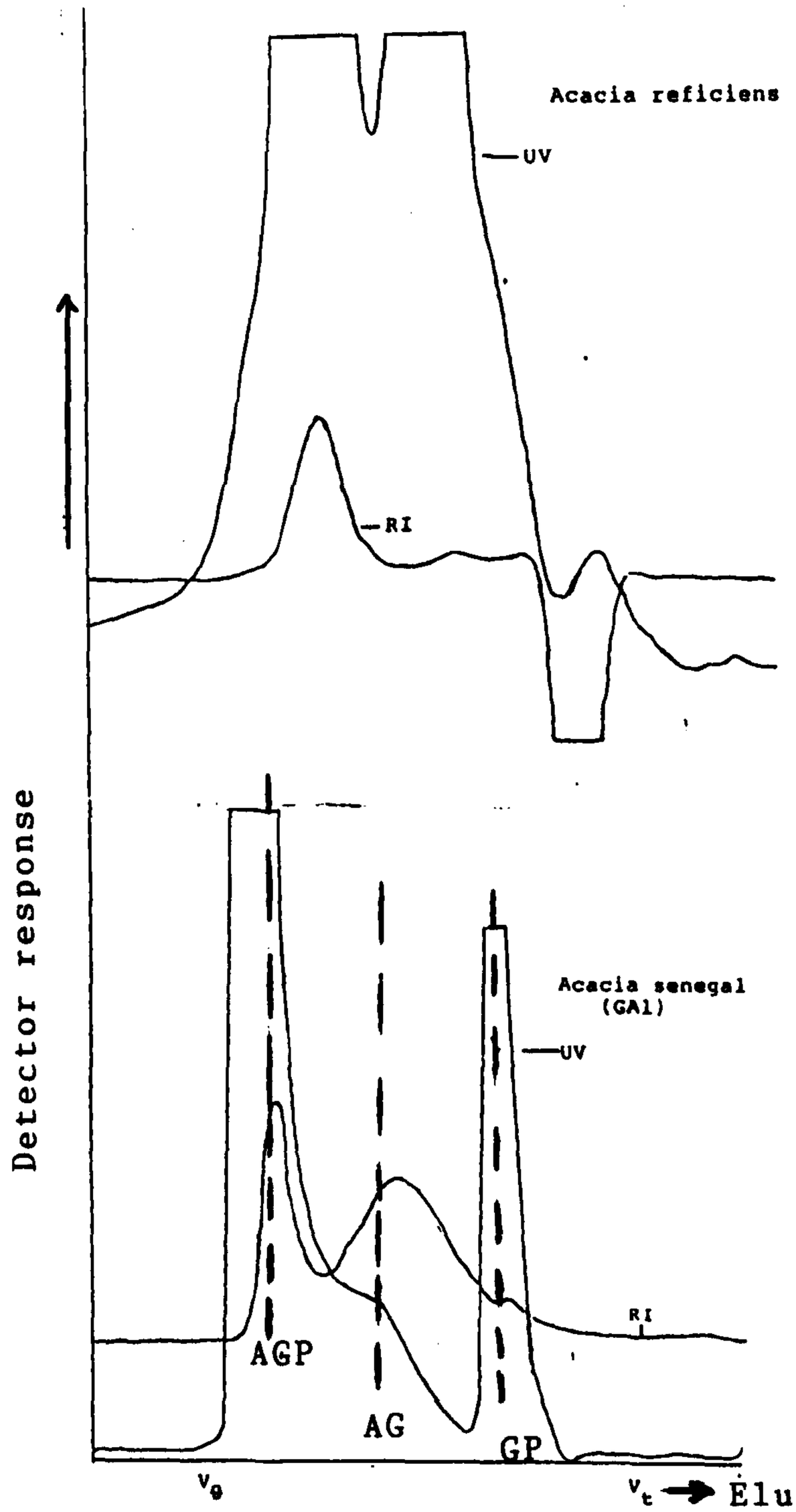


Figure 6.4b: GPC elution profiles of Acacia reficiens and Acacia snegal.

AG/GP complex. The GP component appears to be comprised of two fractions. The presence of more than one fraction of GP in some *Acacia* species has been mentioned previousily (Randall et al, 1989). An examination of the RI profile confirms that *A. tortilis* consists of three molecular mass fractions; the first associated with the AGP and the other two with the AG/GP complex. The main fraction is due to the AG component though the GP fraction is also pronounced. The gum for *A. reficiens* is dominated by two peaks that seem to correspond to the AGP and GP fractions indicating the proteinaceous nature of the gum. Analytical data reveal that it has the highest amount of protein recorded of any gum in this study. The RI gave one molecular mass fraction different in profile from that of *A. mubica* and associated with the first AGP peak in the UV. On the basis of the gpc, the three species can be distinguished from gum arabic and from one another by their different profiles. However, a better explanation is again offered when the profiles are combined with information of analytical data, in particular, specific rotation and nitrogen content.

Results of the immunoassay are presented in Table 6.4. The above three species cross reacted to varying extents that seemed to be related to the protein content. This observation does not accord with Menzies (1992) who did not find direct correlation between protein content and extent of cross reaction despite acknowledging the role of proteins in the interaction with the antibody. However, in these studies the results indicate that, *A. reficiens* gum with the highest level of nitrogen also has the highest value of cross reaction while *A. mubica* with low content of nitrogen gave the lowest extent of cross reaction. This observation is also consistent with results shown in Table 6.2 and is further evidence that the protein plays a major role in providing binding sites for interaction and also that magnitude of interaction is influenced by the amount of protein.

Because of the cross reaction with other *Acacia* gums further work is needed to make it more specific to *A. senegal* gum. This is important since polyclonal antisera were used in this study following injection of whole gum (crude) into the rapid that is known to contain different determinants or different linkages (Luderitz et al, 1966). Pazur et al, (1991) have been able to isolate two sets of antibodies directed against different carbohydrate units of gum arabic (i.e. anti carbohydrate antibodies) and established that the terminal disaccharide moeities of the gum having the structure β-D-glucosyluronic acid-(1-6)-D-galactose and α-L-arabinofuranosyl-(1-4)-D-glucuronic acid were the immunodeterminant groups. Earlier studies on the protein component (Williams et al, 1992) have shown that the antibody shows affinity for the AGP and GP and in particular, the GP (Menzies, 1992). It is thus possible to produce monoclonal antibodies by utilising the protein fractions that have shown immunogenic properties. It is now thought that the antibody recognises the carbohydrate-hydroxyproline junction of the gum as the antigen.

Table 6.4: Cross reaction of other gum exudates

Species	Specific rotation(%)	Nitrogen content(%)	Cross reaction
A. nubica	84	0.2	0.96
A. tortilis	88	2.47	2.57
A. reficiens	76	4.10	5.40
X	88	0.18	0.98
Y	-36	0.80	1.56

Following characterisation of authentic *Acacia* gums, data of the suspected contaminants were examined. To establish the likely source (s), additional data of possible contaminants already analysed were compiled from literature. The results

are shown in Table 6.3. Three of the samples (MK1, MK2 and X) have positive optical rotation and are not therefore from *Acacia senegal* or gum arabic. Samples MK1 and MK2 have identical analytical properties and a comparison with data in the table shows their properties to be closely similar to *A. seyal*. Based on general knowledge of the flora of the area (chapter 3), the samples are likely to be from *A. seyal*. However, sample X has slightly different analytical properties from the two authentic *A. seyal* samples. It has a specific rotation and intrinsic viscosity comparable to *A. tortilis* but nitrogen and sugar contents comparable to *A. nubica* and could not be assigned easily on the basis of analytical parameters. Sample Y had a negative specific rotation and is therefore a member of the *A. senegal* complex. The analytical properties are slightly different from *A. senegal* var. *kerensis* showing higher nitrogen and lower rhamnose content.

The two samples were characterised further using gpc and Elisa methods. The gpc elution profiles are shown in Figures 6.5a and 6.5b respectively. Sample X has a profile similar to that of *A. nubica*. Both have UV profiles that have broad and low AG than GP whilst the RI profile shows only one main component. Sample Y shows three distinct UV peaks which are however, different from *A. senegal*. The peak corresponding to the AGP is smaller than for normal gum arabic while the one for AG is more pronounced. Analytical data shows the sample to be of high nitrogen content. Most of the nitrogen appears to be associated with the AGP/AG complex. Proteinaceous components of low molecular mass are also eluted after GP peak. The RI profile shows two peaks characteristic of gum arabic. From this information, it may be concluded that the sample came from a member of *A. senegal* complex. From knowledge of the flora of the area, *A. senegal* var. *leiorhachis* i.e. a form with struggling branches, is found in Isiolo though *A. mellifferae* also grows extensively but is not known to produce gum on a

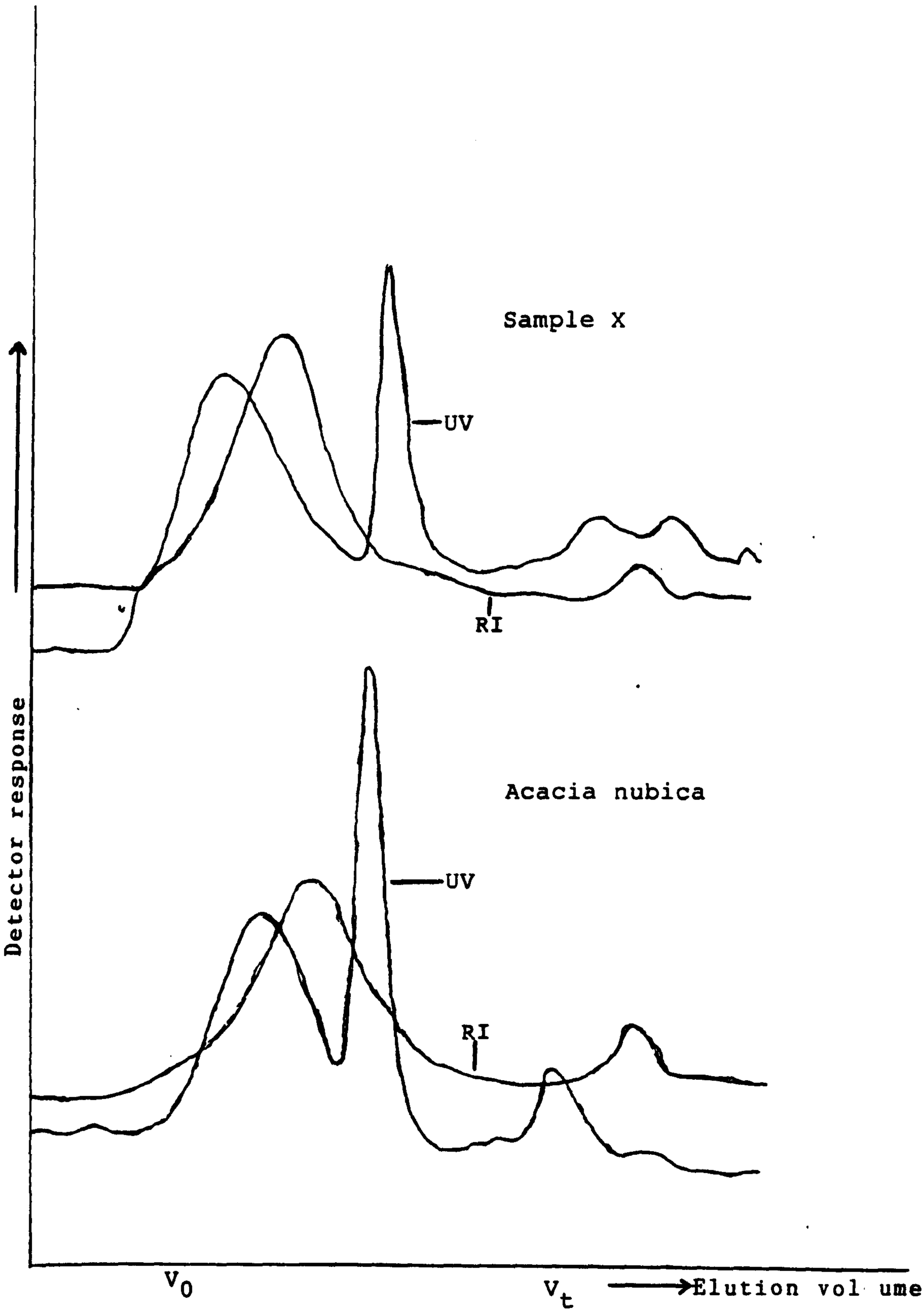


Figure 6.5a: GPC elution profiles for sample X and Acacia nubica

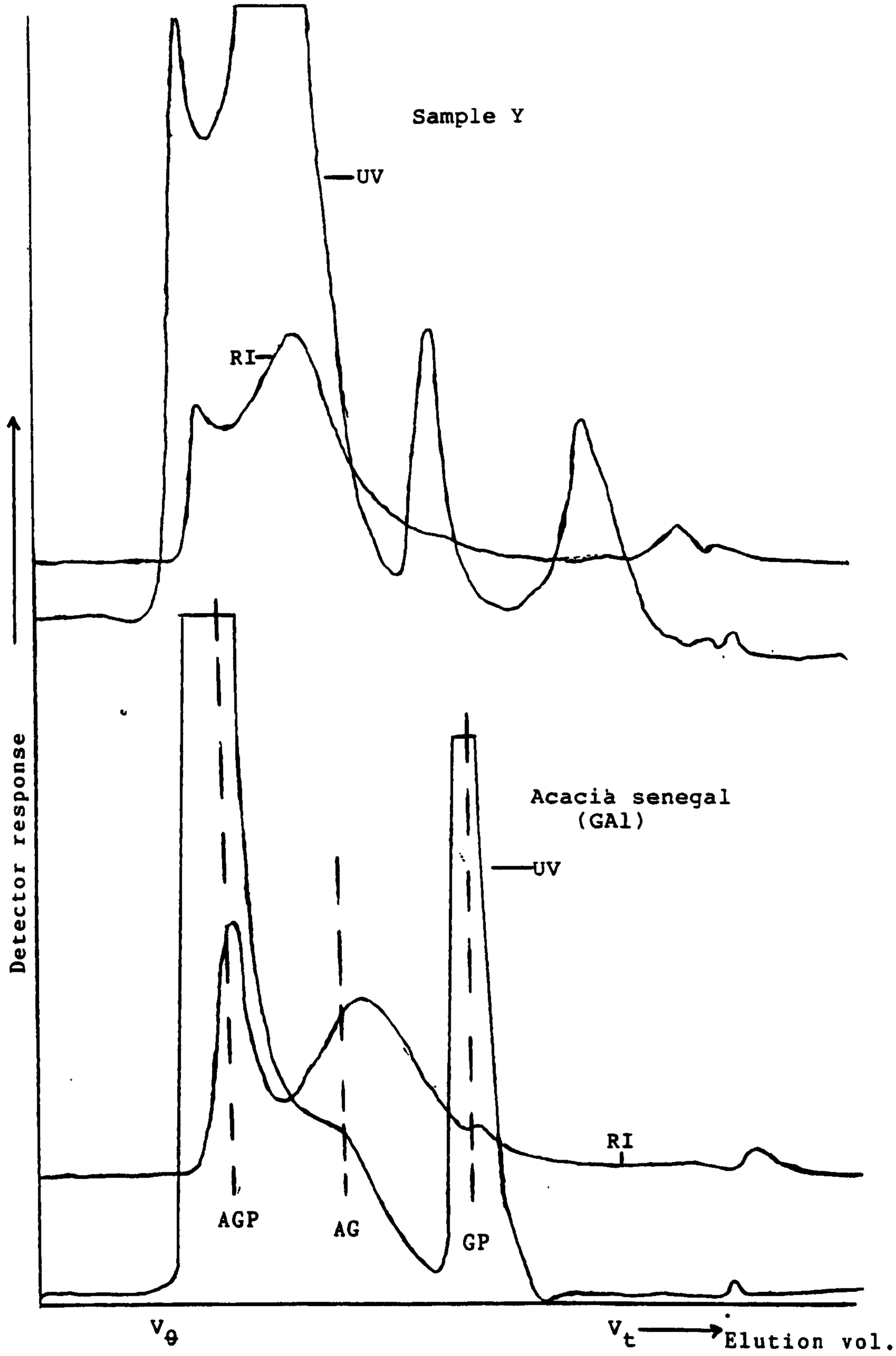


Figure 6.5b: GPC elution profiles for sample Y and Acacia senegal

commercial scale. Results of the Elisa show cross reactions for both samples but they were insufficient to confirm the source.

6.4.3. Evaluation of brown samples of gum arabic

Normal gum arabic is produced either as clear or amber nodules. Sometimes however, trees of *A. senegal* produce brown samples which are occasionally found in commercial gum arabic. Such samples are regarded as adulterants and result in either downgrading or rejection of commercial consignments. An opportunity was presented during the resource survey to collect brown samples from authentic *A. senegal* var. *kerensis* and to examine the chemical nature of the products. Details of the samples are given in section 6.2.2 and appendix IV.

Results of the analytical study are given in Table 6.5. All the samples were exudates either due to insect borer damage or from dying trees. They contained high amounts of foreign matter in the form of insect droppings, wood particles or bark. They were therefore purified by dissolving in distilled water, filteration and freeze drying. A comparison between the samples revealed generally similar properties with slight differences probably reflecting tree to tree differences. It is noteworthy that no tannin was detected in the samples. However, when compared with normal gum arabic, distinct differences were observed in three properties. Specific rotation, intrinsic viscosity and emulsification properties were lower than for normal gum arabic. The low specific rotation is probably linked to colour. All the samples produced coloured solutions even after filtering and freeze drying which could have affected the light passing through the cell path causing a weaker signal and therefore comparatively lower readings. Additionally, the mean values for sugar composition between brown and normal gum arabic show slight differences which could result in the observed differences. The reason for brown colour is not certain but it is thought to be due to several factors (Anderson,

Table 6.5: Physico-chemical and carbohydrate data of brown samples

Sample code	NM16	NM17	NM18	NM19	NM20	I17	I18	KM17	I19	LT	Mean	A. sen.
and number	1	2	3	4	5	6	7	8	9	10		
Loss on drying (%)	8.4	9.2	6.8	8.6	9.4	7.7	7.9	9.9	8.9	9.3	8.6	14.7
Total ash, 550 C(%)	3.1	3.2	2.9	3.7	3.7	4.0	3.2	2.9	3.7	3.1	3.4	3.0
Specific rotation (degrees)	-27	-27	-32	-28	-30	-25	-27	-29	-26	-22	-27	-34
Nitrogen (%)	0.42	0.28	0.39	0.42	0.38	0.44	0.43	0.55	0.43	0.42	0.42	0.44
Hence protein	2.8	1.9	2.6	2.8	2.5	2.9	2.9	3.6	2.9	2.8	2.8	2.9
Intrinsic viscosity mol/dm Nacl (ml/g)	13.5	10.1	15.2	10.4	14.6	10.8	14.5	13.5	15.3	16.1	13.4	21.9
Neutral. eq. wt.	1106	1275	996	1235	845	938	1314	753	961	1088	1051	995
Hence UAA (%)	16	14	18	14	21	19	13	23	18	16	17	18
Emulsification activity (limonine)	1.54	1.41	1.40	1.20	1.51	1.34	1.50	1.41	1.51	1.40	1.42	1.66
Emulsion stablity (%)	85	75	77	65	86	78	80	80	83	84	79	93
Tanin (2% sol.)	0	0	0	0	0	0	0	0	0	0	0	0
Sugar compostion after hydrolysis												
Glucuronic acid	16	14	18	14	21	19	13	23	18	17	17	18
Galactose	40	35	42	40	43	40	40	43	39	40	40	39
Arabinose	24	29	22	23	19	21	28	19	22	23	23	28
Rhamnose	20	22	18	23	17	20	19	17	20	21	20	16

1993). One possible reason could be due to the reaction between the carbohydrate and protein part or production of alkaloids triggered by special mechanisms or enzyme systems following excessive damage to the trees. It is also thought that the presence of large amounts of some metals in the soil (notably iron, manganese or magnesium) and eventually the gum, can result in oxidation and hence colour change though it does not fully explain why it occurs only in the insect infested or dying trees. The reason for the relatively low intrinsic viscosity is not clear based on the analytical data. Poor emulsification suggests that though nitrogen content appears normal, its nature and distribution may be different from that in normal gum arabic. The low values observed in these properties, and in particular, low intrinsic viscosity and emulsification functionality would explain the generally low quality attributed to brown gum. Additionally, the gum has high proportion of foreign matter in the form of sand, bark, wood particles and insect droppings that makes it undesirable for use without special processing facilities.

