Tragacanth Gum: Structural Composition, Natural Functionality and Enzymatic Conversion as Source of Potential Prebiotic Activity

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Tragacanth Gum: Structural Composition, Natural Functionality and Enzymatic Conversion as Source of Potential Prebiotic Activity

Hassan Ahmadi Gavlighi

PhD Thesis

2012
Preface

In the name of Allah, the Most Gracious and the Most Merciful

This thesis is submitted in candidacy for the PhD degree from the Technical University of Denmark (DTU). This PhD thesis contains the result of research undertaken at the Department of Chemical and Biochemical Engineering, DTU from 1st of June 2009 until 30th of November 2012 under supervisions of Prof. Jørn D. Mikkelsen and Prof. Anne S. Meyer. The PhD study was financed by scholarships from the Ministry of Science, Research and Technology of Iran and Tarbiat Modares University.

The most experimental work was done at the Center for Bioprocess Engineering. I thank the department for providing the facilities for conducting my experimental work.

I am immensely pleased to place on record my profound gratitude and heartfelt thanks to my supervisors, Prof. Jørn D. Mikkelsen and Prof. Anne S. Meyer for their guidance during my research at DTU and provided inspiring guidance for the successful completion of my research work. I am forever grateful to my friend Dr. Mohammad Amin Mohammadiifar for their constant support and encouragement throughout my research work. Further, I would like to thanks my colleagues, Dayang N.A. Zaidel who helped me in emulsion part and Malwina Michalak who assisted for prebiotic test.

At this Juncture I think of my parents whose selfless sacrificial life and their great efforts with pain and tears and unceasing prayers has enabled me to reach the present position in life. Especially, I would like to give my special thanks to my wife Farzaneh whose patient love enabled me to complete this work. Finally, I thank all those who have helped me directly or indirectly in the successful completion of my thesis in BioEng.

Hassan Ahmadi Gavlighi

November, 2012
Gum tragacanth derived from the plant (*Astragalus* sp.) has a long history of use as a stabilizing, viscosity-enhancing agent in food emulsions. The gum is mainly produced in the Middle East, and permitted for food use in the US as well as in Europe (E-number E413). Gum tragacanth is known to confer very high viscosities when in aqueous solution, and is described as a complex, highly branched, heterogeneous hydrophilic polysaccharide. The gum contains pectinaceous arabinogalactans and fucose-substituted xylogalacturonans. The objective of this PhD study were to evaluate tragacanth samples from six species of Iranian *Astragalus* for their emulsion stabilizing effects and their detailed chemical composition in order to examine any possible correlation between the make-up and the emulsion stabilizing properties of gum tragacanth. Also, enzymatic modification of highly fucose content of tragacanth gum and separation via membrane technique to get different molecular size. Furthermore, examination of compositional structure and effect of different molecular size on potential prebiotic was evaluated.

The first part of the present study was selected of six different species of *Astragalus* and exudates of gum and fractionated by centrifugation to soluble and insoluble. To examine correlation between composition structure, sugar composition and methoxyl and acetyl content was determined. The six gum samples varied with respect to their levels and ratios of water-soluble and water-swellable fractions, their monosaccharide composition, methoxylation, and acetylation degrees. Emulsion and rheological properties of different gum solution was assessed with WPI as an emulsifier in protein base emulsion and correlation of each composition on emulsion stability was established. Tragacanth gum solution added in emulsion and without emulsion showed shear thinning properties in all gums. The emulsion stabilization effect correlated linearly and positively to the methoxylation degree, and galacturonic acid content of the gums, but not to acetyl or fucose content. A particularly high correlation was found between methoxyl level in the soluble gum part and emulsion stabilization.

The results of this work provide some important clues to the emulsion stabilization mechanisms in relation to the structure composition of tragacanth gums.

From our knowledge and many research for application of this gum in food industry and unique properties of this gum with arabinogalactan and fucoxylogalacturonans in the structure of we decided to evaluate bioactivity of this gum. To date, different commercial of prebiotic compound available but still new compound is needed and interested. The main process for the production of prebiotic is enzymatic process. Thus, the next study of work was using commercial pectinolytic enzyme to get different molecular size and purified with membrane technique and get three different fraction: HAG1 < 2 kDa; 2 kDa < HAG2 < 10 kDa; HAG3 > 10 kDa. HPAEC results shown that these three fractions varied with respect to composition and HAG1 and HAG2 were enriched in arabinose, galactose, and galacturonic acid, but low in fucose and xylose; whereas HAG3 was high in xylose, fucose and galacturonic acid, but low in arabinose and galactose. The structural composition of different fractions with linkage analysis shown that the structure of gum tragacanth fractions was different and included 1,4-bonded galacturonic acid backbone with terminally linked fucose and (1,2)-linked xylose, as well as terminally linked xylose called
fucoxylogalacturonan. In addition, the presence of (1,4)-galactose linkages and 1,5 Ara linkage presumably correspond to arabinogalactan-derived galactan.

Determination of prebiotic effect of different fraction in vitro were assessed on seven different probiotic strains in single culture fermentations on: Bifidobacterium longum subsp. longum (2 strains), B. longum subsp. infantis (3 strains), Lactobacillus acidophilus, B. lactis, and on one pathogenic strain of Clostridium perfringens. The fractions HAG1 and HAG2 consistently promoted higher growth of the probiotic strains than HAG3, especially of the three B. longum subsp. infantis strains, and the growth promotion on HAG1 and HAG2 was better than that on galactan (control). HAG3 completely inhibited the growth of the Cl. perfringens strain.

In summary of this study:

- Emulsion stabilization of the gum is related to the gum composition and structure, and mainly galacturonic acid content and degree of esterification are important
- Low molecular size oligosaccharides produced enzymatically has higher potential prebiotic activity than longer chain gum saccharides
- Tragacanth gum can be a new source for development of innovative functional foods with health claims
Dansk Sammenfatning

forskellig fraktion in vitro blev vurderet på syv forskellige probiotiske stammer i enkelt kultur fermenteringer om: Bifidobacterium longum subsp. longum (2 stammer), B. longum subsp. infantis (3 stammer), Lactobacillus acidophilus, B. lactis, og på en patogen stamme af Clostridium perfringens. De fraktioner HAG1 og HAG2 konsekvent fremmes højere vækst i de probiotiske stammer end HAG3, især af de tre B. longum subsp. infantis stammer, og væksten fremme på HAG1 og HAG2 var bedre end på galactan (kontrol). HAG3 fuldstændigt inhiberede væksten af Cl. perfringens stamme.

I sammenfatning af denne undersøgelse:
• Emulsion stabilisering af gummet er relateret til gummysammensætning og struktur, og fortrinsvis galacturonsyre indhold og grad af esterificering er vigtige
• lavmolekylære størrelse produceres oligosaccharider enzymatisk har større potentielle præbiotisk aktivitet end længere kæder gum saccharider
• traganth kan være en ny kilde til udvikling af innovative funktionelle fødevarer med sundhedsanprisninger
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The present thesis is based on the work contained in the following papers:

I. Stabilization of emulsions by gum tragacanth (Astragalus spp.) correlates to the galacturonic acid content and methoxylation degree of the gum
Hassan Ahmadi Gavlighi, Anne S. Meyer, Dayang N.A. Zaidel, Mohammad Amin Mohammadifar and J. Dalgaard Mikkelsen, *Food Hydrocolloids*, Volume 31, Issue 1, May 2013, Pages 5-14

II. Enzymatic depolymerization of gum Tragacanth: Prebiotic, bifidogenic potential of low molecular weight oligosaccharides
Hassan Ahmadi Gavlighi, Malwina Michalak, Anne S. Meyer and J. Dalgaard Mikkelsen, Submitted to *Journal of Agricultural and Food Chemistry*

III. Compositional analysis and rheological characterization of gum tragacanth exudates from six species of Iranian Astragalus

IV. Tragacanth gum: Functionality and new prebiotics potential
Hassan Ahmadi Gavlighi, Anne S. Meyer and J. Dalgaard Mikkelsen, Submitted to *Agro FOOD industry hi-tech*

Paper not included in the PhD thesis

V. Enhanced enzymatic cellulose degradation by celllobiohydrolases via product removal
Hypothesis and objectives

These PhD study work was built on analysis structural and composition one of exudates gum namely, tragacanth gum, from six different species and explain mechanisms of emulsions stabilization based on structure. Also, because of unique composition on it, healthy beneficial effect of enzymatic product is examined.

The hypothesis of these works is:

- It is different species of gum tragacanth has different chemical composition, physicochemical and functional properties
- It is relationship between the make-up of gum tragacanth and its stabilization in emulsions
- It is possible to produce of different molecular size of gum tragacanth
- Tragacanth gum products has potential prebiotic activity effect
- There is relationship between molecular size and bioactivity of enzymatic products

Aim of these PhD work:

- To analysis of the chemical composition of tragacanth gum obtained from six Astragalus species and also soluble and insoluble fraction
- To make correlations between compositional structure of gums and emulsion stability
- To elucidate of mechanism of the stabilization of six gums in protein based emulsion
- To establish process to produce of different molecular size with different composition via enzymatic method
- To examine enzymatic products for bioactivity effect with pure culture of healthy and pathogen bacteria
1. Introduction

1.1 Emulsion

An emulsion is an immiscible dispersion of one liquid in another. Many food products such as soft drinks, milk, cream, salad dressings, mayonnaise, soups, sauces, dips, butter and margarine exist in the form of an emulsion. Emulsions are classified in two groups: A: A system that consists of oil droplets dispersed in an aqueous phase is called an oil-in-water or O/W emulsion, for example, milk, cream, dressings, mayonnaise, beverages, soups, and sauces. B: A system that consists of water droplets dispersed in an oil phase is called a water-in-oil or W/O emulsion, for example, margarine and butter. An emulsions are produced by homogenizing oil and aqueous phases together in the presence of one or more emulsifiers (Guzey & McClements, 2006). Emulsions are metastable systems that tend to destabilize through a number of mechanisms (creaming, sedimentation, coalescence and flocculation) (McClements, 2004). Therefore, increasing emulsion stability is a key factor for its commercial applications. The use of emulsifiers such as proteins or surfactants is essential for stabilization of emulsions. Many proteins are surface-active molecules that can be used as emulsifiers because of their ability to facilitate the formation, improve the stability and produce desirable physicochemical properties in oil-in-water emulsions. Proteins adsorb to the surfaces of freshly formed oil droplets created by homogenization of oil–water–protein mixtures, where they facilitate further droplet disruption by lowering the interfacial tension and retard droplet coalescence by forming protective membranes around the droplets. The ability of proteins to generate repulsive interactions (e.g., steric and electrostatic) between oil droplets and to form an interfacial membrane that is resistant to rupture also plays an important role in stabilizing the droplets against flocculation and coalescence during long-term storage (Home, 1996).

Furthermore, most of hydrocolloids have been widely used in the food industry for stabilizing emulsions through viscosity effects, steric hindrance and electrostatic interactions but only a few can act as emulsifiers (emulsifying agents). Among the most common stabilizers are such hydrocolloids as xanthan, gum arabic, modified starches, pectin, carrageenan and tragacanth gum (Sima Balaghi, et al., 2011; Eric Dickinson, 2009). It has been shown that presence of some polysaccharides, such as arabic gum, at certain concentration enhances the rate of creaming of dispersed droplets due to depletion flocculation (Chamani & McClements, 2001). As stabilizer in food emulsions, some gums were found to migrate slowly to air–water and oil–water interfaces and exhibit some surface and interfacial activities (Garti, 1999). These researchers have suggested that gums participate or adsorb onto oil droplets and sterically stabilise emulsions against flocculation and coalescence. However, it was shown that both adsorbing and non-adsorbing hydrocolloids are capable of stabilising the system. The term ‘adsorbing’ is related to charged polysaccharides, which can interact with proteins via electrostatic forces and the interaction is highly dependent on pH and ionic strength of solution. In case of using this type of hydrocolloids, stability of system is due to electrostatic repulsion, steric repulsion or both of them. On the other hand, ‘non-adsorbing’ hydrocolloids can prevent serum separation by increasing the viscosity of continuous phase, entrapping water in a network and immobilising the particles (Azarikia & Abbasi, 2010).
In general terms, the stability properties of emulsions prepared with mixtures of proteins and polysaccharides can be related to the thermodynamics of the mixed biopolymer solutions at the corresponding pH, ionic strength and protein: polysaccharide ratio (Eric Dickinson, 2008).

**Figure 1.1** Physical mechanisms of destabilization of food emulsions: creaming, sedimentation, flocculation, coalescence, and phase inversion (McClements, 2004).

### 1.2 Particle size distribution

Particle size of fat globules (oil phase) and their size distribution play a predominant role in deciding the stability of emulsion and emulsion-based products with precisely controlled particle size exhibit better emulsion stability. Creaming, and more specifically the creaming rate, is directly affected by the size of droplets. The stability of an emulsion to gravitational separation can be enhanced by reducing the droplet size. As a rule, large globules tend to coalesce faster than the small ones (McClements, 2004). Many of the most important properties of emulsion-based food products are determined by the size of the droplets that they contain, for example, shelf life, appearance, texture, and flavor. Also, stability of the emulsion in the finished products can be predicted by determination particle size (E. Dickinson & McClements, 1996). Consequently, it is important for food scientists to be able to reliably control, predict, measure, and report the size of the droplets in emulsions. Many techniques have been developed to measure droplet size distribution; the most used techniques are microscopy, light scattering, ultrasonic methods and more recently low-resolution NMR (Denkova, et al., 2004).

If all the droplets in an emulsion are of the same size it is referred to as a *monodisperse* emulsion, but if there is a range of droplet sizes present it is referred to as a *polydisperse* emulsion (Fig 1.2).
Figure 1.2 Schematic representation of monodisperse and polydisperse emulsions. In a monodisperse emulsion all the droplets have the same size, but in a polydisperse emulsion they have a range of different sizes.

A widely used method for determination of particle size is light scattering method that expressing the mean particle size is the area–volume mean diameter ($d_{32}$), which is related to the average surface area of droplets exposed to the continuous phase per unit volume of emulsion. Another commonly used method of expressing the mean particle size of a polydisperse emulsion is the volume–length diameter ($d_{43}$), which is the sum of the volume ratio of droplets in each size-class multiplied by the mid-point diameter of the size-class. It should be noted that $d_{43}$ is more sensitive to the presence of large particles in an emulsion than $d_{32}$, hence it is often more sensitive to phenomenon such as flocculation (McClements, 2004).

1.3 Creaming characterization of emulsion

Creaming or sedimentation process occurring in emulsion can be easily assessed by optical observations. Indeed, in most cases, creaming is characterized by a whitish/yellowish layer at the top of emulsion, while a layer appears at the bottom of an emulsion if sedimentation occurs. Creaming/sedimentation rate can be determined by measuring the volume of cream/sediment in the emulsion with time. This can be done by placing the emulsion in a tube and measuring the height of the cream/sediment. In some cases, visual observations are not accurate enough to measure the creaming then used to measure the creaming rate, using light scattering.

1.4 Emulsion Preparation methods

There are two alternative ways in which emulsion droplets can be stabilized by protein–polysaccharide complexes. These are illustrated schematically in (Figure 1.3). Method A involves first preparing a primary emulsion with the protein as the sole emulsifier, and then adding the charged polysaccharide to the aqueous phase of the emulsion to
produce a secondary emulsion of droplets having a protein–polysaccharide ‘bilayer’ surface coating. Method B involves first preparing a bulk aqueous solution of the protein–polysaccharide complex, and then using the complex as the emulsifying agent during subsequent homogenization. Method A approach has attracted considerable attention recently used as potential for Nano scale encapsulation of nutrients and protection of emulsions against severe environmental stresses (Eric Dickinson, 2008).

![Illustration of two alternative procedures for stabilization of oil droplets by protein–polysaccharide complexes (highly schematic): (A) ‘bilayer emulsion’ preparation, with polysaccharide (Po) added after prior emulsification with protein (Pr); (B) ‘mixed emulsion’ preparation, with both biopolymers present together during emulsification (Eric Dickinson, 2008).]

1.5 Whey protein as emulsifier

A wide variety of proteins are also used as emulsifiers in foods because they naturally have a high proportion of nonpolar groups and are therefore surface-active (Damodaran, Parkin, & Fennema, 2008; Eric Dickinson, 1992). Whey protein is one of the emulsifiers frequently used in foods because of its ability to facilitate the formation and stabilization of oil-in-water emulsions (Eric Dickinson, 1997). The ability of whey protein to form stable emulsions depends on emulsion composition (including pH and mineral content, salt, sugar, surfactant, and polysaccharide contents) and environmental conditions (temperature and pressure) (Demetriades, Coupland, & McClements, 1997; Eric Dickinson, 1992; Ye & Singh, 2000). Whey proteins are therefore suitable for application in food emulsions where the composition and environmental conditions favor a stable product, but not in those products where the conditions promote emulsion instability (Chanamai & McClements, 2002).
1.6 Rheology
Rheology is defined as “the science of deformation and flow of matter.” (Macosko & Larson, 1994). Knowledge of the rheological behavior of food products is essential for process design and evaluation, quality control, and consumer acceptability. In addition, used by food scientists as an analytical tool to provide fundamental insights about the structural organization and interactions of the components within emulsions, for example, measurements of viscosity versus shear rate can be used to provide information about the strength of the colloidal interactions between droplets (Quemada & Berli, 2002).

1.7 Classification of rheological behavior
The major types of fluid flow behavior can be described by means of basic shear diagram of shear rate versus shear stress (Figure 1.4).

**Newtonian Behavior:** With Newtonian fluids, the shear rate is directly proportional to the shear stress and the plot begins at the origin. Typical Newtonian foods are those containing compounds of low molecular weight (e.g., sugars) and that do not contain large concentrations of either dissolved polymers (e.g., pectins, proteins, starches) or insoluble solids. Examples of Newtonian foods include water, sugar syrups, most honeys, most carbonated beverages, edible oils, filtered juices and milk.

**Shear-Thinning Behavior:** With shear-thinning fluids, the curve begins at the origin of the shear stress-shear rate plot but is concave upwards, that is, an increasing shear rate gives a less than proportional increase in shear stress. Most non-Newtonian foods exhibit shear thinning behavior, including many salad dressings and some concentrated fruit juices.

**Shear-Thickening Behavior:** In shear-thickening behavior also, the curve begins at the origin of the shear stress shear rate plot and is concave downwards, that is, an increasing shear stress gives a less than proportional increase in shear rate. This type of flow has been encountered in partially gelatinized starch dispersions (Rao, 2007).
On the other hand, rheological models used to describe the behavior of fluids. There are different models that can be used based on flow behavior such as power law that is widely used as a model for materials of shear thinning fluids behaviour:

$$\eta = m\dot{\gamma}^{(n-1)}$$

Where $\eta$ is shear viscosity, $\dot{\gamma}$ is shear rate $m$ is the consistency index and $n$ is the flow behaviour index (Tischer, Iacomini, & Gorin, 2002). The model can describe a Newtonian, shear-thinning and shear-thickening behaviour, depending on the value of the flow behaviour index, $n$. For a Newtonian material, $n$ is equal to 1, and the equation reduces to the Newtonian model. If $n$ is less than 1, the fluid is shear thinning, whereas if it is greater than 1, then the fluid is shear thickening (dilatant) (Miri, 2011). Shear-thinning behavior is very common in fruit and vegetable products, polymer melts, as well as cosmetic and toiletry products. During flow, these materials may exhibit three distinct regions (Figure 1.5): a lower Newtonian region where the apparent viscosity ($\eta_0$), called the limiting viscosity at zero shear rate, is constant with changing shear rates; a middle region where the apparent viscosity ($\eta$) is changing (decreasing for shear-thinning fluids) with shear rate and the power law equation is a suitable model for the phenomenon; and an upper Newtonian region where the slope of the curve ($\eta_\infty$), called the limiting viscosity at infinite shear rate, is constant with changing shear rates. The middle region is most often examined when considering the performance of food processing equipment.
Figure 1.5 Rheogram of idealized shear-thinning (pseudoplastic) behavior (Steffe, 1996).
2. Gum Tragacanth

2.1 History
Gum tragacanth was first described by Theophrastus several centuries before Christ. The name "tragacanth" comes from the appearance of the exuded gum, which tends to form ribbons similar in appearance to a goat horn (from the Greek "tragos" meaning goat and "akantha" meaning horn). The gum is obtained from small shrubs of the *Astragalus* genus, are small, low bushy perennial shrubs having a large tap root along with branches, and grow wildly in the dry deserts and mountainous regions of South West Asia, from Pakistan to Greece, and in particular, in Iran and Turkey (Whistler, 1993). The main areas of commercial production are the arid and mountainous regions of Iran (accounting for ~70% of the supplies). Fifty years ago, Iran exported annually more than 4,000 t, but in the 1970s and 1980s this amount greatly decreased, due to a number of reasons (Al-Tamimi, Palframan, Cooper, Gibson, & Rastall, 2006). At present, the world market for gum tragacanth is estimated to be no more than 500 t/year (about 300 t) (FAO 1995). Plants develop a mass of gum in the centre of the root, which swells in the summer heat. If the stem is slit, soft gum is exuded. The gum exudes readily from these cuts in the form of ‘ribbons’ or ‘flakes’ which become brittle on drying.

