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Production and characterisation of *Prosopis* seed galactomannan

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LIST OF ABBREVIATIONS

Ara	L-arabinose
ASTM	American Society for Testing and Materials
C2	acetic acid
C3	propionic acid
C4	butyric acid
iC4	iso-butyric acid
C5	valeric acid
iC5	iso-valeric acid
cfu	colony-forming units
d.m.	dry matter
DMSO	dimethyl sulphoxide
ECR	effective carbon response
F1 - F4	fractions obtained by milling *Prosopis* pods
FAO	Food and Agriculture Organisation
FID	flame ionisation detector
Fuc	L-fucose
Gal	D-galactose
GC-MS	gas chromatography coupled with mass spectrometry
GG	sample of guar gum used in this study
GLC	gas-liquid chromatography
Glu	D-glucose
HPAEC-PAD	high performance anion exchange chromatography with pulsed amperometric detection
I1 - I4	fractions of isolated *Prosopis* galactomannan
IPA	isopropanol
JECFA	Joint Expert Committee for Food Additives
LB	sample of carob gum used in this study
LBG	locust bean gum = carob gum
Man	D-mannose

M/G	mannose to galactose ratio
MS	mass spectrometry
NMR	nuclear magnetic resonance
NS	neutral sugars
PA	sample of *Prosopis* gum obtained by acid decoating
PMAA	partially methylated alditol acetates
PN	solution of *Prosopis* gum, extracted from non-treated splits
PR	sample of *Prosopis* gum obtained by roasting
PRx	sample of *Prosopis* gum obtained by roasting and optical sorting
PSp	solution of *Prosopis* gum extracted from milled splits
PSG	*Prosopis* seed galactomannan
PVDF	polyvinylidene difluoride
PW	sample of ground *Prosopis* seeds
R4	insoluble fraction after aqueous extraction of galactomannan
R4c, R4w	seed coat and a white insoluble residue respectively
Rha	L-rhamnose
RT	room temperature
S1 - S4	sieving fractions from splitting of roasted seeds
SCFA	short chain fatty acids
SS	separated seeds
TR	sample of tara gum used in this study
U	units of enzyme activity
UV	ultra-violet
WHC	water holding capacity
WHO	World Health Organisation
Xyl	D-xylose

SUMMARY

The fruits of the "algarrobo" tree (*Prosopis pallida* and *Prosopis juliflora*) have a long tradition as food source in the arid zones of Northern Peru, in addition to their main use as animal feed. Recently, a wide range of food products has been developed on the basis of a detailed characterisation of each part of the fruit. These efforts aim to valorise the fruits as a way to avoid trees to be used for firewood only. The present work is related to the *Prosopis* seed gum (also referred to as mesquite seed gum), a galactomannan polysaccharide with potential applications as thickening agent in the food industry.

Extraction techniques of the seed endosperm have been studied in order to develop an appropriate industrial process. Aqueous extraction of gum has been shown to be suitable for analytical purposes but not for a large-scale production. Two methods, currently used for industrial production of guar, tara and carob gum, were evaluated. Treatment with sulphuric acid and roasting of the seeds have been proven to be effective for the elimination and detachment of the seed coat, respectively. Subsequent splitting and sorting of the endosperm led to the production of two types of technical gum (PA gum = acid process and PR gum = roasting process). Yields were 22% and 3.4% respectively, referring to the weight of the seeds. The large difference is due to different sorting techniques.

Physicochemical characterisation of both technical gums and an isolated galactomannan sample has been performed. PA gum showed a higher amount of impurities derived from the seed coat while PR gum contained proteins originating from cotyledon fragments. Both samples exhibited an M/G ratio of 1.4 and their galactomannan content was approximately 85%, comparable with those of commercial seed gums. The polysaccharide structure was shown to consist of a linear chain of β-1,4-linked mannose units to which single, α-1,6-linked-galactose residues are attached. The average molecular weight was estimated to be 1.1×10^6 in PA gum and 0.6×10^6 in PR gum, while it was 1.8×10^6 in isolated, non-treated galactomannan. This evidences that thermal processing causes considerable depolymerisation and, as a consequence, a reduction of viscosity of gum solutions.

Rheological properties of *Prosopis* gum solutions clearly reflected a non-Newtonian, pseudoplastic flow behaviour, even at low concentrations, which fitted the Ostwald model well. The influence of concentration and temperature on the viscosity has been studied. Solutions have shown to be stable during storage, and not to be affected by pH changes and freeze-thaw processes. The viscosity of 1% solutions of PA gum and PR gum were 1020 and 460 mPa·s respectively (measured at 25°C for a shear rate = 10 s^{-1}).

The *in vitro* digestibility and colonic fermentability of *Prosopis* gum have been evaluated, pointing to its use as soluble dietary fibre. The gum was proven to be indigestible. From fermentation experiments the rate of substrate disappearance, and the amount and proportion of short chain fatty acids produced, provided interesting information, revealing some differences between galactomannans of different M/G ratio. *Prosopis* galactomannan was found to produce higher amounts of propionic acid compared with tara and carob gum during colonic fermentation. On the whole, it has been shown that *Prosopis* gum could be used as a source of soluble dietary fibre.

ZUSAMMENFASSUNG

Die Früchte des "Algarrobo" Baumes *(Prosopis pallida* und *Prosopis juliflora)* haben neben ihrem Hauptverwendungszweck als Tierfutter eine lange Tradition als Nahrungsmittelquelle in den ariden Zonen Nordperus. Basierend auf detaillierten Kenntnissen der einzelnen Fruchtteile, wurde in den letzten Jahren eine breite Palette von Lebensmittelprodukten entwickelt. Ziel dieser Bemühungen ist es, die Früchte aufzuwerten, um die ausschliessliche Verwendung des Baumes als Brennholz zu verhindern. Die vorliegende Arbeit befasst sich mit der potentiellen Anwendung des *Prosopis* Samengummis (auch als Mesquite Samengummi bezeichnet) als Verdickungsmittel für die Lebensmittelindustrie und als potentielle lösliche Nahrungsfaser.

Verschiedene Möglichkeiten zur industriellen Gewinnung des Samenendosperms wurden untersucht. Es konnte gezeigt werden, dass eine wässrige Extraktion des Gummis für analytische Zwecke geeignet ist, für eine Produktion in grossen Mengen jedoch nicht taugt. Zwei Methoden, welche für die industrielle Herstellung von Guar-, Tara- und Caruben-Gummi im Einsatz sind, wurden hinsichtlich ihrer Eignung zur Gewinnung von *Prosopis*-Gummi untersucht. Eine Behandlung der *Prosopis*-Samen mit Schwefelsäure und ein Röstprozess erwiesen sich zur Ablösung und Entfernung der Samenschale geeignet. Anschliessendes Zerkleinern und Sortieren des isolierten Endosperms hat zu zwei Typen technischer Gummis (PA-Gummi = Säureprozess; PR-Gummi = Röstprozess) geführt. Die Ausbeuten betrugen 22% bzw. 3.4% bezogen auf das Gewicht der Samen. Der grosse Unterschied liegt in den Sortierungstechnik verwendet.

Beide technischen Gummis wurden physikochemisch charakterisiert. Der PA-Gummi enthielt grössere Mengen aus der Samenhülle stammender Verunreinigungen, während der Keimling-Reste enthaltende PR-Gummi einen erhöhten Proteingehalt aufwies. Der Galactomannangehalt beider Gummis lag bei ca. 85% und war somit vergleichbar mit dem Gehalt kommerzieller Samengummis (Guar-, Tara- oder Caruben-Gummi). Die beiden Gummis zeigten ein M/G Verhältnis von 1.4. Mit Hilfe der Methylierungsanalyse konnte gezeigt werden, dass die Galactomannane aus einer linearen Kette β-1,4-verknüpfter Mannoseeinheiten bestehen, an welche einzelne Galactosereste α-1,6-verknüpft

sind. Das mittlere Molekulargewicht für den PA-Gummi betrug $1.1x10^6$, jenes des PR-Gummis $0.6x10^6$. Das im analytischen Massstab isolierte, nicht behandelte Galactomannan wies ein mittleres Molekulargewicht von $1.8x10^6$ auf. Diese Resultate machen deutlich, dass eine thermische Behandlung zu einer beträchtlichen Depolymerisierung des Gummis führt und als Folge davon die Viscosität von Gummilösungen herabgesetzt wird.

Der Einfluss der Konzentration und der Temperatur auf die Viscosität von *Prosopis*-Gummilösungen wurden untersucht. Die Viscosität von 1%-igen Lösungen des PA-Gummis und des PR-Gummis betrugen 1020 mPa·s bzw. 460 mPa·s (bei 25°C und einem Schergefälle von 10 s^{-1}). Die rheologischen Eigenschaften widerspiegeln selbst bei tiefen Konzentrationen ein Nicht-Newton'sches, pseudoplastisches Verhalten und erfüllen die Vorgaben des Ostwald Modells gut. Die Lösungen waren lagerungsstabil und wurden weder durch pH Veränderungen noch durch Gefrier-Auftau Prozesse beeinflusst.

Mit Hilfe von *in vitro* Versuchen wurde die Verdaulichkeit von *Prosopis*-Gummi und dessen Fermentierbarkeit im Dickdarm ermittelt. Erwartungsgemäss erwies sich der Gummi als unverdaulich. Der Verlauf des Substratabbaus durch die Dickdarmflora sowie Menge und Verhältnis der produzierten kurzkettigen Fettsäuren lieferten interessante Informationen. Es konnten eindeutige Unterschiede in der Fettsäureverteilung bei Galactomannanen mit unterschiedlichem M/G-Verhältnis beobachtet werden. Während der simulierten Dickdarmfermentation, produzierte der *Prosopis*-Gummi höhere Mengen Propionsäure als Tara- und Caruben-Gummi. Gesamthaft konnte gezeigt werden, dass ein Einsatz von *Prosopis*-Gummi als lösliche Nahrungsfaser durchaus denkbar ist.

1. INTRODUCTION

Located on the north-western coast of Peru, the region of Piura is a semiarid zone where the annual rainfall lies below 100 mm. *Prosopis* tree is almost the only arboreal species that grows wild in these desertic areas and has therefore an enormous importance for the environmental protection and the income of the rural inhabitants of the region. They use the wood as fuel and for rural constructions, and the fruits as animal feed. Nevertheless, due to the excessive commercial extraction of firewood, thousands of hectares have been deforested in the last decades, resulting in widespread desertification.

Since 1984, a research group at the University of Piura has been trying to demonstrate that *Prosopis* could be the base for a sustainable development by utilising its fruits, which have more valuable food uses than simple forage. By involving the local people in the fruit's valorisation, their economic improvement will be encouraged and *Prosopis* forests are expected to be protected and extended.

Several studies have been carried out for the nutritional characterisation and development of food products from *Prosopis pallida* and *Prosopis juliflora* fruits (i.e. soluble powder, syrup, flour, coffee substitute, etc.). The results of these research efforts have been an incentive to small enterprises, which began to elaborate some of these products for the local market.

Recently, a great step towards the goals mentioned above has been done by a European STD-3 research project entitled "New food products from *Prosopis* fruits in Latin America: a base for the extension of the culture and the prevention of desertification in arid zones" involving 6 partners from different countries*. The project aimed to study the pilot plant production of syrup, dietary fibre, and seed

*Partners of the mentioned EU research project were: Universidad de Piura (Peru), Instituto del Frío - CSIC (Spain), Compañía General del Algarrobo (Spain), Centro de Investigación y Estudios Avanzados (Mexico), University of Edinburgh (UK) and Swiss Federal Institute of Technology (Switzerland).

gum, and to characterise each product, not only on its chemical composition but also on its physiological and technological properties.

The present doctoral thesis represents the Swiss participation in the project and is related to the *Prosopis* seed gum. Several leguminous seeds contain galactomannan polysaccharides in the endosperm, which yield highly viscous aqueous solutions and therefore find industrial applications as thickeners. The three major galactomannans currently used by the food industry are: guar gum, locust bean gum (carob gum) and tara gum. Their prices are high and they are demanded in large quantities. Thus, there was a great interest in studying the seed gum from *Prosopis* in those countries where it grows widely, looking for similar possibilities of exploitation.

Only few publications covering the detailed chemical composition and structure of *Prosopis* galactomannan are available. The results are partially different, possibly due to the different species or laboratory extraction techniques used. Some authors have assayed the isolation of endosperm from the seeds with laboratory milling equipment. A complete separation of the seed coat from the endosperm has been reported only by means of a wet, manual operation. Rheological studies are not easily comparable because different methods were used to obtain the gums. Furthermore, the physiological properties of *Prosopis* seed gum, which is expected to behave as soluble dietary fibre, have not yet been studied.

The present work is an attempt to fill some of these gaps. The specific objectives of the study are:

- Development of a procedure to obtain *Prosopis* seed gum at a pilot plant scale
- Physicochemical characterisation of purified and technical-grade *Prosopis* gum
- Determination of digestibility and fermentability of *Prosopis* gum by using *in vitro* methods.

In order to achieve these goals, the following set of experiments will be carried out:

- Different separation techniques will be studied to isolate *Prosopis* gum on a laboratory scale and to obtain an appropriate industrial process.
- The chemical composition of the galactomannan obtained by the different procedures will be determined. In addition, the chemical structure of the

purified galactomannan will be elucidated and the average molecular weight of the polysaccharide will be calculated by intrinsic viscosity measurements.

- Rheological studies will include comparative tests between gums obtained differently and commercial seed gums. pH and temperature stability, effect of the polysaccharide concentration on the viscosity and possible synergism with gelling agents will be evaluated as well. Finally, the effect of a partial enzymatic removal of galactose side-chains from the polysaccharide molecule on viscosity will be checked.
- From a physiological point of view it is interesting to study the behaviour of this potential thickening agent in the human gastrointestinal tract. Therefore an *in vitro* methodology to simulate digestion and colonic fermentation will be applied. The fermentation pattern of *Prosopis* gum will be compared with other galactomannans.

2. LITERATURE REVIEW

2.1. *Prosopis* pods as food source

2.1.1. General description

Prosopis are leguminous trees, from which more than 40 species of *Prosopis* are reported to be distributed around the world [32, 44, 60] in arid or semiarid regions. Although some species are considered as a weed, most of them are trees useful for people and cattle and for improving the ecosystem [64]. *Prosopis* has shown to be one of the most resistant plants in dry zones thanks to its nitrogen fixing capacity and its extremely deep roots [25, 32, 56].

"Algarrobo" is the common name for trees of the species *Prosopis pallida* and *Prosopis juliflora* predominant in Peru, while the fruits are called "algarroba". The name "algarrobo" is also used in Spain for the carob tree (*Ceratonia siliqua*), which sometimes causes confusion. The Spaniards gave *Prosopis* the same name when they arrived to Peru in 1532, due to the resemblance of the fruits. The Peruvian native names for *Prosopis* were "thacco" and "guarango", but they are not often heard today [35]. In North America, *Prosopis* species are known as "mesquite" [132]; their fruits seem considerably different from the Peruvian algarroba. Therefore, in this work, the generic name *Prosopis* will be used for the Peruvian species, and mesquite only for North American species.

P. pallida and *P. juliflora* are very similar species, with especially large and sweet fruits (Figure 1) compared to other species of the genus. The sugar content in the pulp is approximately 50% [20, 36, 133]. This is the reason for the traditional use of the fruits as forage and food.

The fruits are indehiscent pods, pale yellow, 10 to 25 cm long, 1.5 cm wide and 0.8 cm thick, with an average weight of 12 g. A pod consists of 3 separable components: exo- and mesocarp (pulp), endocarp (fibrous hulls) and seeds. The seeds are enclosed in the endocarp, which can hardly be opened by hand. The number of seeds per pod is 25 in average [20, 133]. The seeds are tiny (~5 mm ∅, ~40 mg), ovoid, very hard and

are constituted of the episperm (seed coat, brown and very thin), the endosperm, which lies adhered to the coat, and the cotyledon. Figure 2 shows the structure of the pod and seed, with respective percentages of each component.

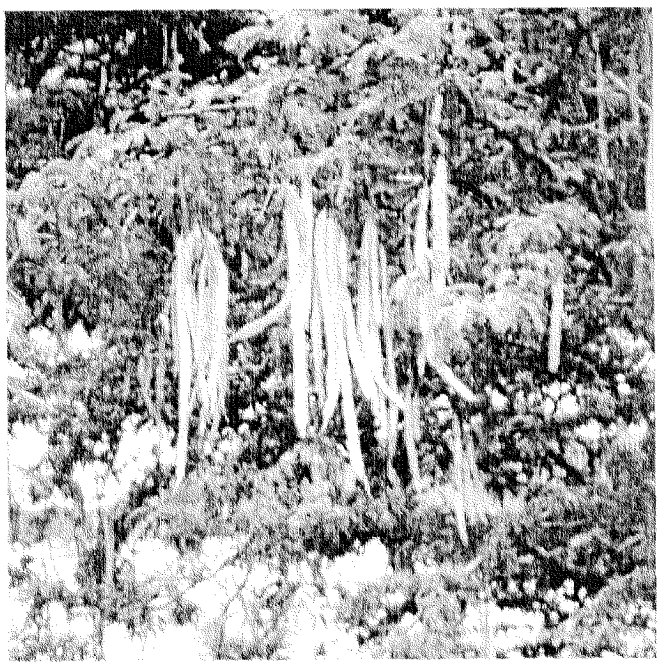

Figure 1: Prosopis pods on the tree

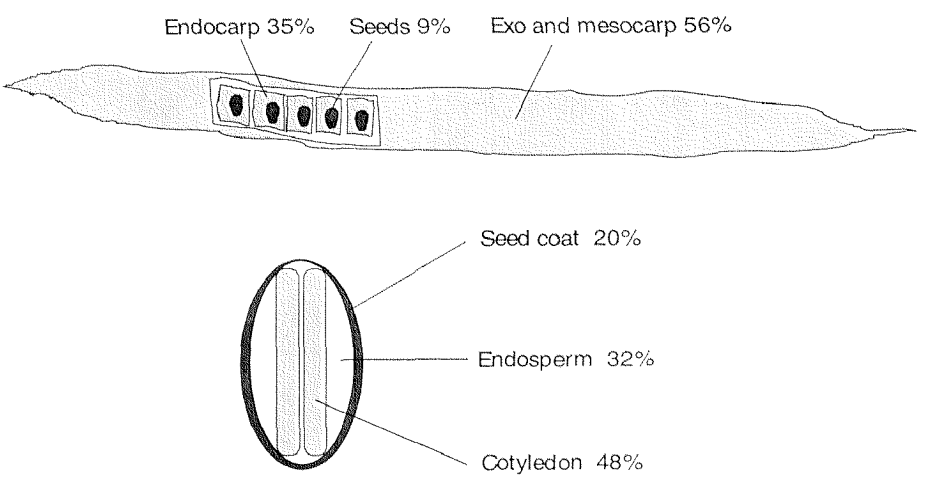

Figure 2: Structure of pod and seed of Prosopis

2.1.2. Production, collecting and market of *Prosopis* pods

Starting in the fifth year the tree bears twice a year: the main pod production occurs in summer (December-March) and a second minor production season occasionally in the middle of the year [74]. The productivity mainly depends on the age of the tree and on the soil quality. Some old trees produce up to 100 kg of pods per year, while the average for adult trees is estimated to be 40 kg/year. A dense forest have approximately 100 trees per hectare, with an expected production of 4 tons/hectare [6, 44]. When fully ripe, the pods fall, and being rather large and indehiscent, they are easily collected from the ground, or eaten there by grazing domestic animals like goats, sheep and donkeys.

Because *Prosopis* in Peru is not a crop but a wild plant growing in desert areas with free access for everyone, it is interesting to describe the pod collection and commercialisation ways.

The collecting is done manually by children and women and sometimes by the whole rural family, as an activity marginal to their home and agricultural tasks. Considering that bearing trees are dispersed and there are no means of transport, one person can collect no more than 150 kg of pods per day, which is equivalent to a daily income of less than US$ 5. During the production season the pods have a very low price due to their abundance (i.e. US$ 27/ton in February 1995). People use the pods for feeding their small livestock, or selling them to merchants. Most of the merchants have storage facilities for supplying ranchers in other regions of Peru during the whole year. They profit from the fact that the price for *Prosopis* pods raises up to 4 times in September, when other forages are also lacking [74]. The amounts of commercialised *Prosopis* pods are not well registered by governmental offices and usually the merchants do not declare the real amounts. A compilation of records reports a total of 12,635 metric tons for 1996, of which 60 % are transported to Lima [6]. This is in any case a very small part of the estimated potential. An estimation based on sampling studies and forestry maps [81, 82] results in 400,000 metric tons of pods annually in the northern region of Peru. Probably more than 50% is lost because it is not collected.

Storage must avoid that insects *(Bruchidae, Pyralidae)* infest the pods [45, 116]. Rustic closed rooms made from adobe are used for storage. Once a room is filled, it is sealed with clay and opened only when the whole batch is to be sold. Chemicals are not often used for insect control during storage, but preferably natural repellent plants. Safe fumigants such as Phostoxin® have been reported to be effective. A considerable amount of pods are already infested while on the tree. Another kind of damage occurs due to rainfall when the pods lie on the ground; then they rot quickly [75].

The way of commercialisation described above makes clear that the main use of *Prosopis* pods in Peru continues to be the feeding of livestock. Ruminants usually eat the whole pods (without processing), but can not digest the seeds because they are enclosed in the fibrous, practically impermeable endocarp. Only few factories produce feed mixtures for livestock including coarse milled *Prosopis* pods; in most of the cases, the mill does not grind the endocarps or the seeds completely [74]. Ranchers seem to prefer *Prosopis* because they believe it has special properties (such as promoting milk production) besides its energetic value, making it irreplaceable.

2.1.3. Traditional human food products

Many references can be found on the food uses of *Prosopis* by ancient cultures wherever it grows. The Sonoran Desert in North America might be one of the locations where mesquite played an important role. Indian tribes cooked green pods with meat. They also made flour and dough with the dried or toasted pulp from ripe pods. A kind of durable cake was prepared by drying dough balls. The pulp was separated from unbroken endocarps by pounding the pods in mortars. If further pounded, the seeds were released from the endocarp hulls, then ground and mixed with water to obtain a fermented beverage [107, 132]. A fermented paste from boiled *Prosopis africana* seeds, called "okpiye" is known in Nigeria [1]. In Northern Argentina, flour made from the sugary pulp is known as "patay", and is still consumed nowadays [117].

Since many years, the inhabitants of northern Peru prepare and consume "algarrobina", a concentrated sugary extract from the pods, and considered a typical confectionery from rural production. To produce this syrup, whole or crushed pods are soaked in water for 2 hours, then the pods are pressed and the juice is filtered.

Finally, this extract is concentrated by evaporation. The dark brown syrup obtained is more viscous than honey and exhibits a peculiar brightness. The process is carried out in very simple -usually rustic- equipment, often in the rural places, and "algarrobina" is sold in reusable glass bottles. No quality standards exist for this product [3, 54].

"Algarrobina" syrup is consumed in different ways. Some people recommend to take a spoon daily as a health practice, directly or added to fruit juices or milk, where it performs as both sweetener and flavouring agent. It is often given in this way to children and elderly persons, because it is believed that the syrup has properties of a fortifier and revitaliser. In urban zones, the syrup is used as ingredient in home confectionery and to prepare a tasty drink, the "cocktail de algarrobina", which is a mixture of a small quantity of "algarrobina" with "Pisco"-brandy and milk.

Another food product from *Prosopis* is "yupisín", a beverage which is also obtained by water extraction of the sugars from the pod. In contrast to "algarrobina" it is consumed directly without concentration or used to prepare puddings with sweet potato flour. "Yupisín" is consumed only in rural zones nowadays, and it is not bottled. A very similar beverage is known in Argentina as "añapa". A fermented beverage, "aloja", can be obtained from "añapa", and used as substitute for beer or wine [32, 117]. In Peru, no fermented beverages are prepared from the sugary pulp nowadays.

2.1.4. Composition of *Prosopis* pods

To evaluate their nutritional properties, several authors have studied pods of *Prosopis* species originated from geographically different regions. An interpretation of the results is, however, difficult, because the terms used to identify the different parts of the fruit are not consistent. For example, the term "pod" is predominantly applied as a descriptor for the whole fruit, but in some cases it refers to the pericarp (pod without seeds). It is therefore important to clearly define the meaning of the terms. In the present study the definitions given in 2.1.1. will be strictly used. The term "pulp" will describe the sum of exo- and mesocarp only, which represent the edible portion of the fruit.

Proximate analyses of whole pods give similar results for several *Prosopis* species, but this offers only limited information for applications in human nutrition. Del Valle *et al.* [41] reported 11% protein, 29% fibre, 4.6% ash and 1.7% fat for whole pods from North American species (*P. glandulosa* or honey mesquite and *P. velutina* or velvet mesquite). Zolfaghari *et al.* [150, 151] analysed green and ripe *P. glandulosa* pods with comparable results. Pak *et al.* [120] reported similar values for *P. tamarugo* pods from Chile. Several authors (reviewed in [32] and [44]) have studied the Peruvian species *P. pallida* and *P. juliflora* as whole fruits for animal feeding; ranges for protein were 9-12%, fibre 14-23%, ash 3-5%, fat less than 1.7%. Pods from Ecuador identified as *P. juliflora* were reported to have almost the same composition with exception of 27% fibre [99]. It is also interesting to note that Figueiredo [61] reported 15% protein and 4.9% fat for *P. juliflora* pods from northern Brazil. Although the values for fibre are strongly dependent on the analytical method used, it could be assumed that Peruvian *Prosopis* pods contain less dietary fibre than all the other species. The protein content for most species is near to 10%.

The compositions of pulp and seeds from Peruvian *Prosopis pallida*, and the properties of syrup obtained by different procedures, have been reported. Sucrose was identified as the main sugar in the pulp [127]. Further studies on each part of the fruit, carried out mainly by the research group at the University of Piura give detailed nutritional information. Their results are described below and compared with data available for other species where possible

Pulp

The pulp represents 56% of the total weight of the fruit. Several studies have been performed to determine, as completely as possible, the chemical composition of the pulp [20, 34, 36, 74, 129]. The results are summarised in Table 1.

The main soluble component of the pulp is sucrose (46%), representing over 90% of total soluble sugars. The reducing sugars glucose, fructose and xylose, are present in very small amounts [36]. The sucrose/glucose/fructose ratio agrees well with the one for mesquite pulp [107]. For pericarp of *P. juliflora* from Ecuador, sucrose has been reported to be 75% of soluble sugars, besides to 12% of fructose, 5% of glucose, 5%

of inositol and 1% of raffinose [99]. A sucrose content of 30% and very low amounts of raffinose, inositol, fructose and glucose have been found in the pericarp of *P. glandulosa* and *P. velutina* from Texas and Arizona respectively. After autolysis of the pericarp, a small increase in glucose and fructose indicated low invertase activity [12].

Table 1: ***Composition of Prosopis pallida pulp*** [20, 34, 36, 74, 129]

Main components *(g/100 g d.m.)*	
Total soluble sugars	48.5
Sucrose	46.1
Fructose	1.26
Glucose	1.02
Xylose	0.27
Total dietary fibre	32.2
Insoluble DF	30.6
Soluble DF	1.6
Protein (Nx6.25)	8.1
Sum of amino acids	7.1
Resistant protein	2.2
Fat	0.77
Ash	3.6
Condensed tannins	0.41
Total soluble polyphenols	0.81

Amino acids *(g/100 g protein)*		
		WHO/FAO pattern
Hydroxyproline	2.13	
Aspartic acid	8.51	
Threonine	4.68	4
Serine	4.96	
Glutamic acid	10.07	
Proline	23.40	
Glycine	4.68	
Alanine	4.26	
Cysteine	0.43	
Methionine	0.57	
Met+Cys	1.00	3.5
Valine	7.80	
Isoleucine	3.26	4
Leucine	7.94	7
Tyrosine	2.84	
Phenylalanine	2.98	
Tyr+Phe	5.82	6
Lysine	4.26	5.5
Histidine	1.99	
Arginine	4.82	
Tryptophan	0.89	1

Minerals *(g/kg d.m.)*	
Potassium	26.5
Sodium	1.1
Calcium	0.76
Magnesium	0.9
Copper	traces
Zinc	traces
Manganese	traces
Iron	0.33

Vitamins *(mg/kg sample)*	
Vitamin A	not detected
Vitamin E	5
Vitamin B1	1.9
Vitamin B2	0.6
Vitamin B6	2.35
Nicotinic acid	31
Vitamin C	60
Folic acid	0.18
Calcium pantothenate	10.5

Dietary fibre represents 30% of the pulp and is largely insoluble (Table 1); more than half of the fibre fraction consists of neutral polysaccharides [20]. Analysed by different methods, higher dietary fibre contents of pulp from other species have been published. Meyer [107] found 35% dietary fibre in mesquite pulp and Zolfaghari *et al.* reported 25% cellulose, 11% hemicellulose and 7% lignin for the pericarp from *P. glandulosa* [151].

The crude protein content of the pulp from *P. pallida* is surprisingly high (8%), considering that seeds are not included. From the amino acid composition shown in Table 1 it can be realised that nearly all the essential amino acids are present in amounts which fulfil the requirements of the FAO/WHO "standard protein" [55], thus indicating an acceptable nutritional quality of the protein. Methionine and cysteine are the limiting amino acids. These results are in agreement with studies on *P. juliflora* from Ecuador [99] and *P. glandulosa* from North America [57, 107, 150]. Compared to other species, remarkably high amounts of proline and hydroxyproline have been determined in *Prosopis* pulp from Peru [34]. The reasons for the observed differences remain to be cleared.

The *in vitro* digestibility of *P. pallida* pulp protein has been determined to be 73% [20], similar to the value for *P. juliflora* pulp from Ecuador [99].

P. pallida pulp contains considerably higher amounts of iron and lower levels of calcium in comparison to the values reported for mesquite [12, 150]. High iron levels have been reported also in *P. juliflora* from Ecuador and Brazil [61, 99] and in *P. tamarugo* from Chile [120], but no figures for its bioavailability are given. With respect to the vitamins, it is interesting to notice that the amounts of vitamins C, B6 and calcium pantothenate are the most relevant in *P. pallida* pulp [75].

Endocarp

The endocarp is a very hard and fibrous hull in which the seeds are enclosed. Its chemical composition is shown in Table 2.