Two samples (I17 and I18) having similar nitrogen content but differing in intrinsic viscosity and emulsification functionality were examined further by gpc, Elisa and ^{13}C-NMR techniques. Results of the gpc are given in Figure 6.6. The UV profile (reflecting the distribution of the protein component) for sample I18 is identical to normal gum (sample KM11) except that it has a more enhanced GP peak probably indicating that it is associated with more protein than in normal gum arabic. The RI profile (reflecting total concentration of the gum) on the other hand shows a higher proportion of the gum associated with the AGP fraction in comparison to normal gum arabic. I17 gives a UV profile with three molecular mass species having profiles that differ slightly from the normal gum. The GP profile is more pronounced than the AGP while AG which usually appears a shoulder in normal gum arabic is lacking. The RI gives two mass fractions associated with AGP and AG as in normal gum. However, both the UV and RI

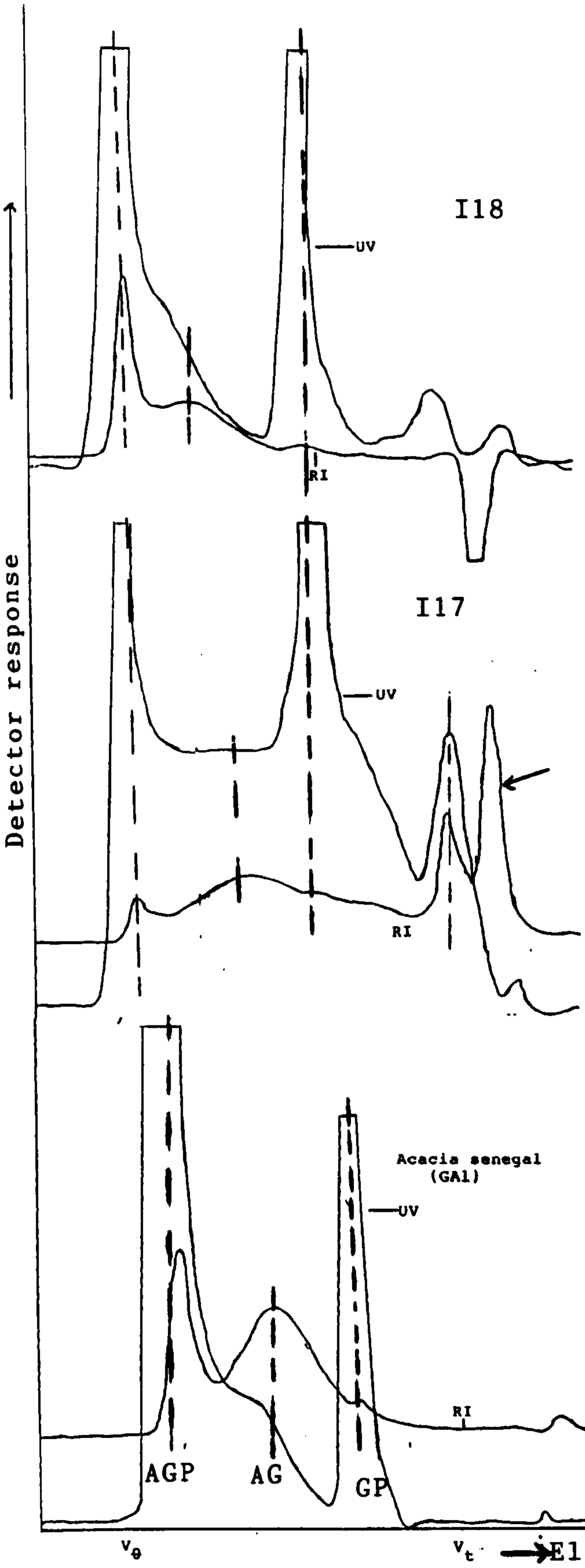
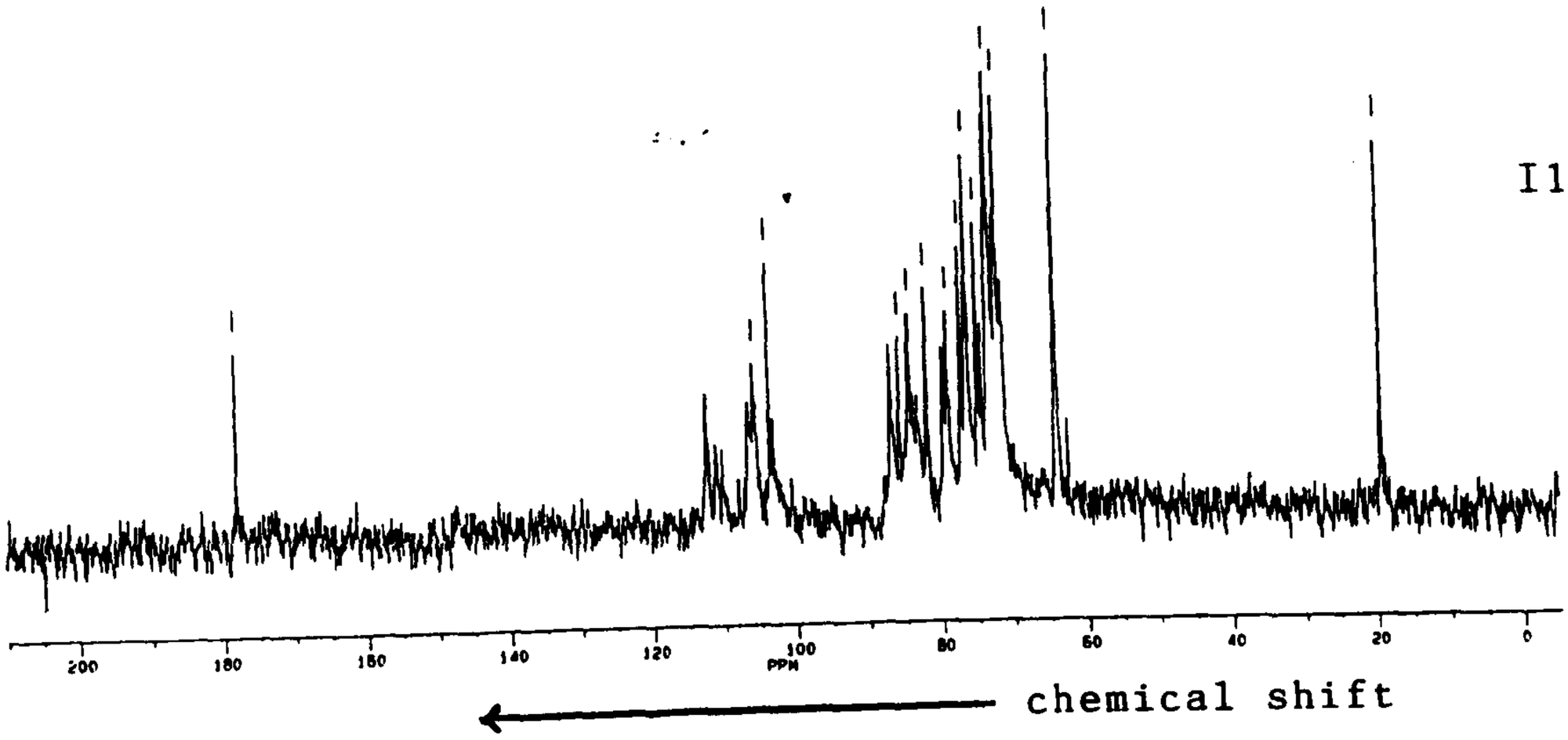
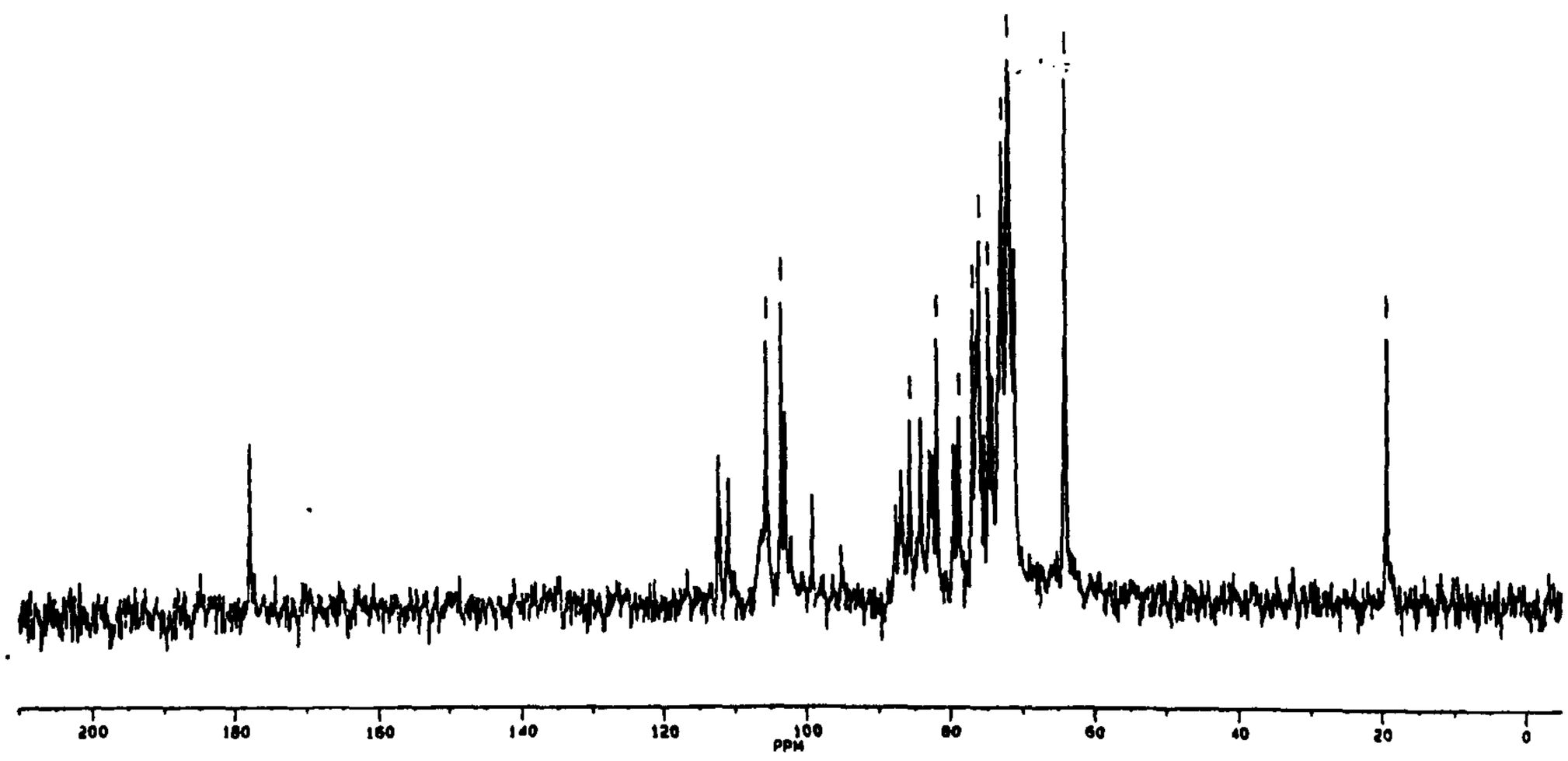


Figure 6.6: GPC elution profiles for brown samples I18 and I17 and normal gum arabic

also show a pronounced peak eluted after GP which probably indicates either an additional component or some sort of degradation. The differences observed in the profiles for brown samples compared to normal gum reflect different distribution of the components that may be affecting intrinsic viscosity and functional properties. Dickinson et al (1991) also found that diferences in the emulsifying properties observed in gum samples having essentially the same nitrogen content could be attributed to the different nature and distribution of the proteinaceous component. Results of the Elisa showed that the gums cross react typically of the all gums studied.

To check whether the analytical and molecular differences observed could be reflected in the structural nature, ^{13}C-NMR spectra were acquired. Results are shown in Figure 6.7. The spectra show vertically identical finger prints of the samples indicating close similarity in their fine structure. All gave resonances at 19.36 ppm attributed to C_6 of α-L-rhamnopyranosyl and at 177.9 ppm attributed to C_6 of β-D-glcpA. The two regions represent extreme fields of the spectra and are due to -C-CH_3 (at 15-23 ppm) and -COOH or -C=O (at 172 + ppm) groups respectively (Weiping, 1993). The slight differences observed reflect different linkages and carbon positions which will affect the chemical shifts. Earlier studies had shown that brown gum arabic is structurally similar to normal gum (Anderson and Dea, 1968). It can be concluded from these results that while the protein content and structure (^{13}C-NMR) might be similar , the low quality of brown samples arise from different distribution of the protein component (results of gpc) which affect the emulsification functionality. Additionally, brown gum results in coloured solutions which limit its application in certain food products beside the higher content of foreign matter. Due attention should therefore be given to colour during grading.





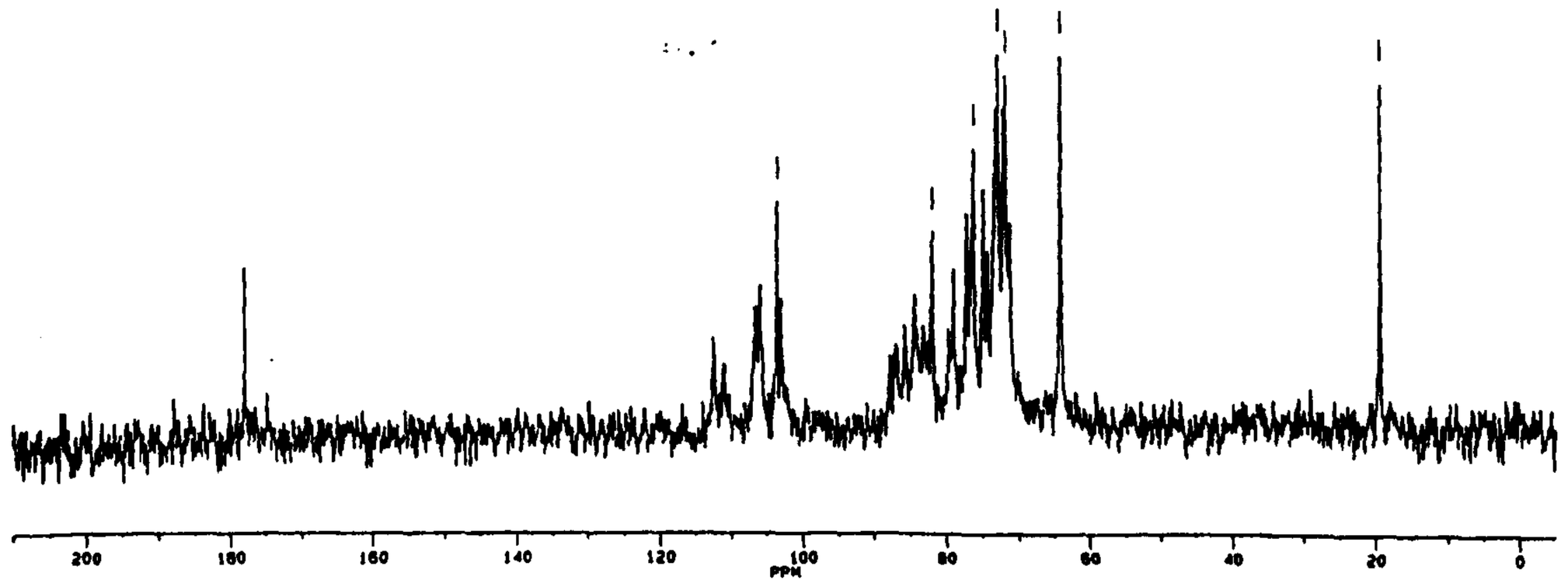


Figure 6.7: ^{13}C-NMR spectra for normal gum arabic (HK8) and brown samples (SSM3 and MI8)

6.4.4. A chemometric evaluation of gum arabic of commerce

6.4.4.1. Introduction

The various methods described above are based on analysis of individual chemical parameters. However, where several sets of analytical data on gums from different sources exist, a chemometric technique can be employed to examine whether they are members of the same or different species. This technique developed recently for gum arabic, is a multivariate statistical method that utilises the Principal Component Analysis (PCA) and Discriminant Component Analysis (DCA) approach (Jurasek et al, 1993a). Physico-chemical, carbohydrate and amino acid data are used as coordinates in n-dimensional space and projected onto a plane spanned by principal components generating scores where each score represents a linear combination of features. In the PCA the first principal component (PC1) of data matrix is the direction with maximum variance in p-dimensional space while the second principal component (PC2) is the direction orthogonal to PC1, also with maximum variance. Plots of scores for PC1/PC2 (or PC1/PC3) provide graphical representation of the PCA results where each point corresponds to a gum sample. The distance and position of points relative to each other indicate similarities or differences between samples and when a 'cluster' is formed it indicates a close similarity to members of related species, sub-genus or variety. Discriminant component analysis on the other hand allows projection onto a plane spanned by the most distinctive features recognised.