2.2 Structure
Gum tragacanth is a highly branched, heterogeneous hydrophilic carbohydrate with polymer. The molecular weight is about 840 kDa. It is a complex, slightly acidic polysaccharide bounded with small proportions of protein (below than 4%) (S. Balaghi, Mohammadifar, & Zargaraan, 2010), and with trace amounts of starch and cellulosic material present. Calcium, magnesium and potassium are the associated cations (Anderson & Grant, 1988). After acid hydrolysis, gum tragacanth commonly produces sugars of D-galacturonic acid, D-galactose, L-fucose (6-deoxy-L-galactose), D-xylose, L-arabinose, L-rhamnose. The exact proportion of each sugar varies between different species and in gums from different locations (Sima Balaghi, et al., 2011). The easy separation of tragacanthin and bassorin suggests that the two polysaccharides are in a physical mixture and not chemically bonded (Lapasin & Pricl, 1995). It has been reported that gum tragacanth consists of two fractions (Figure 2.1) (Phillips & Williams,). One fraction, termed ‘Tragacanthic acid’ or *Bassorin* which represents 60–70% of the total gum with a molar mass of approximately \(10^5\) Da, though insoluble in water, has the capacity to swell and form a gel. Another small fraction, termed *Tragacanthin* is soluble in water with a molar mass of approximately \(10^4\) Da (Elias, 1992) to give a colloidal, hydrosol solution. Bassorin, a pectic component, has a chain of (1-4)-linked \(\alpha\)-D-galacturonic acid units some of which are substituted at O-3 with \(\beta\)-D-xylopyranosyl units and some of these being terminated with D-Gal or L-Fuc (Table 1) (Phillips & Williams, 2009).

The water soluble tragacanth is reported as a neutral, highly branched arabinogalactan (of type II) comprising (1-6)- and (1-3)- linked core chain containing galactose and arabinose (both in furanose and pyranose forms) and side groups of (1-2)-, (1-3)- and (1-5)-linked arabinose units occurring as monosaccharide or oligosaccharides (Table2.1) (Tischer, et al., 2002). Depending on the species, the ratio of the water-swellable to the water-soluble fraction varies (Sima Balaghi, et al., 2011).
Figure 2.1 Gum tragacanth fractions (Sima Balaghi, et al., 2011)

Linkage analysis of the gum tragacanth showed that this material was composed of mostly terminal substituted Fucp, 2-Xylp and 4-GalAp that made up fucoxyllogalactronan structure in gum tragacanth and the results agree with those structure previously presented by Aspinall & Baillie (1963) (Figure 2.2). Also, the presence of 1,5 Araf, 1,4-Galp and 1,3,6 Galp linkages in the structure indicate of occurrence arabinogalactan in the tragacanth gum (Tischer, et al., 2002).

Figure 2.2 Structure of partial part of gum tragacanth structure (Aspinall & Baillie, 1963)
Table 2.1 Linkage analysis of gum tragacanth (Astragalus gossypinus)

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>Linkage type</th>
<th>% peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Fucose</td>
<td>t-Fucp</td>
<td>23.9(69.3)</td>
</tr>
<tr>
<td></td>
<td>1,3-Fucp</td>
<td>2.8(8.1)</td>
</tr>
<tr>
<td></td>
<td>1,4-Fucp</td>
<td>3.2(9.3)</td>
</tr>
<tr>
<td></td>
<td>1,2-Fucp</td>
<td>3.8(11)</td>
</tr>
<tr>
<td></td>
<td>1,3,4-Fucp</td>
<td>0.8(2.3)</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>t-Rhap</td>
<td>0.1(3.3)</td>
</tr>
<tr>
<td></td>
<td>1,2,4 Rhap</td>
<td>2.7(90)</td>
</tr>
<tr>
<td></td>
<td>1,4 Rhap</td>
<td>0.2(6.7)</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>t-Araf</td>
<td>5.2(53.6)</td>
</tr>
<tr>
<td></td>
<td>1,5 Araf</td>
<td>3.4(35)</td>
</tr>
<tr>
<td></td>
<td>1,2,5 Araf</td>
<td>1.1(11.4)</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>t-Galp</td>
<td>1.7(29.8)</td>
</tr>
<tr>
<td></td>
<td>1,4-Galp</td>
<td>2.6(45.6)</td>
</tr>
<tr>
<td></td>
<td>1,6 Galp</td>
<td>0.2(3.5)</td>
</tr>
<tr>
<td></td>
<td>1,4,6 Galp</td>
<td>0.1(1.8)</td>
</tr>
<tr>
<td></td>
<td>1,3,6 Galp</td>
<td>1.1(19.3)</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>t-Glup</td>
<td>0.2(11.8)</td>
</tr>
<tr>
<td></td>
<td>1,4-Glup</td>
<td>1.4(82.3)</td>
</tr>
<tr>
<td></td>
<td>1,2,4-Glup</td>
<td>0.1(5.9)</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>t-Xylp</td>
<td>7.2(21.2)</td>
</tr>
<tr>
<td></td>
<td>1,2,4-Xylp</td>
<td>0.8(2.4)</td>
</tr>
<tr>
<td></td>
<td>1,2-Xylp</td>
<td>24.9(73.5)</td>
</tr>
<tr>
<td></td>
<td>1,2,3-Xylp</td>
<td>1(2.9)</td>
</tr>
<tr>
<td>D-Galacturonic acid</td>
<td>t-GalAp</td>
<td>0.1(3.3)</td>
</tr>
<tr>
<td></td>
<td>1,4-GalAp</td>
<td>2.8(93.3)</td>
</tr>
<tr>
<td></td>
<td>1,4,6-GalAp</td>
<td>0.1(3.3)</td>
</tr>
</tbody>
</table>
2.3 Composition, structure and molecular size

Fully understand of functional and biological properties of polysaccharides needs to use special methods to find relation between structure and functional properties. Hence, the approach to assessment of quantitative of the monosaccharaides composition of gum tragacanth was done by means of acid hydrolysis (TFA 2M, 121 °C for 2 h) where the efficiency of this method has been established previously by Arnous and Meyer (2008). The monosaccharide content of the hydrolysates was determined with the chromatography method HPAEC-PAD. The separation and the quantification method using HPAEC-PAD were accomplished in one single run without the need for pre-derivative treatment.

To determine effect molecular size of oligomers produce via enzymatic reaction on bioactivity properties of gum tragacanth, High-performance size exclusion chromatography (HPSEC) has been done. The separation principle is achieved by the differential exclusion from the pores of the packing material, of the sample molecules as they pass through a bed of porous particles (Rasmussen & Meyer, 2009). The elution of polysaccharide is then achieved by the time that larger molecules takes less time in the pores elute first and the smaller molecules elute later (Cutié & Martin, 1995).

The complete structural elucidation of polysaccharide and enzymatic hydrolysis of oligomers has been done by methylation analysis to reveal essential structural information on structure and linkage. This method (an ingenious approach for determining linkage positions) was developed in the late 1960s and early 1970s. For glycosyl linkage analysis, the sample was permethylated (Ciucanu & Kerek, 1984), depolymerized, reduced, and acetylated; and the resulting partially methylated alditol acetates (PMAAs) analyzed by gas chromatography-mass spectrometry (GC-MS) as described by York, et al., (1986). The PMAAs were analyzed on a Hewlett Packard 5975C GC interfaced to a 7890A MSD (mass selective detector, electron impact ionization mode); separation was performed on a 30m Supelco 2330 bonded phase fused silica capillary column. However, the analyses of these linkage data are sophisticated, usually incomplete and difficult, at least because of the following reasons: (1) most polysaccharides are heterogeneous with high molecular weights, (2) there are many combinations of linkage types between any two monosaccharides, and (3) there is a lack of a complete set of standard materials of all linkage types that could be used as reference standards (Lo, Kang, Wang, & Chang, 2007).

2.4 Functional properties, stability and application

Gum tragacanth solutions are acidic, usually in the pH range of 5-6. Its maximum initial viscosity is at ph 8, but usually exhibited maximum stability near pH 5 (Schwarz, Levy, & Kawagoe, 1958). So compared to other gums, tragacanth gum is fairly stable over a wide pH range down to extremely acidic conditions at about pH 2 (Levy & Schwarz, 1958).

In spite of the availability of alternative materials, the continued use of the gum is the result of its unique functional properties (viscosity and emulsification) combined with a high degree of stability in a range of conditions. The flow behavior of six species of Iranian gum tragacanth dispersions was investigated and results shown that all of the gum dispersions had shear-thinning natures. Depending on the species of the gum, the viscosity of 1.5% solutions range from 1.66 up to 34.6 Pa s (S. Balaghi, et al., 2010).
Gum tragacanth, regarded as a bifunctional emulsifier, is a most efficient natural emulsifier for acidic oil-in-water emulsions. Gum tragacanth has well-defined surface activity properties and produces a rapid lowering of the surface tension of water at low concentration, less than 0.25% and facilitate emulsification (S. Balaghi, et al., 2010; karaya, Phillips, & Williams, 2000). It thickens the aqueous phase and also lowers the interfacial tension between oil and water. Dickinson et al., (1988) working with gum arabic has shown that polypeptide present is involved with the surface activity and emulsification properties.

The use of gum tragacanth in foods has to be in accordance with the FDA Code of federal regulations (title 21, section 184.1351; Table2.2). Gum tragacanth, a highly acid-resistant hydrocolloid, has been accepted since 1961 as GRAS at the level of 0.2–1.3% (Anderson & Bridgeman, 1985) and in Europe, gum tragacanth has E-number E413 on the list of additives approved by the Scientific Committee for Food of the European Community. It has been used for many years as a stabiliser, thickener, emulsifier and suspending agent in the food, pharmaceutical, cosmetic, textile and leather industries as well as in technical applications based on its high viscosity at low concentration, good suspending action, unusually high stability to heat and acidity and effective emulsifying properties. It also is pourable and has a creamy mouth feel and good flavour-release properties (Phillips & Williams, 2009) and very long shelf life (Levy, et al., 1958). Gum tragacanth is used in the food industry in citrus oil emulsions (Taherian, Fustier, & Ramaswamy, 2008), salad dressings, condiments, sauces, bakery emulsions, oil and flavour emulsions, bakery fruit-based fillings and toppings (give a shiny, clear appearance and a creamy texture) (Whistler, 1993), confectionery, soft drinks, jellies, desserts, ice creams (provide texture to the product), flavours, spices (Phillips & Williams, 20). In chewy sweets, such as lozenges, it acts as a thickener and provides texture. The gum is used in fruit tablets, gum drops, and pastilles as a binding agent during compression. It also provides body and mouthfeel and ensures good flavor-release during consumption. In icings, gum tragacanth is used as a water binder, maintaining pliability and preventing cracks and breaks. It also provides consistency, smooth texture, and creamy taste to the product (Verbeken, Dierckx, & Dewettinck, 2003).

Taherian et al. (2008) reported that the emulsions produced based on arabic gum and tragacanth have a greater stability than the emulsions containing arabic gum and xanthan which was related to higher surface activity and greater acid and heat resistances by tragacanth gum. Recently, the use of gum Tragacanth to maintain the quality of bell peppers (one of the most important commercial vegetables) during long-term storage has been recommended (Mohebbi, Amiryousefi, Hasanpour, & Ansarifar, 2012). Gum tragacanth addition to nonfat fermented milk drink (doogh) was found beneficial for improving physical properties and prevents serum separation (Gorji, Mohammadifar, & Ezzatpanah, 2011). Use of gum tragacanth in dairy cream fat showed that fat reduction from 30 wt% to 14 wt% without significant changes to sensory properties, shelf life or packaging requirements (Nasirian, Vaziri, Safekordi, & Ardjmand, 2010).

2.5 Mechanism of tragacanth gum stabilization

There have been different elucidations to how gum tragacanth can stabilize of different emulsion system. Yokoyama, Srinivasan and Fogeler (1988) shown that the stabilization effect of tragacanth is a result of the steric repulsion force and the stability can be controlled by changing pH. (Yokoyama, Srinivasan, & Fogler, 1988). On the other hand, the ability of tragacanth gum in stabilizing the beverage emulsions could be due to its residual surface
activity and enhancement of emulsion viscosity (Rezvani, Schleining, & Taherian, 2012). Azarikia, et al. (2010) stated that tragacanth gum has zeta potential around -21 mV and could stabilize doogh (Iranian yoghurt drink) via electrostatic interactions. This phenomenon has been related to the negatively charged carboxylic groups of galacturonic acid as the main backbone of tragacanth as the soluble part of the tragacanth gum. In addition, they reported that bassorin is probably unable to interact with caseins, and its main effect on the stabilization of doogh is to increase the viscosity of the continuous phase.

Table 2.2 Maximum usage levels (%) of gum tragacanth permitted in accordance with the FDA Code of federal regulations

<table>
<thead>
<tr>
<th>Food (as served)</th>
<th>Percentage</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods and baking mixes</td>
<td>0.2</td>
<td>Emulsifier, emulsifier salt, formulation aid, stabilizer/thickener</td>
</tr>
<tr>
<td>Condiments and relishes</td>
<td>0.7</td>
<td>Emulsifier, emulsifier salt, formulation aid, stabilizer/thickener</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>1.3</td>
<td>Emulsifier, emulsifier salt, formulation aid, stabilizer/thickener</td>
</tr>
<tr>
<td>Gravies and sauces</td>
<td>0.8</td>
<td>Emulsifier, emulsifier salt, formulation aid, stabilizer/thickener</td>
</tr>
<tr>
<td>Meat products</td>
<td>0.2</td>
<td>Formulation aid, stabilizer/thickener</td>
</tr>
<tr>
<td>Processed fruits and fruit juices</td>
<td>0.2</td>
<td>Emulsifier, emulsifier salt, formulation aid, stabilizer/thickener</td>
</tr>
<tr>
<td>All other food categories</td>
<td>0.1</td>
<td>Emulsifier, emulsifier salt, formulation aid, stabilizer/thickener</td>
</tr>
</tbody>
</table>

Anionic hydrocolloids (λ-carrageenan, carboxymethyl cellulose, pectin and gum tragacanth) interact with the positive charges on the surface of casein micelles and reduce the syneresis via the formation of protein–polysaccharide complexes and strengthen the casein network through a bridging mechanism. The electrostatic interactions between gum tragacanth and milk proteins have been studied in both real and model systems. The effect of pH and ionic strength on the formation of complexes between β-lactoglobulin and soluble part of gum tragacanth (exudates form A. gossypinus) suggests an electrostatic nature of their interactions (Mohammadifar, Musavi, & Williams, 2007). It is well-recognized that emulsion stabilization by whey proteins may be improved by polysaccharides (and particularly so at pH near the isoelectric point). For pectin-β-lactoglobulin complexes it has been shown that this effect occurs when the protein associates to the polysaccharide, and soluble, charged
complexes are formed, which have an overall charge as the polysaccharide (Sperber,et al., 2009). The soluble complex is thus stabilized by electrostatic repulsion. From model-studies with dextran-whey protein conjugates it appears that the polysaccharide effect is mainly due to enhanced steric stabilization provided by the bulkiness of the hydrophilic polysaccharides that provides a stabilizing charged layer around the protein stabilized oil droplets which protects the protein stabilized oil droplets against flocculation under conditions where electrostatic stabilization is less favourable (Akhtar & Dickinson, 2003).

In the summary, the tragacanth gum stabilization of protein-emulsified emulsions is probably a result of two mechanisms: Firstly, formation of non-covalent protein–(gum) polysaccharide complexes via electrostatic interaction, and secondly viscosity increase by insoluble fraction (Bassorin) (Figure2.3).

Figure2.3 The scheme of stabilization mechanism of protein-emulsified emulsions by Gum Tragacanth

2.6 Rheological characterization and particle size of tragacanth gum

Rheological characterization of polysaccharides can be importance as it provides fundamental information required for assessment of some of the final properties of a product, such as quality, storage stability, effect of formulation variables on product characteristics (Ramachandran, Chen, & Etzler, 1999). Hence, the flow behavior of six species of Iranian gum tragacanth dispersions was investigated at different temperatures and ionic strengths, within a concentration range (0.05–1.5% w/w) using a controlled shear rate rheometer. The steady shear measurements showed that all of the gum dispersions had shear-thinning natures (Figure2.4) (S. Balaghi, et al., 2010).

In order to have a better understanding about functional properties of different species of gum tragacanth, also the particle size distribution of all gum dispersions was determined. Size measurements were reported as the volume weighted mean diameter (S. Balaghi, et al., 2010):

\[ d_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i} \]

\[ d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \]

where \( n_i \) is the number of particles with diameter \( d_i \).
The results showed that different value on particle size parameters (Table 2.3). The variation in the particle size distribution of different species of gum dispersions may be attributed to differences in the swelling power of different gum particles which seems to be related to ratio of soluble to insoluble part. It was shown that multi-factors such as different amount of neutral sugars, uronic acid, and also methoxyl group content as well as molecular weight and conformational and configurational properties play their own crucial role in rheological properties.

In general, the results indicated that the six varieties of gum tragacanth studied exhibited significantly different rheological properties; therefore, these different gums may find use in a variety of applications as stabilisers, thickeners, emulsifiers and suspending agents depending on their rheological behaviour. Consequently, any research carried out on gum tragacanth or any industrial application of this gum without respect to the plant species will lead to misleading results.

Table 2.3: Particle size characteristics of six species of gum tragacanth dispersions (0.05% w/w) (S. Balaghi, et al., 2010)

<table>
<thead>
<tr>
<th>Species</th>
<th>D [3,2]</th>
<th>D [4,3]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. parrowianus</td>
<td>98.72d</td>
<td>142.50d</td>
</tr>
<tr>
<td>A. fluccosus</td>
<td>98.63d</td>
<td>136.34e</td>
</tr>
<tr>
<td>A. rahensis</td>
<td>97.70d</td>
<td>155.49d</td>
</tr>
<tr>
<td>A. gossypinus</td>
<td>143.63c</td>
<td>357.63c</td>
</tr>
<tr>
<td>A. microcephalus</td>
<td>112.43c</td>
<td>263.69b</td>
</tr>
<tr>
<td>A. compactus</td>
<td>127.60b</td>
<td>196.27e</td>
</tr>
</tbody>
</table>

*Units: micrometer

**Means with different letters within the same row differed significantly (p<0.05)
Figure 2.4 Flow curves of apparent viscosity versus shear rate of gum tragacanth suspensions in different concentrations (w/w %) at 25 °C (S. Balaghi, et al., 2010)
3. Effect of the galacturonic acid content and methoxylation degree of gum tragacanth (*Astragalus* spp.) on emulsion stability

This chapter examines correlation of compositional make-up of six different gum tragacanth exudates from *Astragalus* spp on protein based emulsions (Paper I,III).

**Paper I:** Stabilization of emulsions by gum tragacanth (*Astragalus* spp.) correlates to the galacturonic acid content and methoxylation degree of the gum

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### 3.1 Significance of study

Gum tragacanth has been reported to have both emulsifying and stabilizing properties in emulsions, i.e. being “bi-functional” by having both the capacity to facilitate emulsification as well as providing stabilization of the emulsion after its formation (Weiping, 2000). There are indications in the literature that compositional differences of tragacanth gums obtained from different species of *Astragalus* affect the rheological properties, including the viscosity of tragacanth solutions (Balaghi et al., 2011). However, despite the long and extensive use of gum tragacanth as a stabilizer in emulsion systems, surprisingly little is known about structure function relationships, and the traits of the gum that confer emulsifying properties are presently unknown. It is of course tempting to hypothesize that the composition of tragacanth gum affects its stabilization effects in emulsions, and it has been reported that the terminal deoxyhexoxyl groups (i.e. fucose) or the methoxyl groups of the homogalacturonan in the tragacanth gum structure may play a role in the emulsion stabilization (Stephen, 1990). However, to our knowledge, no unequivocal evidence has up until now been provided for this hypothesis. Hence, the exact significance of specific structural components of tragacanth gum in relation to viscosity and emulsion stabilization properties is unclear. The purpose of this study was to evaluate the stabilization of emulsions by tragacanth gum, and to aim at obtaining an understanding of any relationships between the make-up of gum tragacanth and its stabilization properties in emulsions.