Insoluble dietary fibre represents by far the main component. In a detailed analysis this fraction was shown to contain cellulosic polysaccharides (40%) and lignin (17%) as major constituents [131].

Table 2: ***Composition of Prosopis pallida endocarp*** [129, 131]

Component	g/100 g d.m.
Total dietary fibre	92.3
Insoluble DF	88.9
Soluble DF	3.4
Soluble sugars	1.6
Protein	2.3
Fat	1.3
Ash	1.3
Soluble polyphenols	0.7

Seeds

The seeds of *P. pallida* consist of 32% endosperm, 48% cotyledon and 20% of seed coat (Figure 2). Proteins and lipids from seed cotyledon will be briefly discussed in this section, whereas the literature review on seed endosperm (source of galactomannan) is presented separately in 2.3.

P. pallida cotyledon contains 65% protein, which represents 31% of the seed weight. The amino acid composition of the cotyledon proteins has been determined [36], as shown in Table 3. The content in essential amino acids is remarkably high, cysteine and methionine being the first limiting amino acids. These results are comparable with those reported for *P. juliflora* and mesquite [10, 61, 107, 150].

Data on protein digestibility and protein fractionation are not available for *P. pallida* cotyledon. However, the apparent digestibility of proteins from *P. glandulosa* raw seeds was reported to be 70.9% [150].

The fat content of *P. pallida* cotyledon has been reported to be 7% [84]. The major fatty acids found in the extracted oil were linoleic acid (39%), oleic acid (29%), palmitic acid (13%) and stearic acid (10%). The unrefined oil shows an acidity of 1.7%. Similar values have been reported for mesquite [12, 118] and *P. juliflora* [99]. Fatty acid and sterol compositions of seed oils from several *Prosopis* species in Argentina have been reported as well [92].

Table 3. Amino acid composition of Prosopis pallida seed cotyledon [36]

Amino acids *(g/100 g protein)*		
		WHO/FAO pattern
Aspartic acid	8.30	
Threonine	2.42	4
Serine	4.87	
Glutamic acid	21.31	
Proline	7.49	
Glycine	4.59	
Alanine	4.34	
Cysteine	1.31	
Methionine	0.88	
Met+Cys	2.19	3.5
Valine	4.56	
Isoleucine	3.09	4
Leucine	7.51	7
Tyrosine	1.84	
Phenylalanine	4.29	
Tyr+Phe	6.13	6
Lysine	4.09	5.5
Histidine	3.10	
Arginine	14.63	
Tryptophan	1.37	1

Antinutritional factors

Each fraction of *P. pallida* fruit has been investigated for polyphenols and tannins [20, 129]. Significantly smaller amounts were found when compared to carob pods. These results confirm those reported for *P. chilensis* from India [122, 143].

The presence of further antinutrients has not yet been studied in the Peruvian *Prosopis* pods. Mesquite pods and seeds have been reported to be almost totally devoid of trypsin inhibitor activity (< 6 TIU/mg) [119, 150], although a trypsin inhibitor from *P. juliflora* seeds has been characterised [109]. Cyanogenic glycosides, which occur in some legumes, have not been found, neither in mesquite [12] nor in *P. tamarugo* [120]. It may be expected that *P. pallida* does not contain more antinutritive substances than other *Prosopis* species.

2.1.5. Processing and new products from *Prosopis* pods

As described in 2.1.3, the two traditional food products obtained from *Prosopis* in Peru are mainly based on the soluble sugars of the pulp, which are, in fact, the most palatable components, the rest of the fruit (endocarp, insoluble fibre and seeds) being discarded. To investigate new industrial applications, several studies have been carried out at the University of Piura since 1986. The scheme in Figure 3 shows the diversity of products that could be obtained with a processing that uses all the components of *Prosopis* pods.

Concerning the development of suitable technologies for the milling of the pods and separation of each component, the first complete study was performed by Meyer *et al.* [107, 108, 130] on North American mesquite. It allowed to obtain pulp flour, seeds and endocarp hulls, and proposed techniques for further separation of the seed into endosperm, cotyledon and seed coat. Later on, Saunders' working group preferred to focus on the integral grinding of pods (including seeds) for production of high-protein, low-fibre flours, recoverable by sieving [40, 41, 42].

The currently available process in the pilot plant at the University of Piura allows to separate the pods into four fractions and the recovery of the entire seeds. The process consists basically of drying, milling and sieving operations. By means of the milling, the pulp is finely ground and the seeds are released from the endocarps without being broken. Although this separation had been achieved with a disc mill [32, 107] or even with a meat grinder [121], better results on a larger scale have been obtained with a mill prototype [31], constructed specifically for this purpose. It consists of an improved design of a thresher [66], which has several hammers fixed to the rotating shaft and short hammers mounted on the screening housing. The complete processing technology has recently been published [75].

Syrup from Prosopis pulp flour

Pulp flour containing 46% sucrose is used to obtain syrup. Because the pulp is finely ground, the extraction is faster than for the traditional "algarrobina" processing (from entire fruits), and no heat is required. The filter cake that results from the extraction is washed and dried, and could be used to enrich products with dietary fibre. Syrup and dietary fibre processed under different conditions have been characterised [21].

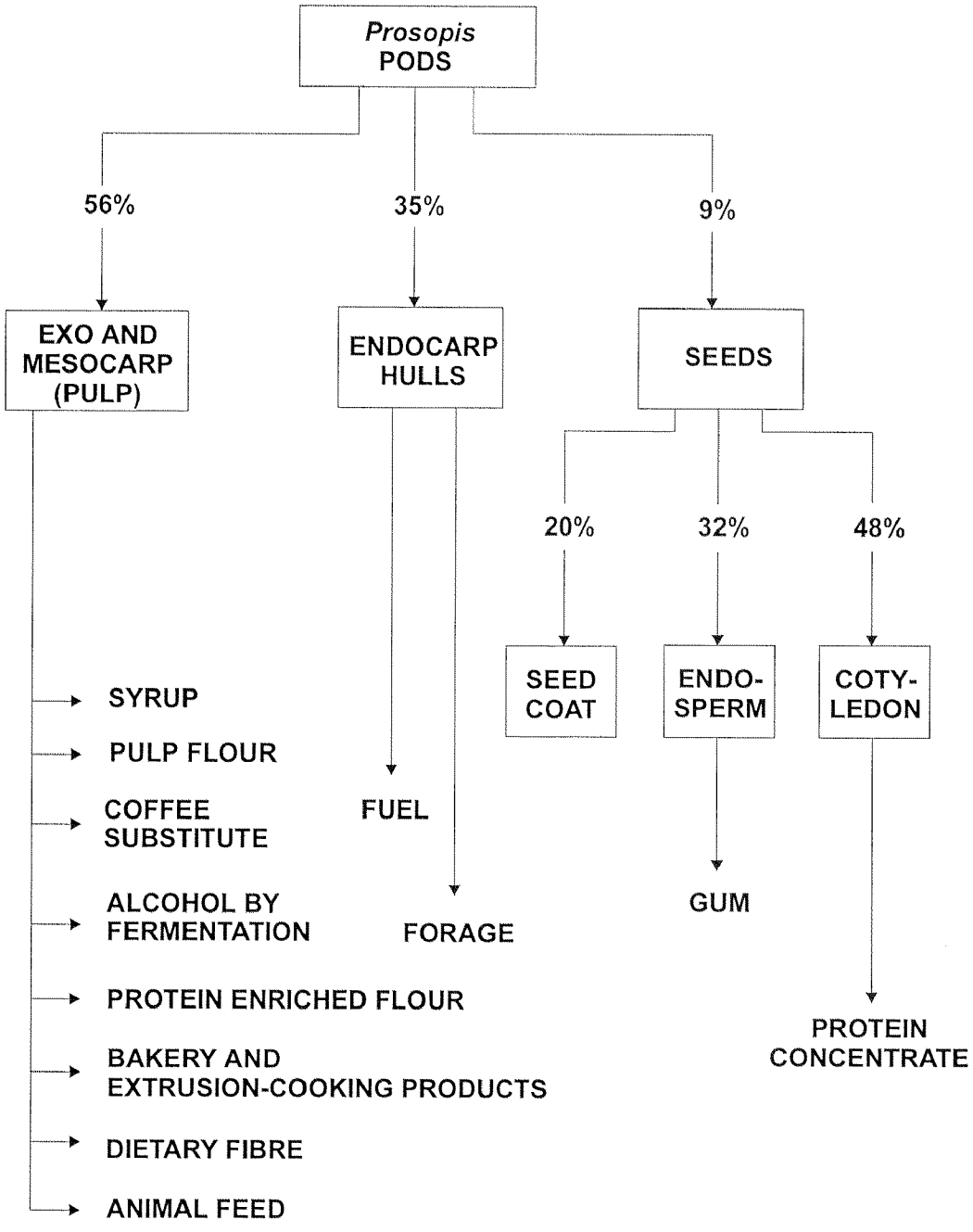

Figure 3: Potential uses of Prosopis pods

Prosopis flours in bread and biscuits

Because of its sugar content, colour and flavour, the use of pulp flour for bakery products seems to be interesting. The absence of starch is, however, a limitation

for *Prosopis* flour levels in the bread formulation. The rheological behaviour of *Prosopis*-wheat composite flours has been studied [33]. *Prosopis* flour causes dough resistance to decrease and dough elasticity to increase, which, from a practical point of view, results in softer leavened bread. Sweet bread containing 5% of *Prosopis* flour is acceptable in texture and taste. In biscuit recipes up to 25% of *Prosopis* flour has been included, lowering the amount of sugar to be added. A slightly bitter aftertaste has been reported, which some people, however, find pleasant.

Instant soluble powder

Prosopis pulp flour can be used as ingredient in many food preparations, such as cakes, ice creams and other desserts. One interesting application could be the use as cocoa powder substitute, as suggested by the current commercialisation of an instant soluble powder derived from carob pulp. A preliminary study [91] has shown that a soluble powder can be obtained from the fine *Prosopis* pulp flour, by re-milling and sieving it through a 100-mesh screen. In order to improve the dispersability in milk, yoghurt and juices, the agglomeration of the fine powder should be studied. Mixes with Andean cereal flours (i.e. *kiwicha*) and cocoa, to improve nutritional or sensorial properties, have been shown to be feasible [75].

Coffee substitute

Manufacture of a coffee substitute from *Prosopis* was studied on the base of some information from Brazil [8]. Roasting of the whole pods was the first process to be considered [26]. In later trials, roasting of only the coarse pulp flour has shown that a better flavour can be achieved. The process is performed at 120°C until the flour becomes dark brown. During roasting, the flour agglomerates into larger granules; thus, it has to be ground again. This product is used in the same way as coffee, i.e. extracted by filtration with boiling water. Compared with other usual coffee substitutes (roasted beans or cereals), it is generally well accepted by the consumers. In addition it has been shown not to be stimulant because it does not contain caffeine [142].

Recently, some small factories in Peru began to manufacture new food products from *Prosopis* pods by adapting the technology developed at the University of Piura. For example, a powder named "garrofina" is being produced from finely ground whole fruits, but it does not have a good solubility. *Prosopis* coffee substitutes or "café de algarroba" are produced and successfully commercialised in the Peruvian market, packed in 250 g plastic bags at a convenient price under the manufacturers own trade names.

Other uses of *Prosopis* pods, for example the production of alcohol and a protein-enriched flour have also been studied in Peru [29, 126] but have not reached the commercial stage so far. In Brazil, the production of a protein isolate from *P. juliflora* seeds and its application in bread has been reported [10].

2.2. Properties and applications of galactomannans

2.2.1. Occurrence and structure

Galactomannans are plant polysaccharides built up of mannose and galactose sugar units. They are hydrophilic and form highly viscous, stable aqueous solutions. They are often referred to as gums [43, 106]. The interactions with water and with other polysaccharides are the basis of the widespread industrial use of certain galactomannans, particularly in the food industry [106].

The distribution of galactomannans in the plant kingdom is limited. Rich sources are the members of the family *Leguminoseae*, but galactomannans have also been found in species of other families such as *Annonaceae* and *Palmae*.[38]. Amongst the leguminous plants, galactomannans are located in the seed endosperm as reserve polysaccharides Reserve polysaccharides are formed by plant cells during the development of the seed, and are degraded to monomers to be used as carbon source by the seedling during germination. The galactomannan deposition does not take place in discrete bodies within the endosperm cells, as in starch granules, but as thickening of the cell wall. Deposition can continue until the cytoplasm is almost excluded. Seed galactomannans therefore belong to the so-called cell wall storage polysaccharides [67, 106].

It is possible that the hard endosperm plays a structural role in protecting the embryo from mechanical damage in the dry state of the seed. After imbibition, galactomannan takes up large amounts of water and the endosperm cell walls become softer. This would also provide a mechanism to preserve the germinating embryo from desiccation if drought follows the seed imbibition [123]. After germination, the polysaccharide is completely catabolised by endogenous enzymes, and its breakdown products are transferred to the embryo where they supply carbon and energy to the growing seedling [23].

Structurally, galactomannans are formed by a linear β(1→4)-linked backbone of D-mannose molecules, to which single units of D-galactose are attached through α(1→6)- linkages [113]. In a minor group of galactomannans from microbial sources, other types of linkages have also been reported [7, 38]. The seed galactomannans from different leguminous species differ in their degrees of galactose substitution, ranging from about 20% to nearly 100%. Thus, the mannose/galactose (M/G) ratio has been proposed as a marker in the taxonomy of *Leguminoseae*, besides to the galactomannan content [23]. In general, high M/G ratios are characteristic for the *Caesalpinoideae*, whereas low M/G ratios are found in the *Faboideae* subfamily. In a review, Dea and Morrison [38] presented M/G ratios of 71 leguminous species. The *Prosopis* genus, which belongs to the *Mimosoideae*, was not taken into account in that study; it was recently surveyed for taxonomic purposes by Buckeridge *et al.* [23].

The galactose substitution ratio alone does not fully describe the galactomannan structure. The pattern of substitution plays a significant role in determining the interaction with other polysaccharides. In some galactomannans the mannan chain has been shown to contain more extended substituted (hairy regions) and non-substituted parts (smooth regions) than would be expected from a random distribution [90]. It has been suggested that the molecular structure is characterised by both ordered and random segments [39, 67, 102].

Galactomannans, like many other natural polysaccharides, tend to be polydisperse and, consequently, can not be accurately described by a single molecular weight but by average molecular weights ($\overline{M}_w$). Values may differ depending on the

determination techniques used. In general, $\overline{M}_w$ of the most known galactomannans have been reported in a range from 200,000 up to 2,000,000 [38].

2.2.2. Galactomannans as food additives

Among the many galactomannans studied, only the three following are currently of industrial importance: (a) carob or locust bean gum (LBG), (b) guar gum and (c) tara gum. They are widely used as thickening agents in food products. In addition they are used in other industries as textiles, paper, etc. [113]. The respective plant sources, the endosperm content in the seed and the M/G ratios of these galactomannans are given in Table 4.

Table 4: ***Sources and basic properties of commercial galactomannans*** [67, 147]

	Carob gum	*Guar gum*	*Tara gum*
Plant source	*Ceratonia siliqua*	*Cyamopsis tetragonolobus*	*Caesalpinia spinosa*
Approx. seed size (mm)	10	4	10
Approx. seed weight (mg)	250	35	250
Seed components (%):			
Endosperm	42-46	38-45	20-22
Cotyledon	23-25	40-46	38-40
Seed coat	30-33	14-16	38-40
M/G ratio	3.5-4	1.5-2	2.5-3

The food uses of carob, guar and tara gum generally depend upon their solution properties, including their interactions in the colloidal state with other molecules. The most important properties of galactomannans are their high water binding capacity and their ability to form very viscous solutions at low concentrations.

The ***solubility*** in water depends on the galactose content. The galactose side chains cause an extension of the very long mannan chains and prevent them from forming hydrogen bonded insoluble associates (pure linear β-mannans devoid of galactose residues are completely water insoluble) [113]. Galactomannans are insoluble in organic solvents. They can be precipitated from aqueous solution by addition of water-miscible solvents like ethanol. The M/G ratios given in Table 4 are only a rough distinction since a fractionated precipitation of carob gum showed that the composition is heterogeneous [147]. Galactomannans show a strong tendency to

form lumps when added to water (and vice versa) [46]. This effect is more pronounced with finely ground galactomannan powders. Lumping is usually prevented by premixing the gum with water-soluble solids (e.g. sugar) or by wetting it with ethanol.

Viscosity is the most important quality criterion for thickening agents. Galactomannans produce highly viscous aqueous solutions at low concentrations (0.5-2%). They exhibit a non-Newtonian behaviour. When the polysaccharide is dissolved in water, the long mannan chain unfolds to form an open, flexible, non-ordered conformation called random coil. At low concentrations these coils are separated and the viscosity increases linearly with the concentration. Above a certain concentration, random coils come into contact, which results in mutual entanglement, and the viscosity rises exponentially. According to this model, the longer the molecule and the more extended its conformation in solution, the sooner entanglement will begin [67]. In commercial galactomannans, the viscosity is usually measured in 1% (w/w) solutions with a RVF Brookfield viscometer (20 rpm, spindle 3), at 25 °C [113, 138].

Stability: Depolymerisation of the mannan chain leads to significant reduction in viscosity. This can occur e.g. when low pH-foods are thermally processed [67]. At room temperature, galactomannan solutions are stable within a pH range from 3 to 8, but they can be degraded by microorganisms. Solutions should be sterilised or preserved for storage. Guar and tara gum exhibit a very good freeze-thaw stability, while LBG becomes partially insoluble as a consequence of freezing [113].

The ***interaction with other polysaccharides*** is one of the most interesting properties of galactomannans. Thus hot solutions of LBG and xanthan gum form strong elastic gels upon cooling, a functional property which neither of the hydrocolloids alone exhibits under similar conditions. The molecular basis of this synergism is believed to be the building of a three-dimensional polysaccharide network by virtue of the formation of junction zones between xanthan and the smooth regions (galactose-deficient segments) of the LBG. This model is supported by the fact that galactomannans with higher contents of galactose show a lower synergistic effect on gelling. Thus tara gum forms only a weak gel and guar gum produces only a synergistic rise in viscosity. Similar observations can be made

with κ-carrageenan, which alone gives a brittle gel, and by addition of LBG can form a much stronger elastic gel [67]. Yet differences have been observed between galactomannans with identical degrees of substitution. They have been attributed to a different statistical distribution of galactose residues along the mannan backbone. Synergistic interactions have been suggested to be favoured by a higher frequency of longer smooth regions [123].

Today carob gum is used only as an additive for foods and pet foods [71, 78]. There are about 15 processing plants in Spain, Portugal, Italy, Greece, Morocco, Switzerland and the Netherlands, which produce a total of approx. 12,000 tons of LBG per year. Because of its lower price, guar gum is produced in much larger quantities (ca. 100,000 tons/year) and is mainly used in technical applications (paper, textiles, explosives, oil well drilling, etc.), but an increasing percentage also as food additive [77]. Tara gum production has been started recently. The tara bush grows wild in the Andean regions of Peru and Bolivia [70]. Its pods are gathered mainly for industrial production of tannins, while the seeds had been considered as a by-product for a long time. It is estimated that today several hundred tons of tara galactomannan are produced in Peru and in Europe [87, 113, 147].

All three galactomannans have been investigated for safety as food additives, and no toxic effects have been found. Thus, the Joint Expert Committee for Food Additives (JECFA) of WHO/FAO has established an acceptable daily intake "not specified". In the European countries the authorised food additives have the so-called E-numbers: E410 stands for carob gum, E412 for guar gum and E417 for tara gum [87, 113].

The principal food uses of galactomannans are in dairy products. The widest use is certainly in the stabilisation of ice cream, for promoting fine ice crystal structure during freezing and for enhancing the creamy mouthfeel [78]. In fresh cheese, galactomannans help in binding the water set free by shear impact and increase viscosity [67]. Galactomannans also find application as thickeners in desserts, as low-energy fat substitutes in e.g. mayonnaises, and as gelling agents in fruit-based water gels and in powdered products (desserts and hot milk puddings). Other uses are in bakery goods (icings and cake mixes), dietary products, coffee whiteners,

baby milk formulations, dressings, sauces and soups, tinned meats, and frozen and cured meat foods [123].

There is generally a clear difference in price between carob and guar gums. This reflects the superior functional properties of LBG, but it may also reflect limits of the LBG supply. Carob is a slow-maturing tree, whereas guar is an annual plant (a crop), whose production can easily be increased in line with demand [123]. This situation causes that the gum industry is continuously looking for new sources of galactomannans with high functionality.

The establishment of a link between structure and functional properties suggested the possibility of "upgrading" guar galactomannan, i.e. to lower its galactose content for improving its synergistic properties. Biotechnological processes to modify guar galactomannan represent an excellent example of the practical approach of the biodegradative hydrolysis that occurs after the seed germination. In that breakdown, three enzymes are involved: α-galactosidase for removal of the galactose side-units, endo-β-mannanase for degradation of the mannan backbone into oligosaccharides, and β-mannosidase for complete hydrolysis of these oligosaccharides into mannose [38].

Many studies have been conducted to isolate α-galactosidases from germinating leguminous seeds and from fungi or bacteria, with the aim of a selective removal of galactose from guar galactomannan [11, 103, 105]. It has been shown that the α-galactosidase from germinating guar seed can remove galactose residues without any requirement for simultaneous depolymerisation of the mannan backbone. Processes for enzymatic modification of guar galactomannan and respective achievements of interaction properties have been described [24, 104].

2.2.3. Technology of the production of seed galactomannans

Two facts may be remembered to understand the technologies for endosperm separation: (a) The endosperm consists almost entirely of galactomannan. Therefore, it is sufficient to separate it from the other two parts of the seed to obtain a commercial gum. (b) The endosperm is hard and compact and adheres to the seed coat, while the cotyledon is friable. Therefore, when the seed is hit, the cotyledon

would shatter while the endosperm, with the coat attached, remains in large pieces [123]. In reality, industrial processes are much more complex and have been kept unrevealed for a long time. Only few authors have described the processes in general terms [113, 147]. In the following sections some technological information is presented briefly.

Tara and carob seeds are difficult to be processed, since their seed coat is very tough and hard. Two processes are known for removing the seed coat without damaging the endosperm and the germ.

Acid process: The seeds are treated with sulphuric acid at high temperature to carbonise the seed coat. The remaining coat fragments are then removed by efficient washing and brushing machines to yield "peeled" seeds. After drying, these are splitted and the crushed germ can be sifted off from the unbroken endosperm halves. These halves can be successively "purified" through swelling-drying-milling steps, and finally milled into a fine powder.

Roasting process: The seeds are roasted in a rotating furnace, where the seed coats more or less pop off from the seeds. Then the roasted seeds are splitted in a mill and the crushed germ and the seed coat are separated as mentioned above. With this process, no sulphuric acid is necessary and therefore no liquid waste is produced. The endosperm powder has, however, a slightly darker colour.

Guar gum is produced by a different milling-roasting process. The seeds are first separated into "splits" (endosperm + seed coat) and fine germ material [123] by means of an attrition mill (or any type of mill having two grinding surfaces rotating at different speed). The splits are heated to soften the seed coat and are fed either into a mill that can abrade the coat away from the endosperm, or into a hammer mill to shatter the hull away.

In all three processes, the obtained endosperm is finally ground and sieved to yield gum powders of various particle sizes (100- to 200-mesh). Because of the particular hardness of both tara and carob seed coat, and because of the smallness of guar seeds, industrial isolation of endosperm is never quantitative. This implies that in commercial products small amounts of protein-rich germ and also of fibre-rich seed coat can be found [115]. The purity criteria and specifications for food grade

galactomannans have been approved by the JECFA of FAO/WHO, as shown in Table 5.

Table 5: FAO/WHO Specifications for galactomannans as food additives [69]

Specification	Carob gum	Guar gum	Tara gum
E-Number	E 410	E 412	E 417
Loss on drying (%)	< 14	< 15	< 15
Total ash (%)	< 1.2	< 1.5	< 1.5
Acid insoluble matter (fibre, %)	< 4	< 7	< 2
Proteins (%)	< 7	< 10	< 3.5
Starch	non detectable		
Arsenic	not more than 3 mg/kg		
Lead	not more than 10 mg/kg		
Heavy metals	not more than 20 mg/kg		

2.2.4. Galactomannans as dietary fibre

Galactomannans are not hydrolysed by the human digestive enzymes. They are not absorbed in the small intestine and are not a source of energy, but they act by modulating digestion and metabolism of other nutrients. The actions of polysaccharides on the body can be classified into their effects on the gastrointestinal tract, postprandial plasma levels of glucose and insulin, plasma lipids and cholesterol, the effects of the products of their fermentation by colonic bacteria, and their effects on satiety and energy balance [48].

The effect of plant cell wall polysaccharides or hydrocolloids in the gut is dependent on their chemical composition as well as on their physical and chemical interactions with the food ingested. For example, intact plant cell walls can protect starch and other nutrients from the digestive enzymes leading to greater resistance to chewing and gastric action.

Behaviour in the upper gastrointestinal tract

In the stomach, food is subjected to the action of gastric acid, to the proteolytic enzyme pepsin and to the grinding action of gastric motility. The pylorus allows chyme to pass into the duodenum only when the particles are small enough. Disruption of food is easier when most of the liquid has been emptied from the

stomach. The pattern of gastric emptying therefore depends on the liquid/solid nature of the chyme. Intact cell walls promote the integrity of the food and slow down disruption. Most soluble polysaccharides increase the viscosity of the chyme and thus reduce the movement of the liquid phase, delaying the gastric emptying. This results in a prolonged satiety.

Digestion in the small intestine progresses as the chyme interacts with enzymes secreted from the pancreas. The products of pancreatic digestion move through the bulk phase and must be finally digested by intestinal enzymes before they are absorbed. Slowing of digestion and absorption in the small intestine by soluble viscous polysaccharides, such as galactomannans, may be due to a combination of a physical entrapment of nutrients, resistance to the mixing movements of intestinal contractions, inhibition of enzymatic activity and an increase in mucin production [48]. The result of a decreased rate of absorption of glucose in the small intestine is reduced postprandial glycemia and insulin response. The glycemic index is a useful parameter to evaluate differences in starch hydrolysis *in vivo*. It relates the postprandial blood glucose level after ingestion of a starchy food to the postprandial glucose seen after ingestion of an equivalent amount of glucose or white bread [83].

In addition to the action of viscous polysaccharides on motility and mixing, some of them are believed to have the ability to sequester bile acids and thus to avoid their re-absorption in the small intestine. This results in an increased demand for cholesterol for bile synthesis and therefore the cholesterol level in the plasma decreases. Bile sequestration can also inhibit fat absorption by inhibiting micelle formation [68].

Behaviour in the large intestine

Carbohydrates escaping digestion in the small intestine are at least partially fermented by the numerous and diverse colonic bacteria (up to 10^{12} cfu/g and 400 different species), which can utilise about 60-70 g of carbohydrates per day [37]. Some polysaccharides, such as gums and pectin, are rapidly fermented. Others, such as cellulose, are resistant to fermentation. The factors that determine fermentability of different polysaccharides are multiple (chemical composition,

structure, etc.). The fermentation products may determine some beneficial effects of dietary fibre on metabolism.

One of the main effects of dietary fibre is the increase of stool output. It is related to the water-holding capacity (WHC), another characteristic physical property of polysaccharides, as well as to the increase of the bacterial mass. Other effects of dietary fibre on stool output are reduction of transit time and stool consistency, and increased frequency [50].

Products of the colonic fermentation and their physiological effects

It is generally accepted that most of the carbohydrates escaping digestion are fermented in the colon by the Emden-Meyerhof pathway to short-chain fatty acids (SFCA), predominantly acetic, propionic and butyric acid, and gases such as hydrogen, carbon dioxide and methane [48]. Amino acid degradation gives rise to branched SCFA (isobutyric and isovaleric acid).

The distribution of SCFA production is used as a fermentation parameter for different substrates. An overall pattern in the proportions of SCFA has been reported for some types of substrates fermented in the colon (Table 6). Nevertheless, the variability of reported values is sometimes high, due to the various fermentation systems, the complex microbial ecology, different analytical methods used, and not least, due to the differences in the chemical composition of the substrate analysed (see for example the values for guar in Table 6).

Acetic acid is the major SCFA produced from all substrates. Resistant starch, starch, oat bran and lactulose are associated with production of large proportions of butyric acid, while arabinogalactan, arabinoxylan and guar gum are related to high propionic acid production.

There has been considerable interest in the effects of the SCFA on the colonic mucosa and on the liver and other tissues after their absorption. Acetic acid rapidly passes into the blood, contributing to the energy needs of the muscles, while butyric acid and partly propionic acid are metabolised by the epithelial cells of the intestinal mucosa. SCFA, and especially butyric acid, play an important role in maintaining health of the colonic mucosa and in modulating cell proliferation. Some studies have proposed

that butyric acid attenuates the growth of cancer cells [37, 128]. Propionate has been mentioned as a possible inhibitor of cholesterol synthesis in the liver [5, 48].

Table 6: Profile of SCFA (molar percentages) after in vitro fermentation of different substrates by human faecal bacteria

Substrate	Acetic acid	Propionic acid	Butyric acid	Reference
Pectin	84	14	2	[52]
Guar gum	58	27	8	[2]
	61	25	14	[101]
	49	41	10	[93]
	53	33	14	[19]
Gum arabic	68	20	8	[2]
Gum tragacanth	67	19	8	[2]
Xanthan gum	71	19	7	[2]
Gellan gum	62	20	7	[2]
Karaya gum	63	10	10	[2]
Arabinogalactan	50	42	8	[52]
Arabinoxylan	56	26	10	[49]
Oat bran	57	21	23	[101]
Wheat bran	52	11	20	[2]
Cellulose	61	20	19	[144]
Starch	50	22	29	[52]
Resistant starch	41	21	38	[51]
Lactulose	67	13	20	[144]

It is difficult to study the fermentation of polysaccharides *in vivo* in humans since most of the fermentation takes place in the proximal colon. Much of the present knowledge is therefore based on animal studies and *in vitro* fermentations.

Applications of galactomannans as dietary fibre

The various beneficial effects on human health attributed to soluble dietary fibre suggest that an increased use of galactomannans in the context of functional food is to be expected [67, 112]. Soluble polysaccharides ingested alone are often unpalatable, giving a viscous mouthfeel and aftertaste. These effects can be reduced by their incorporation into food products such as bread. Most studies have been carried out to evaluate the inclusion of guar gum in bread formulation and its effect as regulator of carbohydrate metabolism in diabetic patients [22] as well as the

reduction of the plasma cholesterol concentration in hypercholesterolemic human subjects [15]. Low doses of galactomannans may have little metabolic effect when used as stabilisers in foods.