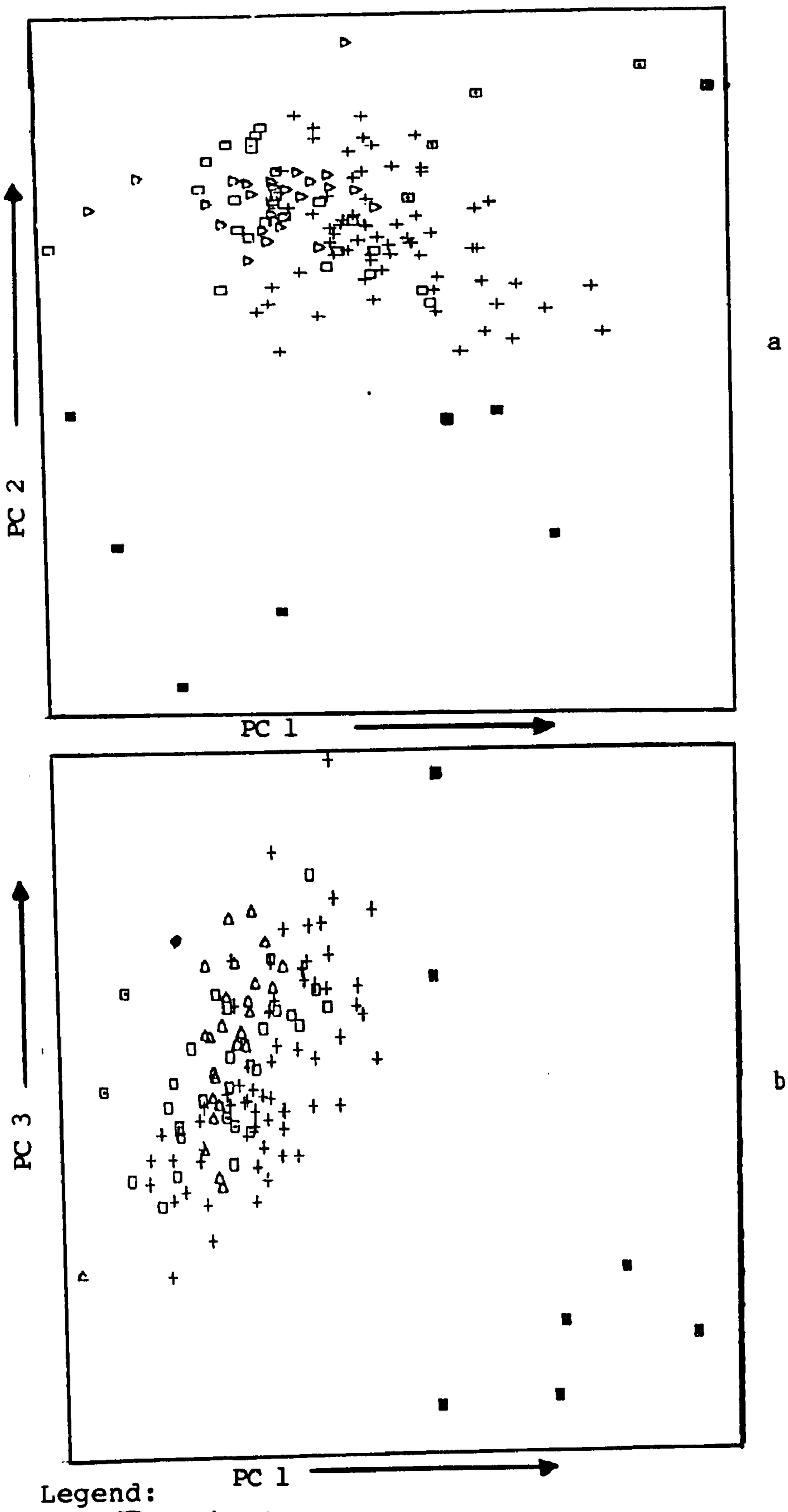
A chemometric study of gum arabic from Kenya was carried out at the Slovak Technical University, Slovakia as a contribution to the on-going studies on the classification of natural gums. Detailed analysis are given in a paper by Jurasek et al (1994). The physico-chemical and carbohydrate data on the 67 Kenyan samples were from Tables 5.6a - 5.6d and 6.6 of the present thesis and were

combined with 65 samples analysed earlier (Jurasek et al, 1993a). Data on 18 amino acids was also combined with 48 samples analysed by Jurasek et al (1993b).

6.4.4.2. Results and discussion

Figure 6.8a are the PCA spanned by planes PC1/PC2 and PC1/PC3 for the physico-chemical and carbohydrate parameters. PC3 was included in the analysis because it gave more elaborate representation. Samples from Kenya were associated with the main *A. senegal* cluster with most falling within the cluster. Some samples formed the periphery and a scrutiny revealed that they were from Turkana, an area with high negative optical rotation and low intrinsic viscosity. When compared with samples from members of vulgares series that are not *A. senegal*, the Kenyan samples were closer to the main cluster suggesting that they are true gum arabic. Interestingly, samples of brown gum arabic which are of inferior quality fell within the main cluster indicating that for purpose of classication, they are true gum arabic. On the other hand, combretum gum fell outside the main cluster reflecting that they are not related.

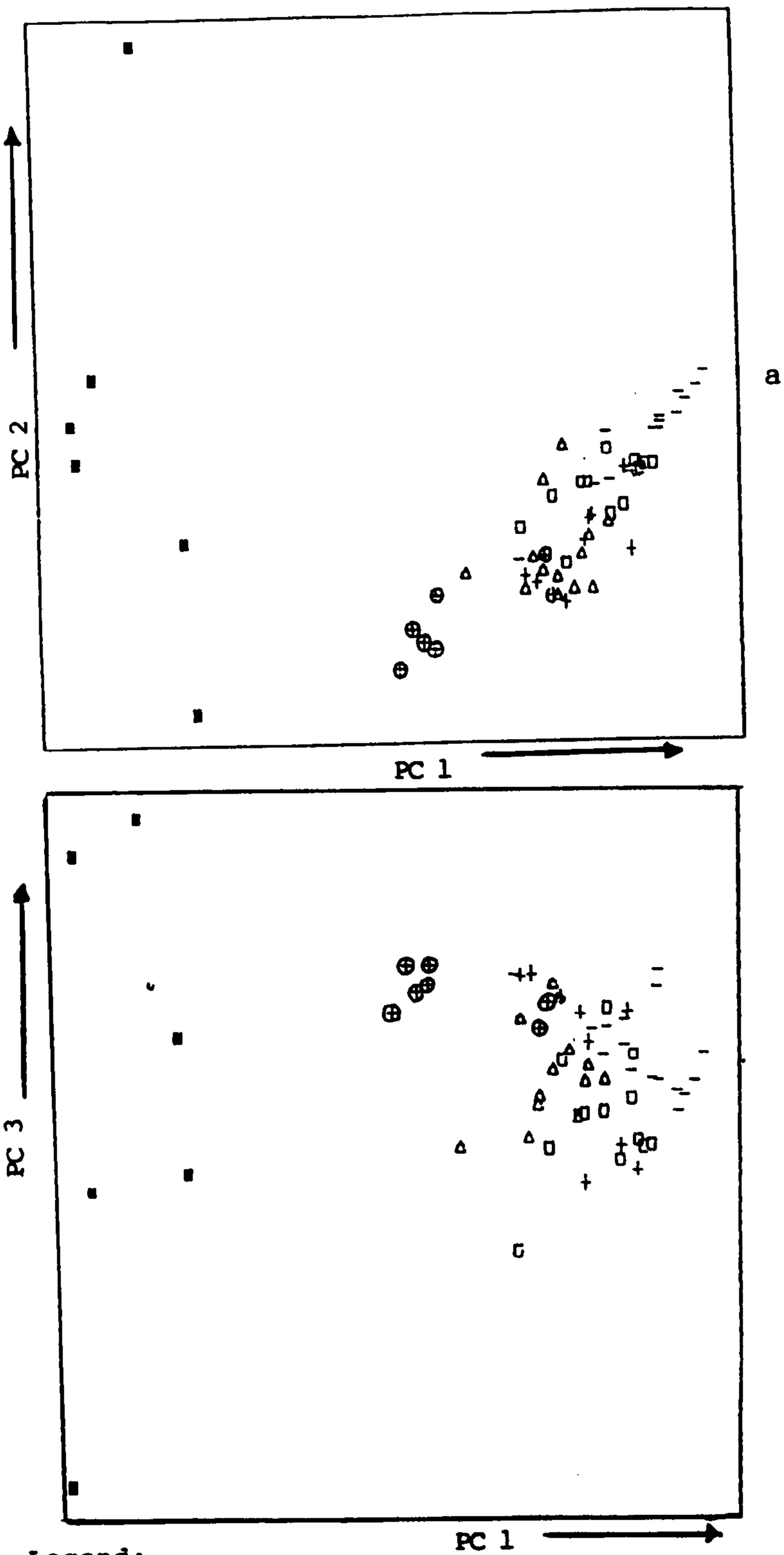
When PCA plots due to the amino acids were examined (Figs. 6.8b), again a number of Kenyan samples fell centrally within the main cluster, more so for PC1/PC3 confirming further that it is true gum arabic. Some samples form a minor cluster on the periphery and were found to be from Kargi, Marsabit attributed to the high nitrogen content. Samples from combretum gum fall outside the main cluster showing that they are not related. A chemometric method is thus a useful technique for identifying gum arabic from other gums. Earlier studies have shown that it can readily distinguish between gum from gummiferae and vulgares series due to the distinct clusters formed by each group while still showing overlap at the boundaries indicating a common taxonomic origin (Jurasek et al, 1993a). As a routine routine quality control technique it has limitations since occasionally outliers may occur in the plots for samples which are truly of a given variety.



Legend:

- ■ Combretum
- ◘ Acacia (vulgares series)
- □ Gum arabic (Sudan and Nigeria)
- △ Authentic A. sengegal gum (Sudan Nigeria and Niger)
- \+ Kenya (A. senegal var. kerensis)

Figure 6.8a: Principal component analysis based on nine physico-chemical and carbohydrate data. a) PC1/PC2 with 30.1% and 22.0% variance, b) PC1/PC3 with 30.1% and 19.2% variance



Legend:
- ■ Combretum A. senegal
- △ Sudan
- + Nigeria
- ● Uganda
- – Kenya (A. sengal var. kerensis)

Figure 6.8b: Principal component analysis based on 18 amino acids and nitrogen content.
a) PC1/PC2 with 52.5% and 10.3%, b)PC1/PC3 with 52.5% and 10.1%

However, the method is powerful and will show changes in trends in gum character appearing within a collection area or from a given source.

6.4.5. Guidelines for production of gum arabic of uniform and good quality

Apart from characterising gum arabic and related gums the above methods were also developed for monitoring and quality control. However, since a single consignment consists of thousands of nodules and taking into account the natural variability that exist in gum arabic of commerce, the effectiveness of even the best method can be considered only as approximate. The main requirement for gum arabic of commerce is that it must be free from contaminating gum that are not permitted food additives. This requirement can easily be made in producer countries if the suggestions given below are followed.

Results of the resource survey revealed that all the gum arabic resources in Kenya are growing in natural stands and in association with some of the gum producing *Acacias*. It was further shown that the main source of gum arabic is *A. senegal* var. *kerensis* and likely contaminants were *A. seyal*, *A. tortilis* and probably *A. paoli* or *A. mubica*. The source of gum arabic and suspected contaminants in other countries are likely to be different depending on ecological conditions and plant community associations. To readily overcome the problem of adulteration, the main source (s) of gum arabic and possible contaminating gums in each country must be identified. Local farmers should then be enlightened on the differences and importance of collecting gum from main source. In a case where one of the contaminating species produces gum on a commercial scale, this should be collected separately. Meanwhile representative samples of gum from main source of gum arabic and contaminant gums should be collected for subsequent characterisation and future visual comparison.

Secondly characterisation of gum arabic from Kenya revealed regional differences with some areas (Marsabit) producing gum having relatively high nitrogen content and intrinsic viscosity while Turkana produced gum with low viscosity but higher negative specific rotation. To minimise variation in a consignment, gum within a country could be marketed by region. In consignments for export, they should indicate source by variety which would eliminate suspicion that the slightly different properties observed are probably due to adulteration. The example where starch is usually marketed by source (potato, corn, etc) could be applied. Meanwhile during handling stringent rules should be observed at grading stage to eliminate samples of different colours or unusual size even if they are true gum arabic as they tend to posses unique properties due to different modes of formation. All gum must be properly dried to avoid deterioration during storage and reduce gelling during processing.

Finally, laboratories in producer countries need to be equipped with modest facilities for routine monitoring and quality control. Important equipment would be for physico-chemical and carbohydrate analysis and a gpc unit. These would be sufficient for generating data on the main source of gum arabic and contaminating gums. Initial analytical data for monitoring and quality control would be on moisture content and specific rotation to provide information on the condition of the gum and whether the suspected sample is from gummiferae. Additional data might then be required on the nitrogen content, intrinsic viscosity and in countries where the contaminant is from either *Albizia* or *combretum* species, data on sugar composition would be useful. Confirmation can then be obtained from gpc profiles.

CHAPTER 7: PROCESSING OF GUM ARABIC AND SOME NEW OPPORTUNITIES

7.1. Introduction

Being a natural product, gum arabic is subject to some variability. Because it is collected from the bush, it contains foreign matter in the form of sand, dust, bark and dead insects as well as micro-organisms whose count grows with time depending on storage conditions. The different nodules also posses different physical and chemical properties that make the material variable. It therefore requires processing to clean it from foreign matter and ensure a uniform product. The most common method of processing involves dissolution of the gum in water and heating the solution at around 100°C for a short period to make it flow easily as well as sterilise it.

Although gum arabic is readily soluble in water and aqueous solutions of over 50 percent concentration can be prepared, some gums behave like a chewing gum (a condition called stringiness) at concentrations of 30 - 40 percent, they do not flow readily and are difficult to filter (Woolen, 1982). The gums become impossible to handle and need special processing. This condition has been experienced among some consignments from West Africa and was also observed in samples in the present study and appears to be related to high viscosity. The tendency to form gel can compound the problem of processing further.

To overcome the high viscosity requires some aspect of breaking down the gum molecule. Various methods exist that breakdown or separate the gum into various components enabling elucidation of its structure. Auto hydrolysis (heating gum at boiling) readily cleaves labile furanoside linkages within the gums (Cree, 1966) resulting in a reduction in viscosity and also in protein content (McDougal, 1987). Mild acid hydrolysis and Ultra Violet (UV) radiation have similar effects, with UV radiation having effects comparable to auto hydrolysis. Mild acid hydrolysis tends to cause more drastic degradation

however (McDougal, 1987). The influence of these treatments on the functional properties of gum has not been adequately studied.

The use of enzymes in breaking down the gum molecule resulting in reduced viscosity has also been reported (Connoly et al, 1987). Randall et al (1988) used protease enzyme to break down gum arabic but found that the protein rich fraction decreased with time and such a degraded gum did not produce stable emulsions indicating the importance of the protein component in emulsion functionality. A recent study using viscozyme (a carbohydrase complex) has shown that there is reduction in viscosity (Weiping, 1993).

The present study was designed to investigate ways of reducing viscosity and gelling of gum arabic from Kenya without drastically affecting its emulsification functional properties using experiments on heating and enzyme hydrolysis.

7.2. Auto hydrolysis and moderate heating

7.2.1. Theoretical considerations

Heating gum solution makes it flow easily. This is due to the lowering of viscosity which is known to be inversely proportional to temperature (Aspinall, 1982). The use of heat is thus common practice in the initial stages of gum processing (Woolen, 1982). However, prolonged heating causes a breaking down of the gum molecule. Earlier studies showed that gum arabic undergoes auto hydrolysis splitting off L-arabinose and L-rhamnose leaving a core of D-galactose and D-glucuronic acid (Whistler, 1959). Subsequent work confirmed that auto hydrolysis breaks down the galactopyranosidic bonds resulting in constituent sugars, some aldobiouronic acids and oligosaccharide material leaving a core that has galactose as the reducing end group (Anderson and Stoddart, 1966). The degraded gum is greatly reduced in molecular weight and viscosity.

Gum arabic is not made entirely of carbohydrate but has also small amount of protein (Anderson and Stoddart, 1966). Biochemical studies have

shown that gum arabic is in fact an Arabinogalactan-protein having a high proportion of carbohydrate (90-95%) with arabinose and galactose as predominant monosaccharides and a low proportion of protein (2-3%) containing high levels of hydroxyproline (Fincher et al, 1983). The protein fraction is the major component responsible for the emulsification functionality (Randall et al, 1988). It is quite sensitive to heating and tends to precipitate out as a brown flocculent on heating (McDougal, 1987; Randall et al, 1989b). However, the extent to which it affects the physical, chemical and functional properties of gum arabic has not been adequately addressed and yet it remains the most common industrial processing practice. It has further been shown that gum solution can change in viscosity without drastically changing the molecular structure of the gum molecule if heated at moderate temperature (Fenyo and Vandevelde, 1990). Understanding these changes, and in particular, a lowering of viscosity with limited loss in functionality was the aim of the present investigation.

7.2.2. Materials and methods

Nodules of gum that remained after experiments in chapters 5 and 6 were used. A 10% unbuffered gum solution (w/v) was prepared and heated in an oil path set at 100°C to asses the effects of auto hydrolysis. The solution was allowed to stand for 96 hours with samples of 100 ml being removed every 3, 6, 12, 24 and 48 hours. The samples were cooled and dialysed against distilled water (regular changes) for 24 hours using a 1200-1400 molecular cut off membrane. Dialysates were concentrated (rotary evaporator) and freeze dried to give a series of fractions labelled as D3, D6, D12, D24, D48, and D96 (i.e. D3 for the product heated for 3 hours, D6 for 6 hours, etc.). For samples which formed brown flocculant, the latter was filtered, collected and freeze dried. Contents remaining in the dialysis bag (residual) were dialysed for a further 48 hours against running tap water, concentrated and freeze dried to give fractions S3, S6, S12, S24, S48 and S96. A similar procedure was

followed for the experiment on moderate heating except that the temperature was set at 65°C and yielded fractions MH3, MH6, MH12, MH24, MH48 and MH96. Meanwhile, sample GA was dissolved, filtered and freeze dried as unmodified control. The analytical and gpc methods used in the analysis of the samples are described in chapters 5 and 6 respectively.

7.2.3 Results

Analytical data on sequential auto hydrolysis are shown in Table 7.1. The dialysate samples (D3-D96) were sticky and could not be recovered readily and only measurements on yield were recorded. The rest of the data presented is therefore for fractions labelled as S3-S96. Interest was centred on changes in intrinsic viscosity, the main parameter under investigation and accompanying changes in the gum molecule as hydrolysis progressed. Intrinsic viscosity decreased progressively with time attaining a value of 8.2 ml g^{-1} after 96 hours. The relationship between viscosity and hydrolysis time was almost linear (Fig. 7.1). A value of 15.4 ml g^{-1} was reached after 12 hours. An examination of the data and figure reveals that changes in other properties had began to be evident at this time, notably nitrogen (hence protein) content and emulsification properties. However, there were no apparent changes in the carbohydrate component as specific rotation and sugar composition remained practically unaltered.