### 3.2 Experimental considerations

In the beginning of work, six different of tragacanth gum from *Astragalus* species (*A. parrowianus, A. fluccosus, A. rahensis, A. gossypinus, A. microcephalus,* and *A. compactus*) growing in different regions of Iran were collected. The dried raw gums were ground with a coffee mill, sieved, solubilized in deionized water, then freeze dried, and finally used in this study. In order to determine the ratio between the water-soluble and the water-insoluble fractions of the gums, each gum sample was re-suspended overnight in deionized water (1 % dry matter gum weight/volume) and the separation of the soluble and insoluble fraction was done by centrifugation. The monosaccharide composition and
methyl and acetyl group of each of the gum tragacanth samples was determined. To confirm our hypothesis about effect of methoxyl content, saponification of tragacanth gum solution has been done (Leroux, et al., 2003).

Emulsion was prepared and stabilization and particle size determined via method explained previously by Zaidel et al. (2012) to investigate correlation between composition structure of gums and creaming index and particle size. Particle size of fat globules (oil phase) and their size distribution play a predominant role in deciding the stability of emulsion and emulsion based products with precisely controlled particle size exhibit better emulsion stability (McClements, 1999). The stability of an emulsion to gravitational separation can be enhanced by reducing the droplet size. As a rule, large globules tend to coalesce faster than the small ones (Chiewchan, Phungamngoen, & Siriwattanayothin, 2006).

WPI was used as an emulsifier because whey proteins, having pH near 5, are amphiphilic molecules near the isoelectric point 4<pH<6. Whey proteins, essentially β-lactoglobulin, are widely used as emulsifiers in food applications because they are inexpensive, natural, readily available, and have the ability to facilitate formation and stabilization of oil-in-water emulsions in systems having pH 4-6 (Dickinson, 2001). In this particular system a pH of 4.5, slightly below the pH of the whey protein, was used to enhance the electrostatic attraction between the (potentially pectic) negatively charged gum tragacanth polysaccharides and the protein.

Also, tragacanth gum rheological properties of different gum solution and emulsions were evaluated to compare any difference in interaction between gums in the emulsion system.

3.3 Highlights
It has been demonstrated that different tragacanth gum samples obtained from different species of Astragalus have different composition, and produce different levels of soluble and insoluble gum fractions. The results shown that the gums from A. parrowianus and A. fluccosus had relatively high tragacanthin:bassorin ratios of ~66:34 and ~75:25, respectively, whereas in the other gums this ratio approached 50:50 (A. rahensis, A. microcephalus, A. compactus) or tipped towards higher bassorin than tragacanthin (A. gossypinus). The monosaccharide make-up of the six gums also varied, but all the gums contained relatively high levels of galacturonic acid (~100-330 mg/g), arabinose (50-360 mg/g), xylose (~150-270 mg/g), and galactose (~40-140 mg/g), and also contained fucose, rhamnose, and glucose. Galacturonic acid was high in the soluble part of all species whereas L-fucose and partially xylose was major in insoluble fraction. A positive correlation between the methoxyl content of the soluble part of the gums and creaming index indicate that methoxyl groups may have properties that play a role in the emulsification properties of tragacanth gums Fig 1. The results obtained after removing the methyl groups from the A. fluccosus gum also confirmed that gum tragacanth with methoxyl was acting better as an emulsion stabilizer than the corresponding gum with no methyl groups.

The viscosities of the emulsions were considerably higher than the gum tragacanth solutions, but the overall flow behavior in the emulsions with gum tragacanth added were quite similar to the behavior observed in the gum tragacanth solutions (See Fig. 4 in paper). Increased viscosity of emulsions may be a result of hydrophobic bonding between tragacanth gum and WPI emulsified emulsion particles. Clearly, the viscosity is a factor, but apparently also the composition, notably the total galacturonic acid content in the gum, the amount of methoxyl groups and probably also fucose in the solubilized part are factors determining how tragacanth gums work to stabilize emulsions.
The flow behavior index was found to be lower than 1 for all tragacanth gum solutions as well as for all the emulsions (See Table 2 in paper); this value of less than 1 confirmed the shear thinning behavior of the samples.

The tragacanth gum stabilization of protein-emulsified emulsions is probably a result of two mechanisms: Firstly, formation of non-covalent protein–(gum) polysaccharide complexes, and secondly viscosity increase. It is well-recognized that emulsion stabilization by whey proteins may be improved by polysaccharides (and particularly so at pH near the isoelectric point).

To conclude, the work provides some important clues to the emulsion stabilization mechanisms in relation to the composition of tragacanth gums mainly soluble methoxyl content and galacturonic acid content.

![Figure 3.1](image.png)

Figure 3.1 Correlation between methoxyl content in the soluble fraction of different gum tragacanth samples from different Astragalus species and Creaming Index.
Compositional analysis and rheological characterization of gum tragacanth exudates from six species of Iranian *Astragalus*

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**Abstract**

The sugar composition and viscoelastic behaviour of Iranian gum tragacanth exuded by six species of *Astragalus* was investigated at a concentration of 1.3% and varying ionic strength using a controlled shearrate rheometer. Compositional analysis of the six species of gum tragacanth by high-performance anion-exchange chromatography with pulsed amperometric detection suggested the occurrence of arabinose, xylose, glucose, galactose, fucose, rhamnose and galacturonic acid residues in the gum structure; however, the proportions of each sugar varied significantly among the gums from the different species of *Astragalus*, and this variation led to interesting differences in functional properties. Rheological measurements performed on dispersions of the six species of gum tragacanth demonstrated viscoelastic properties. The mechanical spectra derived from strain sweep and frequency sweep measurements indicated that the different gum tragacanth dispersions had distinctive viscoelastic behaviours. Investigation of the viscoelastic properties of the different gum dispersions in the presence of NaCl revealed that the addition of NaCl could lead to slight to drastic decreases in the Ĝ', Ĝ'' or η̄' values of the various gums. In general, the results indicated that the six varieties of gum tragacanth studied exhibited significantly different rheological properties; therefore, these different gums may find use in a variety of applications as stabilisers, thickeners, emulsifiers and suspending agents depending on their rheological behaviour.

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**1. Introduction**

Gum tragacanth, a dried exudate obtained from the stems and branches of Asiatic species of *Astragalus*, is a very complex hetero-geneous anionic polysaccharide of high molecular weight (*Weiping, 2000*) and consists of two main fractions: a water-insoluble component called bassorin, which has the capacity to swell and form a gel, and a water-soluble component called tragacanthin (*Balaghi, Mohammadifar, & Zargaraan, 2010*). The easy separation of tragacanthin and bassorin suggests that the two polysaccharides are in a physical mixture and not chemically bonded (*Lapas & Pricl, 1999*). It has been reported that gums tragacanth from different species of *Astragalus* have different ratios of the two fractions, different chemical compositions and also varying physicochemical properties; therefore, different functionalities and applications for each species are expected (*Balaghi et al., 2010*). Gum tragacanth, a highly acid-resistant hydrocolloid, has been accepted since 1961 as GRAS at the level of 0.2–1.3% (*Anderson & Bridgeman, 1985*) and has been used for many years as a stabiliser, thickener, emulsifier and suspending agent in the food, pharmaceutical, cosmetic, textile and leather industries as well as in technical applications based on its high viscosity at low concentration, good suspending action, unusually high stability to heat and acidity and effective emulsifying properties. It also is pourable and has a creamy mouth feel and good flavour-release properties (*Weiping, 2000*) and very long shelf life (*Levy & Schwarz, 1958*). Gum tragacanth is used in the food industry in salad dressings, condiments, sauces, bakery emulsions, oil and flavour emulsions, fillings and toppings, confectionery, soft drinks, jellies, desserts, ice creams, flavours and spices (*Weiping, 2000*).

To date, a wide variety of gums has been extensively investigated for their structural characteristics, functional properties and application properties. A number of studies have been devoted to elucidate the effects of various processes (salting, heating, acidification, ultrasonication, irradiation, high pressure, etc.) and
processing parameters (time, temperature and rate of a process) as well as the ratio, type and concentration of ingredients on the functional properties of a broad range of hydrocolloids (Ahmed & Ramaswamy, 2004; Dogan, Kayacier, & Ic, 2007; Farhoosh & Riazi, 2007; Mu et al., 2010). Dynamic rheology is one of the methods most extensively used to assess the viscoelastic behaviour of polysaccharide solutions/dispersions or gels. The viscoelastic properties of various gums such as xanthan, guar (Mills & Kokini, 1984), pectin (Gigli, Garnier, & Piazza, 2009), gelatin (Gómez-Guillén et al., 2002) and mucilage (Medina-Torres, Brito-De La Fuente, Torrestiana-Sanchez, & Katthain, 2000) have been reported in various researches. Although the physicochemical properties and steady-state rheological evaluation of gum tragacanth have been recently established (Balaghi et al., 2010), only a few studies have dealt with the viscoelastic behaviour of gum tragacanth (Mohammadifar, Musavi, Kiumarsi, & Williams, 2006). However, the viscoelastic properties of gum solutions/dispersions are particularly important for food processors in adjusting processing parameters, monitoring consistency as well as predicting the stability of fluid food systems and the final textural attributes of formulated foods.

One of the factors that seem to have a direct bearing on the rheological behaviour of the gum is the sugar composition. The sugar compositions of many hydrocolloids such as xanthan, locust-bean galactomannans (Lazaridou, Billaderis, & Izydorczyk, 2001) and mucilage gum (Medina-Torres et al., 2000) have been reported in the literature. The determination of sugar composition might be useful in developing microstructure–function relationships for systems of gum dispersions and intermolecular interactions. In 1988, Anderson and Grant determined the sugar composition of several gums tragacanth by formic acid hydrolysis followed by paper chromatography (Anderson & Grant, 1988).

The ionic strength of the medium is a crucial variable influencing the networking mechanism of charged biopolymers. The effects of NaCl addition on the linear viscoelastic properties of various gum dispersions and polysaccharide gels have been previously investigated. Since the addition of salt may alter the chain flexibility and conformation features of a polymer, different phenomena were observed in these investigations. The formation of junction zones in the gel network can be controlled by many factors such as the content and type of the counter ion. However, when gelation does not involve the formation of an ordered structure, the behaviour is strongly influenced by the presence of a counter ion (Silva, Brito, de Paula, Feitosa, & Paula, 2003). To the best of our knowledge, the effect of NaCl on the linear viscoelastic properties of gum tragacanth has not been examined.

Owing to the fact that various varieties of gum tragacanth possess different chemical compositions and physicochemical properties (Balaghi et al., 2010), understanding the rheological properties of gum tragacanth is essential for evaluating the gums from different species and their potential applications and use as food thickeners, stabilisers or emulsifiers. Unfortunately, most of the previous studies have been done on gums tragacanth from unknown botanical sources; moreover, the experimental conditions such as the applied shear rate, temperature and solvent were not clearly explained (Anderson & Grant, 1988; Azarikia & Abbasi, 2010; Chenlo, Moreira, & Silva, 2010; Stauffer & Andon, 1975; Zahedi, Vedadi, & Dollimore, 1979). Therefore, the aim of the present study was to determine the sugar compositions and to characterise the dynamic rheological properties of six varieties of gum tragacanth at different ionic strengths, as this information is necessary to gain insight into the functional properties of this biopolymer and should be useful in the processing of tragacanth gum for scientific research as well as for potential industrial applications.

2. Materials and methods

2.1. Plant materials and standards

Iranian gum tragacanth exuded by various species of Astragalus (Astragalus parrowianus, Astragalus flaccosus, Astragalus rahensis, Astragalus gossypinus, Astragalus microcephalus and Astragalus compactus) was collected from plants growing in different provinces of Iran including Isfahan, Khorasan, Semnan and Shiraz. The plants were tapped with a knife by making careful longitudinal incisions in the taproot and the bark of the branches. The gum readily exuded from these cuts in the form of ‘ribbons’ that became brittle on drying. Taxonomic identification of the specimens was done by Dr. Ali Masoumi, an academic member of the Forest, Range, and Watershed Management Organisation of Iran. Deionised (Milli-Q) water was used for all experimental work. Neutral monosaccharide standards, L-fucose, L-rhamnose, D-arabinose, D-galactose, D-glucose, D-mannose, and D-galacturonic acid were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Trifluoroacetic acid (99%) was from Merck (Darmstadt, Germany). NaOH standard solution HPLC grade was from Fluka/Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Native gum tragacanth sample preparation

The raw gum tragacanth was powdered in a high-speed mechanical blender and later sieved to obtain uniform samples. Powdered gum with a mesh size between 200 and 500 μm was used in this study.

2.3. Sample preparation for high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

Approximately 15–20 mg of powdered gum tragacanth sample (200–500 μm particle size) was transferred to a screw-cap vial plus deionised water for 2–4 h (depends on variety of Tragacanth because some of them is dissolved faster than others) at room temperature. After dissolving samples Trifluoroacetic acid (TFA; 2 M, 600 μL) was added by micropipette. The apparatus was then heated in a drying oven maintained at 121 °C for 2 h without stirring and then allowed to cool at room temperature. Hydrolysates were lyophilised and kept at −20 °C under N2 until analysis. Prior to analysis by HPAEC-PAD, the sample hydrolysates were redissolved in 5 mL of double deionised water and then filtered through 0.20μm nylon syringe filters into the sample vials used for the AS50 auto sampling chromatography system (Arnoux & Meyer, 2008).

2.4. Determination of carbohydrate composition

The monosaccharide contents of the hydrolysates and standards were determined with a BioLC system consisting of a GS50 gradient pump, an EDSO electrochemical detector and an AS50 chromatograph coupled to an AS50 autosampler (Dionex Corp., Sunnyvale, CA). Separations were performed using a CarboPacTM PA20 (3 mm × 150 mm) analytical column (Dionex Corp., Sunnyvale, CA) according to the method of Arnoux and Meyer (2008). The eluent flow rate was 0.5 mL/min. The monosaccharides were separated with adequate resolution using a two-eluent system consisting of deionised water (18.2 mΩ at 25 °C) and 500 mM aqueous NaOH. Neutral monosaccharides were eluted isocratically with 2.5 mM NaOH for 20 min followed by a second isocratic elution at high NaOH (500 mM) for 10 min to elute any acidic monosaccharides present (galacturonic). This high concentration of NaOH simultaneously washed the column. Before each injection (10 μL), a column re-equilibration program was run for 5 min with 100 mM NaOH.

2.5. Sample preparation for high-performance cation-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

The plant materials and standards were formulated foods.

2.5.1. Plant materials and standards

Iranian gum tragacanth exuded by various species of Astragalus (Astragalus parrowianus, Astragalus flaccosus, Astragalus rahensis, Astragalus gossypinus, Astragalus microcephalus and Astragalus compactus) was collected from plants growing in different provinces of Iran including Isfahan, Khorasan, Semnan and Shiraz. The plants were tapped with a knife by making careful longitudinal incisions in the taproot and the bark of the branches. The gum readily exuded from these cuts in the form of ‘ribbons’ that became brittle on drying. Taxonomic identification of the specimens was done by Dr. Ali Masoumi, an academic member of the Forest, Range, and Watershed Management Organisation of Iran. Deionised (Milli-Q) water was used for all experimental work. Neutral monosaccharide standards, L-fucose, L-rhamnose, D-arabinose, D-galactose, D-glucose, D-mannose, and D-galacturonic acid were obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Trifluoroacetic acid (99%) was from Merck (Darmstadt, Germany). NaOH standard solution HPLC grade was from Fluka/Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

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followed by 5 min with 2.5 mM NaOH. The different carbohydrate standards were mixed proportionally to resemble the matrix of the studied samples. The following pulse potentials and durations were used for detections: E1 = 0.1 V, t1 = 400 ms; E2 = −2 V, t2 = 20 ms; E3 = 0.6 V, t3 = 10 ms; E4 = −0.1 V, t4 = 70 ms, data-collection rate: 0.2 Hz (Arnous & Meyer, 2008).

2.5. Preparation of dispersions for rheological analysis

Gum tragacanth powder (1.3 g) was accurately weighed and dispensed into 100 mL pure deionised water and also in 100 mL of 0.2 M NaCl. The whole gum dispersions were kept on a magnetic stirrer at room temperature and gently stirred for 2 h. Later, the gum dispersions were allowed to stand at 3 ± 1 °C for 24 h to enable biopolymer hydration.

2.6. Rheological measurements

Oscillatory shear measurements were performed with a Physica MCR 301 rheometer (Anton-Paar, GmbH, Graz, Austria) using a serrated plate-and-plate system (25 mm in diameter, 0.6 mm gap). Temperature control was carried out with a Peltier system equipped with a fluid circulator. All samples were left standing for five minutes to allow structure recovery and temperature equilibration. The samples were covered with a solvent trap to prevent evaporation. All experiments were carried out at 25 °C. Strain sweep tests were performed (0.01−1000%, 1 Hz) to determine: 1) the limiting value of the linear viscoelastic range (LVE or γL); 2) the structural strength (G' at LVE); 3) the resistance to mechanical force or yield stress (γt); which is also a measure of structural strength and can be determined from the limiting value of LVE range in terms of shear stress (it determined visually or manually from the linear viscoelastic plateau and from the data table at which the G' values are just beginning to deviate noticeably from the previously constant values.; 4) the flow point (γg), the stress at which the internal structure breaks to the extent that it causes the material to flow (G' = G''); and 5) the damping factor (tan δ), or the ratio of loss modulus to elastic modulus, to provide a direct view of whether the samples behaved as liquids or solids (Mezger, 2006). Frequency sweep tests were performed using a frequency ramp from 0.01 to 100 Hz. The experimental data were fitted to a power-law model.

2.7. Statistical analysis

Analytical values are based on the mean and standard deviation of three replicates. For all rheological measurements, the reported values are based on the mean of three replicates. Analysis of variance (ANOVA) was used for the data analysis (SPSS, 16). When the F-values were significant (p < 0.05) in ANOVA, Duncan’s multiple-range test was used to compare treatment means. Spearman correlation-coefficient analyses were used to evaluate potential relations between the sugar compositions of the samples and their rheological parameters and also between the different rheological parameters.

3. Results and discussion

3.1. Sugar composition

The sugar compositions of the six varieties of gum tragacanth determined by HPAEC-PAD are listed in Table 1. The results revealed the presence of galacturonic acid, xylose, fucose, arabinose, galactose, glucose, and traces of rhamnose; however, the proportions of each sugar varied among the gums from various species of Astragalus.

It has been reported that gums with higher viscosity contain high proportions of fucose, xylose, galacturonic acid and methoxyl groups and low proportions of arabinose and nitrogenous fractions (Anderson & Grant, 1988). However, by considering our previous research data regarding the consistency coefficient of gum tragacanth (Table 2) from different Astragalus species (Balaghi et al., 2010), we didn’t find any relationship between consistency coefficient and the amount of each individual sugar; but we found that gums with higher amounts of galacturonic acid and fucose and usually lower amounts of xylose and arabinose had higher values of the consistency coefficients. The Spearman correlation coefficients and p-values between the sum of galacturonic acid and fucose and the consistency coefficient (m) as well as between arabinose and the consistency coefficient, fucose and the consistency coefficient, and between arabinose/(xylose + fucose) and the consistency coefficient were +1 (p = 0), −0.943 (p = 0.005), +0.900 (p = 0.037) and −0.943 (p = 0.005), respectively. The relations between the sugar compositions and the consistency coefficients are illustrated in Fig. 1. According to Fig. 1, gums with higher amount of galacturonic acid and fucose have higher values of consistency coefficients. On the contrary, gums with higher amount of xylose and arabinose have higher lower values of consistency coefficients. Spearman correlation coefficient and p value between consistency coefficient and sum of xylose and arabinose were −0.943 and p = 0.005.