To allow higher amounts of guar gum in formulated foods without an excessive increase of viscosity after ingestion, the native galactomannan is enzymatically treated to reduce the average molecular weight by an order of magnitude. This gives a partially depolymerised guar gum, whose safety in food has been evaluated over the past 10 years [9, 76]. It seems that the physiological effects of guar gum are not diminished by a partial depolymerisation [15].

Finally, it is interesting to mention that several insoluble galactomannan derivatives have been synthesised and evaluated as coating material for drug delivery to the colon (for local treatment of colonic diseases), since they are not digestible in the upper digestive tract but easily fermentable by colonic bacteria [79].

2.3. *Prosopis* seed gum: state of the art

Since *Prosopis* species in North America are known with the common name mesquite, it is necessary to distinguish the seed gum that will be reviewed here from the so-called "mesquite gum", which refers to a trunk exudate. Mesquite gum has been shown to be similar in composition, properties and uses as gum arabic [4, 73]. It seems to have some commercial importance in Mexico, where many application studies have been done [13, 14, 72, 141].

For a clearer identification, the denomination "*Prosopis* seed gum (PSG)" instead of "mesquite seed gum" has been proposed [64]; however, the last one continues to be used by many authors. In the present work, PSG will be used when an abbreviation is required.

Figueiredo [61] reported the occurrence and possibilities of exploitation of a seed galactomannan from Brazilian *Prosopis juliflora* in 1975. This is the first, rather detailed chemical study published. Methods for separation of seeds and endosperm on a large scale were, however, not studied.

In Figueiredo's study, the galactomannan was extracted with water from defatted whole seed flour and purified by repeated precipitation with ethanol. Its chemical structure was investigated by means of different techniques. The monomeric composition was determined by GLC of trimethylsilyl derivatives of the sugars after acid hydrolysis. Mannose and galactose were shown to be present in a M/G ratio of 4, thus similar to LBG. The position of glycosidic linkages was determined after permethylation and methanolysis of the polysaccharide. The resulting methylglycosides were separated and analysed by GLC, NMR and MS, and were found to be the same as those obtained from carob galactomannan. In addition, the galactomannan exhibited an average molecular weight of 250,000.

Galactomannan from Brazilian *Prosopis juliflora* has been studied by Bobbio [17] as well. The extraction of the gum was carried out from the whole seeds as described by Figueiredo [61]. The polysaccharide contained 8% protein, which could be reduced to 0.75% after protein denaturation with chloroform. Quantification of monosaccharides after acid hydrolysis was performed by GLC of their trimethylsilyl derivatives. An M/G ratio of 4 was reported. ^{13}C-NMR analysis of the degraded polysaccharide suggested the presence of β(1→3) and β(1→4) glycosidic linkages in the linear mannan backbone.

In 1984, Meyer [107] studied the production of a seed galactomannan from mesquite (*P. glandulosa* and *P. velutina*). The seeds were split in a cyclone mill to yield "splits" (endosperm + seed coat), cotyledon fragments, and ground fine material. Splits accounted for 40% of the seed weight, and could be separated from the smaller particles by sieving and subsequent air separation. Meyer studied two methods for the further separation of endosperm from the splits. (a) A wet process, in which splits were soaked and stirred in water, and then the mucilage filtered and freeze-dried. (b) A dry process, in which splits were run through a cyclone mill equipped with a rubber rotor at 2200 rpm, resulting in a partial (minimal) decoating of the splits.

Meyer determined the monosaccharide composition of the endosperm by GLC of the aldonitrile acetates of the sugars resulting after hydrolysis. The M/G ratio of 1.6 was similar to the one for guar gum. In a former study of mesquite seeds, a M/G ratio of approximately 1.0 has been reported [12]. The Brookfield viscosity of

1% (w/w) galactomannan solutions was determined to be in a range from 2250 to 3300 mPa·s. Maximal synergistic effect of mesquite gum with xanthan on the viscosity was observed at a 9:1 ratio (mesquite : xanthan gum).

More recent publications by Figueiredo and co-workers [62, 63, 64, 65] on *Prosopis* seed galactomannan reviewed the above mentioned studies and primarily aimed to divulge the possibilities of production and use of PSG in the food industry. The interest of the industry on *P. juliflora* galactomannan has been demonstrated by the registration of a German patent [88] for the extraction process and uses of PSG. However, there is no evidence of an actual application of the patented process.

In more recent years, some studies aimed to determine the rheological properties of the *Prosopis* seed gum described by Figueiredo, i.e. to investigate if they are comparable to those of LBG on the hypothesis of a similar M/G ratio. Thus, an interesting method for endosperm separation was described by Yoo *et al.* [149], consisting of seed coat removal by vigorous agitation of the seeds in a reactor containing an aqueous solution of NaOH, MgO and H_3BO_3, and subsequent washing. The intrinsic viscosity of this PSG was 2.4 dL/g. Synergistic effects of mesquite gum with κ-carrageenan and xanthan gum were reported to be similar to those of guar gum but not as those described for LBG [59].

Some rheological properties of *Prosopis chilensis* seed galactomannan have also been studied [125, 140]. The seed endosperm was manually separated from seeds soaked in a solution of 5% (w/w) NaOH according to a method proposed earlier by the same authors [139]. It was reported that the flow curves fit the Ostwald model for non-Newtonian fluids well. The effect of pH on the viscosity was evaluated and reported to be irrelevant.

First studies on galactomannan from the Peruvian *Prosopis pallida* approached the endosperm separation by acid peeling and wet extraction [27, 134] at a laboratory scale (approx. 20 g of seeds). Gum was obtained from whole seed flour by aqueous extraction and alcoholic precipitation. A 1% (w/w) solution of this gum showed a Brookfield viscosity of 1800 mPa·s. A later study [53] proposed a dry milling process to separate the cotyledon before the wet extraction. By doing so, only

splits (endosperm + coat) are soaked in water-ethanol, then squeezed, dried and air-separated. The M/G ratio of this gum was 1.36, which has been confirmed by other authors [20, 36].

When *Prosopis* seed gum was obtained by wet methods, subsequent drying was necessary, which was reported to cause browning of the product [134]. Only freeze-drying avoided this effect. Several ways to prevent browning (washing with solvents, use of antioxidants, etc.) were studied without success [134]. Furthermore, wet-extracted PSG solutions exhibited low viscosity and poor stability, due to the presence of enzymes from the cotyledon. A method for seed splitting was developed, including a step for elimination of the cotyledon fine particles that remained adhering to the splits. The yield of the process was 15% of dry gum on the basis of the weight of whole seed [134].

A comparative chemical characterisation of endosperm seed gum from *Prosopis pallida* obtained by dry and wet methods has also been performed more recently [137]. No significant differences were found when extraction processes were carried out avoiding contamination with cotyledon fragments.

3. EXPERIMENTAL PART

3.1. Plant material

Raw material for seed separation were mature fruits from *Prosopis pallida* and *Prosopis juliflora*. The pods were collected randomly in the experimental field of the Universidad de Piura, Peru, between January and March 1996. A large amount of pods gathered from the ground was carried in plastic bags to the pilot plant of the Laboratory of Chemistry of the University of Piura. The damaged pods were manually removed, and the good ones were washed to eliminate adhering sand.

3.2. Separation of *Prosopis* seeds

Pods were dried in a gas heated drying chamber with forced air at 80°C for 4 hours. According to the processing technology developed at the University of Piura (see also 2.1.5), the dried pods were fed to a mill prototype that allowed separation of the seeds and simultaneously grinding of the pulp [75]. The milling output was separated in a vibrating sifter (with ASTM sieves) into four fractions F1 (>6 mesh), F2 (10 - 6 mesh), F3 (60 - 10 mesh) and F4 (<60 mesh). Residual coarse fragments of exo- and endocarp contained in F2 were separated by air classification to obtain clean seeds.

3.2.1. Separation of seeds from the milled fraction

To improve air-separation of seeds from exo- and endocarp fragments, a 2 m long tunnel was constructed and equipped with various trays at the bottom, in which different fractions can be collected. Plywood was used to build the tunnel. It had large glass windows on the sides and on the top, which allow to follow the operation visually. A ventilator was installed at the tunnel entry and seeds were fed manually through a funnel from the top.

3.2.2. Improvement of the milling process

Different tests were planned with the objective to improve the yield of seeds in the separation process, including several modifications of the milling conditions, as well as of the design of the equipment itself. Three successive groups of tests were carried out.

Modification of the shape and number of openings on the fixed screen of the mill

The original screen of the mill (A) had been constructed with two slots at the bottom to allow an easy discharge of the milled product. Based on the assumption that the two slots could be too large and let pass whole endocarp hulls without being opened by the hammers, the slots were reduced both in width and length, but several new slots were made (screen B). Later on, a new screen (C) was constructed, in which the round holes were substituted by short slots. These basic screen types B and C are shown in Figure 4. Further modifications of screen C resulted in screen D (wider slots) and screen E (more slots, more open area). Table 7 shows the dimensions of the 5 different versions of screens tested.

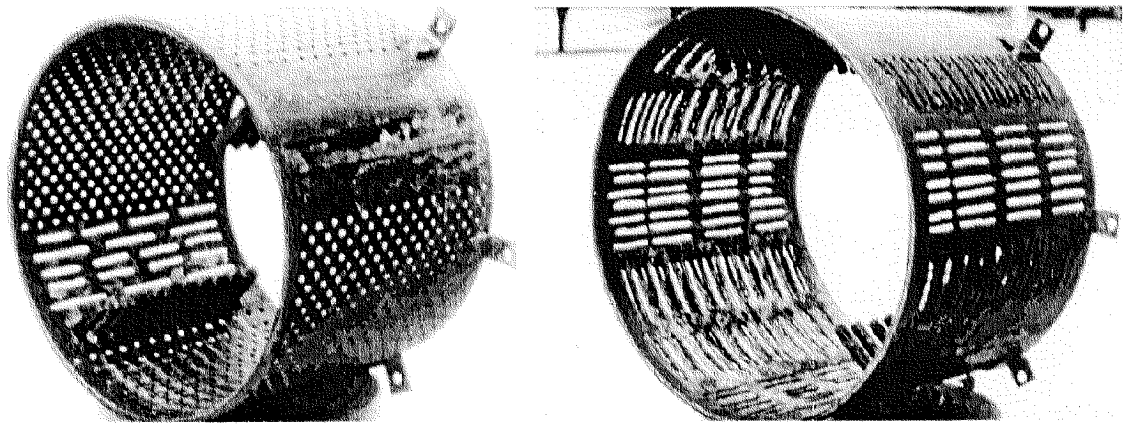

Figure 4: Screens tested to improve seed separation: types B (left) and C (right)

The performance of each screen for milling was evaluated before the next modification was done. Dried whole pods were used. The rotation speed of the mill shaft was 800 rpm, achieved with an electrical motor (1.5 HP, 1800 rpm, 60 Hz) and adequate belt and wheels.

Table 7: Description of the screens used for milling tests

Screen type	Shape of the openings	Dimensions	Open area [cm^2]	Total open area [cm^2]
Screen A	Round holes	2 slots of 254 mm x 6.4 mm 574 holes of 4.8 mm ∅	32.5 103.9	136.4
Screen B	Round holes	20 slots of 51 mm x 4 mm 574 holes of 4.8 mm ∅	40.8 103.9	144.7
Screen C	Slots	16 slots of 51 mm x 3.2 mm 114 slots of 51 mm x 2.4 mm	26.1 139.5	165.6
Screen D	Slots	130 slots of 51 mm x 4 mm	265.2	265.2
Screen E	Slots	242 slots of 51 mm x 4 mm	493.7	493.7

Assessment of the separation efficiency considered the following parameters: percentages of fractions F1 to F4 after milling, percentage of separated seeds (SS) after air-separation of F2, percentages of whole and broken seeds in SS, and percentages of open and closed endocarp hulls in F1.

In one of the trials (with screen C) the effect of using a cyclone dust collector after the mill was compared with the standard procedure for this study.

Variation of the rotation speed of mill hammers

In an attempt to increase the yield of separated seeds, different rotation speeds of the mill were tested. The experiments were carried out with screen C using dry whole pods. Speed was controlled by means of an adjustable control unit Mod. 1XC97A (Dayton Electric Co., Illinois, USA) connected to the motor. The following rotation speeds were evaluated: 800, 640, 560, 480 and 400 rpm.

Milling of bagasse instead of whole pods

In a supplementary set of trials, bagasse consisting of pod fragments after water extraction of the soluble sugars was fed to the mill. The bagasse was a by product from an alternative procedure to obtain *Prosopis* syrup. The efficiency of the process was evaluated by calculating the same percentages and ratios as mentioned above.

3.3. Technological processes for endosperm separation

3.3.1. Acid decoating process

In a first set of experiments, 10 g of seeds were treated with 50 mL of H_2SO_4 of different concentrations (25 to 72% w/w), at various temperatures (25 to 100°C) and for different periods of time (5 to 30 min), under continuous stirring (magnetic stirrer). Attempts to scale up the process were carried out with larger amounts of seeds up to 500 g, using technical H_2SO_4 (72% w/w) and a larger stirrer with plastic shaft and blades.

After stirring, sulphuric acid was quickly and completely drained and then the seeds were extensively washed with tap water. Special attention was paid to this step to prevent excessive local heating by acid-water interactions, which could cause seed damage. During the washing operation, the seeds were manually rubbed to eliminate the burned coat fragments.

The "peeled" seeds were dried in an oven at 60°C and then splitted to get endosperm splits. Different laboratory mills (disc-, roller- and hammer mill) were assayed for the splitting of the seeds. Separation of the splits from the cotyledon fragments was tried by sieving (10 mesh ASTM) and air streaming. However, a final manual selection was necessary to obtain pure splits.

3.3.2. Roasting process

Three different roasters were evaluated for *Prosopis* seeds in this study. A total of 100 kg of seeds were used for the roasting trials. The seeds to be roasted were not absolutely clean but contained approximately 5% of exo- and endocarp fragments.

Rotary roaster

A batch type pilot plant equipment, originally designed for coffee roasting, was used to evaluate the roasting conditions. The roaster was kindly made available by Unipektin AG (Eschenz, CH), and consists of a cylindrical furnace rotating on the vertical axis, where preheated air is blown in from the top. The air is heated with

electrical resistors and the temperature is adjusted by switching them and by opening fresh air valves at the blower inlet. Seeds are loaded in a top funnel and quickly fed into the furnace by opening a valve. The furnace can be opened automatically after a preset period of time or by pressing a button on the panel, allowing the roasted seeds to fall down to a chamber where they are cooled with forced fresh air.

After an initial period of temperature stabilisation (a digital thermometer was installed at the inlet of the furnace), samples of 100 to 400 g of *Prosopis* seeds were fed from the top funnel into the furnace. After a roasting period of 40 to 75 seconds the seeds were cooled down.

40 roasting trials (100 g of seeds each) were carried out at different temperatures (from 170 to 300°C) to establish the optimal roasting conditions for *Prosopis* seeds. The temperature was progressively increased. From each batch, a sample was manually separated to quantify the efficiency of detachment of seed coats.

Finally, 25 kg of seeds were roasted in batches of 400 g (at 260°C for 60 s), and then pooled for splitting experiments.

Fluidised bed roaster

Some trials were performed with a laboratory fluidised bed dryer (Bühler AG, Uzwil, CH). The roaster is equipped with a removable roasting chamber, which consists of a glass cylinder provided with wire meshes at the bottom and at the top, allowing observation of the seeds during roasting. Batches of 400 g of seeds were filled into the chamber, which was then mounted on the hot air outlet of the roaster only when the air temperature had reached the required value. After roasting the seeds at 250 to 280°C for 75-110 s, the chamber was transferred to a fresh air outlet for cooling the seeds.

Laboratory coffee roaster

A laboratory roaster, designed for studying the process of coffee roasting at the food technology pilot plant of the ETH Zurich, was used for further roasting experiments of *Prosopis* seeds. The equipment is basically a fluidised bed roaster,

provided with computer-controlled temperature and airflow at each point of the system. Four roasting trials were performed with 100 g seeds each at 240, 250 260 and 270°C for 60-70 s.

Splitting of roasted seeds

Splitting of the roasted seeds was evaluated with several systems. Preliminary trials were carried out with a micro hammer-cutter mill (Culatti AG, Zurich, CH) and a disc mill (Fuchs Maschinenbau, Granges-Paccot, CH) with samples of 50 g of roasted seeds each. Other initial splitting tests were performed with a manual disc mill (low rotation speed, large gap between discs) and a hammer mill, with samples of 200 g of seeds each. The last two mills had been constructed in a local workshop in Piura.

A cross beater mill model RS2 (Retsch GmbH & Co., Haan, D) with a nominal speed of 2780 rpm at 50 Hz was used for large scale splitting experiments. Different rotation speeds, achieved with a frequency regulator, were tested (30, 40, 50, 60 and 70 Hz). The mill was operated with different outlet ring screens (4 or 6 mm ∅ holes) or without screen. Prior to milling, the seeds were heated in a fluidised bed dryer at 130°C for 5 min. For recovering endosperm splits, the mill output was separated on a laboratory sifter (MLU 300, Bühler AG), and four fractions were quantified: S1 (>2.56 mm), S2 (2.0 – 2.56 mm), S3 (1.8 – 2.0 mm) and S4 (<1.8 mm).

A first series of splitting trials was performed to optimise the rotation speed and to choose the best screen size of the mill. The seeds used for these trials had been roasted in the rotary roaster two months before. Batches of 200 g of roasted seeds were pre-treated by heating them at 130°C for 5 min and then fed to the mill. With the best milling conditions (2780 rpm, 6 mm screen), a second set of trials was carried out with batches of 400 g of seeds, roasted in the fluidised bed roaster just before the milling.

Finally, an industrial soybean dehuller (also called impact detacher) model MJZF (Bühler AG) with a nominal speed of 2900 rpm at 50 Hz was used for splitting of roasted seeds. Batches of 800 g of seeds were pre-treated as above and fed to the mill. Different rotor speeds were tested, equivalent to the frequencies of 40, 50, 55,

60 and 70 Hz. The milled material was sieve-separated into five fractions, as mentioned above.

The experiments with the cross beater mill and the industrial soybean dehuller have been carried out at the pilot plant of Bühler AG in Uzwil.

Sorting of splits with an optical classifier

A colour sorter model 90002 (Sortex Ltd., London, UK), available at the pilot plant of Bühler AG was used. This equipment operates with a 2-sided monochromatic optical inspection system, reliable enough to distinguish between brown (endosperm with seed coat) and white (pure endosperm) splits.

The S2 - fractions obtained from both splitting mills were pooled and fed into the sorter in two successive steps.

In the first step a throughput of 280 g/min was used at a sensitivity of 70%. For the second step the throughput was set at 590 g/min and the sensitivity was 90%. Accepted and rejected material of each step was quantified for calculating the yield of the process.

3.3.3. Preparation of samples for characterisation

Pure endosperm splits obtained by manual sorting from acid decoating and roasting processes were finely ground with a Cyclotec centrifugal mill (Tecator AB, Höganäs, S) provided with a 0.5 mm outlet sieve, and were marked as **PA** (acid) and **PR** (roasting). Endosperm splits from the roasting process obtained by optical sorting were ground in the same way and labelled **PRx.** Particle size analysis of the samples PA and PRx was performed by Unipektin AG using the Alpine air jet sieving technique. This is a sieve sizing technique for powders, which uses an air jet in conjunction with vacuum to continuously lift the particles from the sieve surface and then draw them back down. This technique is particularly useful for sticky powders (like gums), as it prevents particles from agglomerating or clogging the sieve openings.

3.4. Isolation of *Prosopis* galactomannan for structure analysis

The starting material for the isolation of *Prosopis* galactomannan consisted of non-treated splits (endosperm + seed coat), which were obtained from non-treated seeds by splitting with a hammer mill and manual separation.

10 g of splits were boiled under reflux in 50 mL of ethanol at 80°C for 1 h. After cooling, the alcoholic extract was drained, evaporated under vacuum in a Rotavapor® (Büchi Labortechnik AG, Flawil, CH), and the weight of the dry residue determined. The extracted splits were dried at 80°C to eliminate residual ethanol.

The splits were suspended in 1 L of water, stirred at room temperature (RT) during 20 h and finally centrifuged for 20 min at 5200×g (Sorvall RC 5B centrifuge, Du Pont Instruments, Wilmington, USA). The supernatant gum solution was decanted and filtered through a G4 sintered glass filter funnel. The pellet (seed coats and not solubilised endosperm) was re-extracted with 1 L of water at RT for 8 h, centrifuged, decanted and filtered as above. Two further extractions of the pellet were performed at 60 and 80°C for 4 and 2 h respectively. Each supernatant was collected separately (**I1 to I4**). The insoluble residue (**R4**) was freeze-dried (LSL Secfroid Lyolab B-II, Aclens-Lausanne, CH) and its weight was determined.

To isolate the polysaccharide, isopropanol was added to the gum solutions (I1 to I3) at RT under stirring, in a 3:1 (v/v) ratio. The precipitate was recovered by filtration on a G4 sintered glass filter, and freeze-dried. The gum solution obtained after the fourth extraction (I4) was not precipitated with isopropanol but only filtered and freeze-dried.

3.5. Analytical methods for chemical characterisation

Unless otherwise specified, chemicals were purchased from Fluka AG (Buchs, CH) or Merck AG (Darmstadt, D) and were of analytical or *puriss.* quality. Water for laboratory use was of NANOpure® quality (Barnstead/Thermolyne, Dubuque, USA).

3.5.1. General methods

Dry matter content of splits, technical grade gum samples and isolated *Prosopis* galactomannan was determined gravimetrically after drying 1 g of material in an oven at 104°C to constant weight.

Protein content was calculated as the sum of the anhydro amino acids, according to the method described by Lebet *et al.* [95]. The proteins are hydrolysed with 6M hydrochloric acid and the amino acids (except tryptophan) determined by ion exchange chromatography with an automatic amino acid analyser (Alpha Plus, Pharmacia Biotech, Uppsala, S)

3.5.2. Determination of neutral sugars

Neutral sugars were liberated from the polysaccharides either by acid or by enzymatic hydrolysis. Two different chromatographic methods were used to determine the neutral sugars. For comparison, galactose was also determined with an enzymatic method.

Acid hydrolysis: About 20 mg of material was weighed in a 10 mL screw-cap hydrolysis tube with magnetic bar. Insoluble samples were hydrolysed under argon in 0.25 mL of 72% (w/w) (12M) sulphuric acid at RT for 2 h, followed by 3 h at 100°C after dilution with 2.75 mL of water to give 1M H_2SO_4, with continuous stirring (Saeman hydrolysis). Soluble samples (about 20 mg each) were hydrolysed under argon directly in 3 mL of 1M sulphuric acid at 100°C for 3 h with continuous stirring.

Enzymatic hydrolysis: Purified enzymes β-mannanase from *Aspergillus niger* and α-galactosidase from guar seed (Megazyme Ltd., Wicklow, IRL) were used to degrade the galactomannan. 0.25 g of isolated *Prosopis* galactomannan was dissolved in 30 mL of 0.1M sodium acetate buffer (pH 4.5), 40 U of β-mannanase were added and incubated at 60°C for 7 h, then 40 U of α-galactosidase were added and incubated at 40°C for additional 17 h (*).

(*) One unit of enzyme activity (U) is defined as the amount of enzyme that releases one micromole of product per minute at pH 4.5 and 40°C.

Gas chromatography of the alditol acetate derivatives

Preparation of alditol acetates was done according to the method described by Blakeney *et al.* [16]. After cooling, the hydrolysed samples were made alcaline by adding 0.72 mL of 13.5M ammonia solution. 0.2mL *myo*-inositol solution (10 mg/mL) was added as an internal standard. Aliquots (0.1 mL) of each sample were transferred to new hydrolysis tubes, and reduced by addition of 1 mL of a solution of sodium borohydride in dimethyl sulphoxide (prepared by dissolving 1 g of $NaBH_4$ in 50 mL of anhydrous DMSO at 100°C) while stirring at 40°C for 60 min. After reduction, the excess of $NaBH_4$ was decomposed by addition of 0.1 mL of glacial acetic acid. For acetylation of alditoles, 0.2 mL of 1-methylimidazole (catalysator) followed by 2 mL acetic anhydride were added to the tubes and mixed. After standing at room temperature for 10 min, 5 mL of water was added to decompose the excess of acetic anhydride. 1 mL dichloromethane was added to the cooled sample and the mixture was agitated on a Vortex mixer. The tubes were centrifuged at 1600×g for 5 min. The lower phase was removed with a Pasteur pipette, dried over sodium sulphate and transferred to a GC vial.

The alditol acetates were analysed by gas chromatography under the following conditions:

Instrument:	HP 5890 gas chromatograph, with HP 7673A autosampler and ChemStation software (Hewlett-Packard, Palo Alto CA, USA)
Column:	DB 225 capillary column 30 m × 0.32 mm i.d., film thickness 0.25 µm (J&W Scientific, Folson CA, USA)
Carrier gas:	Nitrogen 5.0 (PanGas, Luzern, CH), 1.2 mL/min
Column head pressure:	70 kPa
Septum purge flow:	7 mL/min
Inlet purge flow:	30 mL/min, initially off; on after 0.5 min.
Column temperature:	160°C for 1 min, then raised at 35°C/min to 220°C and kept for 25 min
Injector temperature:	220°C
Detector:	FID, 260°C
Injection volume:	3µL

Quantification was carried out using a reference mixture containing 1 mg/mL of each of the following sugars: L-rhamnose, L-fucose, L-arabinose, D-xylose, D-mannose, D-galactose, D-glucose and *myo*-inositol. The standard mixture was derivatised in the same way as the samples.

Neutral sugars by HPAEC-PAD

Hydrolysed samples of gums were analysed by anion exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) as described by Lebet *et al.* [96]. The hydrolysates were centrifuged (1600×g, 10 min), filtered through a 0.45 µm PVDF membrane filter (Scientific Resources, Inc., Eatontown, USA) and 50 µL of internal standard solution (2-deoxy-D-ribose, 10 mg/mL) was added to 1 mL of filtrate. For calibration of the chromatographic system, L-rhamnose, L-arabinose, D-galactose, D-glucose, D-xylose and D-mannose were dissolved in 1M H_2SO_4 (0.4 mg/mL each, except galactose and mannose: 2.5 mg/mL each) and then centrifuged, filtered and mixed with internal standard solution.

The analyses were performed on a Dionex Bio-LC DX-300 system (Dionex Corp., Sunnyvale, USA) equipped with an autosampler. A pre-column was used for the on-line removal of sulfate ions. Eluants were prepared from a 50% (w/w) NaOH solution (7067, J.T. Baker, Deventer, NL) by dilution with NANOpure® water to the desired concentration and degassed by flushing with helium (degassing module, Dionex Corp.) before using. The chromatograms were integrated with the AI-450 software, version 3.31 (Dionex Corp.). The analyses were performed under the following conditions:

Pre-column:	CarboPac PA1 guard (50 × 4 mm, Dionex Corp.)
Analytical column:	CarboPac PA1 (250 × 4 mm, Dionex Corp.)
Injection volume:	10 µL
Elution:	0.0 - 4.1 min: NaOH 15.4 mM
	4.2 - 35.0 min: water
	35.1 - 45.0 min: NaOH 460 mM (regeneration step)
	45.1 - 60.0 min: NaOH 15.4 mM (equilibration step)
Flow rate:	1 mL/min
Temperature:	20°C
Post-column:	480 mM NaOH, 0.5 mL/min (reagent delivery module, Dionex Corp.)
Detector:	PAD (PED-2 module, Dionex Corp.)
	Applied potentials: E1 = + 0.05V (0.00 to 0.40 s)
	E2 = + 0.75 V (0.41 to 0.60 s)
	E3 = -0.15 V (0.61 to 1.00 s)
	Integration: 0.20 to 0.40 s
Pre-column AG5:	IonPac AG5 (50 × 4 mm, Dionex Corp.)
	0.0 - 0.7 min: on-line, removal of sulphate ions
	0.8 - 45.0 min: off-line, regeneration with 0.6 mL/min of 460 mM NaOH

Enzymatic determination of galactose

On some samples of enzymatically degraded galactomannan solutions, the liberated galactose was determined by using an enzymatic test kit (Boehringer Mannheim GmbH, Mannheim, D) following the instructions of the manufacturer [18]. The method is based on the oxidation of D-galactose to D-galactonic acid by nicotinamide-adenine-dinucleotide (NAD^+) in the presence of the enzyme β-galactose-dehydrogenase at pH 8.6. The amount of NADH formed is stoichiometric to the amount of D-galactose, and it can be measured by means of its UV-absorbance at 340 nm. Absorbances were measured with a Uvikon 940 spectrophotometer (Kontron Instruments AG, Zurich, CH).

3.5.3. Methylation analysis

Samples of isolated *Prosopis* galactomannan (I1, I2) and insoluble residue (R4w) were subjected to methylation analysis. This technique allows the determination of the position of glycosidic linkages present in polysaccharides, after permethylation, hydrolysis and derivatisation to partially methylated alditol acetates (PMAA), which are identified by GC-MS. The analytical procedure developed by Wechsler [145] was used.

Permethylation: Samples of about 2 mg galactomannan were dissolved in 0.4 mL DMSO in closed hydrolysis tubes under argon, with continuous stirring at 50°C for 2 h. After cooling to RT, 0.2 mL of butyllithium (1.6M in hexan) was added and the mixture stirred at 40°C for 1 h. After cooling in a freezer at -20°C, 0.4 mL ice-cold methyl iodide solution was added. The samples were allowed to thaw and then stirred at RT for 1 h. Excess of methyl iodide was evaporated by flushing with nitrogen in a Reacti-Vap® unit (Pierce Chemical Co., Rockfort IL, USA). The methylation step was carried out twice.

Hydrolysis: Permethylated samples were extracted with 3 mL of 2:1 (v/v) chloroform-methanol and then washed three times with 5 mL of water. The tubes were mixed on the Vortex after each addition, then centrifuged (1600×g, 5 min), and the upper phase removed by aspiration. After the final aspiration, the samples were hydrolysed first with 0.4 mL of 90% (v/v) formic acid at 100°C for 1 h and then with

0.4 mL of 2M trifluoroacetic acid containing *myo*-inositol (internal standard, 0.2 mg/mL) at 120°C for 1 h. The acids were removed after hydrolysis by evaporation in a stream of nitrogen at RT.