Table 7.1: Analytical data for gum arabic after autohydrolysis

		GA	S3	S6	S12	S24	S48	S96
Recovery (%)	D	3.0	3.3	3.8	5.1	10.0	15.9	22.0
	S	96.8	96.4	96.0	94.6	89.4	83.6	78.0
Moisture(%)		6.4	8.8	5.5	8.6	9.2	9.4	6.8
Specific rotation (deg)		-33	-33	-33	-32	-29	-27	-24
Nitrogen (%)		0.43	0.42	0.41	0.40	0.37	0.34	0.33
Hence protein		2.85	2.79	2.72	2.65	2.45	2.25	2.19
Intrinsic viscosity		23.8	21.3	18.6	15.4	12.9	9.7	8.2
Emulsion activity		2.74	2.72	2.70	2.61	1.89	1.40	1.38
Emulsion stability		95	94	94	90	70	52	51
Equivalent weight		837	831	885	866	858	850	851
Uronic Acid Anhy.		21	21	20	20	21	21	21
Gel (25% sol.)		++	+	-	-	-	-	-
Sugar composition after hydrolysis								
Glucuronic acid		21	21	20	20	21	21	21
Galactose		37	36	38	38	41	47	47
Arabinose		26	26	26	26	22	18	18
Rhamnose		16	17	16	16	16	14	14

Where:

D -> diffusate (-) -> no gel ++ -> moderate gel

S -> residual + -> light gel

More changes in the gum began to show after 12 hours. Yield of gum recovered (residual) decreased to 78 per cent while the amount of products recovered as dialysate increased from 3 per cent to about 22 per cent. The loss in the protein was more drastic between 12-48 hours representing about 62% of the protein lost. A brown solid which precipitated out of solution on standing was apparent at 12 hours of hydrolysis and the amount precipitated out increased with time. Analysis of the flocculent precipitate after 48 and 96 hours for protein gave values of 21 and 22 per cent respectively. Conversely, protein content of the residual gum decreased by 23 per cent while the emulsion properties decreased by more than half the original values. Changes in the carbohydrate component were also apparent. Specific rotation decreased to

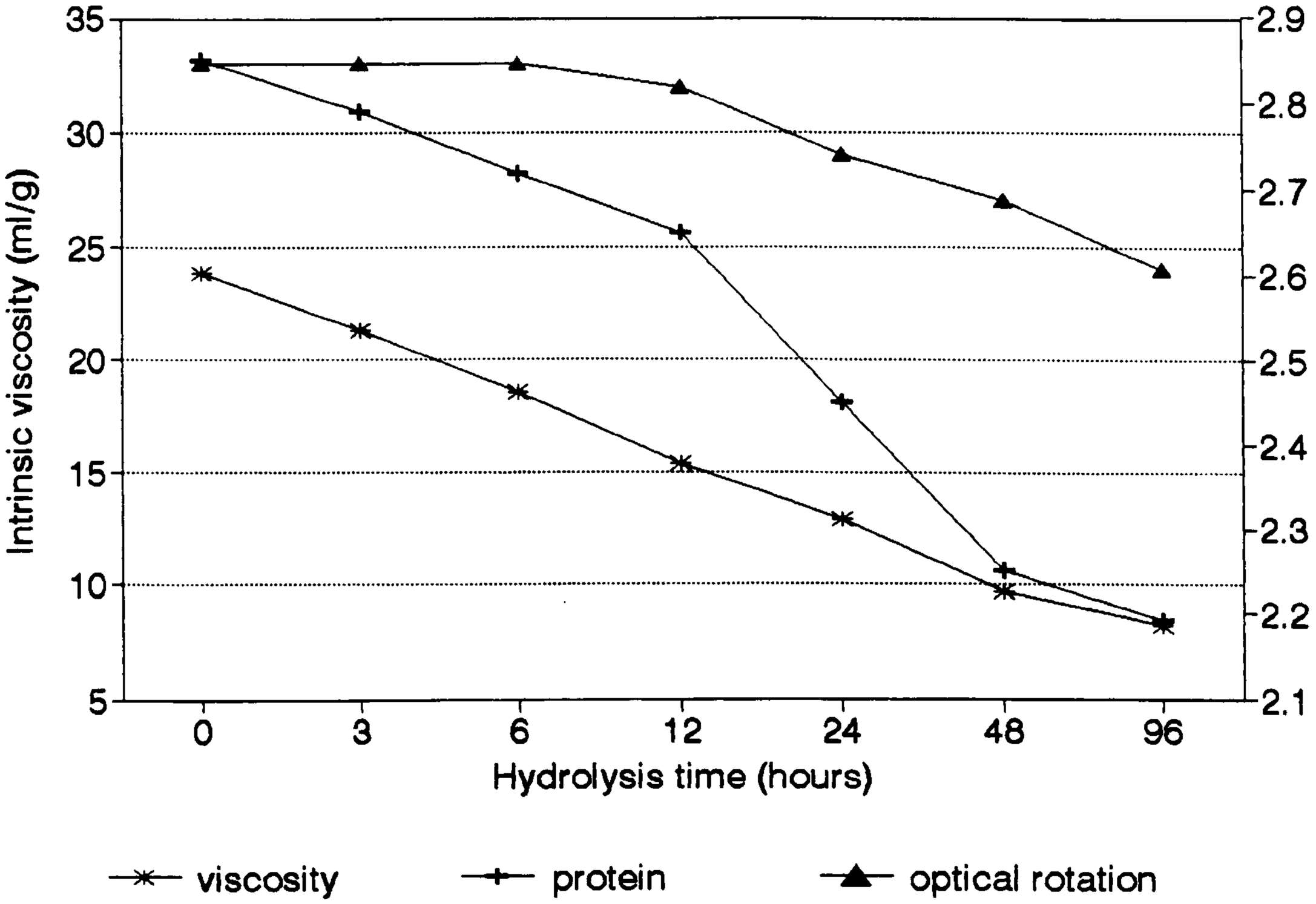


Figure 7.1: Relationship between viscosity and hydrolysis time with accompanying changes in the gum molecule

-24 and most losses were observed in the arabinose and rhamnose with corresponding to increase in galactose. To find out the extent to which loss in the protein content and sugar composition (based on specific rotation) influences intrinsic viscosity, multiple regression based on the general linear models procedure was computed and gave the regression equation below:

$$(\eta) = -25.9 - 0.06X1 + 36.9X2 - 1.70X3;\ R^2 = 0.97;\ p = 0.007$$

where:

(η) = intrinsic viscosity

X1 = hydrolysis time

X2 = protein content

X3 = specific rotation

The changes of the two components had a strong influence on viscosity with the protein fraction imparting a significant contribution.

To gain further knowledge on the structural changes taking place, the unmodified and auto hydrolysed gums were monitored by gpc. The UV profiles (Fig. 7.2) show a substantial decrease in the glycoprotein fraction and gradual decrease in the AGP fraction. The glycoprotein fraction disappeared after only six hours. The RI profiles were more revealing (Fig. 7.3). The AGP fraction decreased gradually with time and was practically totally removed after 48 hours giving way to the major AG fraction.

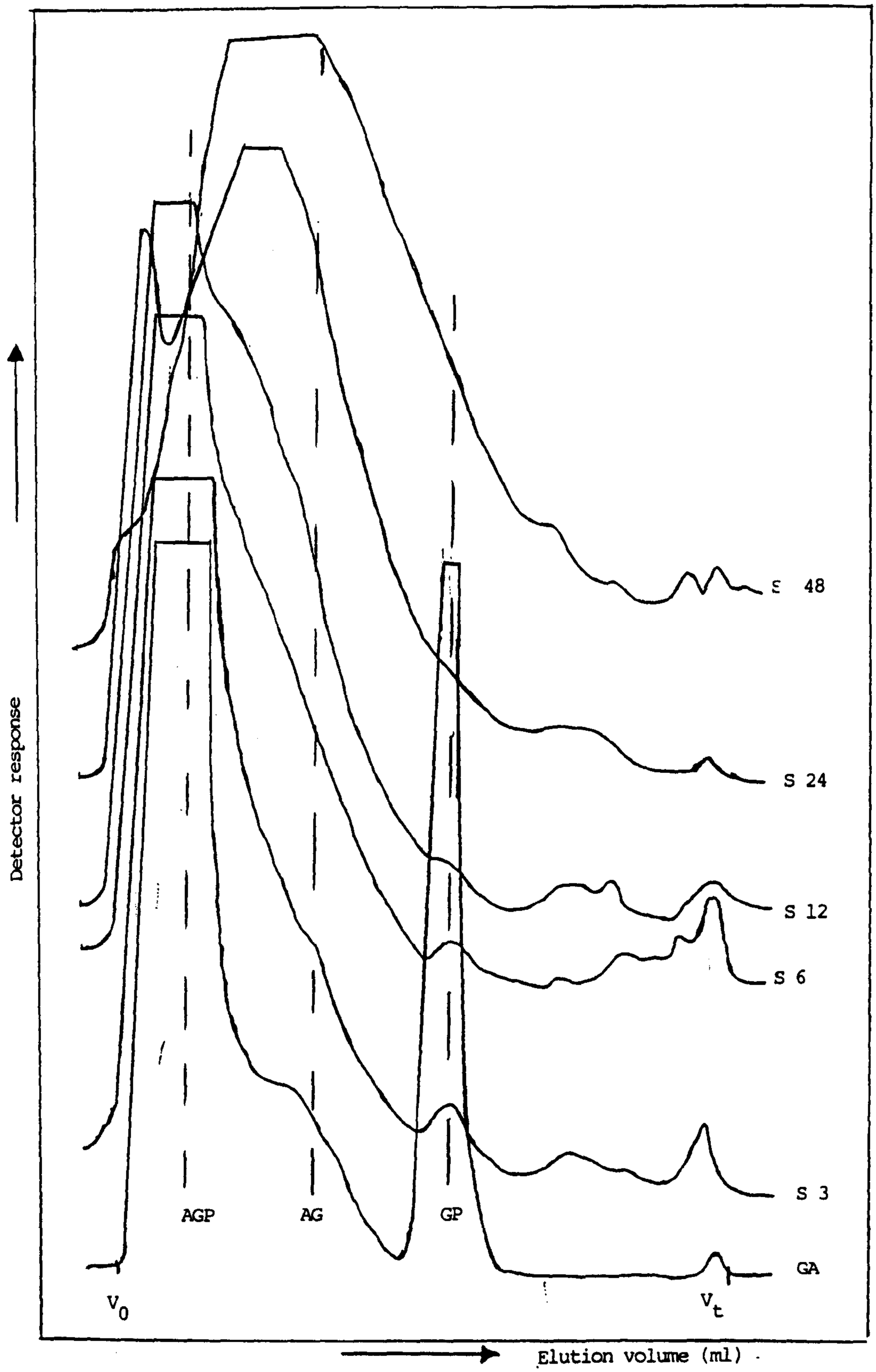


Figure 7.2: UV elution profile of the gum solution after auto hydrolysis

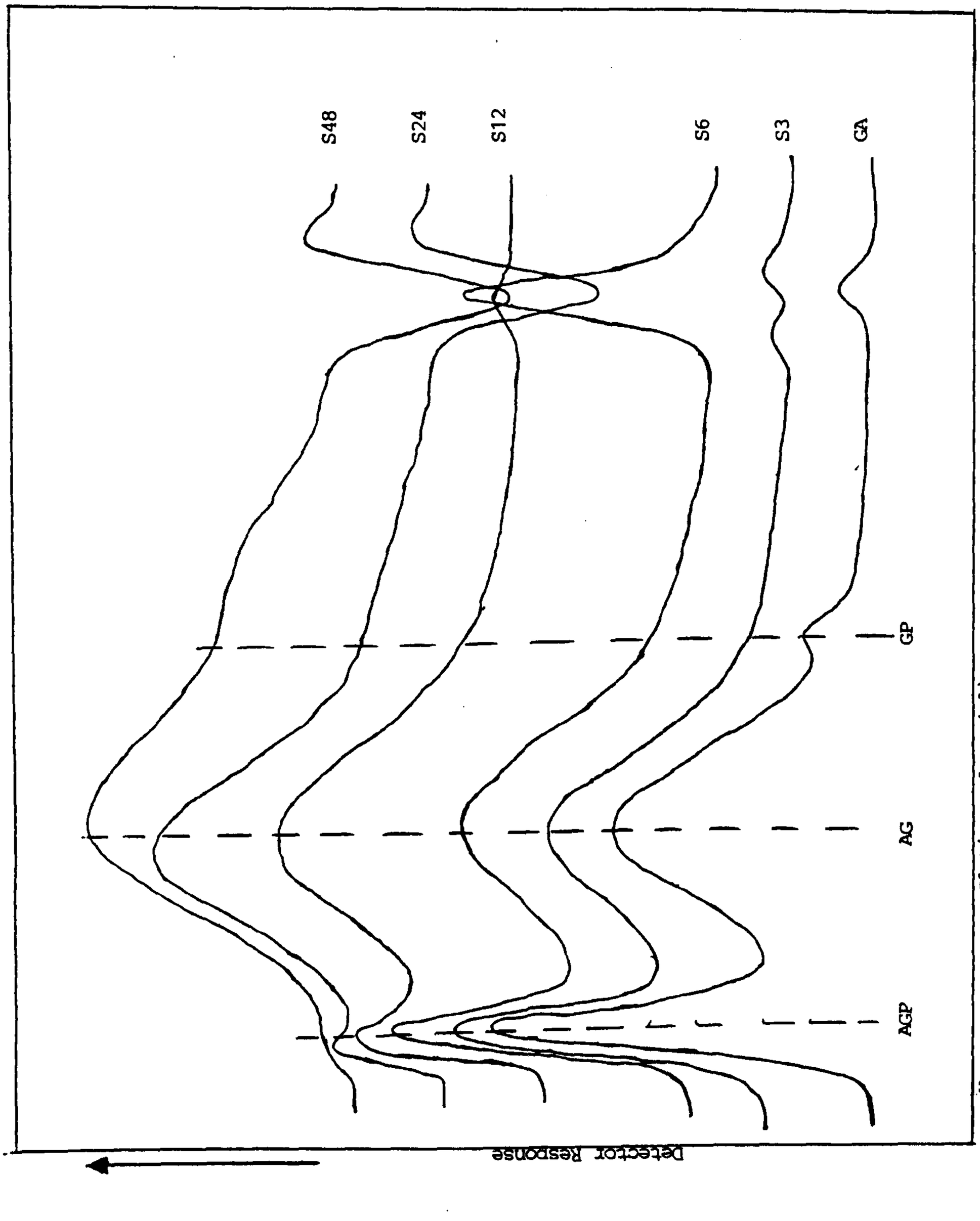


Figure 7.3: RI elution profile of the gum after auto hydrolysis

Moderate heating (mild auto hydrolysis) also brought about a decrease in viscosity with time but this was less pronounced compared to auto hydrolysis (Table 7.2 and Fig. 7.4). Generally, more time was needed to attain a given value of viscosity: for example 24 hours were required to reach a value of 16 ml g^{-1}. This is in line with normal chemical kinetic effects. An important observation was lack of drastic changes in the gum molecule. Only slight changes were noticeable in the protein and emulsion properties after 24 hours of hydrolysis which represented only 5% of the protein lost from the gum. Gelling properties observed in the unmodified gum were lost after heating for 6 hours.

Table 7.2: Analytical data after moderate heating

	GA	MH3	MH6	MH12	MH24	MH48	MH96
Recovery	96.8	96.4	96.4	96.4	96.1	95.7	95.4
Moisture(%)	6.4	9.5	9.7	4.0	4.2	5.3	4.6
Specific rotation (deg)	-33	-34	-33	-32	-33	-33	-32
Nitrogen (%)	0.43	0.43	0.43	0.43	0.42	0.42	0.41
Hence protein	2.85	2.85	2.85	2.85	2.79	2.79	2.72
Intrinsic viscosity	23.8	23.6	22.1	20.2	16.8	15.6	12.3
Emulsion activity	2.74	2.72	2.72	2.72	2.72	2.72	2.71
Emulsion stability	95	94	94	93	92	90	89
Equivalent weight	837	870	875	909	910	890	862
Uronic Acid Anhy.	21	20	20	19	19	20	20
Gel (25% sol.)	++	+	+	-	-	-	-
Sugar composition after hydrolysis							
Glucuronic acid	21	20	20	19	19	20	20
Galactose	37	38	37	38	37	39	38
Arabinose	26	26	26	27	27	26	26
Rhamnose	16	16	17	16	17	15	16

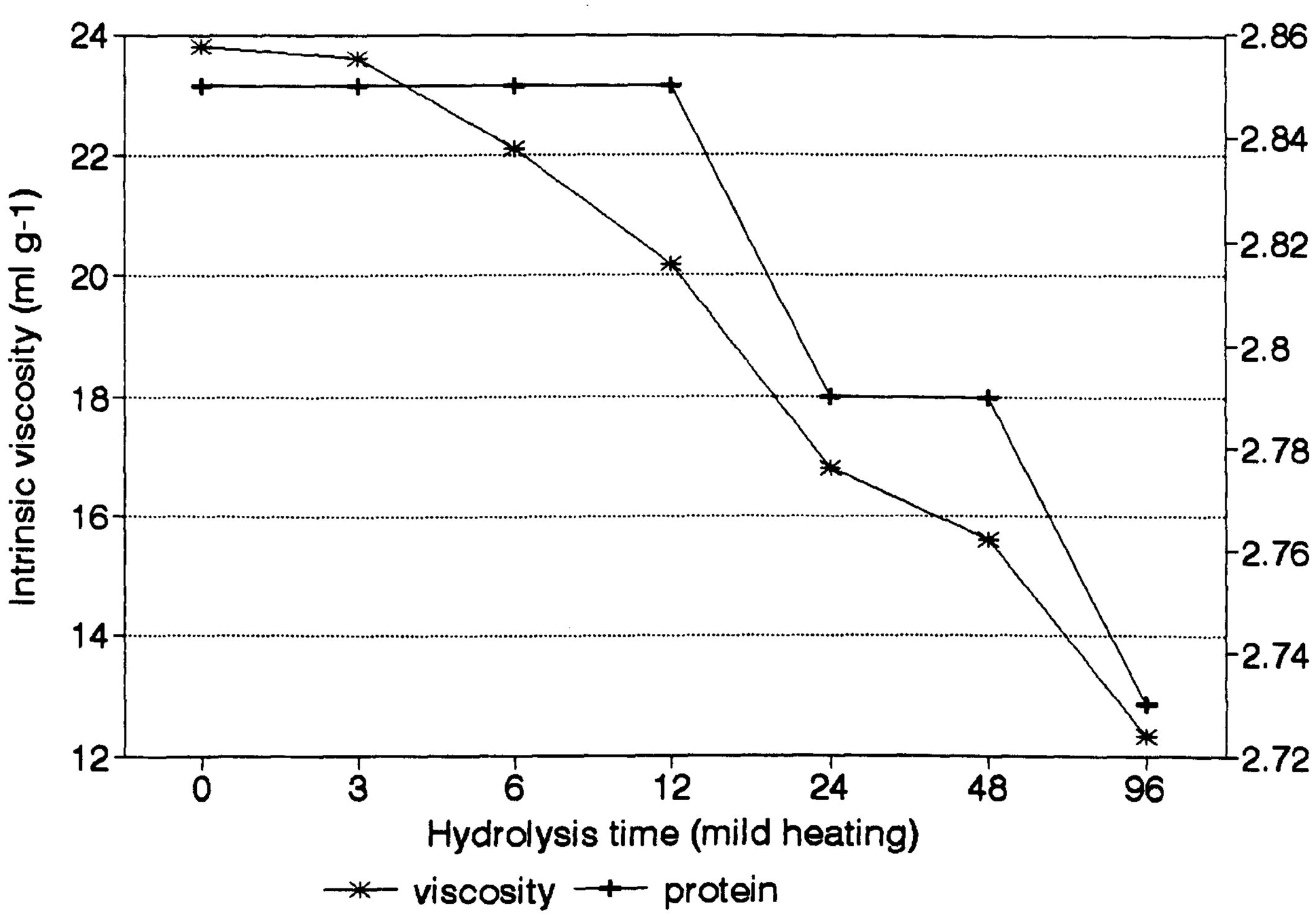


Figure 7.4: Relationship between viscosity and hydrolysis time and accompanying changes in protein content

7.2.4 Discussion

Heating gum solution to allow it flow easily for removal of foreign matter (i.e. filtration) and sterilise it is still the most widely practised industrial method. It is also known that heating results in loss of viscosity (Woolen, 1982). However, prolonged heating has a damaging effect on the gum by affecting its functional properties. For example, while a value of 15.4 ml g^{-1} was attained after only 12 hours, heating for this time was long enough to have damaged the AGP fraction to levels that affected emulsification functionality. Even after 6 hours, the effect on the emulsification properties had become apparent. These results are consistent with earlier studies which showed that emulsification efficiency is affected within 3 hours (Randall et al, 1989b). To avoid loss in the functionality the gum should not be heated for more than 6 hours. Woolen (1982) has shown that from an industrial point, gum can withstand high temperature for short periods but breaks down within 6-8 hours at boiling point. An alternative method would be to heat the gum at a lower temperature even though it takes a longer time to achieve the desired viscosity. There were only slight changes in the protein component until after 24 hours when a reduction in the emulsification efficiency became noticeable. Earlier studies have also shown that moderate heating can reduce viscosity towards usual values for industrial processing without drastic changes to the protein component (Fenyo and Vandavelde, 1990).