The results showed that the content of galacturonic acid varied between 0 and 37% among the six varieties of gum tragacanth. The content of arabinose, which has been reported to be the major component of the side chains of the 1,4-linked α-D-galacturonic acid backbone of gum tragacanth, had a wide percentage range (1−51%) among the six gums. In contrast with A. gossypinus, which contained 1% arabinose and 37% galacturonic acid, gum tragacanth from A. rahensis interestingly had arabinose and galacturonic acid contents of 51% and 9%, respectively. The results also indicated that the fucose contents of the different gums ranged from 0% for A. rahensis to 35% for A. compactus. It was also observed that

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Sugar compositions of gums tragacanth from six species of Astragalus.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. parrowianus</td>
</tr>
<tr>
<td>Arabinose</td>
<td>39b</td>
</tr>
<tr>
<td>Xylose</td>
<td>10a</td>
</tr>
<tr>
<td>Glucose</td>
<td>10b</td>
</tr>
<tr>
<td>Fucose</td>
<td>7a</td>
</tr>
<tr>
<td>Galactose</td>
<td>4a</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>−</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>21a</td>
</tr>
<tr>
<td>Galacturonic acid + fucose</td>
<td>28a</td>
</tr>
</tbody>
</table>

Values with different letters in each row are significantly different (p < 0.05).

In all cases the standard deviations were lower than 2%.
**A. fluccosus** gum had no glucose residues. The contents of xylose, the other constituent of the side chains linked to the galacturonic acid main chain, varied from 10 to 32% among the six species (Table 1). This wide variation in the neutral sugar and uronic acid contents of gum tragacanth from different species of *Astragalus* may imply the occurrence of various numbers as well as different lengths of the side chains, which are involved in the primary structure of gum tragacanth.

The accurate names of the gum tragacanth fractions based on the work of Aspinall and Baillie and Norman has been illustrated in Fig. 2 (Aspinall & Baillie, 1963a, 1963b; Norman, 1931). There has been a large number of publications such as books and articles about polysaccharides and hydrocolloids (Chenlo et al., 2010; Izdorczyk, Cui, & Wang, 2005; Ramsden, 2004; Vebeken, Dierckx, & Dewettinck, 2003; Weiping, 2000; William, Phillips, Stephen, & Churms, 2006), which is for the most part cited by research papers, incorrectly reporting that gum tragacanth consists of a neutral arabinogalactan, in the current study, the arabinogalactan component of gum tragacanth was isolated by extraction with ethanol–water (7:3) (Aspinall & Baillie, 1963a, 1963b) and should not be confused with the water-soluble fraction of the gum (tragacanthic acid), which usually (but not always) seems to be an arabinogalacturonan.

Contrary to the reports which states that soluble fraction of the gum tragacanth is an arabinogalactan, in the current study, the Spearman correlation between the percentages of the soluble fraction (Table 2) and arabinose content (Table 1) was not statistically significant because, for instance, gum exudates from species such as *A. fluccosus*, with 75% soluble part, had just 23% arabinose and gum exudates from *A. gossypinus*, with 50% the soluble part, had only a 1% arabinose content.

According to the extensive studies of Aspinall and Baillie, tragacanthic acid contains residues of α-galacturonic acid, D-xylose, L-fucose, D-galactose and trace amounts of D-glucuronic acid. It was suggested that tragacanthin is based on linear chains of 1,4-linked galacturonic acid residues. It has been reported in the literature that the primary structures of gum tragacanth appear to be arabinogalactans and arabinogalacturonan methyl esters, incorporating lesser quantities of xylose, rhamnose, and fucose (Walter, 1998); however, this is not in complete agreement with our results because for species such as *A. fluccosus* and *A. gossypinus* the major sugar is xylose. Thus, *A. fluccosus* and *A. gossypinus* could be classified as xylogalacturonans; moreover, the major neutral sugar of *A. compactus* is fucose, so it seems to be a fucogalacturan. Therefore, assigning the classification arabinogalacturonan to gum tragacanth from all species would seem to be incorrect.

### 3.2. Strain sweep

From the preliminary step in the analysis of the oscillatory flow data, namely, the strain sweep tests, the limits of the linear viscoelastic domains for the six gum tragacanth dispersions were determined. However, the strain sweep graphs of just two gums in the presence and absence of added NaCl is depicted in Fig. 3. For the six gums, it was possible to discriminate two different regions, namely, a linear viscoelastic region, in which $G' > G''$ were practically constant, and a nonlinear region, in which $G' > G''$ started to decrease with increasing strain, with both moduli finally tending to crossover. The lengths of the LVE indicated that $G' > G''$ values were independent of the oscillation strain (reversible elasticity). The strain sweep tests were performed at a constant frequency of 1 Hz.

As illustrated in Fig. 3, for *A. parrowianus* both in the presence and absence of NaCl, the $G'$ value was approximately equal to $G''$ in the LVE range. In this case, the values of the two moduli were balanced; this behaviour is termed “being at the gel point”. It shows that the sample exists at the borderline between liquid and gel-like states at $f = 1$ Hz (Mezger, 2006).

For *A. rahensis*, *A. gossypinus*, *A. microcephalus* and *A. compactus*, both in the presence and absence of added NaCl, $G''$ dominated $G'$. It has been reported that if $G' > G''$ the test material exhibits a certain rigidity; this is typical for solids or stable pastes. However, there are many dispersions such as coatings or foodstuffs that exhibit low-viscosity flow behaviour at medium and high shear rates but $G' > G''$ in the LVE range. Indeed, *A. rahensis*, *A. gossypinus*, *A. microcephalus* and *A. compactus*, both in the presence and absence of added NaCl indicate a gel-like structure at $f = 1$ Hz; although this is a weak-gel structure, they exhibits a certain stability of form. For *A. fluccosus* in the absence of NaCl, $G'$

![Fig. 1. Consistency coefficient of gum tragacanth as a function of the sum of the galacturonic acid and fucose or as the sum of the xylose and arabinose.](image1)

![Fig. 2. The right and accurate names of the gum tragacanth fractions based on Aspinall and Baillie (1963a, 1963b) and Norman (1931).](image2)

### Table 2

Consistency coefficients for 1.5% dispersions of the six varieties of gum tragacanth in the absence of added NaCl at 25 °C (Balaghi et al., 2010).

<table>
<thead>
<tr>
<th>Gum Variety</th>
<th>Consistency coefficient (Pa s$^{-1}$)</th>
<th>Ratio of soluble to insoluble fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. parrowianus</em></td>
<td>11.29</td>
<td>1.74</td>
</tr>
<tr>
<td><em>A. fluccosus</em></td>
<td>18.46</td>
<td>3.15</td>
</tr>
<tr>
<td><em>A. rahensis</em></td>
<td>1.66</td>
<td>1.30</td>
</tr>
<tr>
<td><em>A. gossypinus</em></td>
<td>23.70</td>
<td>0.51</td>
</tr>
<tr>
<td><em>A. microcephalus</em></td>
<td>13.06</td>
<td>1</td>
</tr>
<tr>
<td><em>A. compactus</em></td>
<td>36.04</td>
<td>0.84</td>
</tr>
</tbody>
</table>
dominated $G''$; however, by adding NaCl, the reverse was observed. In this case, the viscous character dominates the elastic one, so the sample exhibits liquid or sol character in the LVE range. It has been shown that $A. \text{flaccus}$ has a fairly high consistency coefficient (Balaghi et al., 2010); however, even high-viscosity samples with entangled molecular chains but without a chemical or physical-force network show this behaviour. These materials usually are not form-stable at rest because they creep or flow; however, this process can occur at a very low flow velocity (Mezger, 2006).

The limiting values of strain ($\gamma_L$), tan $\delta$ and $\tau$ obtained within the LVE range are presented in Table 3. $\gamma_L$ was high for $A. \text{flaccus}$, $A. \text{parrowianus}$ and $A. \text{compactus}$ (20, 12, and 10%, respectively), which indicates that these samples have longer LVE ranges, implying a higher stability of the viscoelastic material under the $\gamma$-amplitude. The addition of NaCl caused the $\gamma_L$ to increase in dispersions of $A. \text{flaccus}$, $A. \text{gossypinus}$ and $A. \text{microcephalus}$, whereas it caused the $\gamma_L$ to decrease in $A. \text{parrowianus}$, $A. \text{rahensis}$ and $A. \text{compactus}$. It seems that the $\gamma_L$ of the six gums varied in relation to the ratio of the soluble to insoluble fractions (Table 2); that is, gums with higher ratios of soluble to insoluble fractions had higher limiting values of strain (Spearman correlation coefficient 0.829, $p < 0.05$).

### Table 3

<table>
<thead>
<tr>
<th>$G'_{\text{LVE}}$ (Pa)</th>
<th>$\gamma_L$ (%)</th>
<th>$\tan \delta_{\text{LVE}}$</th>
<th>$\tau_{0}$ (Pa)</th>
<th>$G_c$ (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-0</td>
<td>1-0</td>
<td>1-0</td>
<td>1-0</td>
</tr>
<tr>
<td>$A. \text{parrowianus}$</td>
<td>7.34&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.18&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>$A. \text{gossypinus}$</td>
<td>13.50&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.79&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>$A. \text{rahensis}$</td>
<td>2.69&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.81&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.95&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.99&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>$A. \text{microcephalus}$</td>
<td>37.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.69&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>$A. \text{flaccus}$</td>
<td>45.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.52&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>$A. \text{compactus}$</td>
<td>38.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with different letters in each column are significantly different ($p < 0.05$). In all cases the standard deviations were lower than 2%.

### 3.3. Frequency sweep

It has been previously argued that the dispersions of the six gums are polydisperse systems due to the presence of tragacanthin and bassorin (Balaghi et al., 2010); additionally, these polydisperse systems differ in their percentages of bassorin and tragacanthin. These two fractions differ greatly in size (the hydrodynamic diameter of tragacanthin is 0.12 μm vs. the Sauter diameter of 302 μm for bassorin) (Mohammadifar et al., 2006) and this provides a sufficient reason to classify the system as polydisperse. The soluble part of the gum (tragacanthin) readily dissolves in water but the insoluble fraction (bassorin) can swell in water and form a gel.

As shown in Fig. 4, for $A. \text{parrowianus}$ and $A. \text{flaccus}$ dispersions there were transition from a predominantly viscous response at longer time scales ($G'' > G'$) to a predominantly elastic response at shorter time scales ($G' > G''$). At frequencies lower than $\omega_c$, the moduli were within the viscous limit and transitioned to flow region (Barnes, 2000); at $\omega_c > \omega_c$, the moduli approached the rubbery region, $G' > G''$. The crossover point occurred for $A. \text{parrowianus}$ at $\omega_c = 8.11 \text{ rad/s}$ and $G_c = 9.5 \text{ Pa}$, whereas for $A. \text{flaccus}$ it occurred at $\omega_c = 3.4 \text{ rad/s}$ and $G_c = 10.25 \text{ Pa}$. A lower crossover value indicates a larger elastic contribution (de Brito, Sierakowski, Reicher, Feitosa, & de Paula, 2005). For the $A. \text{flaccus}$ dispersion, the crossover point occurred at a lower frequency than with the $A. \text{parrowianus}$ dispersion. For unlocked
polymers, the values of both $G''$ and $G'$ fall constantly towards lower frequencies (Mezger, 2006). It seems that in NaCl-free dispersions both *A. parrowianus* and *A. fluccosus* frequency sweep pattern are similar to that of unlinked polymers (i.e. when the polymer concentration is high enough, the polymer chains begin to form entanglements; however these entanglements seem to be the mechanical interactions and they probably are neither chemical bonds nor physical bonds, therefore, macromolecules are able to move slowly even under the small shear force and glide along each other showing partial or even complete disentanglement). It has been reported that an entanglement network system shows $G''$ and $G'$ curves intersecting at the middle of the frequency range, indicating a clear tendency for more solid-like behaviour at higher frequency (Chamberlain & Rao, 2000). The *A. parrowianus* and *A. fluccosus* dispersions behaved as an entangled network system. The behaviour of these dispersions can be compared to deacetylated *Sterculia striata* polysaccharide (de Brito et al., 2005) and sulphoacetate derivatives of cellulose (Chauvelon, Doublier, Buléon, Thibault, & Saulnier, 2003).

Although the tests were performed within what is known as the linear viscoelastic region, bonds between structural units generally break and reform (Everett & McLeod, 2005). As shown in Fig. 5A and B, the trend of tan $\delta$ curves for *A. fluccosus* and *A. parrowianus* imply that at low frequencies (lower than 0.06 rad/s) the bonds between structural flow units (tragacanthin and bassorin) stretch and relax with very little breakage taking place. Thus, despite the presence of a 60–75% tragacanthin fraction as a dissipating agent in these systems, there would be only slight dissipated energy. In these systems very large particles of bassorin act as an energy-storing agent. Therefore long molecules of bassorin govern the
The addition of NaCl changed the behaviour of the A. parrowianus dispersion. In the presence of NaCl, $G'$ predominated over the investigated frequency window and no crossover point occurred; gel-like behaviour then prevailed. As shown in Fig. 4, it seems that NaCl was involved in the long range rather than the short-range cross-linking of the aqueous dispersion of A. parrowianus. The behaviour of the A. fluccosus dispersion in the presence of NaCl was similar to that of the NaCl-free dispersion; however, the addition of NaCl salt to the A. fluccosus dispersion shifted the crossover point to a higher frequency ($\omega_c = 9.98 \text{ rad/s}, G_c = 1.03 \text{ Pa}$).

As can be seen in Fig. 5A, the tan $\delta$ values of the NaCl-free dispersion of A. parrowianus were significantly higher than that of the same sample with NaCl. Fig. 4 also shows that the added salt decreased both $G'$ and $G''$ over most of the frequency range studied; however, it seems that NaCl decreased the viscous character more significantly than the elastic character, as samples with NaCl displayed more solid-like behaviour ($\tan \delta < 1$). Addition of salt to the dispersions of the gum tragacanth exuded by six species of Atragalus, caused $G'$ and $G''$ values to decrease over the whole frequency range. It seems that external salt concentration plays a large role on the viscosity and elasticity of water-soluble polysaccharide solution having polyelectrolyte properties. This effect might be caused by screening effect on long-range electrostatic repulsion among the uronic acid residues of gum tragacanth. Probably with increasing ionic strength, chain conformations changes from extended conformations to the compact ones with reduced hydrodynamic size. It has been reported that the decrease of viscosity and elasticity is larger when the flexibility of the polymer is larger; this is based on the contribution of electrostatic persistence length ($L_p$) compared to the fractal persistence length ($L_q$). $L_p$ depends only on the ionic concentration, then the larger is $L_p$, the lower is the effect of salt on the rheological properties. It seems that in NaCl-free dispersions, polysaccharide chains are stiffer than NaCl-contained dispersions which results in a higher viscosity and elasticity.

For the A. fluccosus gum, the addition of NaCl enhanced the values of tan $\delta$ significantly (Fig. 5B). Both $G'$ and $G''$ significantly decreased with addition of NaCl. The moduli of A. fluccosus dispersion was higher than those of A. parrowianus, demonstrating a greater degree of elasticity and structural integrity (Khondkar, Tester, Hudson, Karkalas, & Morrow, 2007).

Increasing the frequency increased both the $G'$ and $G''$ values of aqueous dispersions of A. microcephalus and A. compactus gums, both in the presence and absence of NaCl (Fig. 4), with the elastic component dominating over the viscous component throughout the entire frequency range examined ($G'/G'' \approx 3$ for the A. microcephalus aqueous dispersion and $G'/G'' \approx 2$ for A. compactus aqueous dispersion) and the moduli were almost parallel both in the presence and absence of added NaCl. A typical strong gel spectrum consists of two nearly horizontal straight lines, where $G'$ is typically one to two orders of magnitude higher than $G''$. In weak gels, both moduli show a slight frequency dependence, with $G'$ exceeding $G''$ at all frequencies (Martínez-Ruvalcaba, Chornet, & Rodríguez, 2007). Therefore, dispersions of A. microcephalus and A. compactus gums are considered weak gels because their $G'$ and $G''$ values showed slight frequency dependence. However, the rheology of the A. microcephalus dispersion can be compared to a 2% w/w of xanthan gum (Medina-Torres et al., 2000). In the spectra of A. microcephalus and A. compactus, only rubbery regions were observed. The mechanical spectra shown in the figures for these samples are similar to those of sparsely cross-linked polymers (Mezger, 2006). Cross-linked polymers refers to a network of bridges between the macromolecules which are fixed by chemical (primary valency) bonds. This makes it possible for the molecule chains to move along each other without destruction of the chemical network.

For the A. gossypinus aqueous dispersion (Fig. 4), $G'$ and $G''$ increased with frequency and $G'$ was greater than $G''$ throughout the frequency range covered. The $G'$ increased with $\omega$ within the range $0.66 < \omega < 195 \text{ rad/s}$, followed by an almost constant value for range $0.06 < \omega < 0.41 \text{ rad/s}$ indicating frequency independence; a similar response was also observed for $G''$. The tan $\delta$ values of A. gossypinus, A. compactus and A. microcephalus gums, which have bassorin fractions over 50%,
were less than unity; this is indicative of more solid-like behaviour of these samples. It seems that bassorin (the insoluble fraction of the gum), with its swelling power and its greater Sauter diameter than tragacanthin (the soluble fraction), contributed to the gel-like character of the samples. The tan δ curve of these three gums suggests that as the frequency was increased, the energy-dissipating agent of the system increased \( G' \), while the magnitude of \( G'' \) remained greater than \( G' \) due to the dominance of the larger bassorin particles. Indeed, the longer molecules of the bassorin particles are probably less able to glide along each other; therefore, their entanglements began to form a temporary network, resulting in \( G' \) dominating \( G'' \).

The tan δ values of the aqueous dispersion of \( A. \) microcephalus gum in the presence and absence of NaCl (Fig. 5A) were very similar; both were less than unity and had slight frequency dependence (tan δ = 0.2 to 0.4), indicating solid-like behaviour throughout the entire frequency range examined. The similarity of the tan δ values of the NaCl-added and NaCl-free aqueous dispersions implies that addition of NaCl caused \( G' \) to decrease to the same extent as \( G'' \). However, the tan δ values of the aqueous dispersion of \( A. \) compactus gum indicated frequency dependence, ranging from 0.8 to 0.3 with increasing frequency. Therefore, the NaCl-free aqueous dispersion of \( A. \) compactus gum had a more solid-like behaviour at higher frequencies. In contrast, the tan δ values of \( A. \) compactus gum in the presence of NaCl increased from 0.5 to 0.8 up to the frequency of 3 rad/s, indicating a more liquid-like behaviour, and then at frequencies higher than 3 rad/s it began to decrease to 0.45, indicating a more solid-like behaviour.

The ratio of \( G' / G'' \) for \( A. \) gossypinus was greater than 0.1 and less than unity, meaning that this sample was not a true gel. Thus, the \( A. \) gossypinus dispersion had a structure between that of a concentrated biopolymer and a true or strong gel. As this sample had a \( G' \) higher than \( G'' \), with the moduli almost parallel, it could be characterised as a weak gel, like \( A. \) microcephalus and \( A. \) compactus. (Chamberlain & Rao, 2000).

In contrast to the NaCl-free dispersion, the \( A. \) gossypinus NaCl-added aqueous dispersion (Fig. 4) showed a crossover point at a high frequency \( (\omega_0 = 128 \text{ rad/s}, G_0 = 14.78 \text{ Pa}) \). At 0.06 < \( \omega < 122 \text{ rad/s} \), the \( G' \) values were higher than \( G'' \). At frequencies higher than \( \omega_0 \), the \( G' \) and \( G'' \) values were very noisy. The addition of NaCl decreased the \( G' \) and \( G'' \) values (Fig. 4) and \( \tan \delta \) significantly (Fig. 5B), but it is worth noting that in the presence of NaCl the difference between \( G' \) and \( G'' \) values was considerable (\( G' > G'' \)). This implies that NaCl caused the viscous component to decrease more drastically than the elastic component; however, the tan δ values were still greater than 0.1, so the salt-added aqueous dispersion of \( A. \) gossypinus was not a true gel. The features of the \( G' \)-curve and the occurrence of a crossover point at high frequency implied that there might be a sparse cross-linking between the molecules which can be indicative of a flexible gel or dispersions showing low structural strength at rest. The larger effect of the addition of NaCl to \( A. \) gossypinus compared to \( A. \) microcephalus and \( A. \) parrowianus dispersions was probably associated with higher uronic acid content of \( A. \) gossypinus (Table 2).