Reduction and acetylation: 1 mL of freshly prepared solution of sodium borodeuteride (0.1 g in 5 mL of 2M ammonia) was added to the dry hydrolysates and the mixture heated at 60°C for 1 h. 0.5 mL of acetone was added to stop the reaction and the tubes allowed to cool. Samples were then carefully evaporated to dryness in a Rotavapor®. The residue was dissolved in 0.2 mL of 18M acetic acid and in 0.5 mL of ethyl acetate, and then acetylated by adding 2 mL of acetic anhydride and 70 µL of 70% (w/v) perchloric acid. The tubes were cooled on ice and stirred for 10 min and then 5 mL of water and 0.2 mL of 1-methylimidazol were added to decompose the excess of acetic anhydride, mixed and allowed to stand at RT for 5 min. Finally, the PMAA were extracted with dichloromethane, as described for alditol acetates in 3.5.2.

GC-MS: The separation of the PMAA was performed at the following conditions:

Gas chromatograph:	GC-8065 (Fisons-Carlo Erba, Milano, I) equipped with an A200S autosampler (CTC Analytics, Zwingen, CH)
Column:	DB 225 capillary column 30 m × 0.25 mm i.d., film thickness 0.25 µm (J&W Scientific)
Carrier gas:	Helium 5.6 (PanGas), 1.0 mL/min
Column head pressure:	100 kPa
Inlet purge flow:	35 mL/min, initially off; on after 15 s
Column temperature:	160°C for 1 min, then raised at 2°C/min to 220°C, kept for 19 min
Injector temperature:	170°C
Injection volume:	1µL
Interface temperature:	220°C
Mass spectrometer:	SSQ 710 (Finnigan MAT, San Jose CA, USA)
Mode:	EI
Mass range:	40 to 400 Dalton
Ionisation potential:	70 eV

Peaks on the chromatogram were indentified by comparison of the mass spectra of each peak with the spectrum library compiled by Wechsler [145] and by verification of retention times. For quantitative measurements PMAA samples were separated on a HP5890 gas chromatograph (Hewlett Packard) equipped with an FID detector, using the same chromatographic conditions. Peak areas were integrated with the ChemStation-software (Hewlett Packard) and transferred to the Excel macro "Quick

Methylation Analysis" [145] for final calculations. Molar percentages of each substance were calculated from area percentages after correction with the ECR-factors [145].

3.6. Rheological measurements

3.6.1. Preparation of gum solutions

The viscosity studies were performed on solutions of *Prosopis* gum samples obtained by technological processes (PA, PR and PRx, finely ground as described in 3.3.3). For comparison, a commercial guar gum (G-4129, Sigma Chemicals, St. Louis, USA) was used (marked as **GG**). To prepare solutions of a given concentration, the gum sample was precisely weighed in a beaker and deionised water was slowly added under continuous stirring (to avoid the formation of lumps). In some cases, pre-moisturising of the powder with a few millilitres of ethanol was needed. After complete solubilisation of the gum, water was completed to the defined total weight. Solutions in the concentration range from 0.25 to 2% (w/w) were prepared, normally at RT.

Two gum solutions derived from non-treated splits (endosperm + seed coat) were prepared as well. (a) Non-treated splits were finely ground with a Cyclotec mill (Tecator AB). 10 g of the powder were dissolved in 500 mL of water at RT, stirred during 1 h, and centrifuged at 5000×g for 20 min (Sorvall RC 5B, Du Pont Instruments). The supernatant was carefully decanted and marked as **PSp**. (b) 10 g of non-treated entire splits were extracted with 500 mL of water at 50°C for 5 h under stirring, centrifuged and decanted as above, and the pellet re-extracted with 300 mL of water at 50°C for 2 h. Both extracts were combined and marked as **PN**. The actual concentrations of PSp and PN were determined by freeze-drying of an aliquot, and accordingly adjusted to 1% (w/w) by dilution or vacuum evaporation of the solutions.

All solutions were stored at 4°C (except for stability evaluation at RT) in beakers covered with an aluminium foil. Prior to viscosity measurements, evaporation losses were corrected by the addition of deionised water.

3.6.2. Dynamic viscosity of gum solutions

Dynamic viscosity measurements were performed with a shear rate controlled, concentric cylinder rotation rheometer Rheomat 30 (Contraves AG, Zurich, CH) at constant temperature (mostly 25°C). Depending on the viscosity range of the solutions, the sensor cylinder system A, B or C was selected. Each sensor system covers a different range of shear rates (A from 0.09 to 662, B from 0.02 to 158 and C from 0.1 to 100 s^{-1}). Torque readings (%-scale) and speed settings were converted to shear stress (τ) and shear rate (D) respectively, with proper factors specified for each sensor system [30], and recorded (flow curve). Apparent dynamic viscosity ($\eta=\tau/D$) was calculated and recorded as well (viscosity curve).

Supplementary measurements were carried out with a Brookfield viscometer model RVT (Brookfield Engineering Labs, Stoughton MA, USA), with spindle 3, at 20 rpm and 25°C.

Viscosity as a function of concentration and temperature

The dynamic viscosities of *Prosopis* gum solutions (samples PA and PR) of different concentrations (0.25, 0.5, 0.6, 0.8, 1.0, 1.5 and 2.0%, w/w) were measured at 25°C. The viscosities of a 1% solution of PA were measured at 25, 40, 60 and 80°C.

Development and stability of viscosity

Two 1% solutions of PA gum were prepared at RT under stirring for 2 min; after that, their dynamic viscosities were measured. One of them was allowed to complete the solubilisation at RT ("cold-soluble"), while the other was heated at 80°C for 5 min ("hot-soluble"), subsequently cooled to RT and its concentration was adjusted by addition of water. The development of viscosity was evaluated in both solutions at different time intervals (2, 4 and 8 h). To check the stability of gum solutions, these were stored for 23 days at RT, during which the dynamic viscosity was periodically measured.

The pH stability of gum solutions was evaluated with a 1% PA solution in the pH range from 2.3 to 12.5. Alkaline conditions were achieved by adding 2M NaOH

under continuous stirring, acid solutions by adding 1M HCl. Dynamic viscosity of the solution was measured at different pH values after 10 min.

1% and 0.5% solutions of PA gum were used to assess freeze-thaw stability of the gums. The solutions were kept frozen at -20°C for at least 36 h and then thawed (6 h at RT). The freeze-thaw cycle was repeated once, and dynamic viscosity was measured after each cycle at 25°C.

Viscosity of differently processed gum samples

Flow and viscosity curves were recorded for 1% solutions of *Prosopis* gum samples obtained by acid decoating (PA), roasting (PR and PRx), from non-treated ground splits (PSp) and from non-treated entire splits (PN).

3.6.3. Interaction of *Prosopis* gum with xanthan gum

Prosopis seed gum (samples PA and PRx) were dry-mixed with xanthan gum (Fluka AG) at the following ratios: 0, 30, 70 and 100% (% w/w of PSG in the blend). From each blend, 0.5% solutions (w/w) were prepared and heated at 80°C for 15 min. After cooling, the dynamic viscosity of the solutions was measured at 25°C with the Rheomat 30 (Contraves AG). For comparison, mixtures of guar gum (GG, Sigma Chemicals) and xanthan gum were prepared and evaluated in the same way.

3.6.4. Intrinsic viscosity

The average molecular weight of differently processed *Prosopis* gum samples (PA, PRx, PSp, PN) was estimated by measurement of the intrinsic viscosity [η] as a result of the relationship given by the Mark-Houwink equation:

$$[\eta] = K \cdot (\bar{M}_w)^{\alpha} \qquad (1)$$

where $\bar{M}_w$ is the average molecular weight, and K and α are parameters that have to be calibrated for particular polymer-solvent systems [110].

The *Prosopis* gum solutions were diluted to approximately 0.2% (w/w), centrifuged at 5000×g for 10 min, and then filtered through a 0.45 μm membrane filter

(Millipore Corp., Bedford MA, USA). For comparison, a solution of GG was prepared in the same way. The actual concentration of each solution was determined by freeze-drying of an aliquot. From these samples, 0.1% solutions (w/w) were prepared for the viscosity measurements by dilution with NANOpure® water.

The viscosity of each solutions was measured at 25.0 ± 0.1°C with an Ubbelohde viscometer type 50111 (capillary tube No. I, Schott Glas, Mainz, D), which has a flow time for water of 91 s. The solution contained in the viscometer was successively diluted (by addition of exact amounts of water), in order to have viscosity measurements in a range of lower concentrations (0.01 to 0.1%). The intrinsic viscosities of the different samples were determined by using the conventional Huggins' and Kraemer's plots. Average molecular weights were calculated with the Mark-Houwink parameters proposed for guar gum by Robinson *et al.* [124].

3.7. Enzymatic modification of *Prosopis* galactomannan

A solution of *Prosopis* gum obtained by the acid process (PA) was treated with a commercially available purified α-galactosidase from guar seeds (Megazyme Ltd.) at two enzyme concentrations. A solution of guar gum (Sigma Chemicals) was treated in the same way for comparison.

Solutions were prepared by dissolving a powdered gum sample in 0.1M sodium acetate buffer (pH 4.8), to obtain a gum concentration of 1% (w/w). After addition of 5U or 40 U of enzyme to 100 mL of gum solution respectively, the mixtures were incubated at 40°C for 24 h under continuous stirring. Samples of 10 mL were taken at different time intervals, immediately heated to 95°C for enzyme inactivation and finally dialysed in Servapor® tubes (29 mm ∅, Serva Electrophoresis GmbH, Heidelberg, D) against deionised water. From the retentate, a sample of 2 mL was subjected to Saeman hydrolysis and the neutral sugars determined by HPAEC-PAD, as described in 3.5.2, and the mannose/galactose ratio was calculated.

In a parallel assay the α-galactosidase treatment was carried out in the measuring cylinder -system B- of the Rheomat 30 viscometer (Contraves AG) to check the gum stability. The viscometer device was kept under continuous rotation (at a shear rate of 13.55 s^{-1}) at 40°C for 24 h and the viscosity of the incubation mixture was measured at different time intervals at this temperature. Gum concentration was held constant by adding water at regular intervals to compensate the evaporation losses.

Finally, an experiment was performed under continuous elimination of the liberated galactose. For this purpose, 40 U of α-galactosidase were added to 100 mL of *Prosopis* gum solution (PA) and the reaction allowed to proceed inside a dialysis tube placed in a vessel containing 2 L of deionised water, which was replaced every 2 h. After 6 h, the retentate was heated for enzyme inactivation and its M/G ratio determined as indicated above.

3.8. *In vitro* digestion and colonic fermentation of *Prosopis* gum

The procedures used to evaluate the digestibility and fermentability of gum samples will be described below. They are an adaptation of the methods standardised by Lebet [94].

Substrates: Differently processed *Prosopis* gums (PA and PR) were used as substrates. In addition, ground whole *Prosopis* seeds were included in this study. The seeds were ground in a Cyclotec mill (as described for gum samples) and marked as **PW**. For comparative purposes, two samples of commercial galactomannans having different mannose to galactose ratio, namely carob gum (**LB**) and tara gum (**TR**), were studied as well (Vidogum L175 and Vidogum SP175 respectively, Unipektin AG).

Digestion: Two digestion procedures (methods A and B) were used, as shown in Table 8. In method B mucin and bile were added as digestive aids, and the amounts of pancreatin used was changed as compared to method A. 5g of gum samples were dissolved in 500 mL of 20 mM phosphate buffer (pH 6.9). Incubation was carried out stepwise at 37°C under continuous stirring.

Table 8: Parameters for the in vitro digestion procedures

Step	Duration	Method A	Method B
Human salivary α-amylase (Sigma A-1031)	15 min	5 mg	5 mg
pH adjustment to 2.0 with 6M HCl			
Porcine pepsin (Sigma P-7012)	30 min	1.25 mg	1.25 mg
Porcine mucin (Sigma M-2378)		-	1.5 g
pH adjustment to 6.9 with 5M NaOH			
Porcine pancreatine (Sigma P-7545)	A: 90 min	2.5 mg	500 mg
Bovine bile (Sigma B-8381)	B: 3 h	-	12 g

After digestion, suspensions were quantitatively transferred into Servapor® dialysis tubes (29 mm ∅, M_W cut-off: 14000, Serva Electrophoresis GmbH) and dialysed for 24 h against deionised water. During dialysis the tubes were continuously shaken under constant replacement of water. The retentates were concentrated in a Rotavapor®, freeze-dried and weighed. Neutral sugars (by HPAEC-PAD) and protein content (as sum of anhydro amino acids) were determined in the dry material. This material was used as substrate for the *in vitro* fermentation experiments.

Table 9: In vitro fermentation experiments

Fermentation 1	Fermentation 2	Fermentation 3
PA^{da}	PW	LB
PA	PR	TR
PA^{nd}	PR^{nd}	PR

da: digested with method A
nd: non digested
Not specified with a superscript: samples digested with method B

Substrate preparation for fermentation: 100 mg of dry substrates were placed in 50 mL- serum flasks and tightly closed with septum caps. To allow sampling at various incubation times, a series of 12 flasks for each substrate was prepared. Two disposable needles provided with two-way valves (Discofix-2, B. Braun Melsungen AG, Melsungen, D) were installed through the septum of each flask. For hydration and swelling, 8 mL of 0.1M carbonate-phosphate buffer pH 6.9 (prepared as described by Lebet *et al.* [97]) were injected into each flask. Hydration and swelling took place overnight in a CO_2 atmosphere at 4°C. In each fermentation experiment three different substrates and two references (lactulose, an easily

fermentable substrate, and a blank, containing only buffer and faeces) were incubated at the same time. Table 9 shows the set-up of the three *in vitro* fermentation experiments carried out.

In vitro fermentation: The inoculum was obtained from fresh human faeces of two non-methanogenic subjects. Faecal material was collected in an insulated container and immediately flushed with CO_2. One part of faeces was diluted with three parts of carbonate-phosphate buffer solution (pre-warmed to 37°C) and homogenised in a Stomacher® 400 (Seward Ltd., London, UK). After filtration through a screen (1 mm mesh), 2 mL of inoculum were injected into each flask and the headspace flushed with CO_2. After equilibration of the internal pressure, the two-way valves were closed and the flasks were placed in a shaking water bath at 37°C. Duplicates of each substrate were taken at different incubation times (0, 2, 4, 6, 8 and 24 h). Single samples of lactulose and blank were investigated at 2, 4, 6 and 24 h. For each sample taken, the following steps were performed: The overpressure in the fermentation flask was measured at 37°C with a manometer connected to one of the valves to calculate total gas production. A headspace sample was collected in a 60 mL syringe for determination of the hydrogen concentration. A few drops of a saturated $HgCl_2$ solution were injected into the flask to stop the microbial activity. The slurry of each flask was quantitatively transferred to a 30 mL centrifuge tube and its pH measured. Insoluble material was separated by centrifugation at 5000×g for 10 min at 4°C (Sorvall RC 5B, Du Pont). 2.5 mL of the supernatant were acidified with 0.25 mL of 5% (w/v) H_3PO_4 containing 1% (w/v) $HgCl_2$, and stored frozen until the short chain fatty acids (SCFA) were analysed. The sediment of centrifugation was re-suspended in the remaining supernatant and freeze-dried for neutral sugar analyses.

For the determination of the hydrogen concentration in the headspace, 1 mL sample of fermentation gases was injected with a gas tight syringe (1005 TTL with N 722 needle, Hamilton AG, Bonaduz, CH) into a gas chromatograph under following conditions:

Instrument:	HP 5890 gas chromatograph (Hewlett-Packard)
Column:	two glass columns in series, 240 cm × 2 mm i.d., filled with 80/100 HayeSep Q and HayeSep R respectively (Supelco Inc., Bellefonte, USA)
Carrier gas:	Argon (PanGas), 20 mL/min
Column temperature:	30°C

Injector temperature:	105°C
Detector:	thermal conductivity, 115°C
Injection volume:	1 mL
Integrator:	HP 3396 (Hewlett Packard)

H_2 concentrations were quantified using external standards. Mixtures of different hydrogen concentrations (0.1 to 4% H_2 in CO_2) were prepared by volumetric dilution, and a calibration curve was established for each fermentation experiment.

SCFA were analysed by gas chromatography. Previously, frozen samples were thawed and filtered through a 0.45 µm PVDF membrane filter (Scientific Resource Inc.). 0.1 mL of 4-methyl-n-valeric acid was added to 1mL of filtrate as internal standard. The analyses were performed under the following conditions:

Instrument:	HP 5890 gas chromatograph (Hewlett-Packard)
Liner:	4 mm i.d., wool packed (Supelco Inc.)
Pre-column:	50 cm × 0.53 mm i.d. deactivated fused silica
Column:	Nukol® (30 m × 0.25 mm i.d. fused silica, 0.25 µm film, Supelco Inc.)
Carrier gas:	Nitrogen (PanGas), 20 mL/min
Column temperature:	110°C for 2.5 min, then raised at 8°C/min to 150°C, kept for 5.5 min
Injector temperature:	250°C
Detector:	FID, 255°C
Injection volume:	1 µL
Split ratio:	1:30
Integration:	ChemStation software (Hewlett Packard)

A reference mixture of acetic acid, propionic acid, butyric acid, valeric acid, isobutyric acid, isovaleric acid and internal standard was used for calibration [97].

Neutral sugar composition in fermentation residues was determined by HPAEC-PAD after Saeman hydrolysis of the samples, according to the technique described in 3.5.2. Freeze-dried residues had to be ground into fine particles with a ball mill MM 2000 (Retsch GmbH & Co.) before hydrolysis.

4. RESULTS AND DISCUSSION

4.1. Fractionation of *Prosopis* pods

In contrast to other *Prosopis* species, the seeds of *P. pallida* and *P. juliflora* are not easy to be released from their endocarp hulls (see 2.1.1 and Figure 2). The operation principle of the mill protoype used in this work has been proved to be adequate to achieve the separation of the entire seeds. Drying of the pods was indispensable for a successful milling. Because of its high sugar content the pulp powder is very hygroscopic, and this causes the mill to clog up easily. The pods must have a moisture below 6% to be fed to the mill. Drying of *Prosopis* fruits has recently been reported to be effective for the control of insect infestation [100].

The milling output actually consisted of a mixture of seeds, open endocarp hulls, pulp flour and coarse fragments of exo- and endocarp. By sieving, this material was separated into four fractions (F1 to F4), as described in 3.2. The relative amounts of these fractions are given in Figure 5 and correspond to average values at the beginning of this work. The yields obtained with different milling conditions are presented in Tables 10 to 12 and will be discussed in 4.2.

The seeds released from their endocarp hulls were retrieved in fraction F2, which also contained 80% of exo- and endocarp fragments. In the original process a household ventilator was used for a preliminary air-separation of the seeds, however 10% of impurities remained in the separated seeds (SS). With the improved air-separator (see 3.2.1) the amount of exo- and endocarp fragments in SS was reduced to 2%.

4.2. Improvement of the seed separation

At the beginning of the present study, the amount of separated seeds obtained with the prototype mill was very low, meaning that the seeds remained enclosed in the endocarp or were broken. In order to optimise the yield of seed separation, several milling tests were performed. The screen of the mill was successively re-designed.

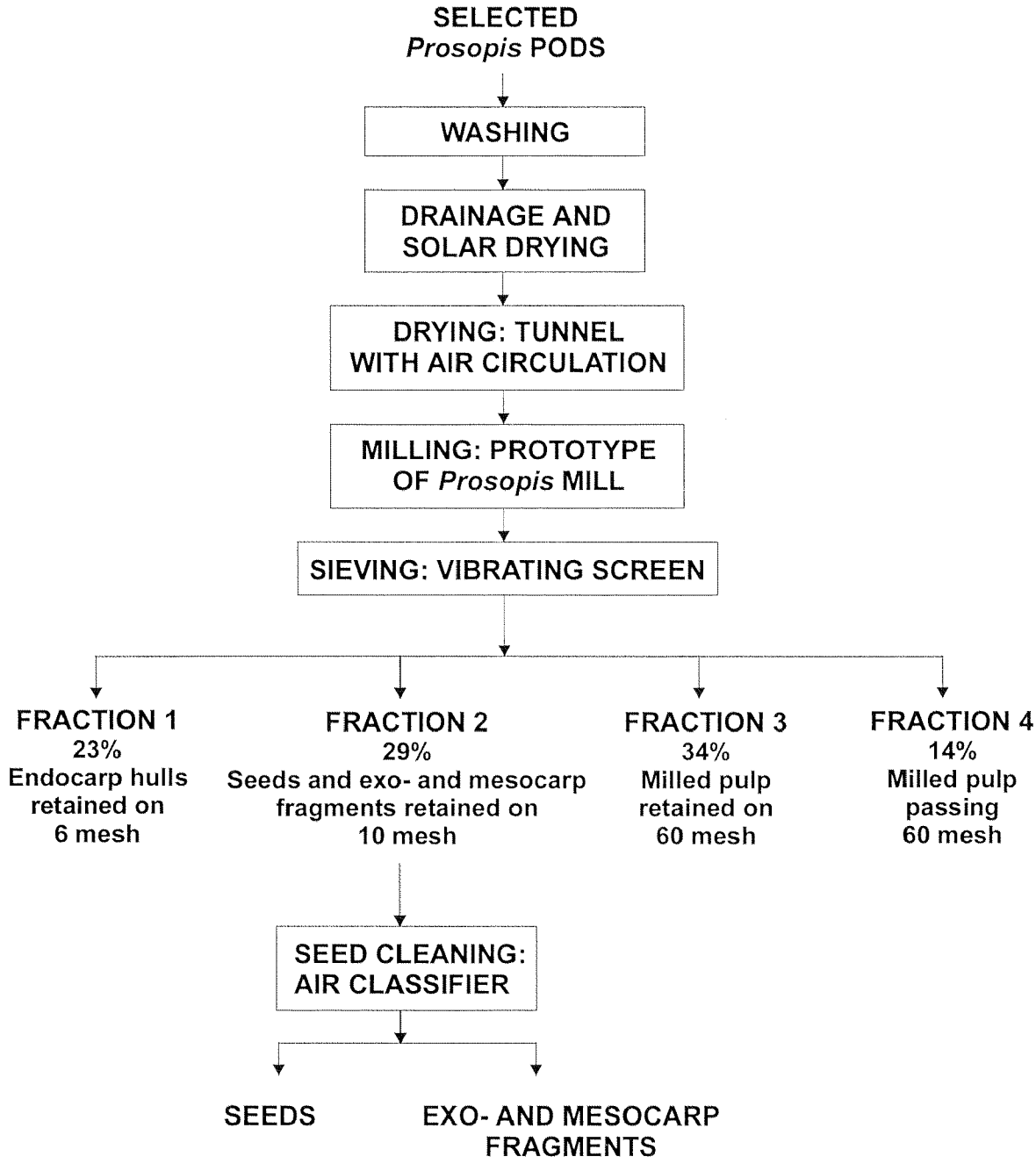

Figure 5. Diagram of the fractionation of Prosopis pods

The results of the experiments with 5 different screens (A-E, see 3.2.1) are presented in Table 10. Using the original screen A (test 1), the milling process yielded 2.5 kg separated seeds/100 kg pods. Considering the fact that the seeds correspond to approximately 9% of the pod weight, the efficiency of extraction was less than

30%. An examination of endocarp hulls in F1 revealed that more than 50% of them were closed and the seeds therefore not released.

Using screen B (reduced slots, test 2) the releasing of the seeds was considerably improved, however the percentage of damaged seeds was higher. This suggested the construction of screen C (slots instead of round holes), which allowed to obtain more separated seeds with lower amounts of broken seeds (test 3). The effect of using a cyclone dust collector, which had been recommended for a convenient handling of the milled product, was evaluated (test 4 vs. test 3). Using the cyclone, the liberated seeds were impacted when passing through a ventilator, and most of them were broken into small fragments and incorporated into the coarse pulp flour (F3). Thus, the cyclone was proven to be strongly negative for the seeds and therefore it was not installed. Results of test 5 revealed that when the screen openings were made uniform and slightly wider, the percentage of open endocarp hulls decreased. This fact was confirmed in the tests 6 and 7, with an even larger open area of the screen. Tests 6 and 7 were done under the same conditions to verify the repeatability.

Table 10: ***Milling tests with whole pods and different screens, at 800 rpm (without other specification, values given as g/100 g of pods)***

	Test 1 screen A	*Test 2 screen B*	*Test 3 screen C*	*Test 4 (*) screen C*	*Test 5 screen D*	*Test 6 screen E*	*Test 7 screen E*
Fraction F1	32.2	17.5	20.3	16.8	18.5	24.2	27.7
Fraction F2	27.6	27.7	33.0	24.5	28.5	28.2	32.4
Fraction F3	31.6	36.1	36.0	44.5	22.5	29.5	26.5
Fraction F4	8.6	18.7	10.7	14.2	30.5	18.1	13.4
Separated seeds (SS)	2.5	6.4	6.8	3.4	6.5	5.5	5.5
Whole seeds (g/100 g SS)	90.0	81.0	86.7	46.6	82.8	85.3	84.2
Broken seeds (as g/100 g SS)	10.0	19.0	13.3	53.4	17.2	14.7	15.8
Broken seeds (as g/100 g F2)	2.0	5.8	1.4	7.6	4.1	0.7	1.2
Open endocarp hulls (g/100 g F1)	42.7	81.4	92.3	90.0	86.4	72.9	75.5
Closed endocarp hulls (g/100 g F1)	57.3	18.6	7.7	10.0	13.6	27.1	24.5

(*) Test 4 carried out with cyclone

The performance of milling whole pods with different rotation speeds is shown in Table 11. The experiments were carried out only with screen C, because it gave the best results in previous tests. Compared with the yield obtained in test 3 (carried out

at 800 rpm), some improvements could be achieved by reducing the rotation speed. The highest amounts of released intact seeds were obtained at 560 rpm (7.7% of separated seeds, corresponding to an efficiency of extraction of 85%).

It can also been noticed that at lower speeds less seeds were broken. The highest yield of whole seeds (93.5%) was obtained at 400 rpm. As a consequence of speed reduction, the milling rate was considerably reduced, e.g. from 30 kg/h at 800 rpm to 20 kg/h at 560 rpm. From an economic point of view, this could be a disadvantage for the production capacity.

Finally, the results of milling bagasse (pod pieces after sugar extraction) are summarised in Table 12. In general, milling of bagasse results in lower releasing rates of seeds than milling of whole pods. It can be suggested that bagasse has a shorter transit time through the mill because it contains less pulp and therefore it is lighter than whole pods. A large quantity of endocarp hulls left the mill before the hammers reached them. By comparing milling tests carried out at the same conditions (tests 12 vs. 13 and tests 14 vs. 15 respectively), significant yield differences were observed. The reason could be a different pulp content in the bagasses used.

Table 11: ***Milling tests with whole pods and different mill rotor speeds, using screen C (without other specification, values given as g/100 g of pods)***

	Test 3 800 rpm	*Test 8 640 rpm*	*Test 9 560 rpm*	*Test 10 480 rpm*	*Test 11 400 rpm*
Fraction F1	20.3	16.9	19.2	18.4	17.8
Fraction F2	33.0	29.3	31.6	30.9	31.8
Fraction F3	36.0	35.1	33.3	34.6	35.5
Fraction F4	10.7	18.7	16.0	16.1	15.0
Separated seeds (SS)	6.8	7.1	7.7	6.9	6.8
Whole seeds (g/100 g SS)	86.7	88.3	89.2	91.5	93.5
Broken seeds (as g/100 g SS)	13.3	11.7	10.8	8.5	6.5
Broken seeds (as g/100 g F2)	1.4	2.2	0.7	0.7	0.7
Open endocarp hulls (g/100 g F1)	92.3	84.8	86.4	88.4	93.1
Closed endocarp hulls (g/100 g F1)	7.7	15.2	13.6	11.6	6.9

Table 12: Milling tests with bagasse and different screens at 800 rpm (without other specification, values given as g/100 g of pods)

	Test 12 screen C	Test 13 screen D	Test 14 screen D	Test 15 screen D	Test 16 screen E
Fraction F1	25.4	17.5	20.4	21.7	21.8
Fraction F2	31.9	35.2	34.3	34.3	30.5
Fraction F3	38.6	39.6	34.1	32.7	33.3
Fraction F4	4.1	7.7	11.2	11.3	14.4
Separated seeds (SS)	5.0	4.3	5.5	6.2	4.3
Whole seeds (g/100 g SS)	71.7	75.0	74.0	80.0	78.8
Broken seeds (as g/100 g SS)	28.3	25.0	26.0	20.0	21.2
Broken seeds (as g/100 g F2)	5.0	5.0	6.6	3.7	2.7
Open endocarp hulls (g/100 g F1)	76.0	76.0	83.4	92.2	90.5
Closed endocarp hulls (g/100 g F1)	24.0	24.0	16.6	7.8	9.5

4.3. Processes for endosperm separation: technological considerations

Laboratory procedures to separate the three components of the *Prosopis* seed (endosperm, cotyledon and seed coat) have been proposed in several studies (reviewed in 2.2.3). However, an efficient method to obtain the three fractions on a pilot plant or even on an industrial scale has not yet been established. Most of the reported methods are wet procedures, in which the seeds were soaked in water for swelling, then manually pressed through a screen to separate the cotyledon and the seed coat, resulting in a gum solution. Wet methods are, however, not appropriate for an industrial process, because large quantities of water must be removed by drying the solutions to obtain gum powders. In addition, the gum could be contaminated with cotyledon proteins during soaking.

The aim of this part of the present study was to develop a method to separate the endosperm from the other components of *Prosopis* seed at pilot plant scale. The goal was to assess methods that could be scaled-up in the future. Thus, the study was oriented to simulate the industrial processes currently used for guar, tara or

carob gum. Nevertheless, major difficulties arise from the small size of Peruvian *Prosopis* seeds and a strong adhesion of the endosperm to the seed coat. Compared to guar seeds they have almost the same weight and size, but they are ovoidal and slightly flat, whereas guar seeds are spheroidal. In addition, *Prosopis* seeds have a considerable lower content of endosperm (28%) than other legume seeds (e.g. carob 42%; guar 38%).