It is worthwhile to examine critically changes that take place in the gum as heating at 100°C progresses. Results of the analysis show that protein is the first component to be affected accounting for a greater part of the gum lost. From the UV elution profiles, the glycoprotein fraction is the first to be affected and was virtually removed after 6 hours. This result is consistent with the work of Menzies (1992). The protein of the AGP fraction is the next to be affected as observed from RI profiles. The AGP fraction is practically lost after 48 hours leaving AG as the main peak. However, after 12 hours, it seems that

decrease in the AGP fraction is a result of degradation of both the protein and carbohydrate components. For example, an examination of the analytical data reveals that after 24 hours, there was loss in the sugars caused by elimination of arabinose and rhamnose. At 48 hours loss in the two sugars was more evident with greater loss occurring from the arabinose. McDougal (1987) analysed the composition of dialysates and found that they contained higher proportions of arabinose and rhamnose indicating that they are the ones mostly removed. Therefore subsequent loss in viscosity was a result of the combined effects of loss in both the protein and cleavage of carbohydrate components.

These observations can now be interpreted in the light of our present understanding about the structure of gum arabic. In the model of Street and Anderson (1983), arabinose and rhamnose constitute the periphery sugars which are labile and hence readily cleaved. The susceptibility of arabinose is also shown in the model by Qi et al (1991) where loss of arabinose might be resulting from the attack of the tri-arabinosides linked to the hyroxyproline. Loss of these sugars (and arabinose in particular) would result in the relative increase per unit mass of the galactose remaining. The AG fraction that remains is therefore rich in galactose and glucuronic acid (whose content remained unchanged throughout the experimental period). However, some arabinose and rhamnose still remain in the gums AG fraction. As for the protein, disappearance of the glycoprotein fraction in the initial stages may indicate that it consists of a proportion which constitutes the periphery amino acids that could be involved in the linkage with periphery sugars. The structure of glycoprotein is said to consist of carbohydrate linked to protein through arabinosyl-hydroxyproline linkages and galactosyl-serine linkages with the carbohydrate fraction comprising of monosaccharides and relatively short oligosaccharide chains (Cho and Chrispeels, 1976) which would be readily susceptible to heat treatment. As heating progresses, amino acids close to the hydroxyproline rich backbone are affected. However, some of the amino acids,

probably those close to the hydroxyproline backbone remain as part of the AG fraction. McDougal (1987) found that the degraded gum is enriched in hydroxyproline, serine, threonine, proline and leucine. Indeed, the gum is not depleted entirely of the protein as observed from the analytical data.

7.3. Enzymatic hydrolysis

7.3.1 Theoretical considerations

Enzymes are biochemical catalysts that accelerate chemical reactions. The effectiveness of these reactions are dependent mainly on concentration, temperature and pH. In principle however, enzymes work under mild conditions of the above factors and in addition they are highly specific in the type of reactions they catalyse, in particular, the substrates they accept (Zubay, 1993).

The enzymes which metabolize polysaccharides (i.e. catalyse bond cleavage) fall in the class of hydrolases. Within it are endo, exo, and debranching enzymes (Norman and Barry, 1985). Endo enzymes depolymerise polysaccharides by random splitting of interior glycosidic bonds and need a region of unbranched or lightly branched glycan chain in the substrate to provide binding subsites for the reaction. However, most endo enzymes do not readily attack more complex polysaccharides that have different linkages or are highly branched. For instance plant gums which are highly branched acidic heteropolymers are resistant to the action of endo enzymes. Exo enzymes remove sugar units from the non-reducing end of the polymer while debranching enzymes on the other hand hydrolyse the β-D-(1-6) bond in the β-D-(1-4) and (1-6) glucans removing the branch chains.

Overall, enzymes have advantages over other methods of hydrolysis because of their great specificity. This property allows polysaccharides to be studied in mixtures and enables complex polysaccharides to be modified sequentially. They have been used in the determination of structures, estimation of individual polysaccharide content, isolation, purification and

characterisation. On a more applied level, enzymes are increasingly becoming important in food processing (Teichgraber et al, 1993). However, studies involving complex polysaccharides were for a long time difficult. Some of the early work involved use of proteolytic enzymes in elucidating the structure of sugar components of proteoglycans and glycoproteins (Spiro, 1966) but inherent problems were encountered. Successful use of enzymes in plant gum studies is thus recent. Connoly et al, (1987) used a proteolytic enzyme in studying the physical and macromolecular characteristics of *A. senegal*. Since then a number of studies involving the enzyme have been carried out (Randall et al, 1988, Menzies, 1992 and Osman, 1993). Use of carbohydrate degrading enzyme is also recent (Reid et al, 1988; Weiping, 1993).

7.3.2 Materials

A sample of gum arabic remaining after experiments on heating was used in the studies. Three were enzymes were examined:

. pronase E (protease type XIV, Sigma Ltd.)

. Viscozyme (120L Novo Nordisk, Denmark)

. β-D-galactosidase (G 3522, Sigma Ltd.)

7.3.3 Methods

7.3.3.1 Pronase E

Pronase E (Protease type XIV) was supplied by Sigma chemical company limited. The enzyme is classified as a research grade, and differs from type XXV only in purity. It is a mixture of at least four enzymes obtained from *Streptomyces griseus* and is known to hydrolyse peptide bonds of the protein producing amino acids.

. Preparation of solution

0.5 M NaCl solution was prepared and pH adjusted to 7.8 at 37°C using 2M NaOH.

. Preparation of enzyme solution

Connoly et al. (1988) and Randall et al. (1989a) have shown that enzyme concentration of 1.5 - 1.6 mg/ml of buffer is optimal for reaction with gum solution. A concentration of 1.5 mg enzyme/ml buffer was found optimal during preliminary experiments.

. Hydrolysis of the gum

From preliminary experiments, 1 ml of enzyme solution (1.5 mg/ml) was required to hydrolyse 100 ml of gum solution. In the main experiment therefore, 300 ml of 10% gum solution was treated with 3 ml of enzyme and pH was adjusted to 7.8 with 2M NaOH. The flasks were sealed and incubated at 37°C in a water bath. Aliquots (samples) of 100 ml were removed every 3, 6 and 12 hours (flask 1) and 24, 48 and 96 hours (flask 2). Enzyme activity was stopped by heating samples at 80^0C for 5 minutes. Samples were cooled and dialysed against tap water (regular changes) for 72 hours, concentrated and freeze dried. The fractions were labelled as PS3, PS6, . . . , PS96.

7.3.3.2 Viscozyme 120L

100 ml of viscozyme (120L Novo) was kindly supplied by Novo Nordisk Bioindustries UK limited. The enzyme is a mixed carbohydrase from a selected strain of the *Aspergillus* group and has a declared activity of 120 FBG/ml at a pH range of 3.3-5.5 and temperature of 40 - 50°C. The enzyme is used to break down cell walls for the extraction of useful components from plant tissues and in the processing of cereal and vegetable materials.

. Buffer

Gum arabic solution has a natural pH range between 4.2-4.6 and hence does not require a buffer medium.

. Enzyme solution

Viscozyme 120L is supplied as a clear brown liquid in a form ready to use.

. Hydrolysis of the gum

A dose of 0.05-0.1% enzyme solution is recommended for preliminary investigation. In the present study a concentration of 0.3% was found optimal. 1 ml of viscozyme was thus added to 300 ml of 10% gum solution (at pH 4) and the solution allowed to stand at 45^0 C in a water bath. 100 ml aliquot was removed every 3, 6 and 12 hours (flask 1) and 24, 48 and 96 hours (flask 2). The solution was inactivated, dialysed and freeze dried as described for pronase E. Fractions were labelled as CS3, CS6, . . ., CS96.

7.3.3.3 β-D-galactosidase

β-D-galactosidase (G 3255) was supplied by Sigma chemical Co. ltd. Literature shows that it is produced from *Aspergillus niger* and is highly specific for the non reducing terminal galactosidic bonds (Bahl and Agrawal, 1969). In addition to β-D-galactosidase, the enzyme has small amounts of α-D-galactosidase (<2%) as well as β-N-acetylglucosaminidase (<1%) and xylosidase (<1%).

. Buffer

0.4 M citrate buffer was prepared from citric acid monohydrate (C6H8O7.H2O) and pH adjusted to 4.0 at 25°C using 2M NaOH.

. Enzyme solution

0.25 units/ml of water as recommended by the supplier was prepared and stored at 4^0C.

. Hydrolysis of gum

Preliminary experiments showed that 1.5 ml enzyme solution was required to hydrolyse 100 ml of gum solution. Hence 300 ml of 10% gum solution (in 0.4 M citrate buffer) was treated with 4.5 ml of enzyme and experiment incubated at 25°C. Aliquots of gum solution were removed and procedure described for the above enzymes followed. Fractions were labelled as B3, B6, . . ., B96.

7.3.4 Results and discussion

7.3.4.1 Pronase E

Data on the effect of pronase are given in Table 7.3. Intrinsic viscosity dropped with incubation time reaching a value of 12.6 ml g^{-1}. The drop was more drastic within the first 3 hours (Fig. 7.5). Beside intrinsic viscosity, changes were observed in the nitrogen (hence protein) content and emulsification properties. Both decreased with incubation time. The relationships between intrinsic viscosity and protein content and protein and emulsification activity were examined by regression analysis (Fig. 7.6a and 7.6b). There was a strong influence of the protein content on intrinsic viscosity accounting for about 97% of the relationship. A strong relationship was also established between protein content and emulsification activity. No changes were observed in the carbohydrate content.

Table 7.3: Analytical data of gum arabic after treatment with pronase

	GA	PS3	PS6	PS12	PS24	PS48	PS96
Recovery	96.8	87.6	85.5	73.8	71.6	70.3	70.3
Moisture(%)	6.4	5.8	6.6	5.5	9.8	5.1	5.9
Specific rotation (deg)	-33	-32	-33	-34	-34	-33	-34
Nitrogen (%)	0.43	0.40	0.36	0.34	0.32	0.30	0.30
Hence protein	2.85	2.65	2.39	2.25	2.12	1.99	1.99
Intrinsic viscosity	23.8	19.5	17.9	15.9	14.7	13.5	12.6
Emulsion activity	2.74	2.10	1.97	1.82	1.77	1.72	1.70
Emulsion stability	95	84	82	80	79	76	74
Equivalent weight	837	857	842	870	851	892	862
Uronic Acid Anhy.	21	21	21	20	21	20	20
Gel (25% sol.)	++	+	-	-	-	-	-
Sugar composition after hydrolysis							
Glucuronic acid	21	21	21	20	21	20	20
Galactose	37	39	39	38	37	39	38
Arabinose	26	24	24	27	26	25	26
Rhamnose	16	16	16	15	16	16	16

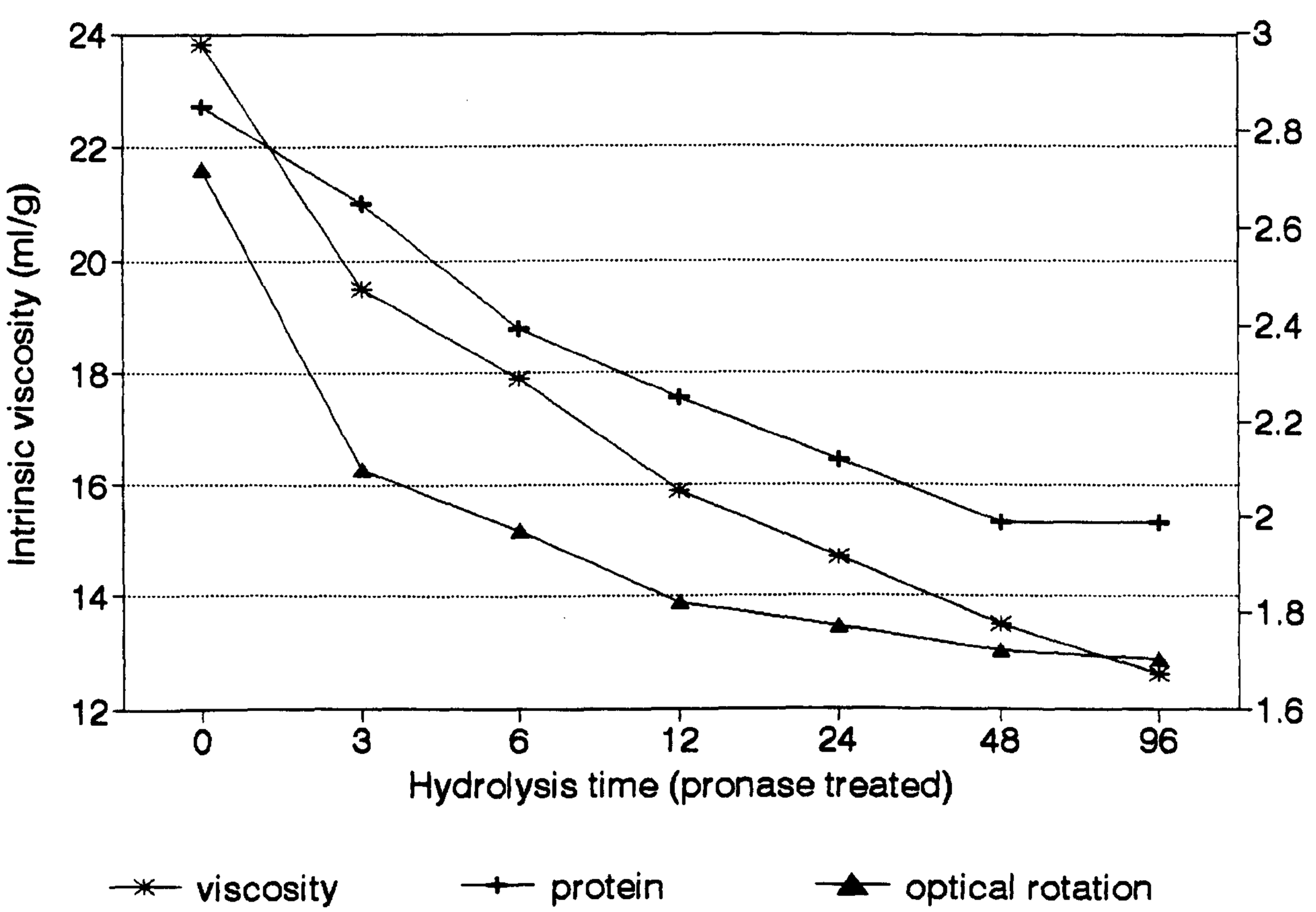
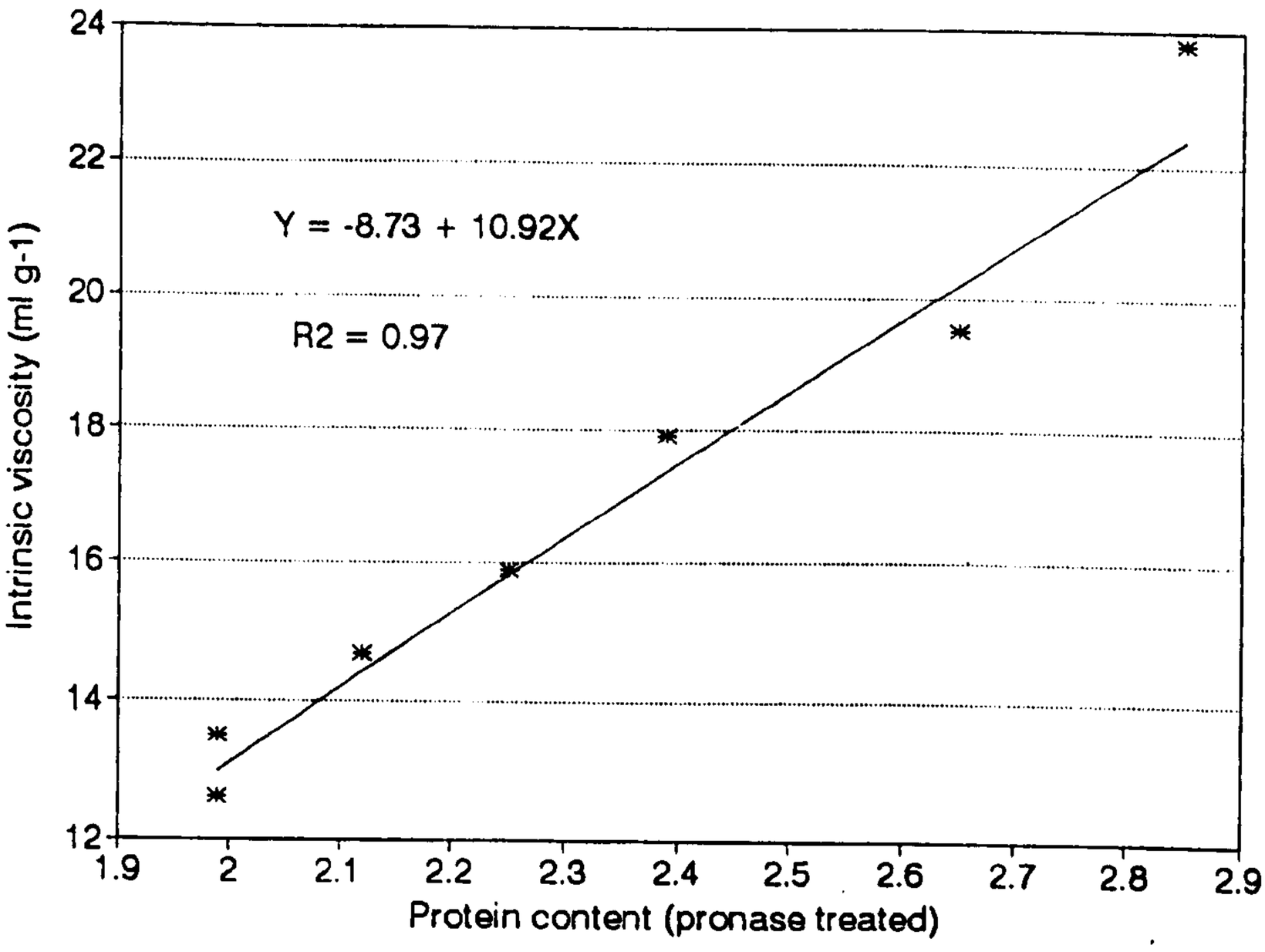
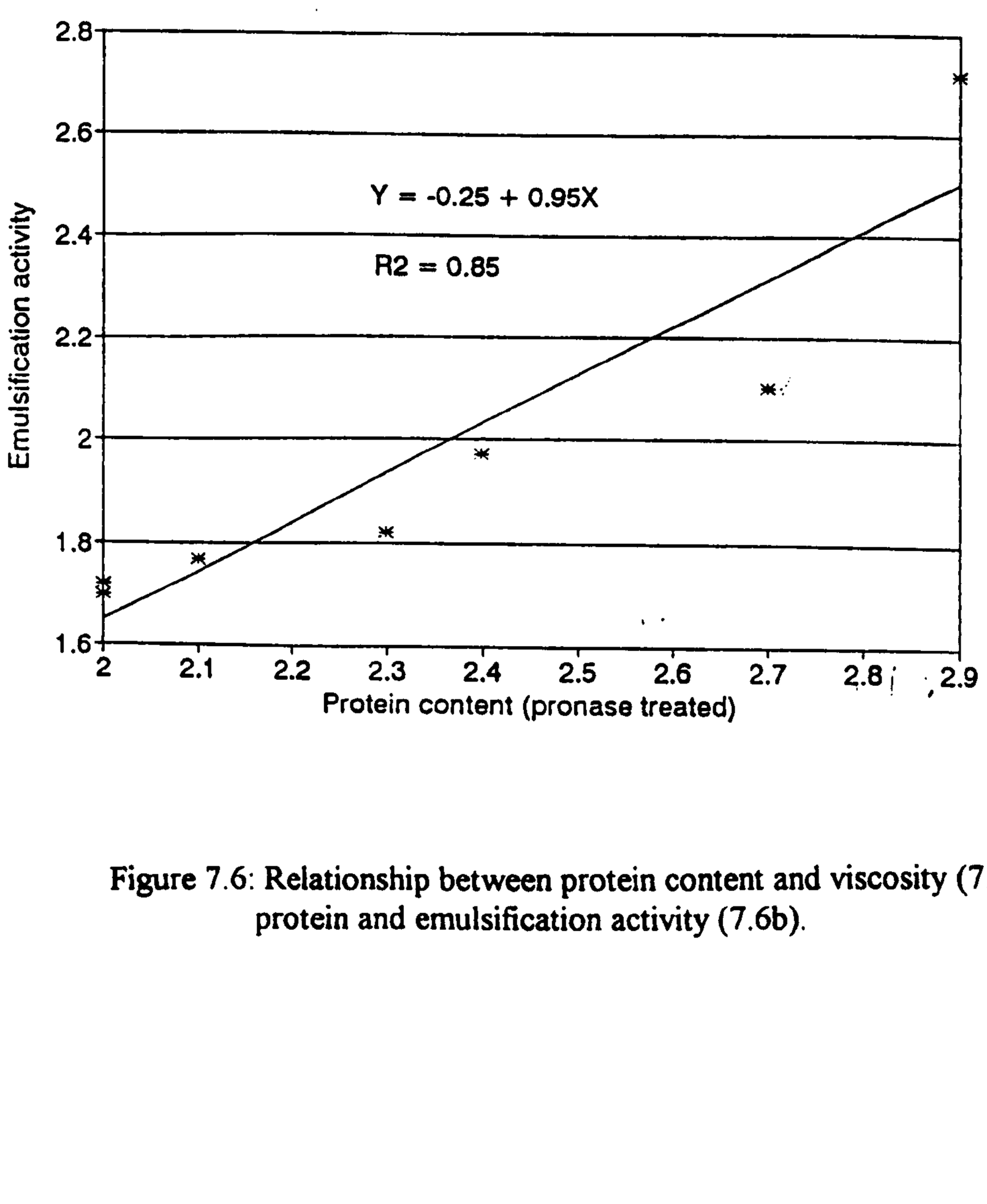