For the \( A. \) rahensis dispersion, increasing the frequency increased both the \( G' \) and \( G'' \) moduli (Fig. 4), with the elastic behaviour dominating up to crossover point \( (\omega_0 = 0.84 \text{ rad/s} \text{ and } G_0 = 0.49 \text{ Pa}) \). At frequencies higher than \( \omega_0 \), the reverse was observed. This supports the fact that a transition from a weak-gel-like structure to a fluid-like one occurred. The tan δ values of the \( A. \) rahensis aqueous dispersion were larger than unity over the entire frequency range and the tan δ values increased with the frequency (Fig. 5A). The \( G' \) and \( G'' \) values of the \( A. \) rahensis salt-added aqueous dispersion were very noisy and did not coincide after running a series of increasing frequency ramps. For the \( A. \) rahensis gum, with a tragacanthin fraction over 55%, a great sensitivity to increasing frequency was observed. Similarly to the \( A. \) parrowianus and \( A. \) flaccus gums, at low frequencies the bonds between structural flow units stretched and relaxed with very little breakage taking place and solid-like behaviour was observed. As the frequency was increased, most of the bonds between flow units broke and did not have enough time to form new linkages before a second oscillation occurred, and the dispersion became increasingly liquid-like as the tan δ values increased from 0.5 to 7. Surprisingly, the liquid-like behaviour of the \( A. \) rahensis dispersion remained at the highest frequencies, and it did not change to a solid-like character at the shortest time scale of the experiment.

The tan δ has previously been argued to be an indicator of bond-relaxation behaviour. The peak values for tan δ correspond to an average bond-relaxation time between structural flow units in the system, which is equal to the reciprocal of the frequency. It must be pointed out that the bonds between structural flow units have a spectrum of relaxation times rather than a single characteristic relaxation time (Everett & McLeod, 2005). The tan δ values and the corresponding frequencies of the peaks of the tan δ curves for the six gums in the presence and absence of added NaCl are presented in Table 4. The peak values differed among the six species. For \( A. \) parrowianus, \( A. \) flaccus, and \( A. \) compactus, the peak values occurred at low frequencies (0.1–0.4 rad/s); however, for \( A. \) rahensis, \( A. \) gossypinus and \( A. \) microcephalus, it occurred at high frequencies. The addition of NaCl to \( A. \) parrowianus, \( A. \) gossypinus and \( A. \) compactus dispersions caused peak values to shift to higher frequencies. The frequency of the peak value was not sensitive to the addition of NaCl with \( A. \) flaccus and \( A. \) microcephalus.

As shown in Fig. 4, the complex viscosity value \( \eta' \) of the six species decreased with increased frequency, showing a non-Newtonian shear-thinning behaviour. With decreasing frequency, the \( \eta' \) of cross-linked polymers rise to an infinitely high value, and the zero-shear viscosity cannot be calculated (Mezger, 2006). This observation in the case of the cross-linked molecules of \( A. \) microcephalus, \( A. \) compactus and \( A. \) gossypinus indicated gel-like character and therefore form stability at rest. For the non-cross-linked molecules of \( A. \) parrowianus and \( A. \) flaccus aqueous dispersions, according to their \( \eta' \) functions (Fig. 4), the plateau values of zero-shear viscosity cannot be calculated. Indeed, for \( A. \) parrowianus and \( A. \) flaccus, both with over 60% tragacanthin fractions, with decreasing frequency it seems that \( \eta' \) would approach the plateau region, but, due to the polydispersity of the system and the presence and impact of the large particles of bassorin, it could not flatten out, and thus rose to a high value with a lower slope than the other gums.

### 3.4. Modelling of the mechanical spectra

The power-law parameters used to model the frequency dependence of \( G' \) for the aqueous dispersions of the six gums are shown in Table 5. Here, the coefficient \( a \) represents the magnitude of \( G' \) at

<table>
<thead>
<tr>
<th>Species</th>
<th>( \tan \delta ) Max</th>
<th>( \omega ) (rad/s)</th>
<th>( \tan \delta ) Max</th>
<th>( \omega ) (rad/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A. ) parrowianus</td>
<td>1.29</td>
<td>0.42</td>
<td>1.84</td>
<td>4.44</td>
</tr>
<tr>
<td>( A. ) flaccus</td>
<td>1.29</td>
<td>0.26</td>
<td>1.78</td>
<td>0.26</td>
</tr>
<tr>
<td>( A. ) rahensis</td>
<td>1.67</td>
<td>0.26</td>
<td>1.78</td>
<td>0.26</td>
</tr>
<tr>
<td>( A. ) gossypinus</td>
<td>0.807</td>
<td>29</td>
<td>0.796</td>
<td>76</td>
</tr>
<tr>
<td>( A. ) microcephalus</td>
<td>0.393</td>
<td>76</td>
<td>0.431</td>
<td>76</td>
</tr>
<tr>
<td>( A. ) compactus</td>
<td>0.789</td>
<td>0.16</td>
<td>0.809</td>
<td>2.85</td>
</tr>
</tbody>
</table>
Particularly, times depending on the species. Among these, the stability of the structure. The cooperative theory of the models, during dynamic oscillatory experiments gel strands may be a physical gel, and the b value is related to the strength and nature of the gel (Khondkar et al., 2007). As can be seen in Table 5, with the addition of NaCl, the a values of the A. compactus, A. gossypinus, A. microcephalus, A. flourensus, A. rahensis gums were reduced by 1.5–3 times depending on the species. Among these, A. gossypinus and particularly A. compactus showed the most sensitivity to the addition of NaCl. Table 5 also shows that the A. microcephalus dispersions in the presence and absence of NaCl had the highest a and a’ values (a = 33.75 Pa s and a’ = 20.62 Pa s), which means that A. microcephalus had a stronger elastic structure than the others. According to the b and b’ values, A. microcephalus (b = 0.16 and b’ = 0.15) had the least sensitivity to frequency variation.

This section concerns with an indirect analysis of microstructures. The viscoelastic spectra are now re-interpreted in accordance with the viscoelastic theory of Bohlin (Gabriele, de Cindio, & D’Antona, 2001). This theory is reported in the literature as the “weak-gel” model. Weak gels and dispersions behave like three-dimensional networks and have limited flow ability. This model considers a “weak-gel” material as a flowing system characterised by weak physical interactions that cooperatively ensure the stability of the structure. The cooperative theory of flow provides a link between the structure of a material and its rheological properties by assuming that in a flowing substance, flow depends on rheological units rather than on single molecular units. Therefore, the real structure of a material is represented by a cooperative arrangement of flow units to form a strand. According to weak-gel models, during dynamic oscillatory experiments gel strands may be considered to be a combination of flow units. According to the theory, the magnitude of G’ is expressed by G’ = A0 z1/2, where, z is the most important parameter introduced in this model and is the “coordination number” and corresponds to the number of flow units interacting with each other in a three-dimensional structure to give the observed flow response. From this point of view, flow units do not break during flow, but strands do. In this context, the variable A refers to a flowing gel and may be interpreted as the “interaction strength” between the rheological units, a sort of amplitude of cooperative interactions (Gabriele et al., 2001). Thus: G’ = A0 z1/2. According to Table 5, A. microcephalus had the highest z value and A value, so, A. microcephalus has the highest number of flow units interacting with each other and the highest “interaction strength” between the rheological units. It is interesting to note that A. gossypinus in the presence of NaCl had a higher z value rather than in absence of NaCl, which was near that of A. microcephalus. It seems that NaCl contributed to enhancing formation of rheological units in the three-dimensional structure of the A. gossypinus dispersion.

4. Conclusions

In summary, the following conclusions can be inferred from the present experimental investigations: i) the sugar-compositional analysis of gum tragacanth from different species of Astragalus by HPAEC-PAD showed different values for each of the sugar components (arabinose, xylose, galactose, glucose, fucose, D-galacturonic acid and traces of rhamnose); therefore, the sugar composition of Astragalus gum exudates is strongly species-dependent and the functional properties of the gums are greatly influenced by their sugar compositions. ii) It is worth reemphasising that the correction of all mentioned errors regarding the names of the components of gum tragacanth as well as the sugar compositions of their structures should be applied to the broad range of literature. iii) The strain sweep and frequency sweep measurements of 1.3% gum tragacanth dispersions from different species exhibited a variety of mechanical spectra, which indicates that from an application-oriented viewpoint, each of the gum varieties can potentially endow distinct rheological and textural attributes to dispersed systems. Moreover, the addition of NaCl caused significant changes in the rheological parameters of viscoelasticity for each sample.

References


Stabilization of emulsions by gum tragacanth (Astragalus spp.) correlates to the galacturonic acid content and methoxylation degree of the gum

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\textbf{A B S T R A C T}

Gum tragacanth samples from six species of Iranian Astragalus bush plants ("goat’s-horn") were evaluated for their emulsion stabilizing effects and their detailed chemical composition in order to examine any possible correlation between the make-up and the emulsion stabilizing properties of gum tragacanth. The six gum tragacanth samples were exudates from the species Astragalus parrowianus, Astragalus flaccosus, Astragalus rahensis, Astragalus gossypinus, Astragalus microcephalus, and Astragalus compactus. The six gum samples varied with respect to their levels and ratios of water-soluble (tragacanthin) and water-swellable (bassorin) fractions, their monosaccharide composition, methoxylation, and acetylation degrees. The gums from A. parrowianus and A. flaccosus had relatively high tragacanthin:bassorin ratios of ~66:34 and ~75:25, respectively, whereas in the other gums this ratio approached 50:50 (A. rahensis, A. microcephalus, A. compactus) or tipped toward higher bassorin than tragacanthin (A. gossypinus). The monosaccharide make-up of the six gums also varied, but all the gums contained relatively high levels of galacturonic acid (~100–330 mg/g), arabinose (50–360 mg/g), xylose (~150–270 mg/g), and galactose (~40–140 mg/g), and also contained fucose, rhamnose, and glucose. The ability of the gums to act as stabilizers in whey protein isolate based emulsions varied. The best emulsion stabilization effect, measured as lowest creaming index ratio after 20 days, was obtained with the A. flaccosus gum. The emulsion stabilization effect correlated linearly and positively to the methoxylation degree, and galacturonic acid content of the gums, but not to acetyl or fucose content. A particularly high correlation was found between methoxyl level in the soluble gum part and emulsion stabilization. The work provides some important clues to the emulsion stabilization mechanisms in relation to the monosaccharide composition of tragacanth gums.

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1. Introduction

The natural plant exudate gum tragacanth is obtained from the stem of the bush like plant “goat’s-horn”, Astragalus species. Gum tragacanth has been used commercially for well over 2000 years and is currently widely used as an emulsifier and thickener in emulsion systems in different food, pharmaceutical and cosmetic applications (Whistler, 1993). Gum tragacanth has a bland flavor and good stability to heat and acid and is allowed for food uses in accordance with the FDA Code of Federal Regulations as a food additive at the level of 0.2–1.3% by weight of the product (FDA, 2006). In Europe, gum tragacanth has E-number E413 on the list of additives approved by the Scientific Committee for Food of the European Community. The main area of commercial production is the Middle East with Iran, supplying 70% of the commercially used gum tragacanth, followed by Turkey and Syria as main producers (Anderson, 1989).

Gum tragacanth is known to confer very high viscosities when in aqueous solution, and is described as a complex, highly branched, heterogeneous hydrophilic polysaccharide that may form complexes with salts, notably salts with divalent cations, such as Ca\textsuperscript{2+} salts (Balaghi, Mohammadifar, & Zargaraan, 2010). The molecular weight of a typical gum has been reported to be about 840 kDa, calculated by Svedberg’s method and formula, and the shape of the flake gum was
originally described as being elongated with dimensions of 450 nm by 1.9 nm (Gralén & Kärholm, 1950). The polysaccharides of gum tragacanth were studied in detail by James and Smith (1945a, 1945b) already almost 70 years ago and intensively by Aspinall and Baillie (1963a, 1963b) in the sixties. Tragacanthin is the water-soluble fraction, and bassorin is the insoluble part which swells in water to form a gel (Aspinall & Baillie, 1963a). It is well known that the ratio between the water-soluble and water-insoluble fraction of gum tragacanth varies significantly among gums obtained from different Astragalus species (Balaghi et al., 2010), but it is unclear to what extent the composition of the polysaccharides affects the ratio of tragacanthin to bassorin.

With respect to the compositional make-up of the gum tragacanth polysaccharides, it has been amply reported that gum tragacanth contains L-arabinose, D-galactose, D-glucose, D-xylose, L-fucose, L-rhamnose, and D-galacturonic acid upon acid hydrolysis (Balaghi, Mohammadifar, Zargarana, Gavlighi, & Mohammadi, 2011; Tischer, Iacomini, & Gorin, 2002). Recently, it has become evident, that the understanding up until now that the soluble fraction (tragacanthin) consists of arabino-galactan and that the insoluble fraction (bassorin) is made up of "tragacanthic acid" is probably not correct (Balaghi et al., 2011). Rather, at least the water-soluble fraction (tragacanthin) appears to resemble pectin, and seems to contain linear chains of galacturonic acid (probably 1,4-linked); hence, gum tragacanth species rich in xylose with minor levels of fucose may contain xylogalacturonans and some fucoxylogalacturonans as the main components of the soluble fraction, whereas those having high fucose levels may mainly contain fucosylxylogalacturonan in the tragacanth fraction (Balaghi et al., 2011). In most Astragalus species, the bassorin and tragacanthin also have been found to contain methyl groups, probably representing methoxylated galacturonic acid. The insoluble bassorin part generally appears to have less methoxyl substitutions than the soluble tragacanthin part (Anderson & Grant, 1988).

Gum tragacanth has been reported to have both emulsifying and stabilizing properties in emulsions, i.e. being "bi-functional" by having both the capacity to facilitate emulsification as well as providing stabilization of the emulsion after its formation (Weiping, 2000). There are indications in the literature that compositional differences of tragacanth gums obtained from different species of Astragalus affect the rheological properties, including the viscosity of tragacanth solutions (Balaghi et al., 2011). However, despite the long and extensive use of gum tragacanth as a stabilizer in emulsion systems, surprisingly little is known about structure function relationships, and the traits of the gum that confer emulsifying properties are presently unknown. It is of course tempting to hypothesize that the composition of tragacanth gum affects its stabilization effects in emulsions, and it has been reported that the terminal deoxyhexosyl groups (i.e. fucose) or the methoxyl groups of the homogalacturan in the tragacanth gum structure may play a role in the emulsion stabilization (Stephen, 1990). However, to our knowledge, no unequivocal evidence has up until now been provided for this hypothesis. Hence, the exact significance of specific structural components of tragacanth gum in relation to viscosity and emulsion stabilization properties is unclear.

The purpose of this study was to evaluate the stabilization of emulsions by tragacanth gum, and to aim at obtaining an understanding of any relationships between the make-up of gum tragacanth and its stabilization properties in emulsions. This was done by comparing the chemical composition of tragacanth gum obtained from six Astragalus species growing in different regions of Iran, and by systematically assessing whether there were any correlations between emulsion stabilization properties and the prevalence of specific chemical components in the gums.

2. Materials and methods

2.1. Materials and gum preparation

Gum tragacanth samples exuded from various species of Astragalus (Astragalus parrowianus, Astragalus flavusus, Astragalus rahensis, Astragalus gossypinus, Astragalus microcepalus, and Astragalus compactus) were collected from plants growing in different provinces of Iran. The plants were tapped by making careful longitudinal incisions in the taproot and the bark of the branches. The gum readily exuded from these cuts in the form of ‘ribbons’ that became brittle on drying. The dried raw gums were ground with a coffee mill, sieved, solubilized in deionized water, then freeze dried, and finally used in this study. The protein contents (g/100 g) of the gum tragacanth samples were 3.0, 2.6, 3.8, 0.31, 2.0, and 1.7 for A. parrowianus, A. flavusus, A. rahensis, A. gossypinus, A. microcepalus, and A. compactus, respectively (Balaghi et al., 2010). Neutral monosaccharide standards, L-fucose, L-rhamnose, D-arabinose, D-galactose, D-glucose, D-xylose, D-galacturonic acid and D-glucuronic acid were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Trifluoroacetic acid (99%) was from Merck (Darmstadt, Germany). NaOH standard solution HPLC grade was from Fluka/Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All chemicals used were of analytical grade. Rapeseed oil was obtained from Inco Denmark and whey protein isolate (WPI), LACPRODAN DI-9224, was purchased from Arla Foods Ingredients (Videbæk, Denmark). The WPI was a neutral and heat stable type of whey protein isolate that according to the supplier is sold as an emulsifier as well as for use in sports and clinical nutrition. According to the supplier’s data sheet, the WPI consisted of 92% protein (N × 6.38), 0.2% lactose, 4.5% ash and approximately 0.5% sodium, 0.2% phosphorus, 1.3% potassium and 0.1% calcium (all by weight).

2.2. Determination of soluble and insoluble gum fractions, and monosaccharide composition

In order to determine the ratio between the water-soluble and the water-insoluble fractions of the gums, each gum sample was re-suspended overnight in deionized water (1% dry matter gum weight/volume) and the separation of the soluble and insoluble fraction was done by centrifugation at 3600 × g for 180 min and the separate fractions were freeze dried and weighed as described previously (Mohammadifar, Musavi, Kiumarsi, & Williams, 2006). The monosaccharide composition of each of the gum tragacanth samples was determined after acid hydrolysis (4 g L⁻¹ substrate, 2 M trifluoroacetic acid, 2 h, 121 °C) and by use of separate recovery factors for each monosaccharide (Arnous & Meyer, 2008). The monosaccharide analyses were done by use of an ICS3000 ion chromatography system consisting of a G550 gradient pump, an ED50 electrochemical detector and an AS50 chromatograph coupled to an AS50 autosampler ( Dionex Corp., Sunnyvale, CA). Separations were performed using a Carbopac™ PA20 (3 mm × 150 mm) analytical column (Dionex Corp., Sunnyvale, CA) and an elution programme principally as described previously (Balaghi et al., 2011).

2.3. Determination of total methyl and acetyl contents of gum tragacanth

The methanol and acetic acid contents of each freeze dried gum tragacanth sample (soluble, insoluble, and full fraction) were determined by HPLC using a Summit LC system ( Dionex Corp., Sunnyvale, CA) equipped with a P680 HPLC pump, an ASI-100 automated sample injector, and an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad Labs, Richmond, CA, USA) in combination with a guard column (AG50WX4. 50 × 4.6 mm, Bio-Rad Labs)
2.4. Saponification of tragacanth gum

Methyl and acetyl groups of the tragacanth gum solution from A. flaccusus (1% w/v) were removed by slowly adding 5 M NaOH in water (cold) until pH 13 and stirring for 24 h at 4 °C. After that, the saponified solution was dialyzed against deionized water at 4 °C using a 5 kDa molecular weight cut off polyethersulfone membrane (Sartorius Stedim Biotech) and crossflow membrane filtration. After the dialysis was completed, the solution was freeze-dried (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003).

2.5. Preparation of O/W emulsions

Oil in water (O/W) emulsions were prepared by mixing rapeseed oil (10% w/w) into a solution of 0.5% w/w WPI of 100 mM sodium acetate buffer (pH 4.5) and stirred overnight at 40 °C. Pure methanol and glacial acetic acid from Fluka were used as standards.

2.6. Emulsion stability

Emulsions, 5 mL sample size, were placed in sealed glass tubes (diameter × height: 2 × 6 cm) at room temperature and the emulsion stability was assessed by measuring the height of the clear serum layer (forming at the bottom of the emulsion systems) relative to the total height of the emulsion after (0), 5, 7 and 20 days. The creaming index was expressed as the height of the serum layer over the total height of the emulsion in the tube.

2.7. Particle size determination

Immediately after emulsion preparation, samples were analyzed for droplet size distribution by use of a laser diffraction particle size analyzer (Mastersizer S, Malvern Instruments Ltd, Worcestershire, UK). All measurements were made at room temperature on at least two freshly prepared samples. Optical properties of the sample were defined as follows: refractive index of rapeseed oil and dispersion medium (water) were 1.46 and 1.33, respectively and absorption was assumed to be 0. The average droplet size was described as the surface area moment mean (also known as the surface area weighted mean diameter) \[ D(3,2) = \sum n_i d_i^3 \sum n_i d_i^2 \] and the volume mean diameter (\(D(4,3)\)) was automatically calculated using the software provided with the apparatus:

\[ D(4,3) = \sum n_i d_i^4 \sum n_i d_i^3 \] where \(n_i\) is the number of particles with diameter \(d_i\) (McClements, 2005).