4.3.1. Preliminary experiments

First attempts to remove the seed coat by mechanical abrasion on sandpaper failed. Because of the shape of the seeds, the abrasion took place only on certain sites. Thus, not only the coat, but a considerable amount of endosperm was peeled off as well. Alternatively the seeds were treated in a roller mill at different speeds and gaps. This treatment, however, caused breaking of the seeds without detachment of the coat. Since no positive results were obtained with these mechanical procedures, chemical peeling was also tried. Soaking in sodium hydroxide solutions and rubbing resulted in discoloration of the seeds, but the seed coat could not be removed by this treatment.

4.3.2. Acid decoating process

This process is based on the destruction of the seed coat by sulphuric acid. From a first set of experiments with small amounts of seeds, the following conclusions could be drawn:

1. Acid concentration of 25% (w/w) caused the endosperm to swell and some seeds split into two halves.
2. H_2SO_4 solutions of concentrations less than 50% (w/w) needed to be heated to achieve seed coat degradation.
3. When the temperature of acid was above 80°C, the seeds burned and the process became too fast and not controllable.
4. The seeds to acid ratio and the stirring intensity also played a very important role: friction between seeds facilitated the detaching of degraded seed coat fragments.
5. The best results for small batches were obtained by treating 30 g of seeds with 30 mL of 72% (w/w) H_2SO_4, at 35°C for 20 min, in a 250 mL beaker, under constant agitation using a magnetic stirrer with a 4 cm magnetic bar.

When the procedure was assayed for seed amounts of 250 g in 3 L beakers, the magnetic stirrer had to be replaced by a conventional stirrer with plastic blades. Consequently, the conditions were adjusted again. The volume of sulphuric acid had to be increased from 250 to 500 mL. The duration of treatment was reduced by increasing the temperature (with larger amounts, the process could be better controlled). Batches larger than 250 g were difficult to stir or yielded low quantities of peeled seeds.

On the whole, the acid decoating process has proven to be effective but strongly dependent on the stirring conditions. The best results were obtained by treating 250 g of seeds with 500 mL of 72% (w/w) H_2SO_4, at 55-56°C for 10-12 min and stirring at 80 rpm.

Seeds obtained by this procedure contained significantly less coat material, but they were not completely peeled. A loss of 24% of the seed weight (on a dry matter basis) has been measured (Figure 6). This means that not only the seed coat (20%), but also part of the endosperm was destroyed by the acid treatment.

With regard to the splitting of the peeled seeds, both a disc mill and a roller mill broke the seeds into too many small and hardly separable fragments. Better results were achieved with a hammer mill. Larger fragments obtained by sieving correspond to 43% of the seed weight. However, sieving and air streaming were not sufficient for endosperm separation. Endosperm splits completely devoid of cotyledon fragments were obtained only after manual sorting and represented 22% of the seed weight (Figure 6). When finely milled, these splits could be considered a "technical-grade" *Prosopis* gum. This slightly beige gum powder was marked as sample **PA** for the characterisation (see also 3.3.3).

4.3.3. Roasting process

Roasting is used by the gum industry especially for the treatment of tara and carob seeds. The aim of roasting is the separation of the seed coat, which pops off from the rest of the seed as a consequence of a rapid and intense heating. Time and temperature are critical variables of the process, the seeds being very sensitive to little changes. Then, the type of roasting equipment is also important to assure the process to be carried out in a correct and narrow range of time and temperature.

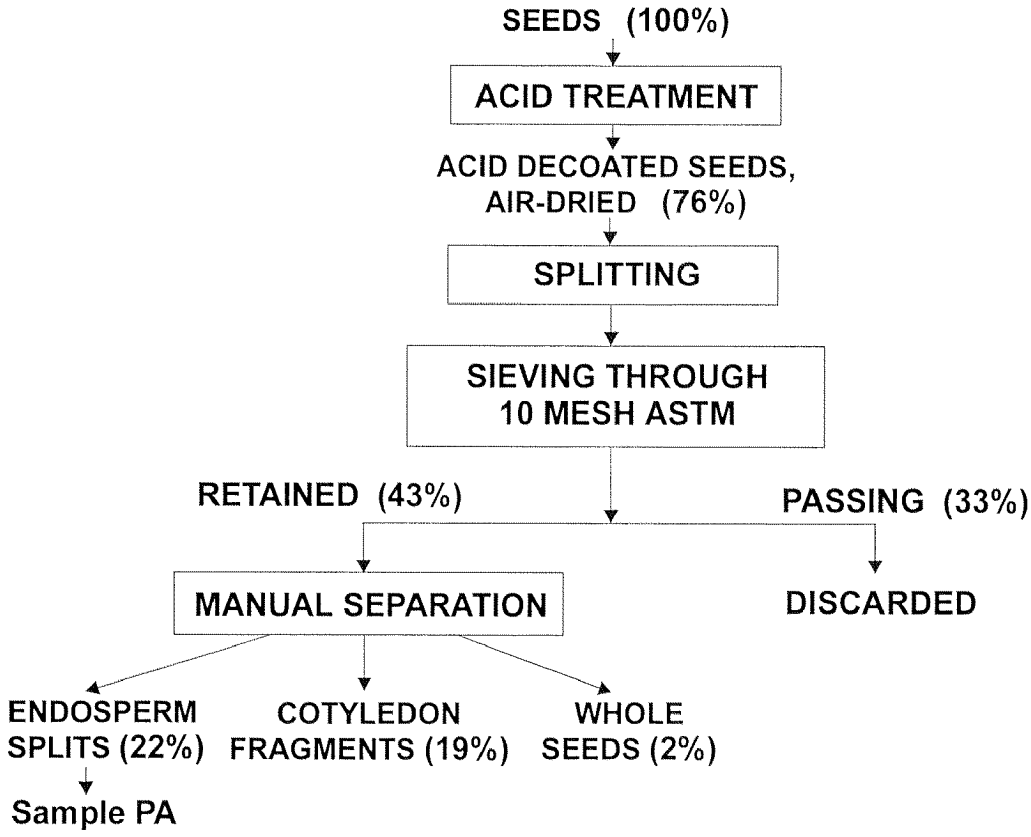

Figure 6: Splitting of acid decoated seeds and separation of endosperm

Optimal roasting conditions were established in the rotary roaster described in 3.3.2, with batches of 100 g of seeds each. To obtain an initial reference, tara seeds were roasted as well. These showed fissures on the coat after roasting at 170-180°C for 60-120 s. The only presence of fissures facilitated the detachment of most of the coat when the tara seeds were split in a laboratory hammer mill. In contrast, the seed coat of *Prosopis* was not affected at these temperatures (no changes in the strong adhesion between the coat and the endosperm occurred, even when roasting time was extended up to 8 min). The results of roasting of *Prosopis* seeds in the rotary roaster at higher temperatures are summarised in Figure 7.

When the temperature was stepwise increased from 170 to 230°C, keeping the roasting time at 40-60 s, some browning of the seed coats was observed but the behaviour of the seeds in the splitting mill was not improved. At 240°C and higher temperatures, a popping effect was observed, i.e many seeds exploded. The explosion of the seeds occurred because the cotyledon expanded, in many cases it

emerged from the seed. Burst seeds showed, in fact, that the coat had been detached, at least in one of the endosperm halves. Although the sample amounts were small and the roasting was carried out with a large flow of hot air that assures homogeneous heating, not all seeds exploded. The percentage of exploded seeds, determined by manual separation, was used as efficiency parameter for the roasting treatment (see Figure 7).

At temperatures above 260°C, roasting times longer than 50 s caused over-roasting of the seeds that were already burst open. Nevertheless, a few not-exploded seeds were still found in all roasting trials, even when the rest was over-roasted. The temperature of 260°C was considered appropriate to achieve a high explosion rate without over-roasting. Several experiments were carried out at 260°C using different roasting times, in order to optimise the duration of the process. The best results were obtained with a roasting time of 30 s for batches of 100 g of seeds, and 60 s for 200 g of seeds.

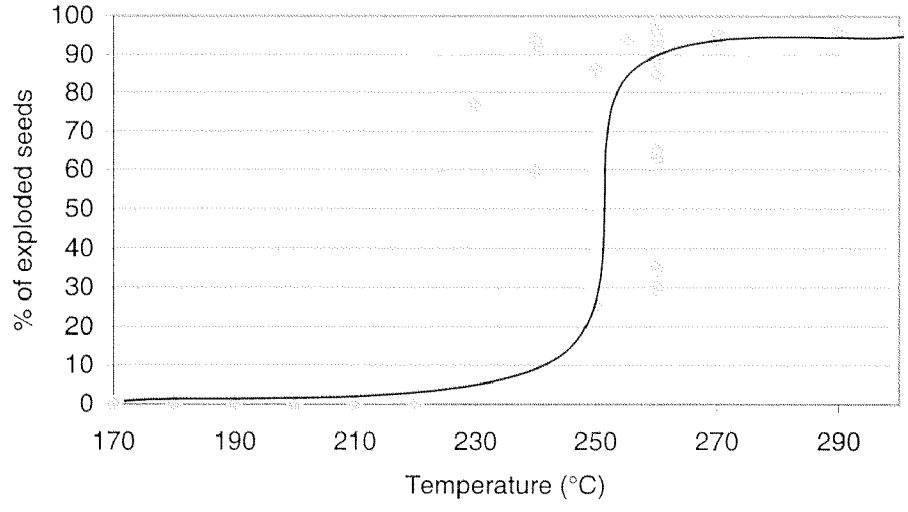

Figure 7: Effect of roasting temperature on the seed burst

At 260°C, the roasting caused a weight loss of 4% of the seeds. This value will be taken into account in the determination of the overall yield.

The other two types of roasters used in this work (fluidised bed roaster and prototype of coffee roaster) also allowed the same popping effect on the seeds, at temperatures

higher than 250°C (results not shown). However, manual feeding of the seeds into the roasting chamber and cooling them was not as easy as for the rotary roaster.

Splitting of roasted seeds

Splitting seeds in the laboratory mills initially tested (see 3.3.2) showed that the cotyledon was easily crushed, but not all seed coats were detached from the endosperm. Most cotyledon particles were separated by sieving the milled material through a 10-mesh screen (ASTM). Manual sorting of pure endosperm splits from the fraction retained on the screen yielded 9 g of pure endosperm/100 g of roasted seeds with the manual disc mill and 6 g with the hammer mill, respectively. Pure endosperm separated manually from roasted seeds was finely ground and marked as sample **PR** for further characterisation.

Endosperm separation trials at a larger scale were carried out with a cross beater mill and a soybean dehuller at the pilot plant of Bühler AG as described in 3.3.2. The milling product was sieved and separated into four fractions, S1 to S4.

Using the cross beater mill, a simple visual inspection of the obtained fractions revealed that most of the endosperm splits were present in S2 (particle size > 2.0 mm). This fraction exhibit the lowest amounts of seed coat and cotyledon fragments. Therefore, the milling trials with different screens and at various rotation speeds were oriented to optimise the yield of fraction S2. The highest yield of fraction S2 was 21.7%, obtained by milling at 2780 rpm with the 6 mm screen (see Table 13). The content of pure endosperm fragments in S2 was determined by manual sorting, revealing that it was not significantly affected by the milling conditions. The average content of pure endosperm in S2 using the cross beater mill was 38.6%. This represents the endosperm fragments free of seed coat, which are, therefore, theoretically separable from S2 as a "technical-grade" gum.

The experiments with the soybean dehuller made clear that besides fraction S2, also fractions S1 and S3 contained significant amounts of pure endosperm fragments. However, the yields of these fractions were lower, compared with those obtained from the cross beater mill (see Table 13). The sum of fractions S1+S2+S3 corresponded to less than 20%. With the optimal rotation speed (3190 rpm), the soybean dehuller yielded 11.1% of fraction S2 whose content of pure endosperm

fragments was 33.5% (determined by manual sorting). Fraction S1 amounted to 3.2% of the seed weight and contained 8% of pure endosperm. The endosperm fragments present in fraction S3 were not quantified because of the small particle size in this fraction.

With both types of mill, fraction S4 basically contained cotyledon, which, being softer, was more easily ground.

Table 13: Results of splitting of roasted seeds

	Cross beater mill	*Soybean dehuller*
Optimal rotation speed (rpm)	2780	3190
Fractions obtained by sieving of milled material (g/100 g of roasted seeds):		
S1 (> 2.56 mm)	8.8	3.2
S2 (2.0 – 2.56 mm)	21.7	11.1
S3 (1.8 – 2.0 mm)	7.9	4.9
S4 (< 1.8 mm)	61.6	80.8
Pure endosperm fragments theoretically separable from S2 (as g/100 g of S2)	38.6	33.5
Pure endosperm fragments theoretically separable from S2 (as g/100 g of roasted seeds)	8.4	3.7

Heating of the seeds immediately before the splitting has been mentioned to facilitate seed coat detachment in other leguminous seeds, e.g. for soybean dehulling [148]. However, heating had no positive effect on *Prosopis* seeds. On the contrary, seeds that had been roasted immediately before splitting showed a poor detachment of the seed coat. The results were significantly better when the roasted seeds were stored for 2 days prior to splitting. Seeds roasted some months before splitting showed a good behaviour as well. Part of this improved splitting might be attributed to the fact that considerable tensions are built up in the seed coat during cooling, which results in a better cracking and splitting only after a certain storage period.

Although fractions S2 from both types of mill contained a considerable amount of pure endosperm splits, it was not possible to separate them from the non-peeled splits neither by sifting nor by air-classification. Consequently, a sorting-by-colour

equipment was evaluated, in an attempt to complete the process to produce pure *Prosopis* endosperm with industrial technologies.

Sorting by colour is used in the food industry for products such as coffee beans, nuts, rice grains or vegetables, in which the defective pieces have the same shape or specific gravity, but differ in colour (e.g. spotted, discoloured or off-colour beans, incompletely peeled nuts, etc.) [98]. The product passes through an optical inspection system, which measures the reflectivity of each particle. Depending on the threshold set for acceptable colour, defective particles are rejected from the product stream by high speed air ejectors, each firing at up to 600 times per second [98]. All colour sorters inevitably remove some acceptable particles and fail to remove some defective particles. Sensitivity is one of the parameters that can be adjusted to optimise the performance of the sorter.

Since the contents of pure endosperm in the fractions S2 obtained with both splitting mills were not very different, the material was combined. 3050 g of S2 from the cross beater mill and 1450 g from the soybean dehuller were pooled and subjected to sorting by colour. The following yields were achieved after two successive steps through the optical sorter (material passing the first step was fed to the second step):

First step (4500 g):	Passed:	1669 g	37%
	Rejected:	2831 g	63%
Second step (1669 g):	Passed:	828 g	49.6%
	Rejected:	841 g	50.4%

Material passing the second sorting step was considered to be acceptable for a "technical-grade" gum. It consisted of endosperm splits with only few remaining fragments of seed coat and cotyledon. These impurities have been quantified by chemical analysis, as will be discussed in the next sections. Fine grinding of these splits yielded a slightly grey coloured powder, which was marked as **PRx** (see also 3.3.3). The yield of the sorting process was 18.4%, on the basis of fraction S2.

4.3.4. Overall yield of the endosperm separation process

The overall yield of the roasting-splitting-sorting process can be summarised as follows:

Using the cross beater mill 21.7 g of fraction S2/100 g of seeds were obtained, from which only 3.4 g of "pure" endosperm was isolated by optical sorting. This means that 420 kg of seeds (equivalent to 6000 kg of pods) are required to produce 1 kg of gum.

It should be emphasised that the optical sorting conditions were not optimised in this study, because the equipment was available only for a short period of time. On the assumption that the sorting parameters can be adjusted and/or the system can be operated with successive feedback, the recovery of pure endosperm is thought to be improved, but a reduction of the sorter throughput is to be expected.

The poor detachment of seed coats during the roasting and splitting operations is yet the limiting factor of the whole process.

The acid decoating process yielded 22 g of "peeled" endosperm splits/100 g of seeds (by manual sorting of the splits). Extrapolated to the raw material, this means that 65 kg of pods are needed to produce 1 kg of gum.

However, a quantitative comparison between the yields of both technical processes presented here is not realistic, because different splitting and sorting methods were used. It may be assumed that splitting of acid decoated seeds can be improved by using a cross beater mill, so that the cotyledon can be disintegrated and separated by sieving only. In such a case, the optical sorting of the decoated splits would not be necessary.

4.4. Characterisation of *Prosopis* galactomannan

4.4.1. Isolation of galactomannan

Water extraction of non-treated splits was performed to obtain native endosperm for structural studies, as described in 3.4. A scheme of the extraction and purification procedure is shown in Figure 8.

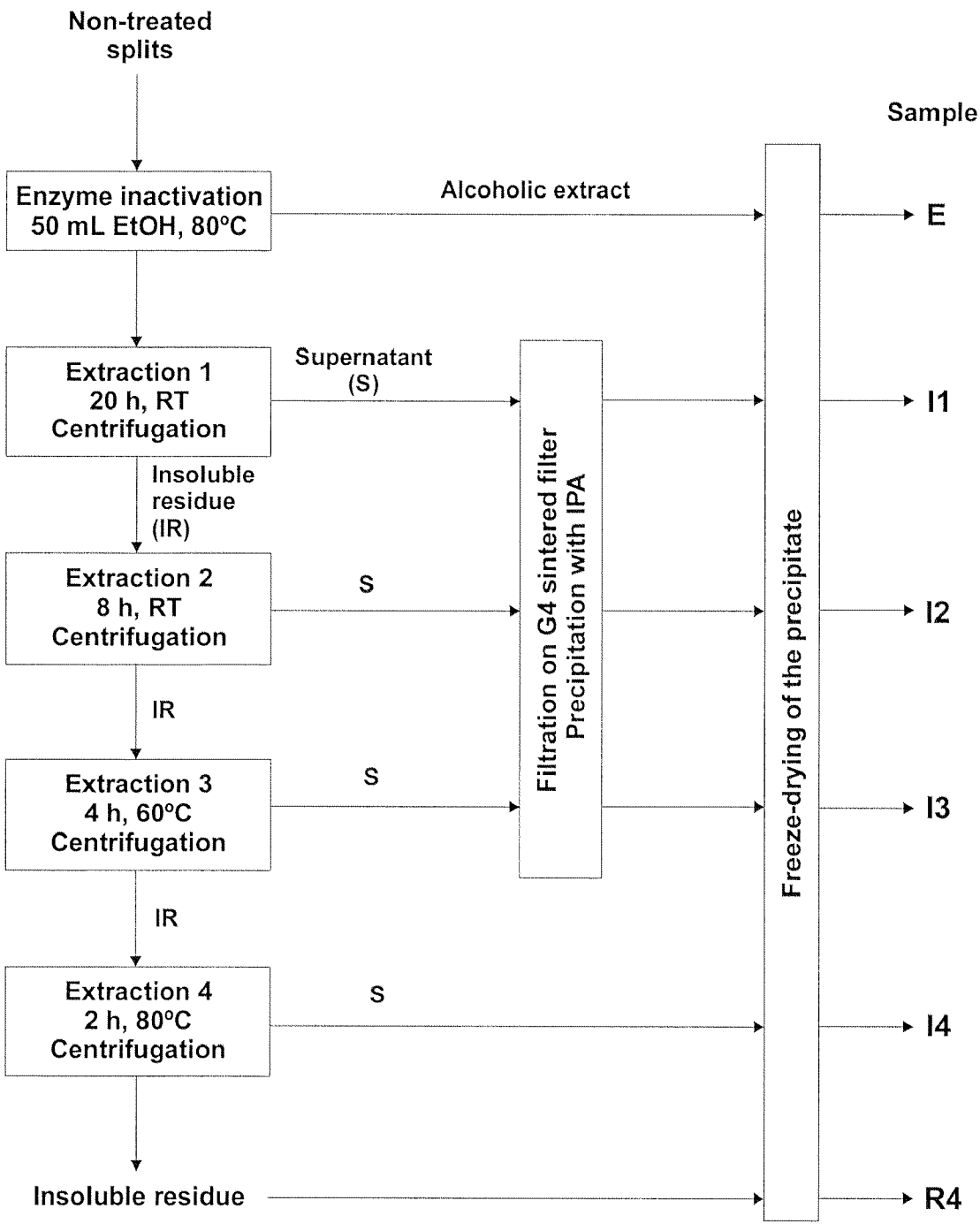

Figure 8: Extraction and isolation of Prosopis galactomannan

Boiling the splits in ethanol was performed to inactivate endogenous enzymes (e.g. β-mannanase) that could cause degradation of the polysaccharide chain during endosperm swelling. The amount of alcohol soluble matter (E) was very low, and corresponded to 0.2% of the weight of the splits. Extraction of gum with water was done under continuous stirring in 4 steps to evaluate the influence of the temperature

on gum solubilisation and to compare the composition of the gum fractions obtained in each step.

The gum solutions were filtered and the polysaccharide precipitated with isopropanol as described in 3.4. By doing so, alcohol-soluble mono- and oligosaccharides possibly present in the aqueous extract were removed from the alcohol-insoluble galactomannan. Yields determined after freeze-drying of the precipitates are shown in Table 14. No further purification steps were needed, since only traces of protein were found in the samples, as will be discussed later.

Water extraction of the splits yielded 50% endosperm gum and 40% seed coat as residue. Approximately 10% of material was lost during the extraction, probably because some part of the mucilage adhered to glassware and could not be recovered. Most of the gum (86%) was obtained at room temperature (fractions I1 and I2).

Table 14: Yield of gum extraction

Fraction	*Dry weight (g)*	*Yield (% of splits)*	*Relative yield (% of extracted gum)*
Splits	9.0	100	
Extracted endosperm (gum)	4.5	50	100
I1	3.5		76
I2	0.5		10
I3	0.2		5
I4	0.3		9
Residue (R4)	3.6	40	
Loss (by difference)	0.9	10	

The residue resulting from the last extraction at 80°C (R4) contained seed coats (**R4c**) and a white, practically insoluble material (**R4w**). Both components were analysed separately.

4.4.2. Monomeric components and impurities

Neutral sugar composition of the isolated polysaccharide fractions (I1 to I4) and of the white material in the residue (R4w) was determined by gas chromatography of alditol acetates. The samples were hydrolysed as described in 3.5.2 for soluble polysaccharides (1M H_2SO_4, 100°C, 3h). The results are shown in Table 15.

Table 15: Neutral sugar composition of isolated Prosopis galactomannan analysed by GLC of alditol acetates (g/100 g sample)

Sample	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Gal+Man	M/G ratio
I1	0.0	0.0	0.4	0.2	41.6	28.8	0.0	70.4	1.45
I2	0.3	0.1	0.5	0.3	40.3	27.2	0.3	67.5	1.48
I3	0.2	0.0	0.4	0.1	41.2	25.9	0.3	67.1	1.59
I4	0.6	0.3	15.5	3.4	23.1	14.7	0.4	37.8	1.58
R4w	0.5	0.2	8.9	4.4	14.8	9.8	0.2	24.6	1.50

The results obtained here confirmed that *Prosopis* seed polysaccharide is a galactomannan. Besides mannose and galactose only traces of other monosaccharides were present in the endosperm solubilised at low temperatures (I1 to I3), whereas the amounts of arabinose and xylose were significantly higher in the gum extracted at 80°C (I4) and in the white residue R4w. This suggests that the endosperm contained minute amounts of other polysaccharides (arabinoxylans, arabinans, arabinogalactans). Although no uronic acid determination was carried out, the rhamnose found in the fractions I2, I3, I4 and R4w suggests the presence of small amounts of pectic substances.

The M/G ratio slightly increased with the extraction temperature of the samples, from 1.45 for the gum extracted at room temperature to 1.6 for the gum extracted at 80°C. The M/G ratios of all the samples of Peruvian *Prosopis* gum analysed in this work were within this range. These results are in agreement with those reported in the literature (section 2.3) except for Brazilian *Prosopis* galactomannan [64]. For comparative purposes, samples of raw *Prosopis* endosperm from Brazil (**BR**)* and from Mexico (**MX**)* were analysed after hydrolysis by gas chromatography of alditol acetates. The M/G ratios were 1.39 for BR and 1.42 for MX. These values were very similar to those determined for the Peruvian samples.

In a later stage of this work, a different analytical technique (HPAEC-PAD) was used for the determination of the neutral sugar composition of *Prosopis* gum samples. This method had been recently introduced at the Laboratory of Food

* Samples BR and MX were kindly provided by Dr. Cristina Andrade (Instituto de Macromoléculas, Universidade Federal do Rio de Janeiro, Brazil) and Dr. Francisco Goycoolea (Centro de Investigación en Alimentación y Desarrollo, Hermosillo, Sonora, Mexico)

Chemistry of ETHZ and proven to give reliable results [96]. Gum samples from acid decoating (PA) and roasting (PR) processes, obtained as described in 3.3.3, were analysed. For comparison, commercial samples of guar, carob and tara gums (GG, LB and TR respectively, specified in sections 3.6.1 and 3.8) were characterised as well. Hydrolysis of the samples was performed with the conditions described in 3.5.2 for insoluble polysaccharides (Saeman hydrolysis), in order to allow the quantification of seed coat as impurity of the "technical-grade" gum samples. The white insoluble endosperm fraction (R4w) and a sample of washed seed coat from the extraction residue (R4c) were analysed as sources of impurities. Additionally, the fractions I1 to I3 of isolated *Prosopis* galactomannan were analysed for comparison with the results obtained by GLC of alditol acetates. The neutral sugar composition of the different samples obtained by HPAEC-PAD analysis are given in Table 16.

Table 16: ***Neutral sugar composition of different galactomannan samples analysed by HPAEC-PAD (g/100 g sample)***

Sample	Rha	Ara	Gal	Glu	Xyl	Man	Gal+Man	M/G ratio
PA	0.1	2.6	29.3	1.7	0.2	42.4	71.7	1.45
PR	0.0	1.2	35.9	0.7	0.0	49.6	85.5	1.38
I1	0.0	0.4	34.7	0.1	0.1	53.9	88.6	1.55
I2	0.0	0.2	31.9	0.2	0.0	48.4	80.3	1.52
I3	0.0	0.3	30.3	0.2	0.0	46.5	76.8	1.53
R4w	0.0	6.7	13.6	6.8	5.6	20.8	34.4	1.53
R4c	1.0	16.1	1.5	16.2	3.2	0.0	1.5	-
GG	0.1	2.5	33.4	1.8	0.5	54.9	88.3	1.64
LB	0.0	1.0	18.2	2.0	0.0	66.5	84.7	3.65
TR	0.0	1.1	21.9	0.9	0.0	64.6	86.5	2.95

The results indicate that *Prosopis* gum obtained by roasting (PR) has a galactomannan content comparable to the commercial gums from carob and tara. The lower galactomannan content of PA can be explained by the incomplete removal of the seed coat, as indicated by relatively high values for glucose and arabinose. These sugars were determined as major components of the seed coat fraction (R4c). Glucose probably derives from cellulose, which is known to be

present in seed coats. The acid treatment of the seeds proved to be less effective in removing seed coats compared to the roasting procedure.

The M/G ratios for both types of "technical-grade" *Prosopis* gums (PA and PR) are similar to those for the main fractions of isolated galactomannan (I1 and I2). In contrast to the gaschromatographic analyses (Table 15), no remarkable differences of M/G ratios were found among the different isolated galactomannan fractions. The values for guar, carob and tara gums agree with those indicated in the literature (Table 4 and [123]).

The protein contents of gum samples were determined to assess for contamination of the gums with cotyledon fragments. The results are presented in Table 17.

Table 17: Protein content as sum of anhydro amino acids

Sample	Protein (%)
PA	1.5
PR	1.5
PRx	4.5
I1	1.4
LB	3.6
TR	1.2

Although the protein content of roasted *Prosopis* gum obtained by optical sorting is high (sample PRx), this value is not very different compared to the one found for the commercial carob gum (LB) analysed in this study. The low protein content of the samples PA, PR and the isolated galactomannan (I1) can be explained by the fact that the cotyledon fragments had been removed manually after splitting.

Methodological considerations

Quantification of the neutral sugars in differently processed gum samples has been performed by two different analytical methods (GLC of the alditol acetates and HPAEC-PAD). The total amounts of neutral sugars quantified by GLC in all isolated galactomannan samples were lower than 70%. Taking into account that water contents determined in the freeze-dried samples were 5% and protein content of pure endosperm was below 1.6%, more than 20% of the material could not be

quantified by the GLC method. On the other hand, HPAEC-PAD gives a galactomannan content of around 85% in most of the samples, even in the "technical-grade" and commercial gums. Taking into account the values for protein, water content and remaining neutral sugars in the samples, an almost complete quantification was possible with this method.

In contrast to the differences in the absolute quantification of sugars, the M/G ratios of the same samples obtained by both methods can be considered similar, i.e. within the range of 1.45 to 1.55 (see values for I1, I2, I3 and R4w).

The reasons for the observed differences in quantification between GLC and HPAEC-PAD can be multiple. To check the influence of the derivatisation procedure on the yield of neutral sugars, a reference mixture of pure monosaccharides was always derivatised together with the samples at the same experimental conditions, and used as basis for the quantification. The procedure turned out to have a good repeatability, as could be demonstrated by many repetitions of the GLC analysis. Incomplete cleavage of galactomannans and decomposition of free monosaccharides during acid hydrolysis have been suggested as error sources in gas chromatographic quantification [80, 89, 114]. Therefore, different hydrolysis times were used, and Saeman hydrolysis as well as hydrolysis with trifluoroacetic acid were carried out. The results obtained (data not shown) did not differ much from the previous analyses, indicating that the hydrolysis conditions used did not account for the observed differences. While the repeatability of the derivatisation procedure has been shown to be satisfactory, the yield of alditol acetates could be the source of losses of neutral sugars, although no direct evidence has been obtained in the present work.

4.4.3. Types of glycosidic linkages

The types of glycosidic linkages in the main fractions of isolated *Prosopis* galactomannan (I1 and I2) and in the insoluble residue (R4w) were investigated by methylation analysis, as described in 3.5.3. A reaction scheme for the methylation analysis of a hypothetical galactomannan is presented in Figure 9 to illustrate the principle of this method.