Figure 7.5: Relationship between viscosity and hydrolysis time with accompanying changes in protein and emulsion activity



7.6a



7.6b

Figure 7.6: Relationship between protein content and viscosity (7.6a), and protein and emulsification activity (7.6b).

The effect of pronase E on viscosity was first reported by Connoly et al, (1988) who found that regardless of the initial value, intrinsic viscosity decreased to 11-12 ml g^{-1}. In the present study a value of 12.6 ml g^{-1} was obtained but after 96 hours of incubation an indication that the reaction continues beyond 12 hours. The loss in viscosity is mainly due to degradation of the protein component. The strong relationship between intrinsic viscosity and protein content in the present study confirms the role of proteins. Earlier studies have demonstrated a gradual disappearance in the AGP fraction (the fraction with higher amount of protein) with time (Randall et al, 1989a; Menzies, 1992 and Osman, 1993). It has further been shown that the glycoprotein fraction is not degraded by the enzyme (Osman, 1993). In this study, the protein content was reduced to about 2.0% accounting for about 31% of the protein lost.

The loss of viscosity is accompanied by loss of the emulsification functionality. A strong relationship between intrinsic viscosity and protein and between protein and emulsification activity have been established. The explanation is straight forward since emulsification has been shown to be directly influenced by the protein component (Randall et al, 1989b). Results of regression analysis in this study further confirm that proteins are strongly involved in emulsification functionality. Therefore, though pronase achieves reduction in the viscosity to levels required for industrial processing it is not suitable where gum is to be used as emulsifier or stabiliser.

7.3.4.2 Viscozyme 120L

Analytical data on the effect of viscozyme are given in Table 7.4. The drop in intrisic viscosity was continuos between 3-24 hours (Fig. 7.7). There was a slight decrease in the protein component between 6 and 24 hours though accounting for only 4% of the protein lost from the gum whilst the emulsification functionality shows gradual decrease after 6 hours. Slight changes were also observed in the sugar composition where arabinose and

Table 7.4: Analytical data for gum arabic after treatment with viscozyme

	GA	CS3	CS6	CS12	CS24	CS48	CS96
Recovery	96.8	89.8	81.8	80.6	72.1	70.9	70.3
Moisture(%)	6.4	8.0	7.9	6.5	6.2	7.0	6.2
Specific rotation (deg)	-33	-32	-32	-31	-31	-31	-32
Nitrogen (%)	0.43	0.43	0.43	0.42	0.41	0.41	0.41
Hence protein	2.85	2.85	2.85	2.79	2.72	2.72	2.72
Intrinsic viscosity	23.8	23.2	21.6	19.7	17.3	16.5	14.8
Emulsion activity	2.74	2.73	2.73	2.70	2.67	2.65	2.63
Emulsion stability	95	92	88	85	80	76	75
Equivalent weight	837	913	880	884	883	855	875
Uronic Acid Anhy.	21	19	20	20	20	21	20
Gel (25% sol.)	++	+	-	-	-	-	-
Sugar composition after hydrolysis							
Glucuronic acid	21	19	20	20	20	21	20
Galactose	37	39	38	40	41	41	41
Arabinose	26	26	26	25	25	24	24
Rhamnose	16	16	16	15	15	14	13

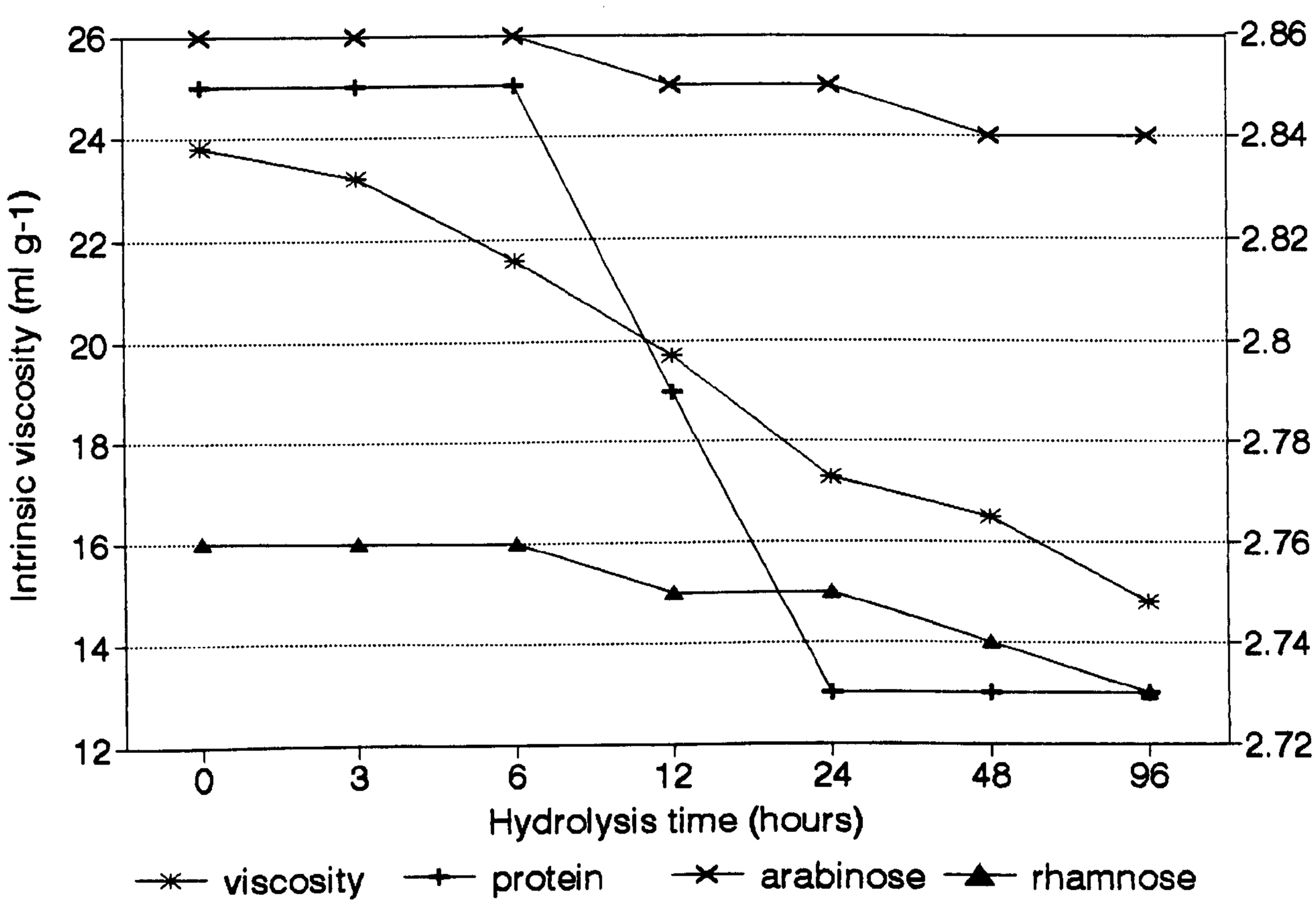


Figure 7.7: Relationship between viscosity and hydrolysis time with accompanying changes in the gum molecule

rhamnose showed a decrease after 6 hours of incubation. Loss of arabinose and rhamnose were 8% and 13% respectively after 96 hours. The combined effects of loss of protein and the two sugars on viscosity were fitted in a multiple regression model resulting in an equation of the form:

$$(\eta) = -65.9 + 31.0X1 - 0.55X2 + 0.90X3;\ R2 = 0.94 \text{ and } p = 0.028$$

where:

X1 = protein

X2 = arabinose

X3 = rhamnose

Hydrolysis time was omitted from the equation since it was highly correlated to the other independent variables. The three parameters accounted for 94% of the total reduction in viscosity and were shown to be highly significant.

The enzyme treated gum was also monitored by gpc using UV and RI detectors. The results are shown in Figures 7.8 (UV) and 7.9 (RI). There was a gradual broadening of the UV profiles for the AGP fraction with eventual masking of the AG fraction. The glycoprotein fraction remained practically unchanged. The RI did not show discernible changes. However, when examined alongside the UV profile, there was gradual shrinking of the AG fraction.

The results show that Viscozyme causes loss of viscosity. A value of 17.3 ml g^{-1} desired for industrial processing was reached after 24 hours. The loss of intrinsic viscosity seems to be due mostly to degradation of part of the carbohydrate component. The UV profile shows that this might be arising from the AG fraction which becomes gradually masked. Analytical data show that the loss is caused by removal of the arabinose and rhamnose sugars. The presence of arabinase in the enzyme mixture explains the loss of arabinose. Probably the periphery arabinose and rhamnose are those attacked in the reaction. Weiping (1993) found rhamnose as the sugar preferably removed. The