2.8. Rheological measurements of emulsions and tragacanth gum solutions

Steady-shear measurements of shear data of the emulsions and tragacanth gum solutions at the same pH of the emulsions (pH 4.5) were carried out at 0.1–500 s⁻¹ on serrated parallel plates (PP60 Ti Serrated) using a Haake Mars rotational rheometer (Thermo Scientific Inc., Germany). All the measurements were carried out at 25 °C with duplication measurement.

2.9. Statistical analysis

Data were analyzed by one-way analysis of variance (one-way ANOVA): Comparisons of mean values were calculated via 95% confidence intervals and compared as Tukey–Kramer intervals calculated from pooled standard deviations (Minitab Statistical Software, Addison-Wesley, and Reading, MA).

3. Results and discussion

3.1. Gum tragacanth composition

The ratio between the soluble and insoluble fractions varied significantly among the tragacanth gums from the different species of Astragalus, but the gums could roughly be divided into three groups: Group 1: The gum from A. flaccusus had the highest soluble:insoluble ratio of 3.16, i.e. with ~75% w/w soluble and ~25% w/w insoluble, and the gum from A. parrowianus had a ratio of ~64% w/w soluble and ~36% w/w insoluble, giving a ratio of 1.77 (Table 1). Group 2: The gums from A. rahensis, A. microcephalus, and A. compactus all had almost 50:50 soluble:insoluble gum fractions giving ratios of soluble to insoluble of ~1, whereas Group 3, represented by the gum of A. gossypinus contained a higher amount of insoluble (~65% w/w) than soluble (~35% w/w polysaccharides), with a ratio of soluble to insoluble of 0.53 (Table 1).

As expected, also the monosaccharide composition varied among the gums from the different Astragalus plant species. In general, all gums contained relatively high amounts of galacturonic acid, arabino, xylose, and to a certain extent galactose. All gums also contained fucose, rhamnose, and glucose, with particularly wide content ranges in the fucose content (varying from 27 to 236 mg/g) (Table 1). Rhamnose content was almost constant in the gums from different species (13–22 mg/g), Galacturonic acid contents ranged from ~100 to 335 mg/g dry matter. Surprisingly, the highest level of galacturonic acid was determined for the gum from A. gossypinus, even though this gum had a lower amount of the soluble fraction than the insoluble bassorin (Table 1). Arabinose levels varied from 48 to 360 mg/g and xylose from ~150 to 270 mg/g, whereas galactose levels varied from ~40 to 140 mg/g (Table 1). Gum tragacanth from A. gossypinus stood out as having the highest content of fucose, xylose, and galacturonic acid, as well as the lowest levels of arabinose, galactose, and glucose, among the samples (Table 1). In all the gum samples, the soluble part consistently contained higher levels...
of galacturonic acid than the insoluble part — but the relative difference between galacturonic acid content in the soluble versus the insoluble fraction varied across the gums from the different Astragalus spp. In contrast, fucose and partly xylose levels were varied significantly in the gums from the different Astragalus species. Blue, filled squares indicate data for the insoluble fraction of gum tragacanth (Balaghi et al., 2010). Among the gum samples, the gum from A. compactus produced the highest viscosity response, up to almost 0.05 Pa s at the lowest shear rate of 25 s⁻¹ (Fig. 3). In contrast, the viscosity of the A. rahensis gum was only 0.01 Pa s at this shear rate (Fig. 3). The gums from the A. fluccosus and A. microcephalus samples also produced high viscosities (0.028–0.029 Pa s at the lowest shear rate of 25 s⁻¹). These two gums had widely different ratios of soluble:insoluble fractions, and also varied widely in their monosaccharide composition (Table 1), so no immediate correlation between the compositional make-up and viscosity properties could be derived. Neither did the quite unique compositional traits of the A. gossypinus gum produce strikingly different viscosity inducing properties, since this gum generally gave the second-lowest viscosity enhancing effect among the samples (Figs. 2 and 3). The power-law functions were calculated for the shear thinning region, i.e. over mid-range shear rates up to 40 s⁻¹ (Table 2): The flow behavior index (n) and consistency coefficient (m) values were obtained by fitting the shear rate versus apparent viscosity data to the power law model (Taherian, Fustier, & Ramaswamy, 2007):
When comparing the $m$ and $n$ values across the different emulsions and tragacanth gum solutions prepared with the six different tragacanth gum species, the $m$-values were found to be consistently higher in the emulsions than in the gum solutions (Table 2). This finding was of course in complete accordance with the observation that the viscosity was always higher in the emulsions than in the gum solutions (Figs. 2–5). The $n$ was found to be lower than 1 for all tragacanth gum solutions as well as for all the emulsions (Table 2); this value of less than 1 confirmed the shear thinning behavior of the samples. The emulsion prepared with the A. flouccosus gum had the highest consistency coefficient ($m$), 0.34 Pa s$^n$, among the samples. This result was in relatively good accordance with the gum solution viscosity measurements since the A. flouccosus gum was the gum giving the second highest viscosity response (Fig. 3), with the viscosity response approaching that of the A. compactus gum at high shear rates. The $n$ for the A. flouccosus sample was also the highest among the gum solutions, but in the emulsion samples the A. compactus gum produced the highest $n$ of 0.68 (Table 2), however for the emulsions, the $n$-values were all in the range 0.5–0.68, the only exception being the emulsion with the A. rahensis gum, which had a lower $n$ of 0.35 (Table 2).

The viscosities of the emulsions were considerably higher than the gum tragacanth solutions, but the overall flow behavior in the emulsions with gum tragacanth added was quite similar to the behavior observed in the gum tragacanth solutions (Fig. 4). The viscosity of the model emulsion without gum tragacanth added (4.4% w/w rapeseed oil, 0.22% w/w WPI, pH 4.5) was very low at all shear rates (control, Fig. 5). These results are similar to those obtained with xanthan gum added to emulsions (Dickinson & Galazka, 1991). In the emulsions, again, the A. compactus gum induced the highest viscosity reaching a viscosity response of 0.09 Pa s at the lowest shear rate of 25 s$^{-1}$. The pattern followed that observed for the gums in suspension, so the lowest viscosity response was observed for the A. rahensis gum, which was only 0.02 Pa s at this shear rate (Fig. 5). The medium-to-high viscosity contributed by the A. flouccosus and A. microcephalus samples increased from just below 0.03 Pa s in the aqueous suspension system to 0.075 Pa s in the emulsion system at the lowest shear rate of 25 s$^{-1}$ (Fig. 5). In general, these viscosity increases did not correlate to the levels or ratios of the insoluble (nor soluble) gum tragacanth fractions, so it was not possible to relate the viscosity responses directly to the swelling ability of the insoluble gum tragacanth fraction.

Rather, the viscosity response may be related to certain structural traits of the tragacanth gum, such as polysaccharide chain length, branching pattern, substituitions, differences in back-bone bond structures or other factors, that are not revealed by the compositional analysis, but which may impact formation of inter and intra molecular interactions between the gum tragacanth polysaccharides, the whey protein, and the emulsion droplets (Azarikia & Abbasi, 2010). The magnitude of the viscosity response and the rheological behavior of the tragacanth gums in emulsions agree with data for xanthan gum behavior in emulsions published by Papalamprop, Makri, Kioseoglou, and Doxastakis (2005).

### 3.3. Particle size distribution of emulsions

The emulsions with gums from A. rahensis and A. flouccosus, respectively, had the smallest average particle size of ~12 μm, measured as $D[3,2]$, and the emulsion with the A. gossypinus gum had the largest $D[3,2]$ of ~38 μm (Fig. 6). The ANOVA indicated that the differences in particle size were statistically significant ($p < 0.05$). Without gum tragacanth added, the emulsion produced a monomodal particle size distribution with an average $D[3,2]$ of ~7 μm (and $D[4,3]$ of ~10 μm) (Fig. 7). Addition of gum tragacanth affected the droplet size distribution to become bimodal (Fig. 7).
The tragacanth gum stabilization of protein-emulsified emulsions is probably a result of two mechanisms: Firstly, formation of non-covalent protein-(gum) polysaccharide complexes, and secondly viscosity increase. It is well-recognized that emulsion stabilization by whey proteins may be improved by polysaccharides (and particularly so at pH near the isoelectric point). For pectin-β-lactoglobulin complexes it has been shown that this effect occurs when the protein associates to the polysaccharide, and soluble, charged complexes are formed, which have an overall charge as the polysaccharide effect is mainly due to enhanced steric stabilization by electrostatic repulsion. From model-studies with dextran and particularly so at pH near the isoelectric point). For pectin-β-lactoglobulin complexes it has been shown that this effect occurs when the protein associates to the polysaccharide, and soluble, charged complexes are formed, which have an overall charge as the polysaccharide effect is mainly due to enhanced steric stabilization by electrostatic repulsion. The soluble complex is thus stabilized by electrostatic repulsion. From model-studies with dextran—whey protein conjugates it appears that the polysaccharide effect is mainly due to enhanced steric stabilization provided by the bulkiness of the hydrophilic polysaccharides that provides a stabilizing charged layer around the protein stabilized oil droplets which protects the protein stabilized oil droplets against flocculation under conditions where electrostatic stabilization is less favorable (Akhtar & Dickinson, 2003).

The observation that the emulsion exhibiting the larger \( D[4,3] \) \((A. gossypinus)\) did not exhibit the largest viscosity despite having the highest galacturonic acid content (Table 1) is in accordance with a recently published observation that the median diameter of particles of tragacanth gum dispersions varied in response to the ratio of the soluble to the insoluble gum fraction. When the same gums as used in the present study were compared in solution the A. gossypinus sample produced the largest particle size and there was no direct relationship between the size of particles, gum composition, and viscosity (Balaghi et al., 2010).

Most of previous studies in which polysaccharides (typically pectin) were used for stabilizing protein-emulsified emulsions have reported that a stable system will have a monodomal distribution with a small average particle size \((\text{e.g. } D[4,3] < 2 \mu m)\) while unstable emulsions will have relatively large particle sizes with \( D[4,3] \) values > 20 μm (Lucey, Tamehana, Singh, & Munro, 1999). Lucey et al. (1999) also reported that higher stability is associated with more uniformity of particle size. However, pectin does not have a fraction with functionality like bassorin in its composition. Therefore, we presume that the second peak \((\text{with higher values for diameter size assessed as } D[3,2]) \) (Fig. 7) is not related to oil droplets, but may be elicited by the gum fraction alone (illustrated in Fig. 7 only for the gum from A. rahensis as an example). Hence, in our opinion the first peak (Fig. 7) is due to a very large amount of small emulsion droplets having a cover layer of whey protein and tragacanthin and the second one represent the presence of small amounts of very large particles of bassorin (Gorji, Mohammadifar, & Ezzatpanah, 2011). We also presume that bassorin may be responsible for the increased viscosity of the continuous phase in the emulsion.

When reported on a volume basis as a volume base mean, \( D[4,3] \), the particle size diameter data of the gum tragacanth stabilized emulsions ranged from \( \sim 100–200 \mu m \) (Table 3). The volume base evaluation also allowed calculation of the volume made up by the smallest particles (calculated from the average area of the first peak in the bimodal, volumetric particle size distribution curve) (Table 3). The \( D[4,3] \) data thus corroborated the interpretation of the \( D[3,2] \) data (Fig. 6), namely that the particle size of the A. gossypinus was biggest because the volumetric fraction contributed by the small particle size peak was significantly lower in the

### Table 2

<table>
<thead>
<tr>
<th>Varieties</th>
<th>A. parrowianus</th>
<th>A. fluccosus</th>
<th>A. rahensis</th>
<th>A. gossypinus</th>
<th>A. microphalus</th>
<th>A. compactus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulsions</td>
<td>( n )</td>
<td>m</td>
<td>( R^2 )</td>
<td>( n )</td>
<td>m</td>
<td>( R^2 )</td>
</tr>
<tr>
<td>Tragacanth gum solutions</td>
<td>0.61</td>
<td>0.17</td>
<td>0.99</td>
<td>0.53</td>
<td>0.34</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>0.08</td>
<td>0.98</td>
<td>0.76</td>
<td>0.06</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>0.68</td>
<td>0.25</td>
<td>0.99</td>
<td>0.55</td>
<td>0.19</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*Power-law parameters m: Pa s\(^{-1}\); n: dimensionless; \( R^2 \): Correlation coefficient for the power law function.*
A. gossypinus gum emulsion than the corresponding volume fractions in the emulsions with the other gums (Table 3). Analogously, the $D_{[4,3]}$ volumetric base data verified the interpretation for the smallest $D_{[3,2]}$ peak average data for emulsions stabilized by A. fluccosus and A. rahensis gums, respectively (Fig. 6), via the finding that the volumetric fraction made up by the first peak in each of their respective volume base particle size distribution curve was higher, making up 24–26% of the total volume, than the corresponding volumetric fractions in the other gum-stabilized emulsions (Table 3). The volumetric percentage calculation based on the volume base particle size mean of emulsions with gums also explained that the emulsion with good stability, namely that with A. fluccosus gum (Table 4, as discussed in the next section) had the highest percentage of small particles, namely 26%, compared to all the other emulsions (Table 3). Also the emulsion with A. rahensis gum had a high percentage of small particles among the emulsions but it was not a very stable emulsion (Table 4, as discussed in the next section). This latter result could be because of the low content of galacturonic acid and methoxyl level in this gum (Table 1).

The $D_{[4,3]}$ data of gums had larger size numbers than emulsion with gums (Table 3) presumably because of bassorin. Hence, $D_{[4,3]}$ data is strongly influenced by the presence of relatively large particles and this is why the $D_{[4,3]}$ particle size data of emulsions with gums produced relatively higher numbers than when assessed via $D_{[3,2]}$ (Fig. 6). The value of $D_{[3,2]}$ is determined mainly by the relatively small particles present in the emulsions, whereas $D_{[4,3]}$ data are strongly influenced by the presence of relatively large particles. Hence, the $D_{[3,2]}$ provides a measure of the mean diameter where most of the particles fall, while $D_{[4,3]}$ provides a measure of the presence of any flocculated droplets.

From the results of the galacturonic acid content and correlation with methoxylation degree in the tragacanthin part of the tragacanth gum samples, it can be concluded that the soluble part of gum tragacanth very much resembles pectin polysaccharides that can be used in combination with WPI for stabilization of acid emulsions (Ganzulevs, Zinoviadou, van Vliet, Cohen Stuart, & de Jongh, 2006; Tippett & Martini, 2012).

### 3.4. Emulsion stabilization by gum tragacanth

Storage stability of the tragacanth gum emulsions was determined after 5, 7 and 20 days (Table 4). The emulsion without tragacanth gum was not stable after 1 h at all (data not shown). During the storage of the emulsions, the creaming index increased gradually with time, except for the emulsion containing the gum from A. fluccosus, which remained stable throughout the 20 days having a creaming index of 0.0 (Table 4). Hence, the A. fluccosus gum induced the best stabilization effect, whereas the lowest emulsion stabilization effect was provided by the A. rahensis gum.

Also the emulsions with the A. gossypinus or the A. compactus gum, respectively, exhibited reasonably acceptable stabilization effects, producing low creaming indexes of only 0.13 and 0.18, respectively, after 20 days (Table 4).

In general, the stabilization effect of gum tragacanth in emulsions may be due to increased viscosity of the continuous phase surrounding the oil droplets. Another stabilization mechanism may be via adsorption/precipitation of the gum at the oil–water interphase causing a reduction in interfacial tension (Huang, Kakuda, & Cui, 2001). Our results showed that the A. rahensis gum produced the lowest viscosity in solution as well as in emulsion.

**Table 3** Comparison of particle size in gum tragacanth stabilized emulsions and suspensions on volume base mean $D_{[4,3]}$ for gum tragacanth samples from different Astragulus species.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>$D_{[4,3]}$ of emulsions with gum tragacanth</th>
<th>Average volumetric fraction made up by first particle size peak in emulsions with gums (%)</th>
<th>$D_{[4,3]}$ of gum tragacanth suspensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. parrowianus</td>
<td>155.2 ± 1.7</td>
<td>15.5</td>
<td>176.6 ± 0.3</td>
</tr>
<tr>
<td>A. fluccosus</td>
<td>113.4 ± 1.6</td>
<td>26.1</td>
<td>157.0 ± 0.2</td>
</tr>
<tr>
<td>A. rahensis</td>
<td>104.0 ± 0.3</td>
<td>24.0</td>
<td>144.7 ± 0.7</td>
</tr>
<tr>
<td>A. gossypinus</td>
<td>192.8 ± 1.3</td>
<td>8.1</td>
<td>212.0 ± 0.8</td>
</tr>
<tr>
<td>A. microcephalus</td>
<td>173.5 ± 1.4</td>
<td>13.1</td>
<td>207.3 ± 2.0</td>
</tr>
<tr>
<td>A. compactus</td>
<td>198.7 ± 1.8</td>
<td>18.9</td>
<td>246.3 ± 0.8</td>
</tr>
</tbody>
</table>

* Calculation based on the average size of the first peak.

**Table 4** Storage stability of emulsions stabilized with different Astragulus gums after 5, 7, and 20 days of storage at 25 °C. For control emulsions without tragacanth gums added, the creaming index reached 0.4 within 1 h.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Creaming index ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 day</td>
</tr>
<tr>
<td>A. parrowianus</td>
<td>0.08 ± 0.01b</td>
</tr>
<tr>
<td>A. fluccosus</td>
<td>0c</td>
</tr>
<tr>
<td>A. rahensis</td>
<td>0.33 ± 0.03a</td>
</tr>
<tr>
<td>A. gossypinus</td>
<td>0c</td>
</tr>
<tr>
<td>A. microcephalus</td>
<td>0c</td>
</tr>
<tr>
<td>A. compactus</td>
<td>1.3</td>
</tr>
<tr>
<td>Saponeifed A. fluccosus</td>
<td>0.1 ± 0.00b</td>
</tr>
</tbody>
</table>

* Values with different letters in each row are significantly different ($p < 0.05$).
(Figs. 2 and 3), and that the A. compactus gum gave the highest viscosity response. Hence, the data obtained agree with the emulsion stabilization through a viscosity increase mechanism for the A. raehens and the A. compactus gums. The data do not fully explain the overall emulsion stabilizing activity of gum tragacanth since for these 6 gums, the correlation between the viscosity and the creaming index was poor ($R^2 = 0.38$ for the linear correlation for the lowest shear rate data with the 20 days emulsion creaming index). The viscosity increasing effects cannot either explain why the gum tragacanth samples (data not shown). Since the methyl groups presumably represent methoxylated galacturonic acids, it might immediately be expected that there would also be a correlation between the galacturonic acid content in the soluble gum tragacanth part and creaming index. However, there was not ($R^2 = 0.0142$). This finding is in fact in good agreement with the observation that the methoxylolation degree of the galacturonic acids in the soluble fraction of the gum tragacanth gum varied widely (Fig. 1). To add to the confusion, a strong linear correlation was found between the total galacturonic acid content (in the full gum) and creaming index, i.e. the more galacturonic acid, the lower the creaming index (Fig. 9). So how can the correlation to the methoxyl groups in the soluble fraction be explained, when at the same time there is no accompanying good correlation to the galacturonic acid content in the soluble gum fraction, but in contrast, a very good correlation between emulsion stabilization and total galacturonic acid content in the gum? The correlation means that the more methoxyl groups in the soluble gum tragacanth fraction, the better the emulsion stabilization effect. Several phenomena may be hypothesized to explain these observations:

1. Methoxyl groups, including methoxylated galacturonic acid residues in “pectin”, may interact with each other or with proteins via hydrophobic interactions, even though the ionic interactions of non-methoxylated galacturonic acids are much more studied in relation to gelation (Zaidel & Meyer, 2012). Hence, it is not unlikely, that the methoxylolation of the soluble saccharides of gum tragacanth stabilizes emulsions may take place via promotion of such hydrophobic interactions in the complex emulsified system which also contained WPI.