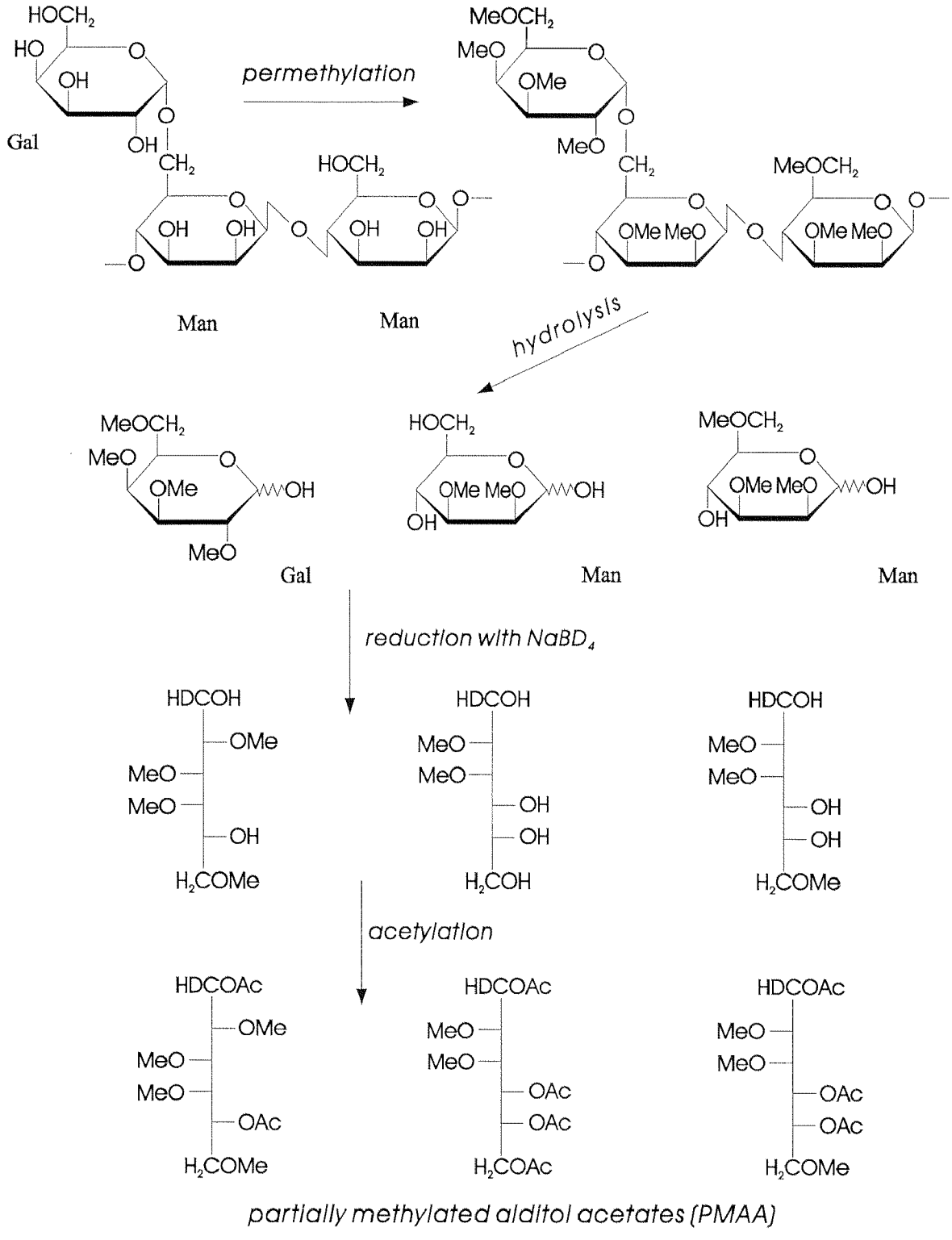

Figure 9: Principle of the methylation analysis applied to a galactomannan

The quantification of monomeric units identified in the samples is summarised in Table 18. It can be clearly seen that terminal galactose, 1,4-linked mannose and 1,4,6-linked mannose are the main units present in the isolated galactomannan. These results confirm that *Prosopis* galactomannan consists of a backbone of 1,4-linked mannose units, to which single galactose units are 1,6-linked. The lack of terminal mannose units is in agreement with the very large size of the polysaccharide. The small concentrations of 1,2,3,4-linked galactose could be due to an incomplete methylation of the hydroxyl groups and do not represent a different monomeric unit.

Table 18: ***Relative amounts (mol %) of single monomeric units of Prosopis galactomannan determined by methylation analysis***

	I1	*I2*	*R4w*
Ara (f) T	0.5	0.3	2.2
Ara (p) T	0.3	0.1	1.2
Ara (f) 1,3	0.4	0.1	1.7
Ara (p) 1,4	0.6	0.3	4.2
Gal (p) T	35.9	34.0	23.1
Gal (p) 1,6	0.7	0.8	0.4
Gal (p) 1,2,3,4	1.3	1.6	1.0
Glu (p) 1,4	0.5	0.2	12.5
Man (p) 1,4	23.8	25.1	19.7
Man (p) 1,4,6	33.9	35.3	25.3
Rha (p) 1,2	0.5	0.5	0.4
Xyl (p) 1,4	0.6	0.5	4.0
Xyl (p) 1,2,4	0.7	0.4	2.5
Ratio branching/terminal	1.0	1.1	1.1

T = terminal; (p) = pyranose form; (f) = furanose form

The residue R4w was found to contain considerable amounts of 1,4-linked glucosyl, 1,4-linked arabinosyl and 1,4-linked xylosyl residues. This could evidence the presence of cellulose as well as arabinoxylans (mentioned in 4.4.1). Cellulose had not been revealed in R4w during the GLC analyses (Table 15) because mild hydrolysis conditions were used, but it could be clearly identified when a stronger hydrolysis of R4w was applied (Tables 16 and 18). The very low amounts of other sugars (arabinose, glucose, rhamnose and xylose) in the samples I1and I2 confirm that the isolation procedure was effective.

The enzymatic hydrolysis of isolated *Prosopis* galactomannan samples (with β-mannanase and α-galactosidase, as described in 3.5.2) caused a degradation of the polysaccharide (evidenced by a viscosity drop) and released considerable amounts of galactose, which was quantified enzymatically and by GLC (data not shown). These results and those from the enzymatic removal of galactose in samples of PA (will be discussed in 4.6), combined with the information provided by the methylation analysis, indicate the presence of β-1,4-glycosidic linkages between the mannose units of the backbone and α-1,6-linked galactose residues at the sides, which are the same type of linkages present in carob, guar and tara galactomannans.

4.4.4. Molecular weight

Estimation of the molecular weight through determination of the intrinsic viscosity [η] is a method widely used for characterisation of galactomannans, especially from guar and carob. Absolute values of $\overline{M}_w$ have been established for these two polysaccharides by light scattering and therefore the Mark-Houwink parameters (see 3.6.4) have been defined [47, 124]. In a dilute polymer solution (before the onset of entanglement), the fractional increase in viscosity per unit of concentration is related to the intrinsic viscosity by the following equations [46, 110]:

Huggins $$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c \qquad (2)$$

Kraemer $$\frac{\ln(\eta_{rel})}{c} = [\eta] + k''[\eta]^2 c \qquad (3)$$

where c is the concentration in g/dL, and k' and k'' are the so-called Huggins and Kraemer constants. Specific viscosity is defined as $\eta_{sp} = (\eta_{solution}-\eta_{solvent})/\eta_{solvent}$ and relative viscosity as $\eta_{rel} = \eta_{solution}/\eta_{solvent}$. It can be seen that both equations represent nearly linear functions and have a common intercept at [η]. This means that intrinsic viscosity [η] can be experimentally obtained by extrapolation of either η_{sp}/c or $\ln(\eta_{rel})/c$ versus c, to an infinite dilution ('zero'-concentration).

The intrinsic viscosities of both "technical-grade" *Prosopis* gum samples (PA and PRx) were determined. For practical reasons, the isolated galactomannan (I1, I2) was not used to prepare various solutions, but its native properties were instead emulated by solutions obtained from non treated splits (PN and PSp, defined in 3.6.1). For compartive purposes, guar gum (GG) was included in the determinations. The intrinsic viscosities shown in Table 19 represent the averages of [η] calculated from both Kraemer's and Huggins' plots for each sample. The [η] values from both experimental plots actually exhibit only a little difference, as illustrated in Figure 10 for PA.

Table 19: Estimated average molecular weight of Prosopis gum

Gum sample	*Intrinsic viscosity (dL/g)*	$\overline{M}_w$ (*)
PN	12.6	1.79×10^6
PSp	9.5	1.21×10^6
PA	9.0	1.12×10^6
PRx	5.9	0.63×10^6
GG	11.3	1.54×10^6

(*) Using the Mark-Houwink parameters calculated for guar by Robinson *et al.* [124].

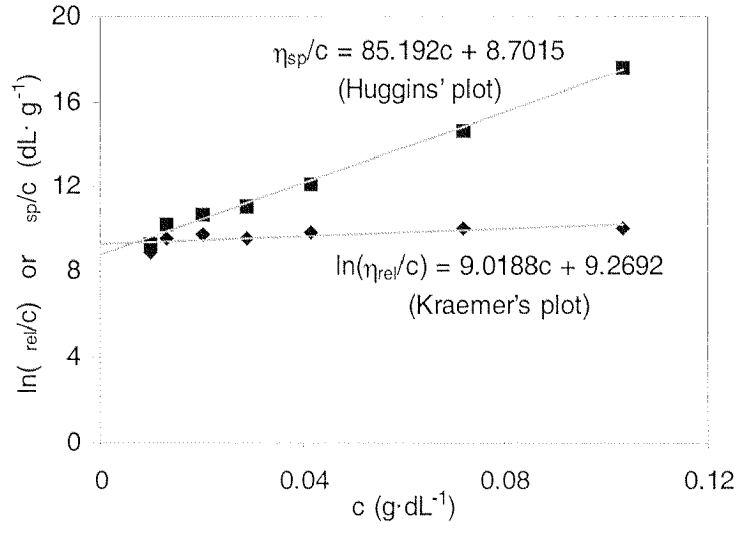

Figure 10: Example of Kraemer's and Huggins' plots for the calculation of intrinsic viscosity, corresponding to the sample PA

An examination of the average molecular weights reveals that any form of processing used led to a more or less marked depolymerisation of the galactomannan. The strongest effect was observed for the roasting process (PR). The intrinsic viscosity of PSp was lower than the one of PN, although the only difference between these samples is that the splits were milled to obtain PSp. It can be expected that milling does not lead to a partial degradation of the polysaccharide chain, but it could cause conformational changes in the supramolecular structure, leading to a lower viscosity and therefore to an apparent drop in molecular weight.

The intrinsic viscosities of all the analysed *Prosopis* samples were higher than the one reported by Yoo *et al.* [149] for mesquite seed gum obtained by a wet procedure ($[\eta] = 2.4$). This means that the processes used in the present work to obtain *Prosopis* gum, although being acid or heat treatments, are milder than other proposed methods.

4.5. Rheological properties of *Prosopis* gum solutions

As discussed in 2.2, the rheological properties of galactomannans are the most important aspect for their practical applications. In the present work, the dynamic viscosity of *Prosopis* gum samples was evaluated under different conditions, in order to check possible industrial uses.

Solubilisation is an important step in the use of hydrocolloids. In order to fully develop their functional properties, a careful dispersion of the gum powder to prevent the formation of lumps is required. This is a critical point since it is almost impossible to dissolve lumps. As a rule, water uptake should be relatively slow and agitation as strong as possible [46]. The finer the gum powders, the higher their tendency to form lumps. On the other hand, coarse gum particles take longer time to become completely solubilised. Thus, it is important to know the particle size when gum solutions are prepared for comparative viscosity measurements. The particle size distributions of "technical-grade" *Prosopis* gum samples (PA and PRx) were determined by the Alpine sieving technique as described in 3.3.3, and the results are presented in Table 20.

Table 20: Particle size distribution of gum powder samples (%)

Sieve	PA	PRx
> 140 mesh	36.0	42.0
170 – 140 mesh	2.0	2.6
200 – 170 mesh	4.6	3.7
< 200 mesh	57.4	51.7

PA and PRx showed similar particle size distributions, more than 50% of the particles passing 200-mesh (being finer than 74 μm). Although the other sample of roasted gum (PR, obtained after manual sorting) was not analysed, it may be assumed that its particle size distribution would have been similar to those for PRx and PA because it was ground with the same laboratory mill.

PA dissolved readily whereas PR and PRx tended to form lumps. Thus, pre-moisturising of the powdered samples PR and PRx with ethanol was required to avoid this problem. It may be concluded that roasting modified the structure of *Prosopis* endosperm so that it became much more hygroscopic and moisture sensitive. Solutions of non-treated *Prosopis* endosperm (PSp, PN) were prepared by direct extraction of splits, as described in 3.6.1, and no problems arose during dissolving.

All concentrations are referred to a dry-matter basis of gum. Concentrations of PSp and PN were calculated by freeze-drying of an aliquot of gum solution, followed by drying at 104°C to constant weight.

4.5.1. Flow behaviour

Flow curves, defined as the plot of shear stress (τ) versus shear rate (D) [146], were obtained for solutions of the differently processed *Prosopis* gum samples from the dynamic viscosity measurements with the Rheomat 30 (Contraves AG), as described in 3.6.2. Figure 11 shows, as an example, the flow curves for PA at three different concentrations. In order to know the extent of deviation of each flow curve from the Newtonian behaviour, the power law of Ostwald and de Waele:

$$\tau = k \cdot D^n \qquad (4)$$

was applied, where: n = flow behaviour index (dimensionless), and k = consistency index ($Pa \cdot s^{-n}$). The calculated constants of the power law for several solutions measured are compiled in Table 21.

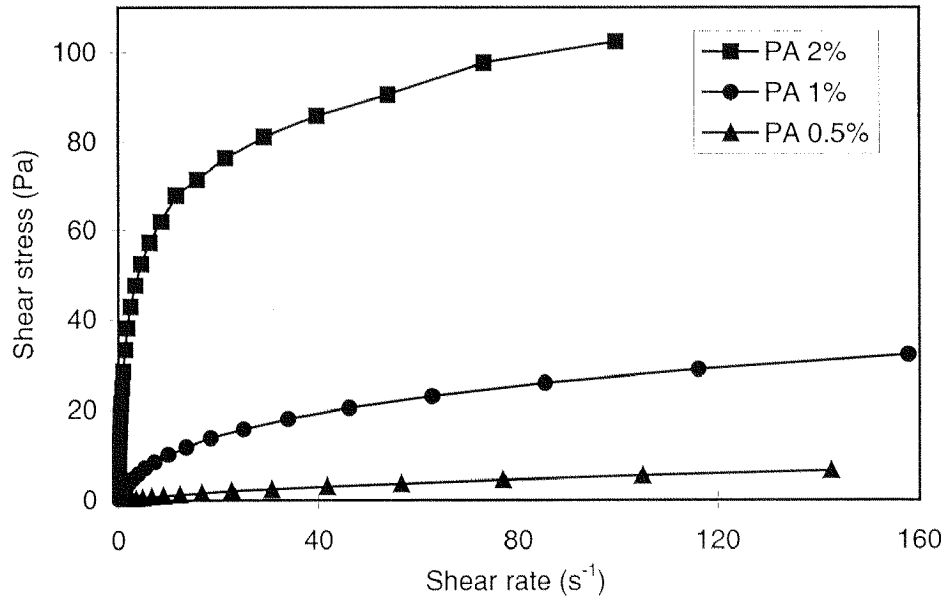

Figure 11: Flow curves for Prosopis gum solutions (PA) at 25°C

Table 21: Flow constants for solutions of Prosopis gum solutions at 25°C

Sample	k	n
PA 2%	34.35	0.25
PA 1%	2.73	0.52
PA 0.5%	0.26	0.64
PR 2%	9.76	0.50
PR 1%	0.89	0.67
PR 0.5%	0.08	0.79
PRx 1%	0.49	0.69
PSp 1%	4.84	0.42
PN 1%	18.11	0.26

As expected, *Prosopis* gum solutions exhibited a pseudoplastic behaviour. The value of n was less than 1 at all concentrations investigated. For the same type of gum, values of k increased with higher concentrations. A 0.5% solution of PR exhibited the closest profile to a Newtonian fluid (n close to 1). The flow constants

for PN are very similar to those determined by Vásquez *et al.* [140] for *P. chilensis* galactomannan obtained by aqueous extraction.

4.5.2. Development and stability of viscosity

Viscosity development depends on the hydration ability of the gum particles to achieve an homogeneous solution. Heating the solution usually contributes to a more rapid dissolution. Figure 12 illustrates for PA that heating at 80°C for 5 min was equivalent to 5 days of static hydration of a cold-solubilised sample for developing the same viscosity. Both cold and heated solutions were stable without sterilisation or addition of preservatives during storage at room temperature for 15 days. The viscosity decreased to 80% of the maximum value after 23 days. Growth of some fungi was visible on the surface of the solution after 20 days.

Variation of pH of the gum solution did not influence the viscosity within the range of pH-values from 2 to 12, at 25°C and within a short time (10 min), as shown in Figure 13 for the gum sample PA. In contrast, the viscosity clearly dropped for pH values lower than 2 or higher than 12 in a few minutes, probably because of a partial depolymerisation of the galactomannan. The results obtained here suggest that *Prosopis* gum is stable in a pH-range from 3 to 10 at RT.

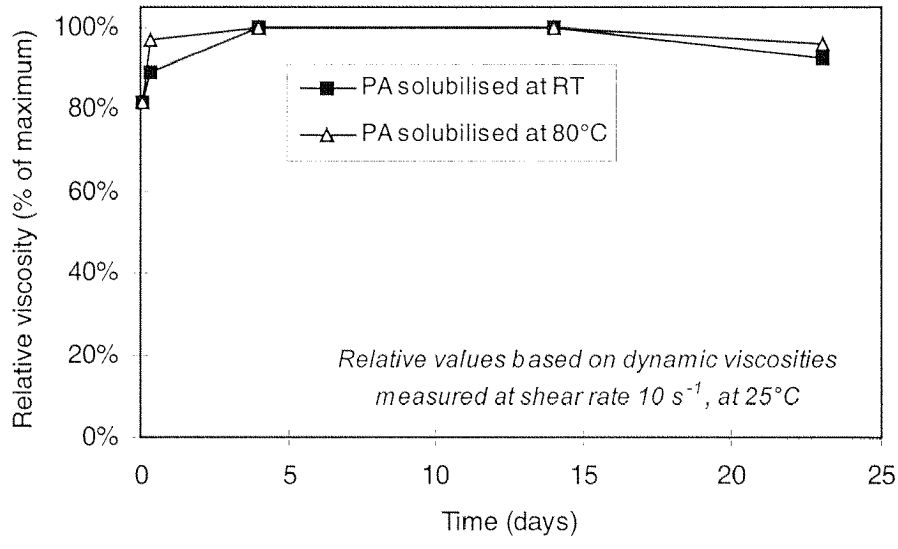

Figure 12: ***Development and stability of viscosity of 1% Prosopis gum solutions (PA)***
t = 0 corresponds to just prepared solutions -before heating-

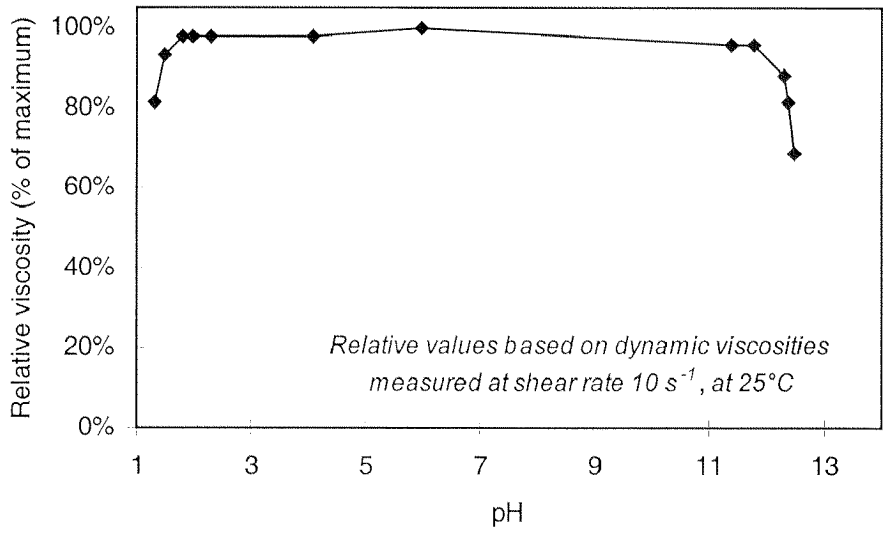

Figure 13: Influence of pH on the viscosity of Prosopis gum solution (PA)

The use of galactomannans in frozen foods requires good freeze-thaw stability. For example, tara and guar gum exhibit this behaviour, while carob gum shows irreversible loss of viscosity after freezing. *Prosopis* gum (samples PA and PR) was investigated for this property. No differences in dynamic viscosity were found after two freeze-thaw cycles with respect to the initial value (results not shown). This allowed to draw the conclusion that *Prosopis* galactomannnan has a good freeze-thaw stability.

4.5.3. Effect of processing on the viscosity

The viscosity curves of differently processed *Prosopis* gum samples are shown in Figure 14. The measurements were carried out as described in 3.6.2. The roasting process (samples PR and PRx) had a strongly negative influence on the viscosity and therefore, on the thickening capacity of the gum. As mentioned before, this could be a consequence of a partial depolymerisation of the galactomannan by the roasting process. The lower values observed for PRx with respect to PR could be due to the presence of cotyledon impurities.

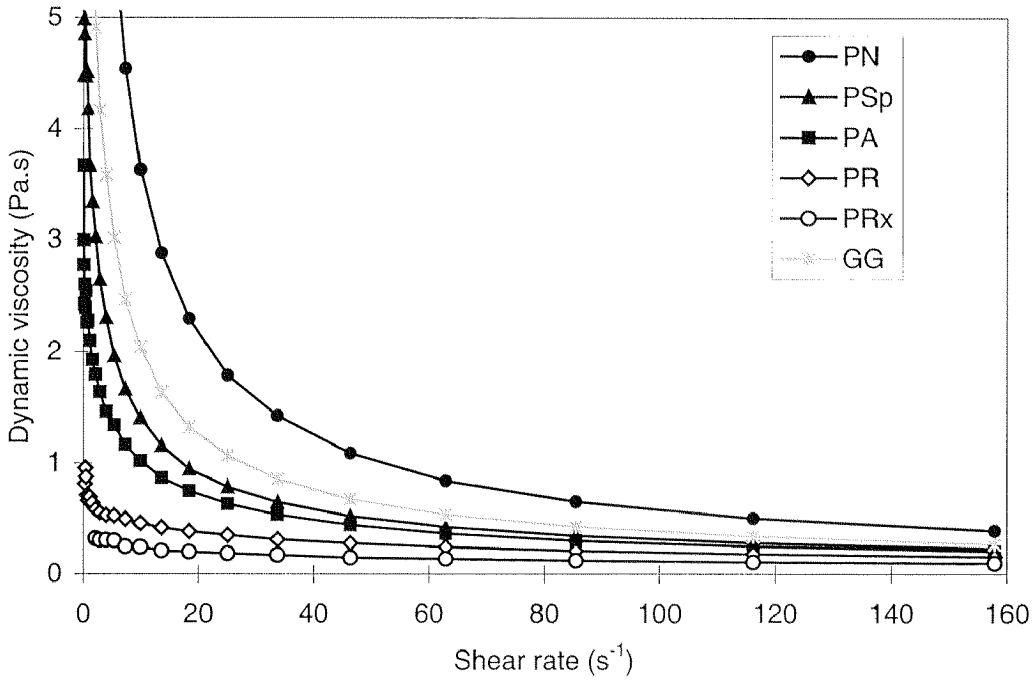

Figure 14: Effect of processing on the dynamic viscosity of 1% Prosopis gum solutions at 25°C

Prosopis gum obtained by the acid decoating process (PA) exhibited a better profile, even if the values were below those of guar gum (GG). Gum obtained from milled splits (PSp) showed similar viscosity as GG.

It is interesting to notice the large difference between the curve for PSp with respect to PN, although both samples were obtained from native splits. Since it is improbable that milling causes a depolymerisation, the reason for this difference could be related to supramolecular changes (as mentioned in 4.4.3) or to the presence of very fine particles of seed coat in PSp (escaping centrifugation), which due to their insolubility, do not account for high viscosity. It must be remembered that the concentrations of solutions of PSp and PN were determined by freeze-drying and adjusted by dilution (i.e. concentration is based on dry matter and not only galactomannan). Another important fact is that PN was extracted from entire splits, from which only a part of the endosperm was recovered, i.e. the most readily soluble fraction. This fraction seems to contain the longest galactomannan molecules and therefore the viscosity of PN is higher than of all the other samples, containing the whole endosperm.

The viscosity reduction due to processing can be quantified by comparison of the various curves at the same shear rate. For example, at 10 s^{-1}, the following relative values can be obtained (PN = 100%): PSp 38%, PA 28%, PR 13%, PRx 7%. At higher shear rates the differences are less pronounced.

4.5.4. Viscosity as a function of concentration and temperature

The dynamic viscosity profiles of *Prosopis* gum (types PA and PR) at different concentrations were determined over a wide range of shear rates, as described in 3.6.2. For comparison purposes, viscosity values at only two shear rates (12 and 80 s^{-1}) have been plotted in Figure 15.

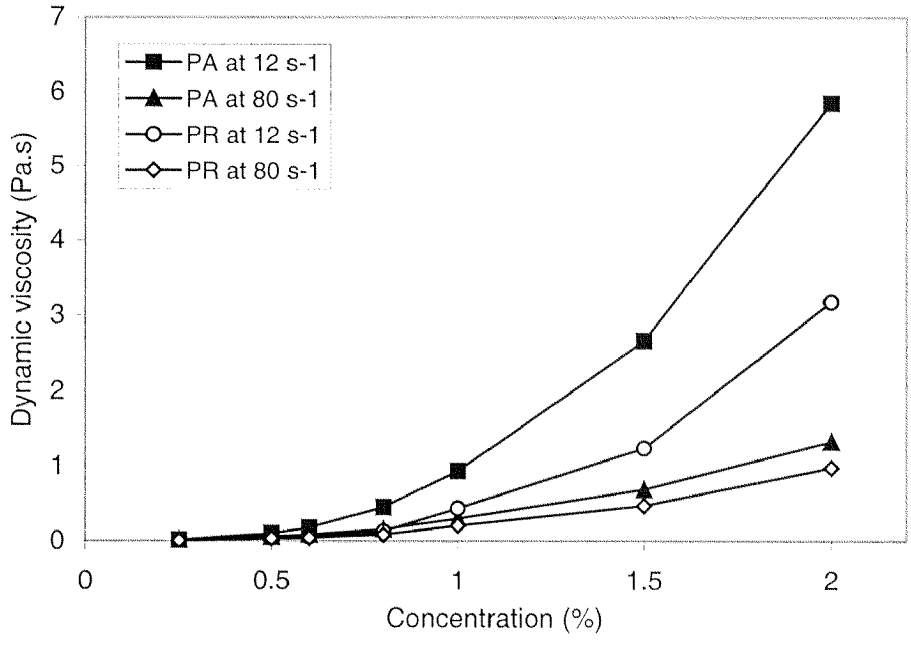

Figure 15: Influence of concentration on the viscosity of Prosopis gum solutions at 25°C

Both PA and PR samples show an exponential relationship between viscosity and concentration. This behaviour is in agreement with the one reported for gum from *Prosopis chilensis* [140]. The slope of these exponential curves is less pronounced at higher shear rates. For food application as thickener, PR should be used as 1.5% solution for giving approximately the same viscosity as PA at 1%.

The knowledge on the rheological behaviour of galactomannans at different temperatures is important for food and non-food applications (e.g. when salad dressings have to be pasteurised or when gums are used for oil well drilling). The relationship between dynamic viscosity and temperature was investigated for *Prosopis* gums PA and PR at different concentrations. As an example, the results for a 1% PA solution, at two shear rates, are shown in Figure 16. In general, the viscosity of *Prosopis* gum solution decreased considerably when the temperature was increased from 25 to 80°C, with a nearly linear function. This phenomenon is reversible, as confirmed by viscosity measurements after cooling some samples. A similar linear decrease, however with a less pronounced slope, has been reported for guar gum [86].

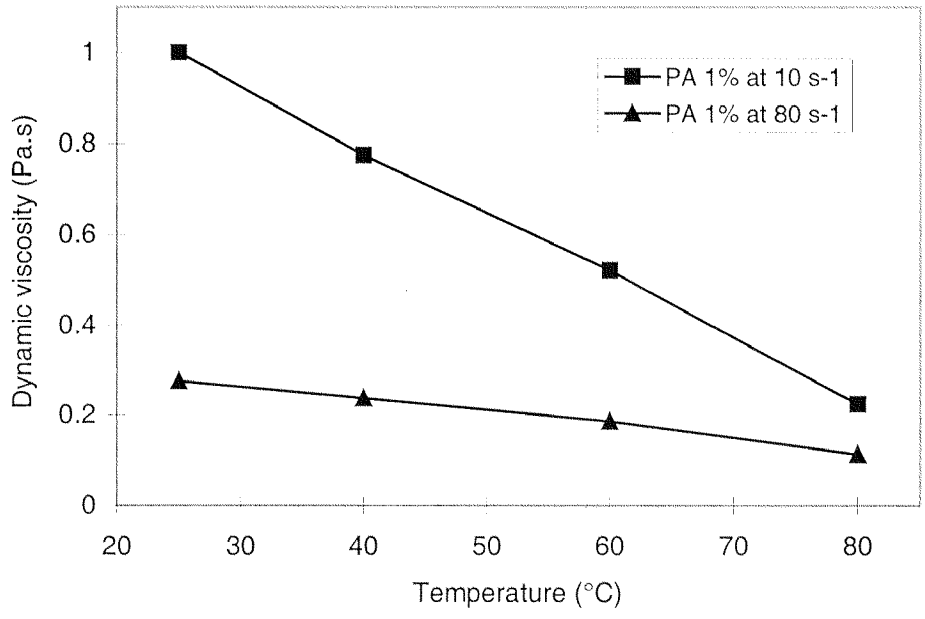

Figure 16: Influence of temperature on the viscosity of Prosopis gum solutions (PA)

4.5.5. Brookfield viscosity

The viscosity of most commercial galactomannans is often specified with values measured in a Brookfield viscometer on 1% solutions at 25°C. Since this instrument does not allow the determination of shear rates, values are referred to a rotation speed of 20 rpm. Measurements under these conditions were carried out for

Prosopis samples and guar gum, and the results are shown in Table 22. Brookfield viscosities were obtained using spindle 3, except for sample PN, which required spindle 4.