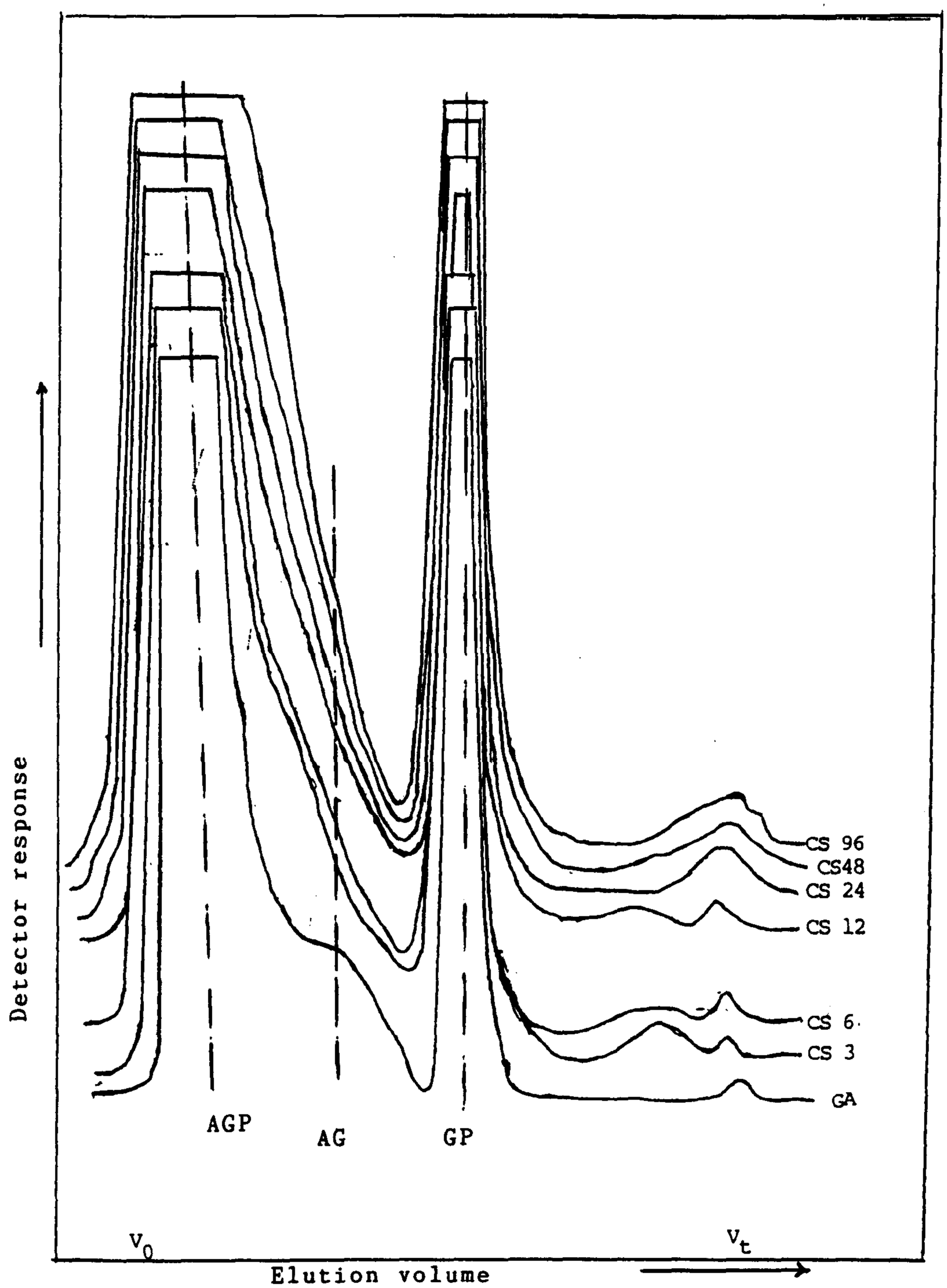


Figure 7.8: UV elution profile after treatment with Viscozyme

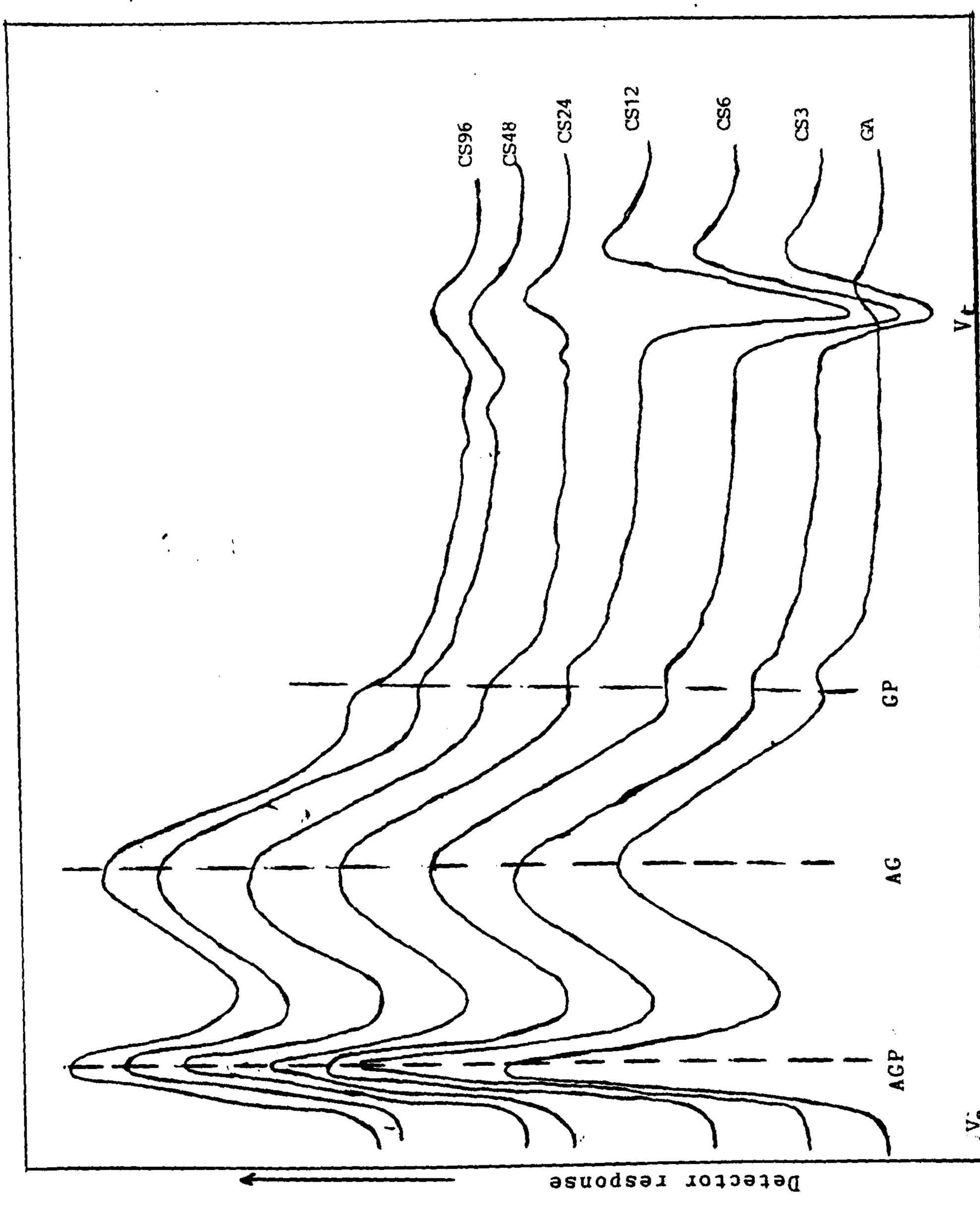


Figure 7.9: RI profile of the gum after treatment w

protein component appears to have been affected slightly in the reaction process. This is seen from the loss of some of the protein and decline in the emulsification properties in the table. The AGP fraction seems to be that most affected. Relatively large emulsions were observed during measurements. Weiping (1993) also found that the diffusate fraction was enriched in alanine, aspartic acid, cystine, glutamic acid, glycine, isoleucine, tyrosine and valine that have been suggested as terminal amino acids and could be probably associated with the AGP component. The enzyme may be useful in the processing of gum. However, process conditions need to be optimised to allow production of stable emulsions alongside the desirable property changes.

7.3.4.3: β-D-Galactosidase

Data are presented in Table 7.5. Again loss in viscosity was observed with increasing incubation time with a drastic drop occurring between 3-24 hours (Fig. 7.10). A final value of 14.7 ml g-1 was reached after 96 hours. Interestingly loss in viscosity was accompanied by increase in the protein content from 2.9% to 3.7% representing a 27% increase. Emulsification properties showed a gradual increase. An examination of the sugars showed that some degradation had occurred with a decrease in the ratio of galactose relative to arabinose and rhamnose. Galactose decreased by about 8%.

Table 7.5: Analytical data for gum arabic after treatment with β-D-galactosidase

	GA	BS3	BS6	BS12	BS24	BS48	BS96
Recovery	96.8	90.4	87.9	81.6	75.0	73.4	73.1
Moisture(%)	6.4	6.4	5.9	6.6	6.0	4.1	6.5
Specific rotation (deg)	-33	-33	-34	-34	-35	-34	-35
Nitrogen (%)	0.43	0.44	0.45	0.47	0.51	0.54	0.55
Hence protein	2.85	2.92	2.98	3.12	3.38	3.45	3.45
Intrinsic viscosity	23.8	23.4	21.2	19.2	15.5	14.9	14.7
Emulsion activity	2.74	2.73	2.74	2.74	2.74	2.75	2.75
Emulsion stability	95	90	92	92	94	95	94
Equivalent weight	837	875	877	871	891	861	883
Uronic Acid Anhy.	21	20	20	20	20	20	20
Gel (25% sol.)	++	+	-	-	-	-	-
Sugar composition after hydrolysis							
Glucuronic acid	21	20	20	20	20	20	20
Galactose	37	37	36	35	35	34	34
Arabinose	26	26	26	28	28	29	28
Rhamnose	16	16	16	17	17	17	18

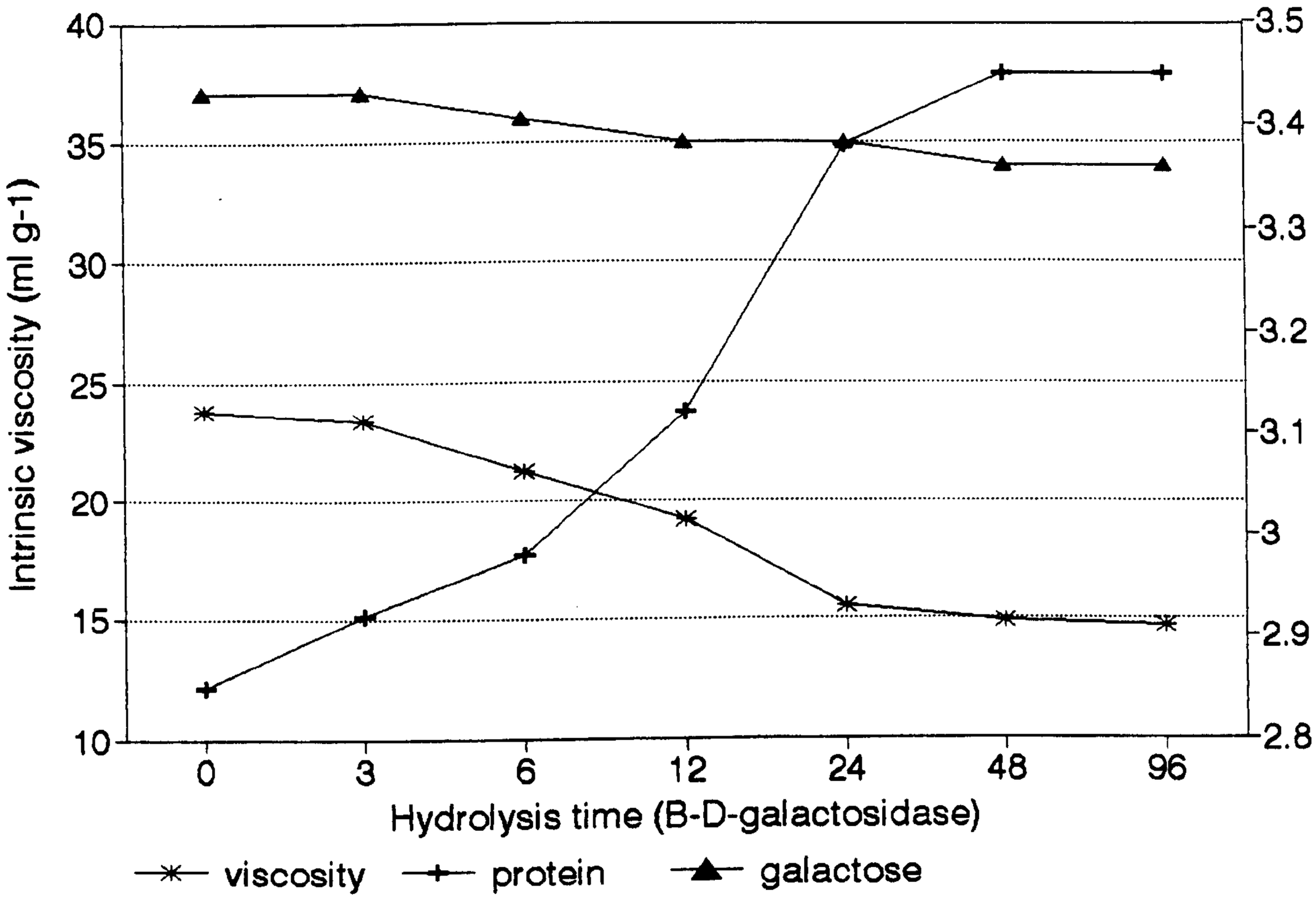


Figure 7.10: Relationship between viscosity and hydrolysis time with accompanying changes in protein and galactose contents

The action of the enzyme was also monitored by the UV (Fig. 7.11) and RI detectors (Fig. 7.12). There was gradual broadening of the AGP fraction with eventual masking of the AG fraction in the UV profile. This became more apparent after 24 hours. Smaller changes were observed in the GP fraction. A close examination of the RI profiles show gradual reduction of the AG fraction.

β-D-galactosidase causes a reduction of viscosity in gum arabic to values desired for industrial application. A value of 15.5 ml g^{-1} was achieved by incubating the gum solution for a day (24 hours). The loss in viscosity is less marked after 24 hours probably indicating that under conditions of the experiment, the hydrolysable part of the gum is more or less exhausted. One of the characteristics of the enzyme is its high specificity for the non reducing terminal galactosidic bonds (Bahl and Agrawal, 1969). The reduction of the ratio of galactose to arabinose and rhamnose reveals that some of the galactose units are being lost from the gum molecule. This may be associated with the AG fraction of the gum explaining the slight reduction in the AG component of the RI profile. The enrichment effect of the protein component shows the high specificity of the enzyme for the carbohydrate fraction. Practically, the protein component seems to remain unattacked resulting in a higher proportion per unit mass of the gum sample over time. This results in better emulsion functionality making the enzyme suitable in future processing of gum arabic. This enzyme has advantage as it works under natural pH condition for gum arabic and at room temperature.

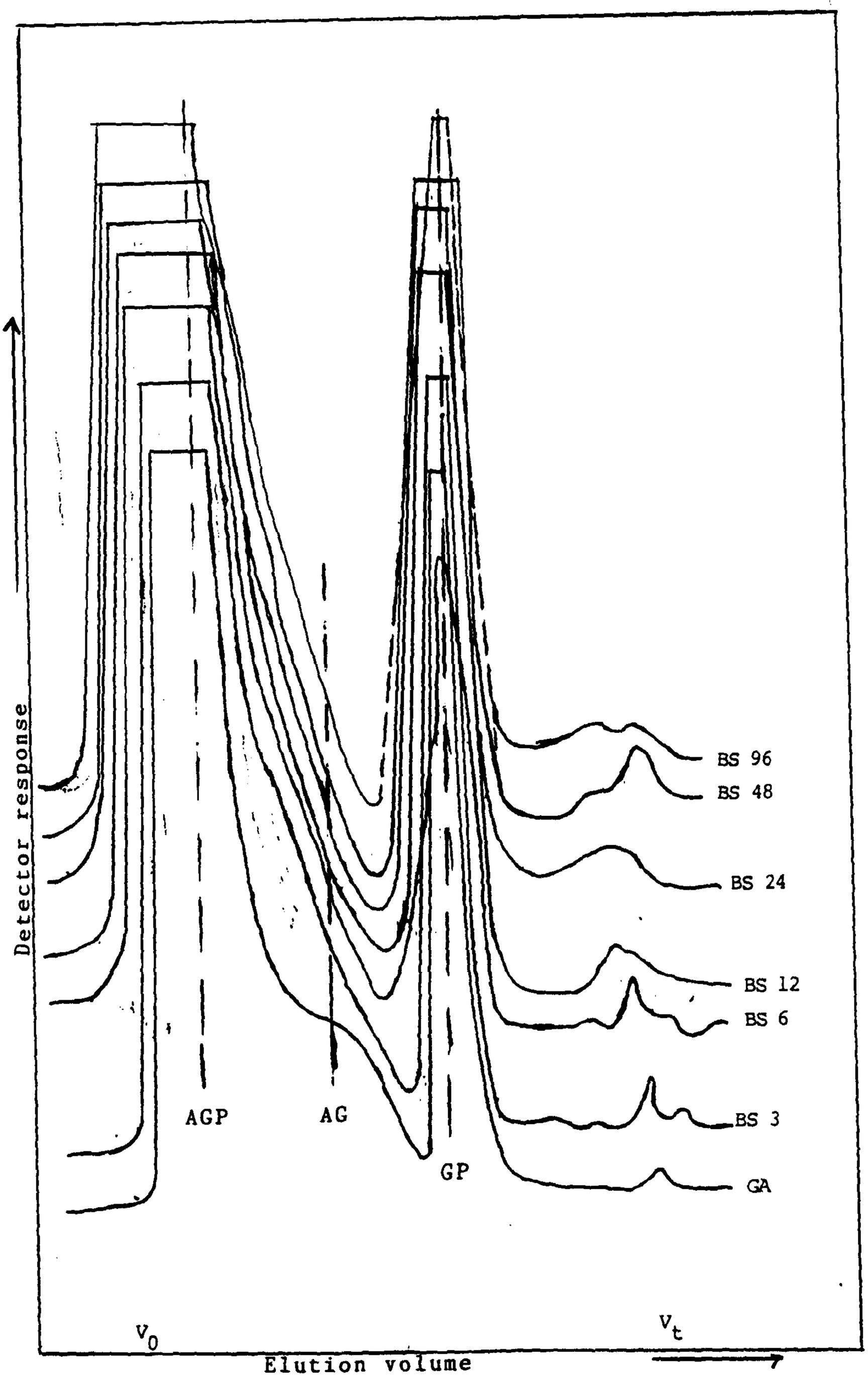


Figure 7.11: UV Elution profile of the gum after treatment with β- D-galactose

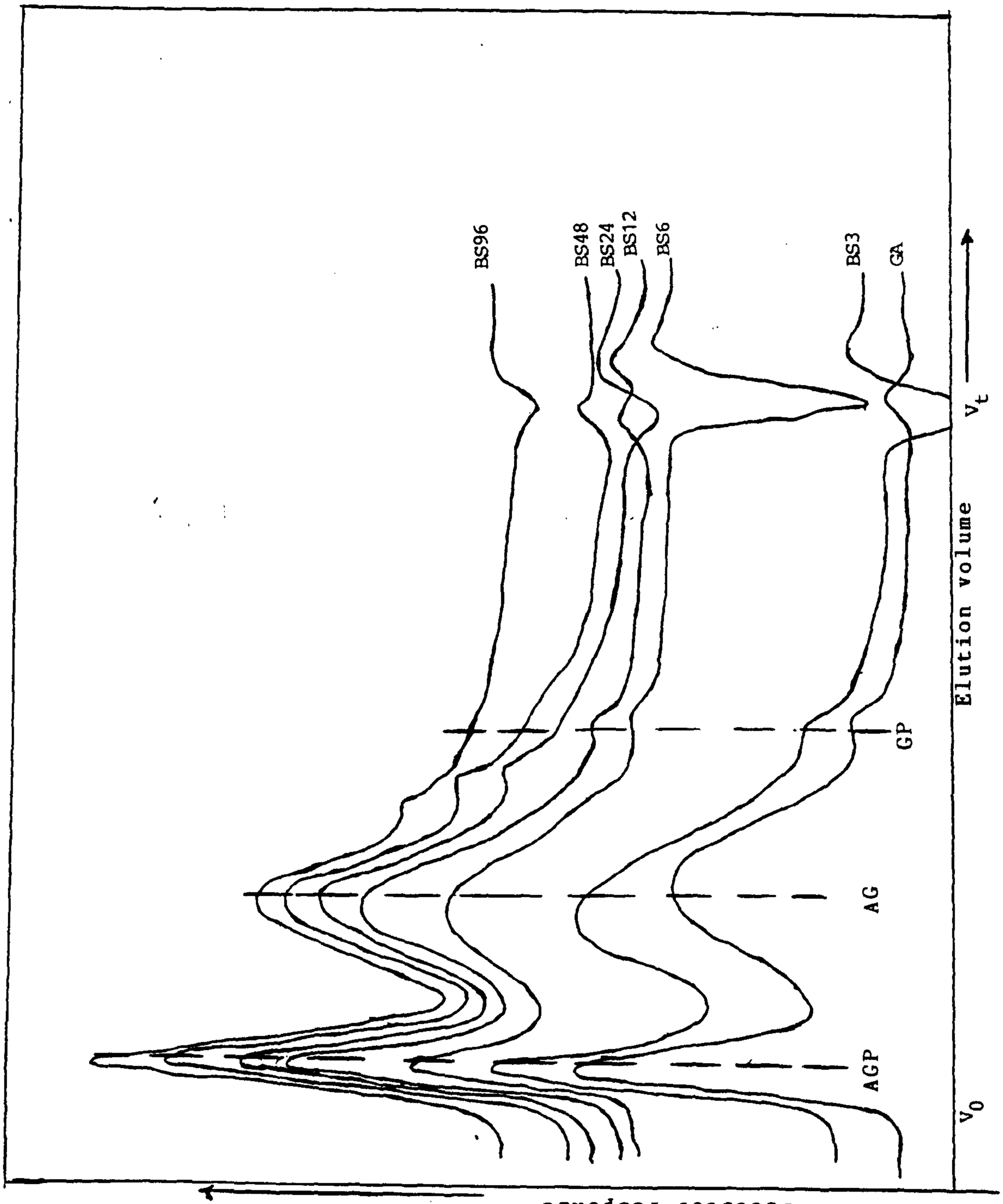


Figure 7.12: RI elution profile after treatment with β-D-galactosidase

CHAPTER 8: SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK.

8.1. Summary and conclusions

Studies carried out on resource availability have led to the production of provisional *A. senegal* maps showing source locations in Isiolo, south western Marsabit and Turkana Districts. The resource occupies about 14%, 20% and 37% of the three districts respectively. *A. senegal* var. *kerensis* was identified as the main source of gum arabic. It grows as a shrub or bush varying in height from under 1 m in environments that are more arid and with poor soil development to about 5 m on deeper soils. It exhibits greatest variation in Marsabit District probably reflecting an extreme range of environments. The variety was observed growing mostly in patches though in the case of Ranges (Isiolo), Kurkum (Marsabit), Lorgum and Kakuma (Turkana) it formed pure stands that were confined on hills or luggas. Topography was identified as the main factor influencing stocking with higher densities associated with hills but occasionally along luggas. The human factor, through harvesting and grazing was as another important variable which partly explained variation in stocking observed in Isiolo and Marsabit Districts. In general, Isiolo District has higher proportion of younger stands while Turkana District offers better representation in the three size classes considered.