2. The distribution pattern of the methoxyl groups on the galacturonic acid backbone, i.e. whether in blocks or randomly distributed, plays a crucial role. Also, chains of demethoxylated galacturonic acids may exist in the soluble fraction of gum tragacanth, which would help explain why the methoxylolation degree varied so widely among the soluble gum tragacanth fractions (Fig. 1). It may well be that a random, but high degree of substitution, will act to stabilize emulsions (and not cause gelation) as observed for enzymatically modified pectin (Willats et al., 2001). This explanation would agree with a mechanism by which the methoxyl groups in the soluble part of the gum tragacanth hydrocolloid structure would allow more stable interactions with the WPI emulsifier through hydrophobic bonding. This mechanism agrees with the increased emulsion stabilization

3.5. Relation between gum tragacanth composition and emulsion stabilization

Considering the significantly different emulsions stabilization effects of the gum tragacanth samples, their relatively different viscosity inducing effects, and their wide compositional variability, we hypothesized that there might be a correlation between composition and functionality, especially with respect to emulsion stabilization. Whereas the acetylation is known to partially prevent gelation of sugar beet pectin, the ability of sugar beet pectin to stabilize emulsions has been suggested to be related to the presence of acetyl groups in the pectin structure (Endress & Rentschler, 1999). However, when evaluating the correlation between acetyl content of the gum tragacanth samples and the creaming index, no significant relationship could be established. No direct correlation between acetyl in the soluble or insoluble fractions and creaming index existed either (data not shown). In contrast, a tendency toward a linear correlation was found between the acetyl content in the (full) gum tragacanth samples and creaming index, with $R^2 = 0.4$ (data not shown). When assessing this deeper, a very good linear correlation of $R^2 = 0.92$ was found between the total acetyl content in the soluble gum fraction, and these data thus support the second highest viscosity response when assessed separately (Figs. 2 and 3). According to Azarikea & Abbasi, (2010), the soluble tragacanthin and full gum prevented serum separation at concentrations of 0.1 and 0.2%, respectively, and these data thus support a main role of the tragacanthin in emulsion stabilization. Whereas the acetylation is known to partially prevent gelation of sugar beet pectin, the ability of sugar beet pectin to stabilize emulsions has been suggested to be related to the presence of acetyl groups in the pectin structure (Endress & Rentschler, 1999). However, when evaluating the correlation between acetyl content of the gum tragacanth samples and the creaming index, no significant relationship could be established. No direct correlation between acetyl in the soluble or insoluble fractions and creaming index existed either (data not shown). In contrast, a tendency toward a linear correlation was found between the acetyl content in the (full) gum tragacanth samples and creaming index, with $R^2 = 0.4$ (data not shown). When assessing this deeper, a very good linear correlation of $R^2 = 0.92$ was found between the total acetyl content in the soluble gum fraction, and these data thus support the second highest viscosity response when assessed separately (Figs. 2 and 3). According to Azarikea & Abbasi, (2010), the soluble tragacanthin and full gum prevented serum separation at concentrations of 0.1 and 0.2%, respectively, and these data thus support a main role of the tragacanthin in emulsion stabilization. However, in addition, the insoluble bassorin may boost stabilization by increasing the viscosity. There was no correlation between the $D[H3.2]$ data and creaming index across the gum tragacanth stabilized emulsions.

**Fig. 8.** Correlation between methoxyl content in the soluble fraction of different gum tragacanth samples from different Astragalus species and creaming index.

**Fig. 9.** Correlation between creaming index and galacturonic acid of gum tragacanth samples from different Astragalus species and creaming index.
stabilization obtained in dairy beverage (Doogh) to which soluble gum tragacanthin was added (Azarikia & Abbasi, 2010).

3. There was no correlation between the fucose content in the gum samples and the creaming index. When grouping the fucose in the soluble fraction with creaming index, a weak linear trend was evident ($R^2 = 0.54$, but not statistically significant). In contrast, the methoxyl and the fucose levels in the soluble gum fractions correlated highly significantly, and positively linearly ($R^2 = 0.96$) (Fig. 10). Fucose is a methylpentose being slightly more hydrophobic than other monosaccharides. Apparently this hydrophobicity could contribute to the positive stabilizing effect via hydrophobic interaction (re hypothesis 2, above). In practice, the data revealed that good emulsion stabilization mainly occur via the soluble gum tragacanth fraction (tragacanthin). Hence, particularly good emulsion stabilization properties will occur if gum tragacanth is divided into a soluble and an insoluble gum fraction, and the soluble fraction is rich in fucose and high in methoxyl content.

4. Conclusion

It has been demonstrated that different tragacanth gum samples obtained from different species of Astragalus have different composition, and produce different levels of soluble and insoluble gum fractions. Galacturonic acid was high in the soluble part of all species whereas $L$-fucose and partially xylose was major in insoluble fraction. A positive correlation between the methoxyl content of the soluble part of the gums and the creaming index indicate that methoxyl groups may have properties that play a role in the emulsification properties of tragacanth gums. Increased viscosity of emulsions may be a result of hydrophobic bonding between tragacanth gum and WPI emulsified emulsion particles. Clearly, the viscosity is a factor, but apparently also the composition, notably the total galacturonic acid content in the gum, the amount of methoxyl groups and probably also fucose in the solubilized part are factors determining how tragacanth gums work to stabilize emulsions.

Acknowledgment

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References


4. Prebiotic

This chapter explains assess effect of different molecular weight of tragacanth gum produced via enzymatic process on growth of pure culture to get potential prebiotic examination (Paper II).

The concept of prebiotics was introduced in 1995 by Gibson and Roberfoid as an alternative approach to the modulation of the gut microbiota (Glenn R. Gibson & Roberfoid, 1995). This definition of Prebiotic was later revised to: a selectively fermented component that causes significant changes, in both concentration and activity of gastrointestinal microbiota. These changes confer well-being and health benefits on the host (G.R. Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). Currently, the target genera are lactobacilli and bifidobacteria; however, prebiotic success has primarily been achieved with bifidobacteria. This may be due to the fact that more bifidobacteria usually reside in the human colon than lactobacilli and they exhibit a preference for oligosaccharides (Brownawell, et al., 2012). In addition, worldwide interest in prebiotics have been increasing extensively both as food ingredients and pharmacological supplements, since they have beneficial properties for human health. Thus, they can be interesting and useful ingredients in the development of novel functional foods.

4.1 Source and production of prebiotics

Traditional dietary sources of prebiotics include: soybeans, bananas, garlic, barley, onion, Jerusalem artichoke tuber, wheat, asparagus, rye, and chicory root. There are several types of common prebiotics; inulins, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), soya-oligosaccharides, xylo-oligosaccharides, pyrodextrins, isomaltoligosaccharides and lactulose used in human nutrition. The majority of studies carried out to date have focused on inulin, FOS and GOS (Macfarlane, Steed, & Macfarlane, 2008).

Prebiotic can be produced by the transgalactosylation activity of the respective carbohydrate by both chemical and enzymatic methods by using various raw materials such as lactose, sucrose (Adamczak, Charubin, & Bednarski, 2009), starch (Zhang, et al., 2010) and xylan (Panesar, Kumari, & Panesar, 2012). Recently, advances in the enzyme technology have been used to synthesize novel oligosaccharides. By enzymatic synthesis oligosaccharides can be produced in large scale. Production of oligosaccharide via using specific extraction methods and specific enzyme from plant cell wall polysaccharide as a novel source of potential prebiotics has been started. Arabinogalacto-oligosaccharides can be made from soybeans by endogalactanases, arabino-oligosaccharides can be made from sugar beet by endoarabinanases, rhamnogalacturonol-oligosaccharides can be made from apple by rhamnogalacturonases, arabinoxylo-oligosaccharides can be made from wheat by xylanases and galacturonol-oligosaccharides can be made from polygalacturonic acid by endogalacturonases (Olano-Martin, Gibson, & Rastall, 2002; Oosterveld, Beldman, & Voragen, 2002; Van Laere, Hartemink, Bosveld, Schols, & Voragen, 2000). Also, Pectic oligosaccharides have been observed to have bifidogenic, prebiotic properties (Olano-Martin, Gibson, & Rastall, 2002). Differences in structure, such as changes in size and the presence of branch(s) and differences in the complexity of the substituents, can cause significant changes in the prebiotic properties of oligosaccharides (Holck, et al., 2011b; Kabel, Korteneoven, Schols, & Voragen, 2002). In addition, prebiotic oligosaccharides may confer health benefits is via their inhibiting the adherence of bacteria to epithelial cells (Figure 4.1) (Shoaf, Mulvey, Armstrong, & Hutkins, 2006).
On the other hand, recently, exudate gums with arabinogalactan structure such as gum Arabic has been considered as a very well tolerated dietary fiber with bifidogenic properties believed to benefit intestinal health (Meance, 2004).

From this view, Tragacanth gum made up of fucoxylogalactronan and arabinogalactan in the structure has a unique properties. From one side has an arabinogalactan that can be hydrolysis by arabinanas and already mentioned has a bifidogenic properties and from another side has a highly fucosylated that can be use as an anti adhesion properties.

Figure 4.1 Mechanisms of prebiotic action against pathogens. adapted from (Charalampopoulos & Rastall, 2009).

4.2. Enzymatic depolymerization of gum Tragacanth: Prebiotic, bifidogenic potential of low molecular weight oligosaccharides

Paper II: Enzymatic depolymerization of gum Tragacanth: Prebiotic, bifidogenic potential of low molecular weight oligosaccharides

Hassan Ahmadi Gavlighi, Malwina Michalak, Anne S. Meyer and J. Dalgaard Mikkelsen, Submitted to Journal of Agricultural and Food Chemistry

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4.3 Significance of study

From previous finding based on chemical composition of six different composition of gum tragacanth, Astragalus gossypinus had specific composition (High fucose). On the other hand, enzyme technology with membrane technology has benefic in large scale production of oligosaccharides. Also, because of interesting area to produce novel ingredient with health beneficial is increasing in the world. Hence, the hypothesis of the present work was that gum tragacanth with highly fucose-substituted xylo-galacturonans could be a new natural source of (food grade) prebiotics. Secondly, It could be possible to produce different molecular weight with different composition from tragacanth gum with enzyme and membrane separation. The aim of this work, therefore, was threefold: A. To investigate whether pectinolytic depolymerization of tragacanth gum could reduce the viscosity of the gum in solution and allow the separation of the
products into different molecular sizes via membrane filtration. B: To investigate whether the resulting fuco-xylo oligosaccharides (FXO) from this gum had potential prebiotic activity. C: To evaluate whether the molecular size and/or structure (type of linkages) of the enzymatically produced gum tragacanth polysaccharides would influence their prebiotic potential.

4.4 Experimental considerations

Tragacanth gum has α-1,4-β-D-galacturonan linkages as a backbone with xylose side chain some of which are substituted at O-3 with β-D-xylopyranosyl units and some of these being terminated with D-gal or L-fuc (Phillips & Williams, 2009). So, pectinase enzyme (Pectinex® BE Color enzyme) has been chosen for hydrolysis in 50 °C. In the first step, continuously enzymatic reaction with 10 kDa cut off membrane was done and then separation completed with 2 kDa. To examine efficiency of membrane separation molecular size with HPSEC. To examine structure of oligomers produced by enzyme, linkage analysis and HPAEC have been done.

For evaluation of prebiotic activity of different enzymatic production, the bacterial strains were Bifidobacterium longum subsp. longum (Danisco Global Culture Collection DGCC 232), Bifidobacterium longum subsp. infantis (DGCC 233), Bifidobacterium longum subsp. infantis (DGCC 1497), Bifidobacterium longum subsp. infantis (DGCC 2238), Lactobacillus acidophilus (NCFM, ATCC 700396), Bifidobacterium longum subsp. longum (BI-05, DGCC 9917), Bifidobacterium lactis (HN019, DGCC2013), and Clostridium perfringens (ATCC 13124). Growth of pure cultures was determined as a function of OD$_{600}$ and time. Galactan from potato (Megazyme International LTD, Bray, Co. Wicklow, Ireland) was used as an established prebiotic standard control (Mäkeläinen, 2010; Michalak, et al., 2012).

4.5 Highlights

Enzymatic hydrolysis was shown that after 10 min viscosity of solution was decrease and indicating the depolymerization of the sample. The monosaccharide composition of each fraction of membrane separation of product was different. Also, the yield of highly fucose content products was higher than others (HAG3 > 10 kDa (~67 % w/w). The smaller fractions, HAG1 and HAG2, became very rich in arabinose and galactose. The enzymatic process thus seemed to cleave the pectin-resembling, polygalacturonic acid parts of the gum tragacanth polysaccharide backbone(s), leaving the suspected xylo-galacturonan, and especially the fuco-xylo-galacturonan stretches in the gum tragacanth intact. Hence, the enzymatic deconstruction using the pectinase can produce different molecular with significantly different composition.

The linkage analysis of each fraction was confirmed the structure of tragacanth which has backbone of galacturonic acid with α (1,4) -linked galacturonic acid with side chain of xylose and 2-linked xylose. Also fucose was terminally linked to xylose. Furthermore, highly content of arabinose and galactose content in the structure can approve arabinogalactan exists in the tragacanth gum structure.

The growth of bacteria in different fractions was significantly higher in the small molecular size compared on high molecular size. Although, fraction higher than 10 kDa (HAG3) was less metabolized by strain but had almost similar activity compared with galactan and there was not significantly different (P<0.05) between two substrates (Figure 5.1).
Also, different composition of each fraction and linkage are also one of factors that could be effect on growth of bacteria such as HAG1 which had high galactose may degraded simpler than by bifidobacteria because they are generally capable of expressing β-galactosidase (EC 3.2.1.23), α-galactosidase (EC 3.2.1.22), endo-β-1,6-galactanase (EC 3.2.1.164), exo-β-1,3-galactosidase (EC 3.2.1.145), α-1,3-galactosidase (EC 3.2.1.-) and endo-β-1,4-galactanase (EC 3.2.1.89) (Michalak, et al., 2012).

In summary, enzymatic process with membrane separation can make different molecular size of oligosaccharides from tragacanth gum in large scale. Also, results from bacteria growth has shown that tragacanth can be use as potential prebiotic ingredient but further investigation is needed to approve prebiotic effect.

Figure 5.1 Differential growth of bacterial strains on enzyme catalyzed (Pectinex BE Colour) degradation products from gum tragacanth (please see text for details) against potato galactan used as control.
Paper II

Enzymatic depolymerization of gum Tragacanth: Prebiotic, bifidogenic potential of low molecular weight oligosaccharides
Enzymatic depolymerization of gum Tragacanth: Prebiotic, bifidogenic potential of low molecular weight oligosaccharides

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Abstract
Gum tragacanth derived from the plant “goat’s horn” (Astragalus sp.) has a long history of use as a stabilizing, viscosity-enhancing agent in food emulsions. The gum contains pectinaceous arabinogalactans and fucose-substituted xylogalacturonans. In this work, gum tragacanth from Astragalus gossypinus was enzymatically depolymerized using Aspergillus niger pectinases (Pectinex® BE Colour). The enzymatically degraded products were divided into three molecular weight fractions via membrane separation: HAG1 < 2 kDa; 2 kDa < HAG2 < 10 kDa; HAG3 > 10 kDa. Compositional and linkage analyses showed that these three fractions also varied with respect to composition and structural elements: HAG1 and HAG2 were enriched in arabinose, galactose, and galacturonic acid, but low in fucose and xylose; whereas HAG3 was high in (terminal) xylose, fucose and 1,4-bonded galacturonic acid, but low in arabinose and galactose. The prebiotic potential of the three enzymatically produced gum tragacanth fractions was evaluated via growth assessment on seven different probiotic strains in single culture fermentations on: Bifidobacterium longum subsp. longum (2 strains), B. longum subsp. infantis (3 strains), Lactobacillus acidophilus, B. lactis, and on one pathogenic strain of Clostridium perfringens. The fractions HAG1 and HAG2 consistently promoted higher growth of the probiotic strains than HAG3, especially of the three B. longum subsp. infantis strains, and the growth promotion on HAG1 and HAG2 was better than that on galactan (control). HAG3 completely inhibited the growth of the Cl. perfringens strain. Tragacanth gum is thus a potential source of prebiotic carbohydrates that exert no viscosity effects and which may find use as natural functional food ingredients.

Keywords: Gum tragacanth, molecular size, prebiotic potential, enzymatic modification, Bifidobacterium longum subsp. infantis.
Introduction

There is an increasing interest in the development and identification of new carbohydrates having prebiotic effects. Prebiotic carbohydrates are defined as selectively fermented compounds that cause specific probiotic changes in the gastrointestinal microbiota (currently mainly understood as stimulating the growth of lactobacilli and bifidobacteria), which in turn confer benefits upon well-being and health of the host (1,2). The beneficial growth stimulation of specific probiotic colonic bacteria is generally explained by the capability of these bacteria to cleave the glycosidic linkages in the prebiotic carbohydrates. A primary example of natural prebiotics is the group of human milk oligosaccharides, which constitutes a structurally diverse family of galactose-, glucose, N-acetyl-glucosamine, sialic acid, and fucose-substituted lactose-based structures containing different types of glycosidic linkages, and which are almost unique to human breast milk. Human milk oligosaccharides were thus originally recognized as being responsible for the “bifidus factor” of human milk, and involved in the relationship between the intestinal bacteria and lower incidences of infectious diarrhea in breast-fed infants (3). Galacto-oligosaccharide (GOS) and fructo-oligosaccharide (FOS) are two main types of oligosaccharides that are recognized as prebiotics and which have been tested in human trials and which are already available in the market (4,5). Foods containing these compounds are generally categorized as functional foods (6).

Gum tragacanth is an exudate from the stem of the bush like plant “goat's-horn”, Astragalus species, and is one of the few natural plant sources of L-fucose-substituted polysaccharides. The gum has been used commercially for well over 2000 years, is approved for food use in both the US and Europe (7, 8), and is widely used as an emulsifier and thickener in emulsion systems in different food, pharmaceutical and cosmetic applications (9).

Gum tragacanth is made up of highly substituted, heterogeneous hydrophilic polysaccharides containing L-arabinose, D-galactose, D-glucose, D-xylene, L-fucose, L-rhamnose, and D-galacturonic acid, and confers high viscosity when in aqueous solution (10). When solubilized in water, the gum is usually categorized in a “soluble” fraction, tragacanthin, and an “insoluble” fraction, bassorin, respectively (11). The water-soluble tragacanthin fraction appears to resemble pectin, and contains linear chains of galacturonic acid (probably 1,4-α-linked) and arabinogalactan structures (12) as well as fuco-xylogalacturonans, whereas bassorin is believed to be mainly composed of a mixture of xylo- and fuco-xylo-substituted polysaccharides (11). Terminal, fucosyl residues are also found in some human milk oligosaccharide structures (as α1-2, α1-3, and α1-4 substitutions), hence gum tragacanth saccharides could have prebiotic effects mainly because of the L-fucose-substituted polysaccharides. However, the high viscosity inducing effects of gum tragacanth partly prevent unfolding of this potential, because the viscosity means that only low levels are used, since inclusion of
even very low levels of gum tragacanth in food products changes the physico-chemical properties of the products significantly.

Recently, enzyme technology has been used to produce various potentially prebiotic oligo- and polysaccharides from plant fiber polysaccharides for food applications (13,14,15). In particular, the combination of enzymatic depolymerization of various natural polysaccharides and membrane reactor separation has proven useful for lowering the viscosity of hydrocolloid polysaccharides and for obtaining prebiotic compounds of different molecular size and structural composition (6,16,17). In these previous studies it has been shown that both the molecular size and the structural make-up of the resulting poly- and oligosaccharides have a significant influence on their potential prebiotic bacterial growth promoting properties (13,14,18).

The hypothesis driving the present work was that gum tragacanth, notably via its content of highly fucose-substituted xylo-galacturonans, could be a new, natural source of putatively food grade prebiotics. A second hypothesis was that the gum tragacanth could be processed into differently sized poly- and oligosaccharide fractions via enzymatic treatment and membrane purification.

The aim of this work, therefore, was threefold: A. To investigate whether pectinase catalyzed depolymerization of tragacanth gum could reduce the viscosity of the gum in solution, and allow separation of the products into different molecular sizes via membrane filtration. B: To investigate whether the resulting fuco-xylooligosaccharides (FXO) from this gum had potential prebiotic activity. C: To evaluate whether the molecular size and/or structure and type of linkages of the enzymatically produced gum tragacanth polysaccharides would influence their prebiotic potential.