Table 22: Brookfield viscosities of 1% Prosopis gum solutions at 25°C

Sample	Brookfield viscosity (mPa.s)
PN	6650
PSp	2250
PA	1850
PR	250
GG	3425

Viscosity values reported for commercial guar gum usually range from 2000 to 4000 mPa·s depending on the production process [86]. *Prosopis* gum samples PA and PSp showed viscosities within or near this range, whereas the viscosity of the sample PR clearly matched the range of depolymerised guar gum [86].

4.5.6. Interaction with xanthan gum

The synergistic effect of mixing xanthan gum with galactomannans is widely used in the food industry and is caused by physical association between the ordered xanthan molecules and the galactomannan chains [58, 138]. Depending on their structure (practically the M/G ratio), some galactomannans show synergism with xanthan in gel formation (carob gum), while others only in viscosity (tara and guar gums), as described in 2.2.2. The blending proportion of galactomannan and xanthan, for which the maximum interaction occurs, has been established for most of the commercial gums. For example, highest interactions in 0.5% hydrocolloid solutions were reported to occur at a 50/50 proportion of carob gum and xanthan, but at a 70/30 for guar gum and xanthan [58, 138].

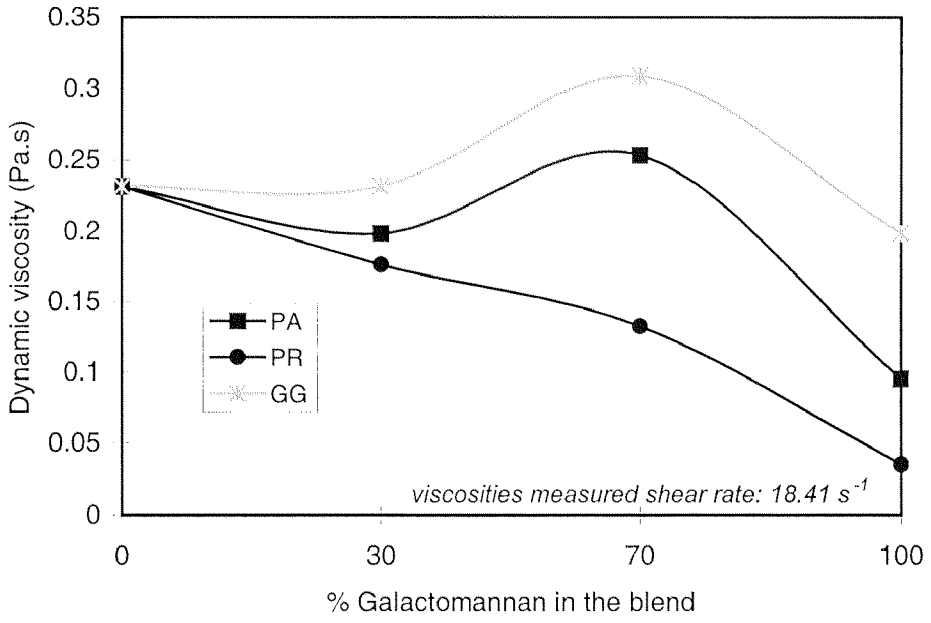

Figure 17: Synergistic effect of Prosopis gum + xanthan on the viscosity of a 0.5% solution, measured at 25°C

Prosopis gums, as expected from their M/G ratio, did not form gels with xanthan gum. The dynamic viscosity of 0.5% solutions of xanthan and PA, PR and GG respectively, mixed in different proportions, is shown in Figure 17. *Prosopis* gum obtained by acid treatment (PA) exhibited considerable synergism with xanthan gum, and the highest viscosity was reached for a proportion 70/30 (PA/xanthan). Compared with the effect measured for GG under the same conditions, the synergistic action of *Prosopis* gum is lower. This can be explained by the lower M/G ratio of *Prosopis* gum.

4.6. Enzymatic removal of galactose from galactomannan

As mentioned in 2.2.2, enzymatic modification of guar galactomannan by α-galactosidase has been proposed to reduce its galactose content (i.e. increase the M/G ratio), and consequently, to imitate the rheological properties of carob gum, e.g. its gelling synergism with xanthan gum. In order to achieve this modification, the use of a highly pure α-galactosidase, especially devoid of endo-β-mannanase activity, is required to exclude depolymerisation of the galactomannan.

In the present work, some experiments have been carried out to assess the treatment of *Prosopis* gum with a commercial α-galactosidase purified from guar seeds. The reaction conditions were taken from the optimal ranges recommended by the manufacturer (Megazyme Ltd.) and are described in 3.7. Two enzyme concentrations (5 U and 40 U per 100 mL of substrate) were tested on 1% solutions (w/w) of *Prosopis* gum (PA) and guar gum (GG).

Figure 18 shows the changes in the M/G ratio in gums PA and GG at the two enzyme levels, during a reaction time of 24 h, as well as the respective loss in viscosity of *Prosopis* gum solutions.

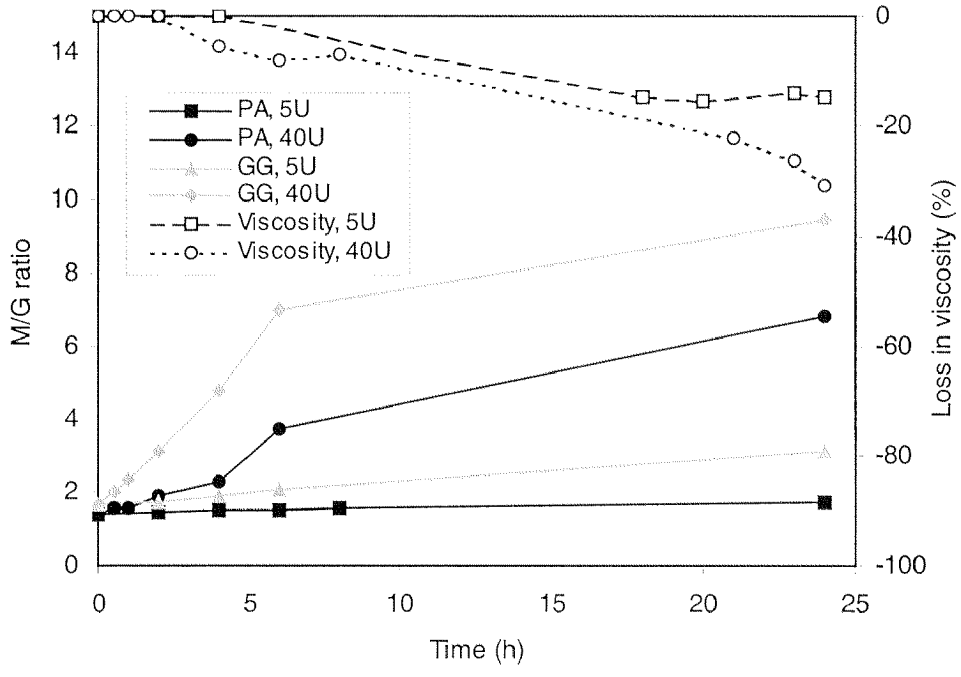

Figure 18: ***Increase of the M/G ratios and loss in viscosity for PA and GG during enzymatic removal of galactose***

The experiment clearly showed that galactose could be removed from *Prosopis* gum, but at a lower rate and at higher enzyme concentrations than for guar gum, although both galactomannans have similar M/G ratios. The reasons for the observed differences can be attributed to the substrate specificity, a well-known property of enzymes.

For *Prosopis* gum, an enzyme load of 40 U/100 mL was necessary to reach an M/G ratio of 3.7 after 6 hours of reaction, while 5 U/100 mL did not release measurable amounts of galactose during the same period of time. During the enzymatic reaction, the viscosity of *Prosopis* gum solutions was not affected significantly within the first six hours of reaction. It decreased by 30% during 24 h, indicating that some depolymerisation of galactomannan occurred. The viscosity was not monitored for guar, however no visual changes were noticed as long as the M/G ratio did not exceed 4, whereas M/G values above 5 led to a loss in solubility and phase separation.

In an additional experiment, the role of the galactose released during the enzymatic reaction was assessed for *Prosopis* gum PA. When the enzymatic reaction was carried out in a dialysis tube for continuous elimination of galactose, an M/G ratio of 7.1 was achieved after 6 h of incubation. This value is actually much higher than the one obtained for PA in the batch experiments at the same enzyme concentration (40 U/100 mL). This result reveals that galactose acts as an end-product inhibitor for α-galactosidase in the debranching of *Prosopis* galactomannan.

On the whole, the results of these preliminary experiments indicate that enzymatic modification of *Prosopis* gum might be a tool to change its physicochemical properties. In fact, when a simple test was carried out by mixing a sample of enzymatically modified PA (M/G = 4.85) with κ-carrageenan, some improvement in gel formation was observed. However, further systematic experiments and gel strength measurements are necessary to confirm this observation. The conditions used for the enzymatic reactions in this study would be unsuitable for large-scale application, because the use of gum solutions at low concentrations leads to large reaction volumes and high water-removal costs. In addition, purified plant enzymes are still very expensive. It is known that industrial research groups are searching for potential solutions for these problems, using guar as substrate. For example, an effective action of α-galactosidase at guar concentrations close to 30% (w/w), even at higher temperatures (50°C), has been reported [24]. Furthermore, progress in the production of microbial α-galactosidases and their application have been achieved [28].

Systematic investigations of the influence of gum concentration, temperature and origin of α-galactosidase will have to be carried out in order to optimise the

enzymatic modification of *Prosopis* galactomannan for the improvement of its physicochemical properties.

4.7. *In vitro* digestibility and fermentability of *Prosopis* galactomannan

The physiological behaviour of *Prosopis* gum as soluble dietary fibre has been studied through several *in vitro* digestion and colonic fermentation experiments, performed as described in 3.8.

Most of the literature on the physiological effects of soluble fibres only refers to guar gum as a typical galactomannan. It is accepted that soluble polysaccharides are easily and completely fermentable, and their monomer composition determines the fermentation pattern. However, the influence of the chemical fine structure, impurities and viscosity on the fermentability within the group of galactomannans has not yet been reported.

The purpose of this part of the study was to assess possible variations in the digestibility and fermentability of differently processed *Prosopis* gum samples (PA and PR), and to compare them with other galactomannans. From a compositional point of view, *Prosopis* galactomannan is similar to guar gum (M/G = 1.4 and 1.6, see Table 16) and therefore, an analogous fermentative behaviour was expected. For comparative purposes, other galactomannans were included in the experiments, namely carob (LB) and tara (TR) gums, which exhibited M/G ratios of 3.6 and 2.9 respectively (Table 16). The content of galactomannan in the samples was nearly 90%, as discussed previously, with exception of PA, which exhibited a lower value because of the presence of seed coat residues.

Additionally, ground *Prosopis* seeds (PW) were considered to be an interesting substrate for the physiological evaluation, because they are simultaneously a good source of protein (from cotyledon) and soluble fibre (from endosperm). The utilisation of whole seed flour as a supplement for bread production could be an interesting application.

4.7.1. Effects of *in vitro* digestion

In order to evaluate the effects of possible changes in the physicochemical properties of galactomannans during the passage through the upper gastrointestinal tract, the gum samples were subjected to an *in vitro* digestion prior to the fermentation experiments. Two different digestion methods (described in 3.8) were assessed for gum sample PA, while other samples were digested with method B only. Neutral sugars, protein content and total weight were determined in the samples before and after digestion. Some compositional differences were observed, as shown in Table 23.

Table 23: ***Differences in weight (g of d.m.) and composition (g/100 g d.m.) before and after in vitro digestion of the various substrates***

Sample	Weight		Protein		Neutral sugars		Gal+Man		M/G ratio	
	before	after	before	after	before	after	before	after	before	after
PA[da]	4.9	4.9	1.5	1.6	76.2	70.3	71.7	66.5	1.45	1.45
PA	4.9	8.8	1.5	4.9	76.2	36.6	71.7	34.9	1.45	1.25
PR	4.9	9.0	1.5	4.1	87.4	40.7	85.5	39.9	1.38	1.18
PW	4.9	7.8	23.9	10.6	n.a.	20.6	n.a.	15.8	n.a.	0.92
LB	4.9	9.3	3.6	n.a.	87.8	41.5	84.7	40.3	3.65	2.74
TR	4.9	9.4	1.2	n.a.	88.6	40.9	86.5	39.9	2.95	2.28

(*) PA[da] indicates digested with method A; otherwise, method B was used. n.a. = not analysed

A first view of the values suggests an apparent reduction of the galactomannan content; however, it must be taken as a relative decrease. With the digestion method A, practically the whole amount of sample was recovered. Considering the fact that addition of 14 mg of enzymes represents only 0.3% of the total weight, it can be concluded that the sample was not digested. On the other hand, digestion of gums with method B caused the samples to gain approximately 80% of extrinsic material. This is clearly reflected in the relative content of galactomannan, which became 40% after digestion, and can be explained by the fact, that large amounts of mucin (1.5 g) and bile (12 g) have been added and could not be completely removed by dialysis. Digestion method B also caused that the protein content of the substrates to increase by 2.5% compared to the original gum samples. The neutral sugar composition of the substrates (results not shown) did not change

during digestion with method A, while method B caused the M/G ratio slightly to decrease. Whether mucin and/or bile contain galactose that could disturb the M/G determination or if this method really modify the galactomannan slightly, remains to be elucidated.

In vitro digestion of whole seed material (PW) reduced the protein content from 24% to 10%. Taking into account that residual mucin and bile contributes to an increase of about 2.5% of protein, the corrected protein content of PW after digestion would be 7.5%. This indicates that the enzymatic degradation of cotyledon proteins in the complex mixture of seed components was not complete.

On the whole, the *in vitro* digestion experiments have shown that digestive enzymes neither degrade nor modify the galactomannan significantly. Although method B introduced other substances to the substrate, it reproduces the physiological digestive process better, and therefore it has been chosen for the preparation of most of the substrates for the *in vitro* colonic fermentation experiments. Digestion method B has previously been studied and proposed as standard method in our laboratory [85].

4.7.2. *In vitro* colonic fermentability

The *in vitro* fermentation system used has been well standardised and allows to follow the production of some intermediate and end products of fermentation [97]. Fresh human faeces were used as inoculum, and strict anaerobic incubation was allowed to proceed for 24 hours. At given time intervals, total gas and hydrogen productions were measured. The pH was monitored along the fermentation. The amount of short chain fatty acids (SCFA) as main fermentation products and the disappearance of substrate were analysed as well.

The fermentability of different galactomannan samples (PA, PR, PW, LB and TR), digested with method B as discussed above, is summarised in Figures 19 to 22. The values plotted for PR are those obtained from fermentation 2. As reference, average values for the blanks and for lactulose samples have been included in the diagrams. For comparison, non-digested samples of PA and PR were also subjected to fermentation (results not plotted). Detailed information on the experiments including all the substrates is given in the Appendix (Tables A1 to A3). In the

following paragraphs, the various parameters evaluated will be discussed separately.

Total gas and hydrogen production

As seen in Figure 19, carob and tara gums (LB, TR) led to higher rates of hydrogen accumulation in the first four hours of fermentation, compared with *Prosopis* gums (PA, PR). Some differences in total gas accumulation can also be observed among the four gum samples, though with another order. Divergences in H_2 and total gas profiles mostly indicate qualitative differences from one to another *in vitro* fermentation experiment, attributed to variances of the microbial flora of the inoculum. In addition, these profiles give an estimation of the fermentability of a substrate.

In any case, the measured values for H_2 and total gas accumulation in all substrates were largely below those for lactulose (see also Tables A1 to A3 in the Appendix) and clearly above the blank. The pH profile for all samples was very similar, showing a decrease of 0.2-0.3 units during the fermentation. The pH deviations observed for PR and PW could be due to technical problems with the pH measurement in one of the experiments and do not reflect anomalous behaviour.

Formation of short chain fatty acids

The production of SCFA was monitored by gas chromatography in the supernatants of fermentation residues (see 3.8). Concentration of acetic, propionic, butyric, valeric, isobutyric and isovaleric acids were measured at each sampling time. Figure 20 shows the profiles for the three main acids, namely acetic, propionic and butyric acid. It can be seen that acetic acid was formed in similar amounts in all substrates. Both *Prosopis* gum samples clearly yielded higher values for propionic acid than carob and tara gums. In contrast, butyric acid formation was lower in *Prosopis* than in carob and tara. Although *Prosopis* seeds (PW) showed the lowest production of SCFA, the values were clearly far above those of the blank. While acetic acid is known to be completely absorbed by the epithelial cells of the colon and enters the enterohepatic pathway, the physiological significance of propionate and butyrate are under discussion. Propionate could be related to the inhibition of

cholesterol biosynthesis *in vitro* when present in large amounts [5]. It was therefore proposed that fibres, which yield high levels of propionate upon fermentation, could reduce serum cholesterol levels by this mechanism. However, experiments with animals have recently shown that the inhibition mechanism does not occur *in vivo* [136]. On the other hand, there is some evidence that butyrate is involved in the prevention of the formation of colon cancer [111].

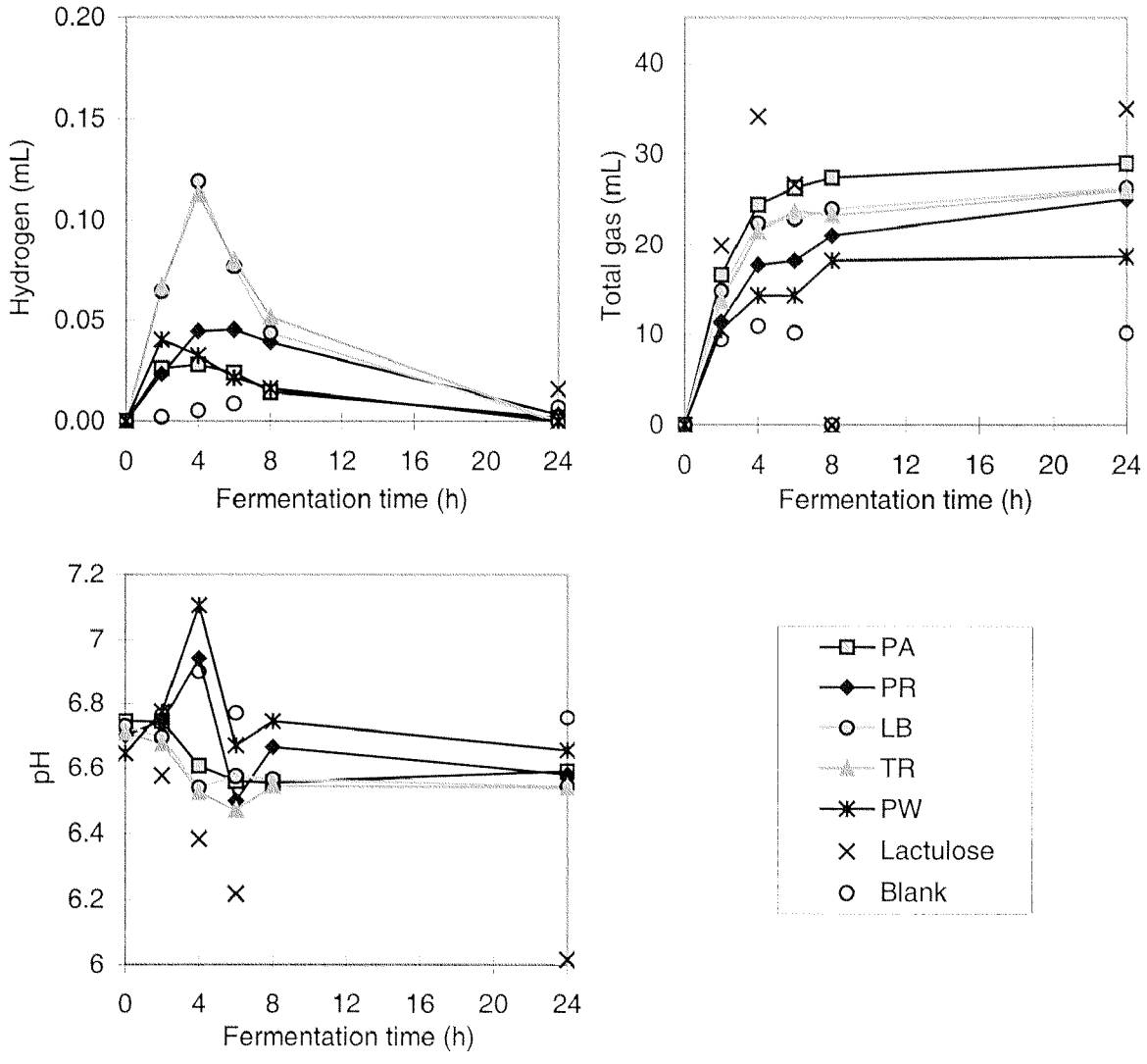

Figure 19: ***pH, total gas and hydrogen accumulation during in vitro fermentation of different galactomannan samples***

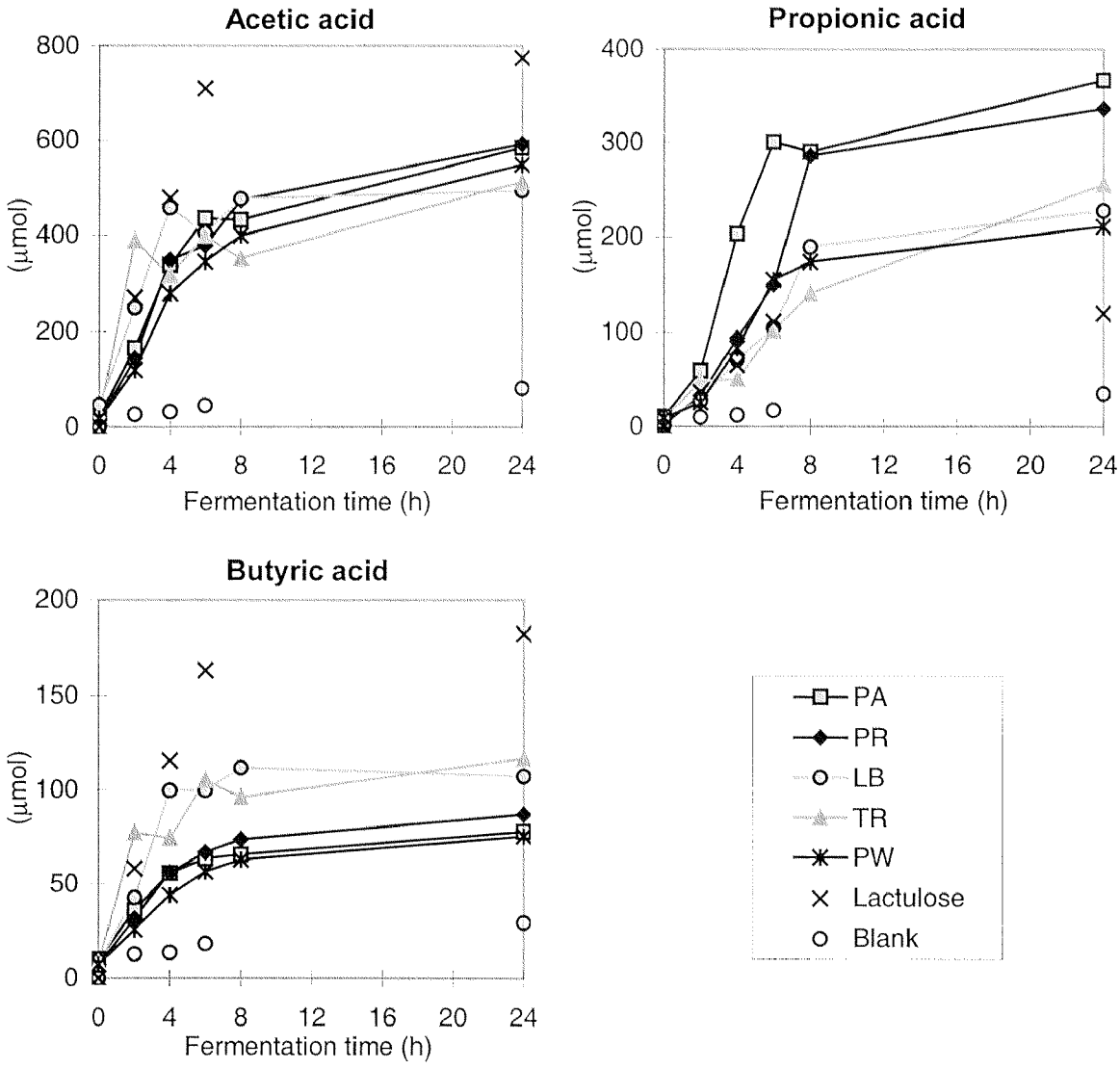

Figure 20: Formation of short chain fatty acids during in vitro fermentation of different galactomannan samples

Qualitative differences in polysaccharide fermentation are reflected in the proportion of the three main SCFA formed after 24 h of fermentation (Figure 21). Values for guar gum taken from the literature [101] have been included as well.

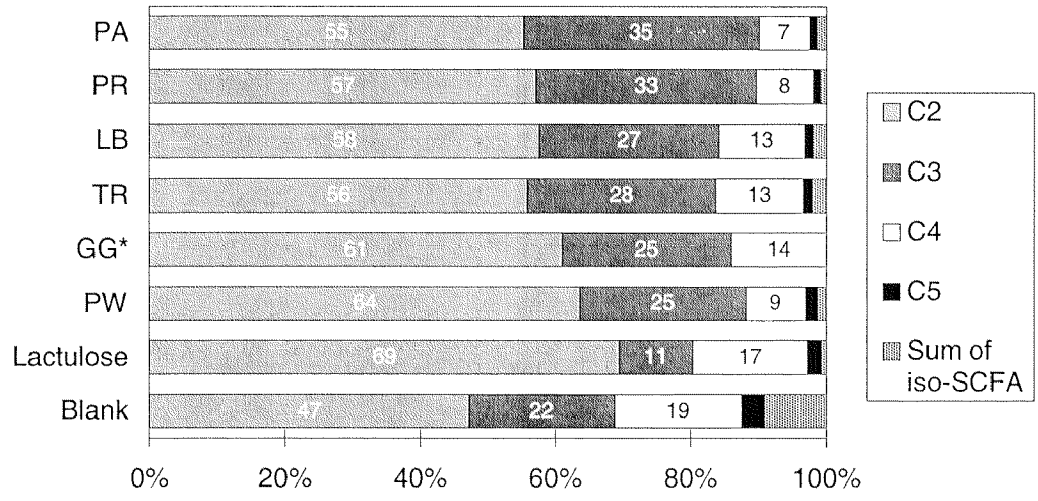

Figure 21: ***Proportion of short chain fatty acids after 24 h of fermentation***
**Values for guar gum taken from the literature* [101]

It is interesting to notice that the *Prosopis* gum samples exhibited molar ratios for acetic acid : propionic acid : butyric acid near to 55 : 35 : 10, irrespective of the production method of the gum (acid treatment or roasting). The SCFA pattern obtained for *Prosopis* gums was considerably different compared to the one obtained by fermentation of carob, tara and guar gums. The reasons for these differences could be attributed to the different M/G ratio or fine structure.

Degradation of substrate

The neutral sugar composition of fermentation residues at each sampling time was determined by HPAEC-PAD and allowed the monitoring of the rate and extent of substrate degradation. Figure 22 shows the degradation patterns of the main monomeric constituents of the studied substrates. The results are expressed as percentage of the weight of the total neutral sugars at the beginning of the fermentation. This offers a standard basis for all samples, independent from the amount of other substances in the fermentation residues. The residues actually contain not only polysaccharides but also bacterial biomass from the inoculum, which grows during the fermentation.

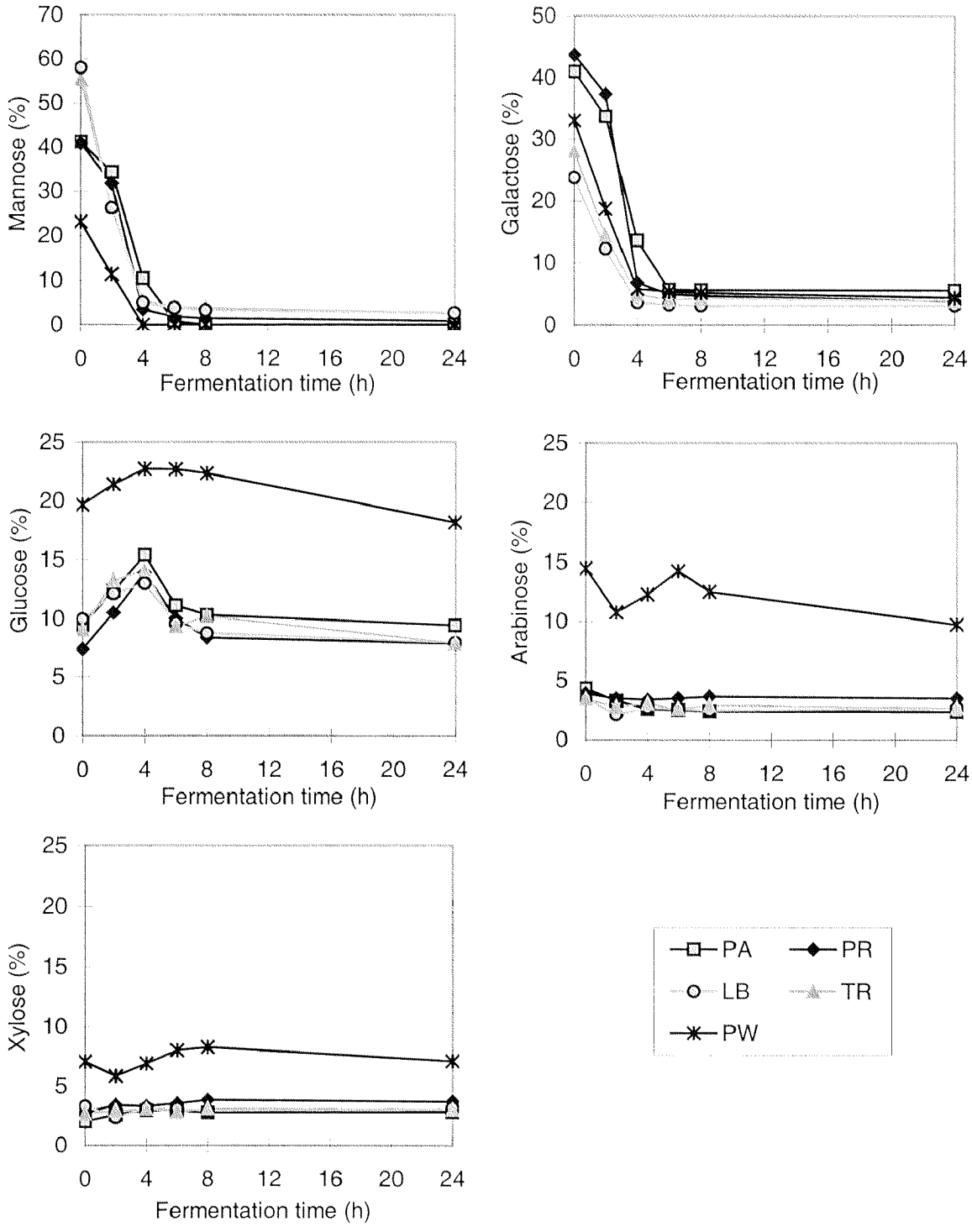

Figure 22: Degradation of substrate during in vitro fermentation. Concentration of each monosaccharide given as % of the sum of neutral sugars at t = 0

The main components of the gum (galactose and mannose) were degraded in an extent of approximately 50% in the first two hours for LB and TR, and 30% for the *Prosopis* samples. This could be due to the different degree of substitution in the galactomannans, which could suggest that galactose side-chain units rendered the bacterial action difficult. However, a further evaluation of the data of the three assays (see Tables A1 to A3 in the Appendix) reveals that these differences could arise also from the particular fermentation behaviour in each experiment.