Acacia circummarginata, earlier thought of as *A. senegal* var. *leiorachis* was observed in Isiolo and Marsabit Districts as a tall tree growing up to 14 m high with a rounded crown. It is confined mostly to luggas and on riverine alluvium. Tapping trials to collect gum were unsuccessful. Ironically, var. *senegal* which is the main source of gum arabic in Sudan was not observed in the study area but grows on sites that receive slightly higher rainfall. It was observed in West Pokot, Baringo, Nakuru and Kajiado Districts.

Results on associated species revealed that *Acacia tortilis*, *A. reficiens* and *C. africana* were the most common associates observed among the 24 species recognised. The former two species were mainly associated with *A. senegal* on the plains and along luggas. Three species of *Commiphora* (*C. confusa*, *C.holtiziana* and *C. pseudopaolii*) were also common associates with the former two being restricted in either Isiolo or Isiolo and Marsabit Districts and are characteristic of hilly terrain. Among the gum producing species, *A. tortilis* produces gum that is distinctly dark brown in colour. However, the greatest threat to adulteration is gum talha from *A. seyal* which, though it was not encountered in the samples during the survey is known to grow in Isiolo and Marsabit Districts on alluvial soils or areas with impeded drainage.

Results on characterisation revealed that gum arabic from *A. senegal* var. *kerensis* differs from var. *senegal*. The former variety exhibits high values of specific rotation, nitrogen and intrinsic viscosity. These differences show that var. *kerensis* is a distinct variety from var. *senegal*. Differences were further observed between samples and regions and were attributed to genetic differences exhibited in the population considering that the gum is still collected from natural stands. Differences between regions were attributed to local adaptations.

A comparison of the methods revealed that gpc is a quick and unambiguous technique in characterising gum arabic of commerce. It was able to separate the three components AG, AGP and GP that have been shown to be characteristic of gum arabic. The technique was also able to distinguish between var. *senegal* and var. *kerensis* due to the enhanced AGP and GP peaks associated with the higher overall protein component. Elution profiles from other *Acacia* gums were distinct from that of *A. senegal* and it was possible to identify contaminants. The analytical methods (physico-chemical and carbohydrate) are also useful in distinguishing between gum arabic and other gums as well as

between the two varieties; specific rotation, nitrogen content and viscosity being particularly useful. However, natural product variability renders the method incapable of adequately characterising gum arabic from even a single source and reliance on the method for specification of gum arabic of commerce can result in rejection of otherwise authentic gum arabic. It was observed that combining information from the two methods provides a powerful way of characterising gum arabic of commerce. The Elisa method as presently developed is able to recognise the protein component in *A. senegal* but also in other gums and the degree of interaction seems to depend on the amount of protein present. It was not able to distinguish unequivocally gum arabic from other gums and needs further refining to make it specific to gum arabic from *A. senegal*.

Results on heating experiments showed that heating gum at 100°C for more than 6 hours results in significant degradation of the protein component and subsequent loss of emulsification properties though reduction in viscosity and gelling to desired levels are achieved. Heating at 65°C achieves reduction in viscosity and gelling within 24 hours without drastic loss in emulsification properties. The three enzymes examined in the study resulted in loss in viscosity and gelling but with different implications on the other gum properties. Pronase degraded the gum to 16 ml g^{-1} within 12 hours but there was loss in protein and emulsification properties. It is therefore not suitable for processing gum in applications where emulsification and stabilisation are important. Both viscozyme and β-D-galactosidase attained reduction in viscosity and gelling within 24 hours. However, the former enzyme caused some degradation of the protein component giving rise to unstable emulsions while β-D- galactosidase resulted in increase in overall protein component and subsequent emulsification properties.

8.2. Suggestions for further work

8.2.1. Resource aspects

Preliminary surveys reveal that the entire region of northern Kenya has resources of *A. senegal* and therefore capable of producing gum arabic. Exploratory surveys could be extended into other districts not covered in the present study, especially in Samburu, Wajir and Mandera to map out source locations. Meanwhile detailed resource inventories are needed to accompany mapping to determine existing resource and actual potential for gum arabic.

The present work being carried out on the taxonomy of *A. senegal* should be extended to include variation in habit of var. *kerensis* which has been noted to be variable while ecological preferences of the two varieties of *A. senegal* in Kenya may need attention. Members of the *A. senegal* and other gum producing species within the gum region in Kenya should be properly identified by area. Tapping of gum arabic from the resources and general management should also be developed.

8.2.2 Monitoring and quality control of gum arabic

Differences observed between samples and regions need to be examined further to find out the extent to which they are influenced by genetic differences and local adaptations. In both cases selection for improved yield and quality could be developed for future dry land agroforestry. Regions exhibiting unique qualities like high specific rotation, nitrogen content and viscosity should be identified and gum sold separately by region to minimise undue variation in consignments.

Gums from members of the *A. senegal* complex and other gum producing species should be collected and analysed to provide data for comparison with gum arabic from *A. senegal*. Representative samples should be kept as reference material for quick visual comparison. Meanwhile knowledge of local farmers about gum arabic and gums from other species should be taken into account and incorporated in extension packages on production and handling of gum arabic. The

above information would be useful in the development of simple quality control and monitoring procedures.

8.2.3. Processing of gum arabic

Process temperature and time were shown as important parameters during heat processing. Processing optimisation studies are needed to establish desired conditions for processing in the light of the present knowledge about molecular structure and some of the functional properties.

The two carbohydrate degrading enzymes have shown promising results in process biotechnology of gum arabic. However, additional work is still required to determine the mechanisms of enzyme degradation and rates of reaction. Some information is also needed on retarding the slight protein degradation by one of the enzymes - Viscozyme.

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Appendix I: Density of *Acacia senegal* (stems per hectare) by sample site and vegetation unit

Ia: Isiolo District

1. Vegetation unit: 4B (ENGARE NTARE)

Sample site	Terrain type	Soil type	Dens	Density by diameter class in cm ≤2	2 - 3	4 - 5	6 - 7	≥8
1	hilly	sand	180	10	57	60	30	23
2	plain	rock	87	30	23	7	10	17
3	plain	rock	168	0	37	27	27	77
4	lugga	rock	273	50	40	87	33	63
5	lugga	rock	56	27	10	13	3	3
6	hilly	rock	227	80	53	30	37	27
7	plain	sand	150	30	54	30	23	13
8	plain	sand	213	103	57	30	20	3

Mean density = 169 std. dev. = 69 coef. of var. = 40%

2. Vegetation unit: 5WBS (Ranges)

1	hilly	rock	643	507	70	26	20	20
2	hilly	rock	693	437	147	53	33	23
3	hilly	rock	397	100	96	77	87	37
4	hilly	rock	500	37	140	210	83	30
5	hilly	rock	223	80	63	23	37	20

Mean density = 491 std. dev. = 170 coef. of var. = 35%

3. Vegetation unit: 5WBG (MALKADAKA)

1	lugga	rock	120	30	30	27	13	20
2	hilly	rock	164	33	11	33	37	50
3	plain	rock	30	0	3	3	10	14

Mean density = 105 std. dev. 56 coef. of var. = 53%

4. Vegetation unit: 4WBG (BISANI BILIKU)

1	plain	sand	506	0	93	193	137	83
2	lugga	rock	513	107	93	203	107	3
3	lugga	rock	257	50	60	77	30	40

Mean density = 425 std. dev. = 119 coef. of var. 28%

5. Vegetation unit: 5BS (HOT SPRINGS)

1	lugga	rock	417	103	113	130	44	27
2	lugga	rock	300	77	67	67	67	22
3	plain	sand	250	23	27	56	67	77
4	lugga	rock	192	73	23	23	23	50

Mean density = 290 std. dev. = 83 coef. of var. = 29%

6. Vegetation unit: 5BG (TIMUT)

1	lugga	rock	67	20	17	13	17	0
2	plain	sand	33	7	3	17	3	3
3	plain	sand	56	11	15	14	11	4

Mean density = 52 std. dev. = 17 coef. of var. = 34%

7. Vegetation unit: 5B (KOM)

1	lugga	rock	347	23	77	103	74	70
2	lugga	rock	327	107	60	80	47	33
3	lugga	rock	523	163	77	183	80	20

Mean density = 399 std. dev. 94 coef. of var. = 23%

Ib. Density of *A. senegal* in Marsabit District

1. Vegetation unit: 18G (OLTUROT)

Sample site	Terrain type	Soil type	Dens	Density by diameter class in cm <2	2-3	4-5	6-7	>8cm
1	lugga	rock	520	40	73	107	137	163
2	lugga	rock	300	90	73	77	40	20
3	lugga	sand	474	81	80	60	113	140

Mean density = 431 std. dev. = 95 coef. of var. = 22%

2. Vegetation unit: 9K. (HEDAD)

1	plain	sand	407	40	57	117	120	73
2	plain	sand	213	33	33	87	27	33
3	plain	sand	110	7	23	27	13	40
4	lugga	sand	207	10	27	37	56	77
5	plain	sand	153	0	0	47	46	60

Mean density = 218 std. dev. = 102 coef. of var. = 47%

3. Vegetation unit: 18G (OLTUROT)

1	lugga	rock	200	67	50	33	33	17
2	plain	sand	78	37	20	11	10	0
3	plain	sand	88	16	41	14	9	8

Mean density = 122 std. dev. = 55 coef. of var. = 36%

4. Vegetation unit: 10F (KURKUM)

1	hilly	sand	683	143	137	187	120	97
2	hilly	rock	757	127	214	193	90	133
3	hilly	rock	530	57	150	133	87	103
4	lugga	rock	650	197	243	117	60	33
5	lugga	rock	407	93	90	90	77	57
6	lugga	rock	307	117	37	33	37	83

Mean density = 556 std. dev. = 158 coef. of var. = 28%

5. Vegetation unit: 20C (NGURUNIT)

1	lugga	sand	80	0	0	0	3	77
2	plain	sand	87	0	3	7	0	77
3	lugga	sand	180	23	23	50	37	50
4	plain	sand	167	3	10	13	3	138
5	lugga	rock	70	0	0	27	23	20
6	lugga	rock	337	93	90	53	57	44

Mean density = 154 std. dev. 93 coef. of var. = 60%

6. Vegetation unit: 23X (ILAUT)

1	lugga	rock	227	53	97	33	17	27
2	lugga	rock	320	160	60	37	13	50
3	lugga	rock	270	80	95	45	20	40

Mean density = 272 std. dev. = 47 coef. of var. = 17%

1c: Density of *A. senegal* in Turkana District.

1. vegetation unit: 1S (LOKICHAR)

1	plain	sand	437	90	80	90	80	97
2	hilly	rock	523	103	86	57	100	177
3	lugga	rock	440	97	87	63	77	117

Mean density = 467 std. dev. = 40 coef. of var. = 9%

2. Vegetation unit: B (LOKORI)

1	lugga	sand	383	53	93	73	80	84
2	lugga	sand	303	27	63	63	97	52
3	hilly	rock	390	60	85	75	90	70

Mean density = 359 std. dev. = 40 coef. of var. = 12%

3. Vegetation unit: DS (LORUGUM)

1	hilly	rock	563	157	150	110	83	63
2	hilly	rock	353	103	100	77	47	26
3	hilly	rock	237	50	53	67	37	30
4	hilly	rock	503	97	80	97	83	180

Mean density = 414 std. dev. = 128 coef. of var. = 31%

4. Vegetation unit: 2S (KAKUMA)

1	lugga	sand	486	100	133	93	80	80
2	hilly	rock	587	130	140	117	127	73
3	lugga	sand	433	57	130	103	80	63

Mean density = 502 std. dev. = 64 coef. of var. = 13%

Appendix II.1: Basal diameter distribution by unit and district

a. Isiolo District

Unit	Mean density	Diameter distribution 2cm	2-3cm	4-5cm	6-7cm	> 8 cm
4B	169	41	41	36	23	28
5WBS	491	232	103	78	52	26
5WBG	138	21	48	21	20	28
4WBG	425	52	82	158	91	42
5BS	290	69	58	69	50	43
5BG	52	14	10	15	10	2
5B	399	98	71	122	67	41

b. Marsabit District

18G	431	70	75	81	97	108
9K	218	18	28	63	52	57
8E	122	40	37	19	17	9
10F	556	122	145	126	79	84
20C	154	20	21	25	21	68
23X	272	107	79	35	15	39

c. Turkana District

Lokichar	467	97	84	70	86	130
Lokori	359	40	78	68	89	69
Loima	424	102	96	88	63	75
Naipeililum	502	96	134	104	96	72

II.2: Basal diameter distribution as a percentage of the total

District	Diameter class		
	<3cm	4-7cm	>7cm
Isiolo	43	38	19
Marsabit	40	29	31
Turkana	35	32	33

Appendix III. Associated species (stems per hectare)

a: Isiolo District

Species	Transect						
	1	2	3	4	5	6	7
Acacia senegal	169	491	138	425	290	52	399
Acacia tortilis	80	50	360	80	28	0	93
Acacia reficiens	20	0	43	0	13	155	0
Acacia mellifera	0	25	0	0	0	0	0
Commi. african	94	190	3	107	180	110	287
Commi. erythre	24	98	0	90	98	40	50
Commi. confusa	0	45	0	120	68	90	137
Maerua grassif.	0	20	0	70	35	30	27
Boscia coriacea	27	10	0	0	0	0	0
Balanites aegyp.	0	0	7	0	0	0	0
Boswe. neglecta	17	0	0	0	0	0	57
Lanea elata	0	0	0	0	0	0	43
Cordia sinensis	12	0	0	0	0	0	0
Salva. persica	11	0	0	0	0	0	0

b: Marsabit District

Species	Transect					
	1	2	3	4	5	6
Acacia senegal	431	218	122	556	154	272
Acacia tortilis	120	0	0	12	170	40
Acacia reficiens	170	152	290	38	44	25
Acacia mellifera	15	16	100	0	0	0
Acacia nubica	0	10	0	0	120	0
Commi. africana	0	96	10	0	0	0
Commi. paoli	10	0	0	33	46	170
Commi. confusa	0	0	0	0	0	25
Commi. flaviflora	45	62	40	8	0	0
Commi. erythrea	0	0	0	0	34	0
Commi. campestris	0	0	0	0	10	0
Commi. incisa	0	0	0	0	0	50
Boswellia hilde.	0	0	0	108	22	20
Balanites aegy.	0	0	10	26	0	0
Maerua grassif.	12	13	20	30	30	50
Salva. persica	0	0	0	0	25	0
Cordia sinensis	0	26	13	20	0	0
Grewia tenax	90	0	17	48	0	0
Euphorbia cuneata	30	54	0	45	18	0
Cadaba farinosa	10	66	2	0	0	0

c: Turkana District

Species	Frequency by transect			
	1	2	3	4
Acacia senegal	467	359	414	502
Acacia tortilis	160	25	23	80
Acacia reficiens	147	35	247	250
Acacia mellifera	0	0	53	70
Commiphora africana	10	10	43	80
Commiphora paoli	10	15	168	60
Boswe. hildebrandtii	0	0	50	0
Balani. orbicularies	63	0	0	0
Boscia coriacea	0	0	0	10
Salvadora persica	13	5	20	0
Euphorbiia cuneata	17	10	0	29
Cadaba rotundifolia	0	0	23	30

Appendix IV: Details of gum samples from single trees

Sample code and no.	grade	Location and date	Mode of exudation	Other remarks
I1	Clear	Isiolio Sep/92	Natural	
I2	Ambe	,,	,,	
I3	,,	,,	,,	
I4	Clear	,,	,,	
I5	,,	,,	,,	
I6	,,		,,	
I7	Amber	,,	,,	Not dry
I8	Amber	,,	Natural	
I9	Clear	,,	Damage by game	
I10	Amber	,,	,,	
I11	,,	,,	Natural	
I12	Clear	,,	Tapping	Not dry
I13	Amber	,, Sep/91	Natural	
I14	Clear	,, Sep/92	,,	
I15	Amber	,,	,,	
I18	Brown	,,	Insect borer	
I16	Amber	,,	Natural	
I17	Brown	,,	Insect borer	
I18	Brown	,,	Insect borer	
I19	Brown	,,	Insect borer	
NM1	Clear	Marsabit Sep/92	Natural	
NM2	Amber	,,	,,	
NM3	,,		,,	
NM4	Clear	,,	,,	
NM5	amber	,,	Natural	
NM6	,,	,,	,,	
NM7	,,	,,	,,	
NM8	,,	,,	,,	
NM9	Clear	,,	,,	Not dry
NM10	Amber	,,	Natural	Not dry
NM11	Clear	,, ,,	,,	
NM12	Clear	,, ,,	Natural	
NM13	,,	,, ,,	,,	Not dry
NM14	,,	,, ,,	,,	
NM15	,,	,, ,,	,,	
NM16	Brown	,,	Insect borer	
NM17	Brown	,,	Insect borer	
NM18	Brown	,,	Insect borer	Sand/bark
NM19	,,	,, ,,	,,	
NM20	,,	,, ,,	,,	
KM1	Amber	,, Sep/91	Insect borer	
KM2	Clear	,, ,,	Natural	
KM3	Amber	,,	Tapping	
KM4	,,	,, Sep/92	Natural	
KM5	Clear	,,	,,	
KM6	,,	,,	,,	
KM7	,,	,,	,,	
KM8	Amber	,,	,,	
KM9	Clear	,,	,,	

KM10	Amber	„		„
KM11	„	„		„
KM12	Clear	„		„
KM13	Amber	„	Sep/91	Damage by game
KM14	Amber	„	Sep/92	Natural
KM15	Clear	„		Natural
KM16	„	„		„
KM17	Brown	„		Insect borer
LT1	Clear	Turkana	Sep/92	Tapping
LT2	„	„		„
LT3	„	„		„
LT4	„	„		„
LT5	Amber	„		„
LT6	Brown	„		Insect borer
KT1	Clear	„		Tapping
KT2	„	„		„
KT3	Amber	„		„
KT4	„	„		„
KT5	„	„		„

Appendix V: List of publications

1. Gum arabic from *Acacia senegal* (L.) Willd. in Kenya. (1993). Food Hydrocolloids, 7, 521-534.

2. The classification of natural gums. Part VI. Gum arabic derived from *Acacia senegal* var. *kerensis* from Kenya. (1994). Food Hydrocolloids, (In press).

4. Survey of *Acacia senegal* resources for gum arabic in northern Kenya. (1994). Commonwealth Forestry Review. (Accepted).

4. An evaluation of the methods for characterising and monitoring gum arabic of commerce and related *Acacia* gums. (1994). (Submitted).

5. Processing of gum arabic and some new opportunities. (1994). Prepared for a conference.