In general, the galactomannan in all the samples was almost completely degraded within the first four hours of fermentation, despite the high viscosity of the substrates. This fact demonstrates that the microorganisms can act directly into the backbone of the galactomannans already from the beginning of fermentation.

Minor components such as glucose, arabinose and xylose (derived from the insoluble fibre of seed coat and also from the inoculum), showed very low degradation rates. Small amounts of these polysaccharides remained undegraded after 24 h in all substrates. This behaviour is clearly evidenced during the fermentation of the whole seed material (PW) containing considerable amounts of cellulose as component of the seed coat and probably arabinoxylans from the outer layers of the endosperm.

The glucose content of the residues at the beginning of the fermentation was higher than the original glucose content of the respective substrates (~2% vs. 1%, see Tables A1 to A3 compared with Table 16). This difference can arise from the inoculum that very likely contains cellulose. An apparently increasing profile of glucose during the first four hours of fermentation can be observed in Figure 22, confirmed by the glucose concentration found in the fermentation residues at each sampling time (see Tables A1 to A3). Considering that the NS analyses were performed on fermentation residues (that consist not only of substrate but also of bacterial mass from inoculum), a further investigation was carried out. The weight of the fermentation residues showed a decrease of 290 mg to 260 mg in average in the first four hours (Figure A1 in Appendix), and therefore a factor 0.9 should be applied to the NS concentrations at 4h to obtain the absolute values. Even by doing so, a small increment of glucose concentration persists. The correct identification and concentration of glucose was verified by enzymatic determination (method

61B/1.1, Swiss Food Manual [135]) in the hydrolysed fermentation residues. Whether glucose originates from a bio-conversion of other sugars, or if the increase arises from methodological sources, remains open.

When the fermentation of digested gums was compared with the non-digested gums (PA vs. PA^{nd}, and PR vs. PR^{nd} respectively), a clear difference was observed in the rate of substrate degradation (see Tables A1 and A2). In the non-digested samples the galactomannan needs 6 h to be degraded to an extent of 50%, while in digested samples this occurs in 2-4 h. Sample digested with method A (PA^{da}, Table A1) showed an intermediate degradation rate between the PA^{nd} and PA. The easier degradation is probably not an effect of the digestion itself, but rather due to the fact that digestion B adds other substances to the galactomannan (as discussed in 4.7.1). This leads to a lower relative concentration of galactomannan in the substrate to be fermented and therefore, a lower viscosity.

On the whole, it can be concluded that *Prosopis* gums are easily fermentable substrates. While no significant differences in the degradation rates of substrates were found between the various types of galactomannan, the proportion of SCFA produced after 24 h were clearly different. It may be suggested that the relative amounts of propionic acid correlate well with the M/G ratio.

5. CONCLUSIONS

The aims of the present research work were:

1. Development of a procedure to obtain *Prosopis* seed gum on a pilot plant scale in a sufficient degree of purity to be used as food additive
2. Physicochemical characterisation of the differently processed *Prosopis* gum samples and comparison with the galactomannan isolated by a laboratory procedure, for which the molecular structure had to be elucidated
3. Study of the *in vitro* colonic fermentability of *Prosopis* gum

The production of *Prosopis* gum comprised both the separation of the seeds from the pod and the separation of the endosperm from the seed. Since *Prosopis* is not yet industrialised for gum production neither in Peru nor in other countries, a prototype milling process for seed separation had to be developed and optimised. This was successfully achieved, i.e. the yield of intact seeds extracted from their endocarp hulls was increased from 2.5% to 7% with respect to the weight of fruits. This is equivalent to approximately 75% of the seeds contained in the pods.

The production of *Prosopis* endosperm with the technologies used for commercial galactomannans (carob, tara and guar gums) proved to be a very difficult task. Two types of processes have been assessed (roasting and acid treatment of the seeds) and their respective parameters established. In both cases, pilot plant equipment was used, including an optical sorter to separate the gum from seed coat and cotyledon fragments. Treatment of seeds with sulphuric acid results in the elimination of most of the seed coat, but inevitably causes some losses in endosperm. On the other hand, roasting causes the seed coat to pop off and facilitates its detachment by splitting.

Actually, the most critical step for both procedures is the splitting of the seeds. Acid decoated seeds can be split with a hammer mill and the endosperm splits recovered from the larger fragments after sieving. Difficulties arise from distinguishing endosperm splits and cotyledon particles of the same size and similar specific weight. When an exhaustive, manual separation was performed, 220 g of

endosperm splits were obtained from 1 kg of seeds. It can be assumed that a similar yield could be obtained by an air-separator with better performance. Splitting of roasted seeds is yet more complicated, since it should detach the popped seed coats and grind the cotyledons in the same operation. Although different roasting temperatures and heat treatments were assayed, less than 40% of the popped seeds allowed the coat to be detached during splitting. Sieving separated a part of the cotyledon fragments, and the separation could be eventually improved by fine air-separation. However, it is extremely difficult to distinguish pure endosperm splits from those adhered to seed coat fragments. The advised solution for this operation is an optical sorting, which was actually proved to give an efficient separation. The yield of pure endosperm splits from the roasting process was very low, namely 34 g/kg of seeds. Even if the removal of impurities during the optical sorting could be improved, the yield would not exceed 75 g/kg of seeds, because of the limited detachment of the seed coats.

Both methods will have to be improved in order to enhance efficacy of gum extraction, yield and purity of the gum. The procedures developed so far did not take into account the economical aspects of gum production, but were focused on the feasibility of the technological processes. Despite these limitations, it may be considered that the first objective has been achieved.

Both technological processes investigated yielded gums with high galactomannan contents as confirmed by its chemical composition. The acid decoating process led to a gum containing approximately 10% insoluble impurities derived from the remaining seed coat. The protein content of the sample PA was 1.5%, which is very similar to the value for the isolated galactomannan. In contrast, gum obtained by roasting and optical sorting (PRx) contained 4.5% of protein, due to cotyledon fragments accompanying the endosperm splits. Despite these impurities, both types of *Prosopis* gum comply with the specifications for guar, tara or carob gum as food additives regarding protein and insoluble fibre. It is therefore proposed to consider both *Prosopis* gums obtained in this work as "technical-grade" gums.

A detailed chemical analysis of *Prosopis* galactomannan, isolated in several steps from non-treated seeds, revealed that small amounts of polysaccharides apart from the predominant galactomannan are present in the endosperm as well, e.g.

arabinoxylans, which are probably located in the outer layers of the endosperm. The galactomannan is almost completely soluble at room temperature, and nearly 70% of it is extractable with water in a single step.

The M/G ratio of *Prosopis* galactomannan was repeatedly found to be in the range of 1.4 to 1.6 (even with different analytical techniques), which is in agreement with previous studies and is also similar to values reported for other *Prosopis* species. A sample of *Prosopis juliflora* from Brazil, which was believed to exhibit a higher M/G ratio, was analysed as well. The results were similar to the Peruvian *P. pallida* and *P. juliflora* species investigated throughout this work. The M/G ratio of gum samples obtained by acid decoating and by roasting were very similar, showing values of approximately 1.4. Thus, *Prosopis* gum is compositionally clearly closer to guar gum (M/G = 1.6, see Table 16), rather than to tara or carob gum. The M/G ratio was not affected considerably by the processing, as evidenced by comparing the values for PA and PR with those of isolated *Prosopis* galactomannan.

Methylation analyses of *Prosopis* galactomannan, complemented by the results from the enzymatic hydrolysis and enzymatic modification, revealed this polysaccharide to consist of a backbone of β-1,4-linked mannose units, to which single galactose units are α-1,6-linked. The substitution degree, calculated from the M/G ratio, is 41%. The distribution of galactose residues on the mannan chain has not been investigated. The average molecular weight, estimated through the intrinsic viscosity, ranges from 0.6×10^6 for the roasting-processed gum to 1.8×10^6 for the non-treated galactomannan. As reference, molecular weights in the range from 0.3×10^6 to 2.1×10^6 have been reported for guar and carob galactomannnans, values depending on gum type and analytical methods used [47, 124].

Viscosity and flow properties of gum solutions showed some differences depending on the treatment during extraction of the gum. Roasting was shown to degrade the polysaccharide chain considerably, and therefore the viscosity strongly dropped. In contrast, acid decoating led to solutions exhibiting higher viscosities, thus proving that it is a milder process, even if the gum contains seed coat impurities. The decrease in the viscosity of PSp with respect to PN, initially thought to be caused by the milling of the splits, has been explained based on the differences during the extraction procedures. In fact, PN contains only the most readily soluble fraction of

the endosperm (probably including the largest molecules of galactomannan), which leads to a higher viscosity. In some way, sample PN should be equivalent to the first fractions (I1, I2) of isolated *Prosopis* galactomannan. These fractions have been chemically characterised; however, their molecular weights were not determined due to the extremely difficult solubilisation.

The flow behaviour of *Prosopis* gum has been clearly proven to be pseudoplastic, even at low concentrations. Solutions prepared from powdered samples of *Prosopis* gum develop their maximal viscosity within a short time at room temperature. The viscosity was not considerably affected by storage for several days at room temperature or by freezing-thawing, not also influenced by changes within a wide range of pH (2-12). A synergistic effect on the viscosity was observed for mixtures of 70% *Prosopis* gum with 30% xanthan. This effect was more pronounced for gum obtained by acid treatment. Gum obtained by roasting showed a weak interaction with xanthan gum, probably because of the depolymerisation of the galactomannan occurred during this treatment.

An enzymatic treatment of *Prosopis* gum with a purified α-galactosidase from guar seeds led to an increase in the M/G ratio (i.e. a partial removal of the galactose side units from the polysaccharide). However, the efficacy of the enzymatic modification on the possible synergistic gelling properties of *Prosopis* gum remains to be systematically evaluated.

On the whole, the results obtained with respect to the use of *Prosopis* seed gum as a food additive (thickener) allow to draw the following conclusions:

a) the acid decoating process is more effective compared to the roasting process, concerning both the yield and the rheological properties of the gum,

b) further studies are needed for improvement of splitting and endosperm separation,

c) the properties of *Prosopis* gum are similar to those of guar gum, and therefore any future industrialisation study should consider these two gums as market competitors.

The nature of galactomannan as dietary fibre (i.e. resistant to the human digestive enzymes) was confirmed. *In vitro* fermentation studies have shown that *Prosopis* gum is completely fermented by the human faecal flora, irrespective of the method of production. The most interesting fact with regard to the colonic fermentation of *Prosopis* gum is the ratio of the SFCA determined for various samples, in which a high proportion of propionic acid contrasts with tara and carob galactomannans. The reason for the different fermentative behaviour could be related to the different M/G ratio or, finally, to the fine structure of each galactomannan.

Considering the beneficial effects on health claimed for soluble dietary fibres based on their viscosity, such as reduction of glucose and insuline response, or reduction of the plasma cholesterol level, it may be suggested that *Prosopis* galactomannan has a potential use as an ingredient for functional foods. It is interesting to notice that the gum obtained by the roasting process offers advantages with respect to its inclusion in the human diet, since it yields solutions of intermediate viscosity and therefore it would allow higher amounts to be incorporated into formulations. This would be of particular interest for the preparation of functional drinks.

The digestion and fermentation experiments of milled *Prosopis* seeds lead to suggest the use of this flour as an interesting component for bakery formulations. Because of the presence of the protein-rich cotyledon, it would increase the protein content of bread and simultaneously contribute, by means of the endosperm gum, to the soluble dietary fibre intake. On the other hand, the possible presence of protease inhibitors in the cotyledon could be a limitation for the use of *Prosopis* seed flour. From the results obtained in the present work, roasting should be the choice for processing of the seeds, since heat causes a partial depolymerisation of the galactomannan and can inactivate possible protease inhibitors. Further studies (animal nutrition assays, acceptable levels, etc.) are necessary.

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7. APPENDIX

Table A1: Results of Fermentation 1: gum sample PA, digested with methods A ([da]) and B, and non-digested ([nd]). Concentrations of neutral sugars are referred to dry matter of the fermentation residue at each sampling time

Substrate	Time	H_2	Gas	pH	Short chain fatty acids (μmol)						Neutral sugars (%)						Total	M/G
	(h)	(ml)	(ml)		C2	C3	iC4	C4	iC5	C5	Rha	Ara	Gal	Glu	Xyl	Man	NS (%)	Ratio
PA[da]	0	0.00	0.0	6.7	28.7	11.7	0.0	11.1	0.0	8.1	0.3	1.3	11.7	2.2	0.6	14.4	30.4	1.23
	2	0.01	10.3	6.8	165.7	62.2	0.0	30.6	0.0	0.0	0.3	0.6	8.9	2.7	0.5	9.9	22.8	1.11
	4	0.04	22.2	6.6	296.1	127.6	0.0	46.4	0.0	0.0	0.4	0.6	5.3	4.2	0.5	4.9	15.9	0.94
	6	0.05	29.9	6.4	440.4	272.3	0.0	65.1	0.0	3.1	0.5	0.6	1.4	5.0	0.5	0.0	8.1	0.00
	8	0.04	32.7	6.4	390.2	333.3	2.1	66.1	5.7	7.7	0.5	0.6	1.2	3.1	0.6	0.0	6.0	0.00
	24	0.01	35.2	6.4	512.1	406.8	10.5	94.0	12.4	21.0	0.6	0.5	1.2	1.9	0.5	0.0	4.7	0.00
PA	0	0.00	0.0	6.7	24.5	10.3	0.0	10.0	0.0	0.0	0.3	0.7	6.6	1.5	0.3	6.6	16.0	1.00
	2	0.03	16.7	6.7	165.9	59.5	0.0	35.9	0.0	0.0	0.3	0.5	5.4	2.0	0.4	5.5	14.2	1.02
	4	0.03	24.5	6.6	338.2	203.1	0.0	55.7	0.0	3.1	0.3	0.4	2.2	2.5	0.5	1.7	7.6	0.77
	6	0.02	26.3	6.6	434.7	300.9	0.0	63.4	0.0	7.2	0.4	0.4	0.9	1.8	0.5	0.1	4.1	0.14
	8	0.01	27.4	6.6	431.4	290.2	0.0	65.6	0.0	7.9	0.4	0.4	0.9	1.7	0.4	0.0	3.8	0.00
	24	0.00	29.0	6.6	584.2	367.5	5.4	77.9	9.5	12.1	0.4	0.4	0.9	1.5	0.5	0.0	3.6	0.00
PA[nd]	0	0.00	0.0	6.8	21.4	10.1	0.0	9.4	0.0	0.0	0.3	1.3	10.8	1.9	0.5	13.4	28.2	1.25
	2	0.01	14.2	6.7	104.4	42.0	0.0	23.0	0.0	0.0	0.3	1.1	10.5	2.4	0.5	12.6	27.4	1.20
	4	0.03	21.9	6.6	176.7	84.0	0.0	34.0	0.0	0.0	0.4	1.0	8.4	3.3	0.4	9.2	22.8	1.10
	6	0.05	31.3	6.4	405.1	242.1	0.0	59.4	0.0	2.8	0.5	0.9	2.4	4.0	0.5	2.0	10.2	0.82
	8	0.04	28.6	6.5	355.5	253.4	0.0	48.5	0.0	10.1	0.5	1.0	2.5	2.1	0.5	2.0	8.5	0.79
	24	0.00	31.1	6.4	433.3	344.2	9.0	76.4	11.7	19.7	0.5	0.8	0.8	1.7	0.5	0.0	4.4	0.00
Lactulose	2	0.60	25.6	6.5	231.7	37.3	0.0	65.9	9.3	0.0	0.4	0.4	7.5	5.4	0.5	0.0	14.1	
	4	1.31	41.1	6.2	350.0	56.6	0.0	130.3	0.0	0.0	0.5	0.4	1.2	5.9	0.4	0.0	8.3	
	6	0.80	41.1	6.1	600.1	83.0	0.0	181.4	0.0	0.0	0.5	0.4	1.2	4.4	0.5	0.0	7.1	
	24	0.00	38.9	6.0	689.9	92.9	0.0	188.6	0.0	14.6	0.5	0.3	1.1	1.9	0.4	0.0	4.2	
Blank	2	0.00	11.4	6.7	23.9	10.7	0.0	12.3	0.0	0.0	0.4	0.5	0.7	1.6	0.5	0.0	3.6	
	4	0.00	15.1	6.7	28.9	12.3	0.0	13.4	0.0	0.0	0.4	0.4	0.6	1.3	0.4	0.0	3.1	
	6	0.01	13.7	6.7	39.2	15.6	0.0	18.9	0.0	0.0	0.4	0.4	0.7	1.3	0.5	0.0	3.1	
	24	0.01	14.6	6.7	54.4	26.4	5.1	26.8	7.7	5.0	0.4	0.5	0.6	1.2	0.5	0.0	3.3	

Table A2: Results of Fermentation 2: gum sample PR, digested with method B and non-digested (nd); whole seed flour (PW), digested with method B. Concentrations of neutral sugars are referred to dry matter of the fermentation residue at each sampling time

Substrate	Time	H_2	Gas	pH	Short chain fatty acids (μmol)						Neutral sugars (%)						Total	M/G
	(h)	(ml)	(ml)		C2	C3	iC4	C4	iC5	C5	Rha	Ara	Gal	Glu	Xyl	Man	NS (%)	Ratio
PR	0	0.00	0.0	6.7	15.7	2.1	0.0	7.1	0.0	0.0	0.2	0.5	6.0	1.0	0.4	5.6	13.6	0.94
	2	0.02	11.4	6.7	144.2	32.7	0.9	31.6	1.2	1.4	0.2	0.5	5.1	1.4	0.5	4.3	12.0	0.85
	4	0.04	17.8	6.9	348.2	93.9	0.9	55.8	0.9	4.3	0.3	0.5	0.9	1.9	0.5	0.5	4.5	0.51
	6	0.05	18.3	6.5	377.6	149.9	1.0	66.9	1.0	5.8	0.3	0.5	0.7	1.4	0.5	0.3	3.5	0.38
	8	0.04	21.0	6.7	472.8	285.9	1.2	73.6	1.3	7.1	0.3	0.5	0.6	1.1	0.5	0.2	3.3	0.31
	24	0.00	25.1	6.6	592.2	336.8	3.8	86.9	6.4	10.0	0.3	0.5	0.5	1.1	0.5	0.1	3.0	0.20
PW	0	0.00	0.0	6.6	17.4	8.7	0.0	7.1	0.0	0.0	0.2	1.2	2.7	1.6	0.6	1.9	8.1	0.70
	2	0.04	10.5	6.8	119.6	25.5	0.0	25.7	2.2	2.6	0.2	0.9	1.5	1.7	0.5	0.9	5.7	0.61
	4	0.03	14.4	7.1	279.2	83.1	0.0	44.1	0.0	4.8	0.2	1.0	0.5	1.8	0.6	0.0	4.1	0.00
	6	0.02	14.4	6.7	344.7	154.6	2.4	56.5	2.9	8.4	0.2	1.1	0.4	1.8	0.6	0.0	4.3	0.00
	8	0.02	18.3	6.7	399.2	173.9	2.7	63.0	3.6	10.2	0.2	1.0	0.4	1.8	0.7	0.0	4.1	0.00
	24	0.00	18.7	6.7	548.0	210.9	4.3	75.3	7.0	15.0	0.2	0.8	0.4	1.5	0.6	0.0	3.4	0.00
PRnd	0	0.00	0.0	6.7	14.8	5.9	0.0	5.3	0.0	0.0	0.2	0.7	10.3	1.2	0.4	12.4	25.2	1.19
	2	0.02	6.9	6.7	58.1	16.9	0.0	14.9	0.0	0.0	0.2	0.4	9.5	1.6	0.3	11.7	23.7	1.23
	4	0.08	15.8	7.1	191.5	52.6	0.0	34.0	0.0	0.0	0.2	0.4	7.3	3.3	0.4	7.7	19.4	1.06
	6	0.09	21.9	6.4	306.0	119.0	0.0	44.3	0.0	0.0	0.3	0.5	5.0	2.0	0.4	5.3	13.5	1.07
	8	0.08	24.7	6.5	410.6	220.9	0.0	55.1	0.0	5.6	0.3	0.5	3.2	1.8	0.4	3.1	9.4	0.97
	24	0.01	32.0	6.3	498.4	353.6	7.0	76.6	13.0	17.8	0.3	0.5	0.8	1.4	0.5	0.3	3.7	0.37
Lactulose	2	0.54	17.4	6.6	160.5	32.0	2.0	46.1	2.3	2.2	0.2	0.4	8.6	2.6	0.4	0.0	12.2	
	4	0.50	27.9	6.8	520.4	72.5	1.5	107.7	1.7	2.6	0.3	0.4	4.0	3.9	0.4	0.0	9.0	
	6	-0.01	34.3	6.3	832.8	126.9	1.8	168.9	2.2	3.7	0.4	0.4	0.8	2.6	0.4	0.0	4.7	
	24	0.04	29.7	6.0	1027.1	147.6	4.5	195.2	5.8	20.9	0.4	0.4	0.7	1.3	0.4	0.0	3.2	
Blank	2	0.01	7.8	6.8	21.3	6.7	1.2	8.5	2.7	1.4	0.2	0.4	0.5	1.0	0.4	0.0	2.5	
	4	0.01	8.7	7.2	21.1	9.0	1.6	11.5	3.5	1.7	0.2	0.4	0.5	0.9	0.4	0.0	2.4	
	6	0.01	6.9	6.8	40.9	17.5	2.4	18.3	4.9	2.4	0.2	0.4	0.4	0.9	0.4	0.0	2.4	
	24	0.01	8.2	6.8	54.9	31.5	5.5	26.1	9.2	4.4	0.2	0.4	0.4	0.9	0.4	0.0	2.3	

Table A3: Results of Fermentation 3: gum samples LB, TR and PR, digested with method B. Concentrations of neutral sugars are referred to dry matter of the fermentation residue at each sampling time

Substrate	Time	H_2	Gas	pH	Short chain fatty acids (µmol)						Neutral sugars (%)						Total	M/G
	(h)	(ml)	(ml)		C2	C3	iC4	C4	iC5	C5	Rha	Ara	Gal	Glu	Xyl	Man	NS (%)	Ratio
LB	0	0.00	0.0	6.7	46.2	9.2	0.0	8.7	1.2	5.1	0.3	0.7	4.6	1.9	0.7	11.3	19.5	2.44
	2	0.06	14.9	6.7	249.5	27.2	14.6	42.5	4.2	8.0	0.2	0.4	2.4	2.4	0.5	5.1	11.0	2.14
	4	0.12	22.4	6.5	457.3	71.1	2.9	99.6	25.3	12.6	0.3	0.5	0.7	2.5	0.6	1.0	5.7	1.36
	6	0.08	22.9	6.6	404.4	104.7	1.9	99.8	28.1	7.9	0.3	0.5	0.6	1.9	0.6	0.7	4.6	1.15
	8	0.04	24.0	6.6	475.6	189.3	2.5	111.5	26.8	8.3	0.3	0.5	0.6	1.7	0.6	0.6	4.3	1.03
	24	-0.02	26.3	6.5	493.1	227.4	3.8	107.1	13.8	10.6	0.3	0.5	0.6	1.6	0.6	0.5	4.1	0.85
TR	0	0.00	0.0	6.7	26.5	8.3	0.5	7.9	1.1	4.6	0.3	0.7	5.4	1.7	0.5	10.6	19.1	1.98
	2	0.07	13.7	6.7	374.3	46.5	15.6	71.9	23.5	14.6	0.2	0.5	2.8	2.5	0.6	5.1	11.7	1.82
	4	0.11	21.5	6.5	316.7	50.7	0.7	74.1	29.5	6.0	0.3	0.6	1.0	2.7	0.6	1.1	6.2	1.11
	6	0.08	23.8	6.5	399.3	101.6	2.4	105.3	39.0	8.3	0.3	0.5	0.8	1.8	0.5	0.7	4.6	0.94
	8	0.05	23.3	6.5	351.9	140.6	2.8	96.1	21.3	8.3	0.3	0.6	0.8	2.0	0.6	0.7	4.9	0.84
	24	-0.02	26.1	6.5	511.6	255.2	4.8	116.6	14.9	12.5	0.3	0.5	0.7	1.5	0.6	0.5	4.1	0.66
PR	0	0.00	0.0	6.7	24.6	8.1	1.0	7.5	1.3	3.5	0.3	0.7	6.9	1.8	0.6	7.9	18.1	1.14
	2	0.02	11.4	6.7	135.7	22.5	1.6	29.7	5.4	6.1	0.2	0.4	4.3	1.9	0.4	5.1	12.3	1.19
	4	0.05	18.1	6.6	299.2	45.9	1.5	66.5	31.2	7.4	0.2	0.4	1.0	3.1	0.4	1.0	6.2	0.91
	6	0.03	21.0	6.6	336.6	84.6	2.0	75.0	37.4	7.4	0.2	0.5	0.7	1.8	0.5	0.5	4.1	0.73
	8	0.02	22.2	6.6	373.4	139.5	2.3	77.9	33.6	8.0	0.2	0.5	0.7	1.6	0.5	0.5	4.1	0.69
	24	-0.02	25.1	6.5	559.7	307.0	5.0	105.9	15.6	12.5	0.2	0.5	0.7	1.4	0.5	0.4	3.8	0.62
Lactulose	2	0.64	16.9	6.6	420.1	39.8	12.9	62.7	0.0	3.8	0.2	0.5	8.9	3.3	0.5	0.7	14.1	
	4	1.63	33.4	6.2	563.7	66.7	0.0	108.4	11.9	5.3	0.3	0.5	1.8	4.1	0.6	0.4	7.7	
	6	0.13	4.6	6.3	696.2	122.5	2.1	138.9	34.2	7.2	0.3	0.5	1.1	2.6	0.5	0.4	5.4	
	24	0.00	36.6	6.0	606.0	119.6	6.6	163.1	9.1	28.7	0.3	0.5	1.1	1.8	0.6	0.4	4.7	
Blank	2	0.00	9.1	6.8	34.2	12.5	0.7	17.1	2.9	1.9	0.2	0.5	0.8	1.7	0.6	0.4	4.2	
	4	0.00	9.1	6.8	46.4	15.6	1.7	15.4	3.2	2.2	0.2	0.6	0.7	1.4	0.6	0.4	3.9	
	6	0.01	10.1	6.8	55.4	18.6	1.2	17.3	3.9	2.9	0.2	0.5	0.7	1.2	0.5	0.3	3.5	
	24	0.01	7.8	6.8	135.8	47.2	6.0	35.4	8.8	6.2	0.2	0.5	0.6	1.1	0.5	0.3	3.2	

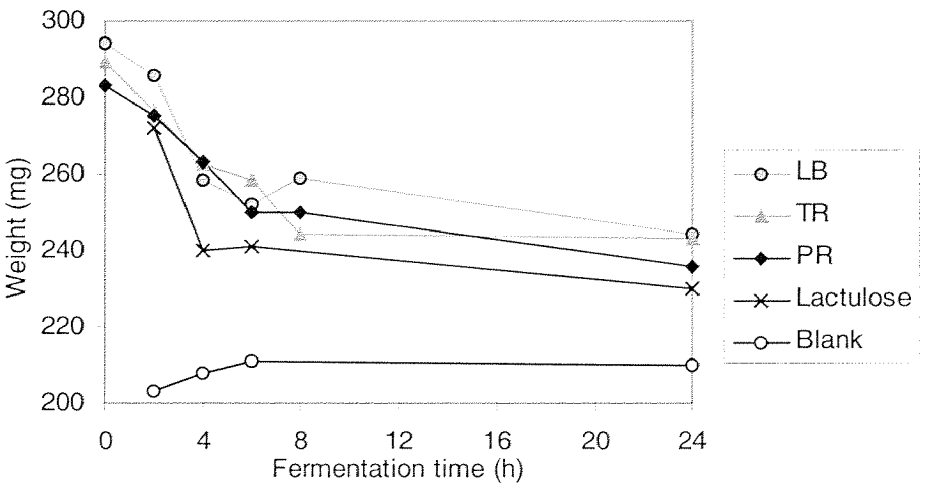

Figure A1: Weight of fermentation residues (corresponds to Fermentation 3)

Curriculum Vitae

1963	Born on June 2nd in Lima, Peru
1971-1980	Primary and secondary school in Sullana, Peru
1981-1986	Studies in Industrial Engineering at the University of Piura, Peru, leading to the degree of "Ingeniero Industrial". Degree's thesis carried out at the Department of Chemistry
1987	Single courses in Food Sciences at the University of Milan, Italy
1988	Research assistant at the Laboratory of Chemistry, Univ. of Piura
1989-1993	Faculty lecturer in chemistry and principles of food engineering at the University of Piura
1994-1995	Research assistant at the Institute of Food Engineering, University of Karlsruhe, Germany, as participant of the 30th International Seminar for Research and Teaching in Physical Chemistry and Chemical Engineering
1995-1999	Ph.D. student and research assistant at the Institute of Food Science of the ETH Zurich - Laboratory of Food Chemistry, Prof. Dr. R. Amadò. A part of the work was carried out at the University of Piura