Materials and Methods

Materials. The gum tragacanth sample, Astragalus gossypinus, was selected for this work because of its particularly high fucose content (8). The dried raw gum was ground using an OBH Nordica coffee mill (OBH Nordica A/S, Copenhagen, Denmark) to pass through a 500 µm sieve. Dextran standards with molecular weight of 10, 40, and 110 kDa were from Pharmacia (Uppsala, Sweden). Pullulan standard with molecular weight of 1.3 kDa and 400 kDa, and L-fucose, L-rhamnose, D-arabinose, D-galactose, D-glucose, D-xylose, and D-galacturonic acid were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Trifluoroacetic acid (TFA) (99%) was from Merck (Darmstadt, Germany). Sodium hydroxide (NaOH) standard solution HPLC grade was from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).
**Enzyme.** Pectinex® BE Colour, 3600 MOE units/mL, pH optimum 3.5-4.0, temperature optimum 45-55 °C, was obtained from Novozymes A/S (Bagsværd, Denmark). This enzyme preparation is derived from *Aspergillus niger*, and mainly contains pectin lyase and polygalacturonase activity, and is approved as a processing aid for industrial food applications (19).

**Enzymatic digestion and membrane separation of gum Tragacanth.** Tragacanth gum (10 g/L) was dissolved in deionized water (4 L, milliQ water) during stirring for 2 h at room temperature (22 °C). After heating the tragacanth gum solution to 50 °C, using a Sartorius Biostat heating cap set-up, 10 mL Pectinex® BE Colour enzyme (equivalent to addition of 9000 MOE units/L) was added, and after 2 h of reaction, when the viscosity of the solution had dropped from 1.2 Pa·s to 0.57 Pa·s, continuous filtration of the enzymatically treated gum tragacanth solution was initiated by using a cross-flow Hydrosart 10 kDa membrane (Millipore, Sartorius). This filtration was continued overnight (18 h). Then, the retentate was heated to 100 °C for 10 min to halt the enzyme reaction. This retentate (~1 L) was defined as HAG3 > 10 kDa. The permeate (~3 L), i.e. the enzymatically degraded product, was then separated further using another cross-flow Hydrosart membrane set-up with a 2 kDa membrane; this second filtration was stopped after collection of ~1.5 L of permeate, and this permeate was defined as HAG1, and the retentate remaining was defined as HAG2. Finally, the three collected fractions: HAG1 < 2 kDa; 2 kDa < HAG2 < 10 kDa; and HAG3 > 10 kDa, were freeze dried and stored frozen at -20 °C until further analysis and microbial growth testing.

**Compositional analysis.** The monosaccharide composition of the *A. gossypinus* gum tragacanth starting material and of the membrane separated products from the enzymatic reaction were determined after TFA hydrolysis (2 M, 2 h, 121 °C) (20) by use an ICS3000 ion chromatography system equipped with a CarboPacTM PA20 (3 mm × 150 mm) analytical column (Dionex Corp., Sunnyvale, CA), using an elution programme described previously (8).

**Molecular Weight Distribution (HPSEC).** The lyophilized fractions from the membrane filtration and the gum tragacanth starting material were dissolved in 0.1 M sodium acetate buffer (NaOAc) (pH = 6), filtered through a 0.20 µm syringe-tip nylon membrane filter (VWR International, USA), separated for high performance size exclusion chromatography on a Shodex OHpak SB-806 HQ (8.0 mm × 300 mm) column (Showa Denko KK, Kawasaki, Japan), and eluted with 0.1 M NaOAc (pH = 6) at a flow rate of 0.5 mL/min at 30 °C on a system consisting of a P680 HPLC pump, an ASI-100 automated sample injector using a refractive index detector Shodex RI-101 (Showa Denko KK, Kawasaki, Japan). The injection volume was 25µL. The column was calibrated with Pharmacia Dextran (T10, T40, and T110 kDa) and pullulan (1.3kDa and 400 kDa) as standards (21).
**Linkage Analysis.** The samples (2 mg) were permethylated by the method of Ciukanu and Kerek (22) involving treatment with sodium hydroxide and methyl iodide in dry DMSO. For glycosyl linkage analysis, after permethylation, the samples were depolymerized, reduced, and acetylated. Finally, the resulting, partially methylated alditol acetates (PMAAs) were analyzed by gas chromatography-mass spectrometry (GC-MS) (23) on an Agilent6890N GC interfaced to a 5975B MSD (mass selective detector, electron impact ionization mode); separation was performed on a 30 m RTX 2330 bonded phase fused silica capillary column.

**Bacterial strains and growth conditions for single culture fermentations**

The bacterial strains were *Bifidobacterium longum subsp. longum* (Danisco Global Culture Collection DGCC 232), *B. longum subsp. infantis* (DGCC 233), *B. longum subsp. infantis* (DGCC 1497), *B. longum subsp. infantis* (DGCC 2238), *Lactobacillus acidophilus* (NCFM, ATCC 700396), *B. longum subsp. longum* (BI-05, DGCC 9917), *B. lactis* (HN019, DGCC2013), and *Clostridium perfringens* (ATCC 13124). These strains were incubated in mono-cultures at 1% (v/v) cell suspensions with each of the individual substrates at a final concentration of 1% (w/v) in a glucose-free MRS-medium base (de Man, Rogosa and Sharpe medium without glucose) in designated Micro titer plates in a Bioscreen® C system (Labsystems, Helsinki, Finland), and incubated anaerobically as described previously (24). Prior to the growth tests, the dissolved substrate solutions were sterilized (in 10% w/v solutions) by UV-radiation for 3 minutes. Anaerobic growth was measured by monitoring the increase of the biomass by optical density measurement at 600 nm (OD\textsubscript{600}) using Biolink® software (Labsystems) according to the protocol detailed in (24). The bacterial growth was determined as a function of OD\textsubscript{600} and time. The baseline growth in the glucose-free MRS medium without addition of carbohydrates was used as the blank control and the growth obtained was subtracted from the growth data obtained in the presence of substrate. Galactan from potato (Megazyme International LTD, Bray, Co. Wicklow, Ireland) was used as an established prebiotic standard control. The experiments were done in three replicates for each strain and carbohydrate substrate. Data are given as mean values ± standard error.

**Statistical and data analysis**

Reported data are given as average values determined after a minimum of duplicate determination. Data were analyzed by one-way analysis of variance (one-way ANOVA): Comparisons of mean values were calculated via 95% confidence intervals and compared as Tukey-Kramer intervals calculated from pooled standard deviations (Minitab Statistical Software, Addison-Wesley, and Reading, MA).

**Results and Discussion**

**Enzymatically produced gum tragacanth polysaccharides**
Modification of the selected, highly fucosylated, gum tragacanth with the Pectinex enzyme preparation was established to produce potentially prebiotic poly- and oligosaccharides having different molecular size. The enzyme treatment caused a sharp (50%) decrease in viscosity within 10 min. (data not shown), indicating the depolymerization of the gum tragacanth sample. The enzymatic reaction products were separated by membranes with 10 and 2 kDa to get three fractions (yields given in parenthesis as dry matter weight/weight of the starting material): HAG1 < 2 kDa (yield ~10 % w/w); 2 kDa < HAG2 < 10 kDa (~23% w/w); HAG3 > 10 kDa (~67 % w/w). HPSEC analyses confirmed the depolymerization of the gum into different molecular weight fractions, and revealed that the average size of the high molecular weight fraction population, HAG3, was approximately 110 kDa (Fig. 1). By a rough estimate, the HAG3 fraction thus contained branched polysaccharides having degrees of polymerization (DP) ~600-650 monosaccharides; the HAG2 polysaccharides were DP ~12-60; whereas the HAG1 fraction contained oligomers of maximum 10-12 DP. The HPSEC also indicated that there were no free monosaccharides in any of the three fractions (Fig. 1), and this was also confirmed by HPAEC analysis (data not shown). The monosaccharide composition of the three fractions turned out to vary significantly, and to differ from the starting material (Table 1). The data confirmed the dominance of galacturonic acid, xylose, and fucose in the starting material (Table 1). The high molecular weight fraction (HAG3) in essence got enriched in fucose during the enzymatic depolymerization, with the final product containing 290 mg fucose/g dry matter. This fraction, HAG3, was also rich in galacturonic acid and xylose, ~380 and 320 mg/g, respectively (Table 1). This result agrees with the expected attack pattern of the pectinases, since the pectinases are not able to attack the substituted fuco-xylo-galacturonan acid structures. Apparently, the enzymatic degradation thus depleted the gum tragacanth polysaccharides in rhamnose, arabinose, galactose, and glucose, which is in accord with previous findings that have indicated that, in addition to polygalacturonase and pectin lyase, the enzyme preparation contains side-activities that can catalyze the cleavage of several different kinds of bonds in complex plant cell wall structures (19). Analogously, the smaller fractions, HAG1 and HAG2, became very rich in arabinose and galactose; hence, the arabinose content was 293 mg/g in HAG1, 125 mg/g in HAG2, and 27 mg/g in HAG3, but HAG1 and HAG2 had significantly lower fucose and xylose contents than HAG3. The level of galacturonic acid was high in all three fractions, ranging from 196-380 mg/g, although lowest in the HAG1 (196 mg/g) (Table 1). The enzymatic process thus seemed to cleave the pectin-resembling, polygalacturonic acid parts of the gum tragacanth polysaccharide backbone(s), apparently leaving the suspected xylo-galacturonan, and especially the fuco-xylo-galacturonan stretches in the gum tragacanth intact, i.e. left in the HAG3 fraction. Hence, the enzymatic deconstruction using the pectinase in
combination with membrane separation produced three significantly different gum tragacanth polysaccharide fractions with respect to both molecular size and composition.

**Linkage analysis**

The linkage analysis confirmed the dominance of terminally linked fucose and (1,2)-linked xylose, as well as terminally linked xylose in HAG3 (Table 2). Taken together with the high galacturonic acid content (Table 1), these findings underscored that HAG3 contained the typical fuco-xylo-galacturonan structural elements of gum tragacanth (Figure 2), first discovered by Aspinall and Bailile (25). The low detection of galacturonic acid linkages is due to the linkage analysis method. In general the frequency of the gum tragacanth hallmark fucose- and xylose-linkages were lower in HAG2, whereas the (1,4)-galactose linkages, and notably the (1,2,4), rhamnose-linkages were higher in HAG2 than in HAG3. The presence of these latter linkages in HAG2 appeared to indicate enrichment of rhamnogalacturonan 1-galactan-like structures in the HAG2, a trend which became more evident in HAG1. In HAG1, the frequency of the 1,4-linked galactose bonds was dominating (Table 2); we presume that these bonds are due to the presence of β1,4-linked galactan structural elements; e.g. β-Galp-(1→4)-[β-Galp-(1→4)]2-Galp, β-Galp-(1→4)-β-Galp-(1→4)-Galp, that presumably correspond to arabinogalactan-derived galactan, which is in agreement with previous interpretations (12). In addition to the possible presence of β-bonded glucan- and galactan structures, e.g. β-GlcP-(1→4)-[β-Galp-(1→4)]2-Galp structures, that have been proposed previously to be present in gum tragacanth (12), the presence of 4-glucose linkages in all three fractions, HAG1, 2, and 3, also indicated the presence of 1,4-β-glucan structures and/or starch oligomers, which is in accordance with the comprehension that gum tragacanth may contain some cellulose and starch elements (26). With regard to the high arabinose levels in HAG1 and HAG2 (Table 1), the linkage analysis supported that the gum fractions contained mainly 1,5 and terminally linked arabinoses, which is in accordance with the presence of side-chain structures with α-Araf and Araf in arabinogalactan, and short stretches of 1,5-α-bonded arabinan. The results thus indicated that the lower molecular weight oligosaccharides were rich in arabino-galactan and also contained short galacturonic acid stretches.

**Growth of pure strains on tragacanth fractions**

The growth of the strains in single cultures varied for all three gum tragacanth fractions, HAG1, HAG2, and HAG3, and the growth responses also differed from those on pure galactan used as control (Fig. 3). The highest growth response was observed for *B.longum subsp. infantis* (DGCC 2238) on the HAG1 fraction <2 kDa (Fig. 3). There was a significantly increasing growth in response (p < 0.05) to lowering of the molecular weight of the gum tragacanth samples for both of the *B. longum subsp. longum* strains (DGCC 232 and B1-05, DGCC 9917), for the *B. longum subsp.*
infantis strain DGCC 2238, and the B. lactis strain, whereas the growth of the B. longum subsp. infantis strain DGCC 233 was equally good on HAG1 and HAG2, but still lower on HAG3 (Fig 3.). The growth profile results for the HAG2 gum tragacanth fraction, which had molecular size of 2-10 kDa, were similar to those obtained on HAG1. The growth of the B. longum subsp. longum strain B1-05, DGCC 9917 was suppressed by the HAG3 as well as the galactan (Fig. 3).

The HAG1 and HAG2 thus generally produced higher growth responses than galactan, the only exception being for L. acidophilus which grew equally well on all three gum tragacanth fractions and on galactan (Fig. 3). Although the HAG3 fraction, having molecular weight higher than 10 kDa was less metabolized by the majority of the strains, the growth responses obtained for HAG3 were the same as those on galactan, i.e. there were no significantly different effects (P > 0.05) between these two substrates, except for the Cl. perfringens growth which was suppressed by HAG3. Hence, Cl. perfringens used as a pathogenic control did not grow at all on the HAG3 fraction, but was able to grow (and grew equally well) on the HAG1 and HAG2 fractions, and on the galactan.

**Discussion**

The stimulation by prebiotic carbohydrates of specific, probiotic, colonic bacteria is explained by the capability of these bacteria to degrade the glycosidic bonds in the putative, prebiotic carbohydrates, and metabolize at least some of the saccharide components. This growth stimulation may provide a selective advantage when competing with other bacterial species in the mixed bacterial community prevailing in the human colon. The glycosidic bonds, the molecular size, as well as the monosaccharide composition of the prebiotic carbohydrates thus define the effect and the eventual relative increase in beneficial bacteria, including certain Bifidobacterium and Lactobacillus species. Gum tragacanth, contains some unique substituted saccharides, notably the highy substituted fuco-xylo-galacturonan and the arabino-galactan structures. As well, the long use of gum tragacanth in foods, albeit in low concentrations due to the marked viscosity effects, would indicate that the human gut microflora might be able to “recognize” this natural gum, and possess genes encoding enzymes that can degrade the structural elements in this gum. The sequencing of the genome of the B. longum subsp. infantis (ATCC15697), considered as “the archetypical” human milk oligosaccharide utilizing bacterium, has thus shown that genome possesses a large (43 kbp) gene cluster within which several specific glycosyl hydrolase encoding genes are located, including a fucosidase encoding gene (27). It has also recently been demonstrated that B. longum subsp. infantis (ATCC15697) will proliferate on human milk oligosaccharides (28), and mass spectrometry-based glycoprofiling of this oligosaccharide consumption has revealed that B. longum subsp. infantis appears to exhibit a specific preference for fucosylated oligosaccharides (28), although, as yet, no specific relations
between the microbial consumption, the saccharide structures, linkages, and e.g. fucosyl-substitutions have been proven unequivocally.

In the present work, in order to provide for a measurable response, and to test the hypothesis that gum tragacanth saccharides might be prebiotic, the viscosity of the gum had to be lowered in order to enable the supply of higher dosages of the gum saccharides. Pectinase assisted degradation provided a route to retain the unique fuco-xylo-galacturonans as well as the xylo-galacturonans, and partly the available arabinogalactans, while at the same time cause a lowering of the viscosity. The data obtained confirmed that the gum tragacanth could be processed into differently sized poly- and oligosaccharide fractions via the enzymatic treatment and membrane purification. The size of the tragacanth gum oligomers had an impact upon the growth of prebiotic bacteria, whereby a lower molecular size (HAG1), of an estimated DP of 10-12, as well as the HAG2, of DP ~12-60, co-inciding with the compounds being richer in arabinogalactans, were supporting a much higher growth than the significantly higher molecular weight fuco-xylo-galacturonans (HAG3) (Fig. 3).

The putative fucosidase activity of the selected *B. longum subsp. infantis* was expected to be induced and allow these bacteria to grow on the HAG3. However, the highly fucose-substituted xylo-galacturonans did not support the growth of prebiotics to the extent expected, but despite the relatively modest growth, the three different *B. longum subsp. infantis* strains responded significantly differently, with the *B. longum subsp. infantis* strain DGCC 1497 growing significantly less (*P*<0.05) than the two other *infantis* strains, but significantly more (*P*<0.05) than both of the tested *B. longum subsp. longum* strains (Fig. 3). We ascribe the general low growth on HAG3 as most likely being due to the large molecular size of these polysaccharides (Fig. 1), but the differential response of the *B. longum subsp. infantis* strains may be related to differences in the expression of fucosidase activity by these strains, whereas the lack of response of the *B. longum subsp. longum* growth may indicate a deficiency fucosidase encoding genes. Nevertheless, the results clearly confirmed that the molecular size, structure, and type of linkages of the enzymatically produced gum tragacanth polysaccharides impacted their prebiotic potential. In conclusion, the results showed that enzymatically produced, oligomeric structures from gum tragacanth had potential prebiotic activity. The data are a first step indicating that gum tragacanth may be a source of functional poly- and oligosaccharides that may exert beneficial bioactivity as prebiotics. The eventual application of enzymatically hydrolyzed gum tragacanth oligomers in foods would be a new, value-added application of a classic food gum.
Acknowledgement. The authors would like to acknowledge Tarbiat Modares University and the Ministry of Science, Research and Technology of Iran for their financial support.

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References


Figure Legends

**Fig. 1.** Molecular size profiles of gum tragacanth samples of different molecular size: HAG1 < 2 kDa; 2 kDa < HAG2 < 10 kDa; and HAG3 > 10 kDa, obtained after enzymatic modification and membrane separation. Tra Control indicates the track of the original gum tragacanth starting material.

**Fig. 2.** Structural motif of a putative fuco-xylo-galacturonan structural element (with galacto-xylo-substitution) present in gum tragacanth and presumed to be elevated in the HAG 3 fraction after enzymatic treatment.

**Fig. 3.** Differential growth of bacterial strains on enzymatically produced gum tragacanth samples of different molecular size fractions: HAG1 < 2 kDa; 2 kDa < HAG2 < 10 kDa; and HAG3 > 10 kDa, obtained after enzymatic modification and membrane separation, against potato galactan used as control. Growth responses for the 4 substrates are shown for a substrate concentration 10 g/L for all bacterial strains. Data are given as average values of 3 growth assay replicates and shown ± S.D. For the one-way ANOVA of the bacterial growth the pooled S.D.s were: *B. longum subsp. longum* (DGCC 232): 14.11, *B. longum subsp. infantis* (DGCC 233): 19.46, *B. longum subsp. infantis* (DGCC 1497): 10.67, *B. longum subsp. infantis* (DGCC 2238): 15.15, *L. acidophilus* (NCFM, ATCC 700396): 21.03, *B. longum subsp. longum* (BI-05, DGCC 9917): 19.46, *B. lactis* (HN019, DGCC2013): 17.99, and *C. perfringens* (ATCC 13124): 30.72. For the total ANOVA (level: 32) the pooled S.D. was: 18.75.
Table 1. Tragacanth gum, A. gossypinus, monosaccharide composition of different molecular size fractions: HAG1 < 2 kDa; 2 kDa < HAG2 < 10 kDa; and HAG3 > 10 kDa, obtained after enzymatic modification and membrane separation.

<table>
<thead>
<tr>
<th>Sugar Composition (mg/g dry matter)</th>
<th>A. gossypinus*</th>
<th>HAG1</th>
<th>HAG2</th>
<th>HAG3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fucose</td>
<td>236</td>
<td>26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>102&lt;sup&gt;b&lt;/sup&gt;</td>
<td>290&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arabinose</td>
<td>48&lt;sup&gt;c&lt;/sup&gt;</td>
<td>293&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galactose</td>
<td>39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>123&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucose</td>
<td>4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Xylose</td>
<td>273</td>
<td>56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>227&lt;sup&gt;b&lt;/sup&gt;</td>
<td>321&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galacturonic Acid</td>
<td>335</td>
<td>196&lt;sup&gt;b&lt;/sup&gt;</td>
<td>370&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>379&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*Starting material</sup>
Table 2. Glycosidic linkage analysis of different gum tragacanth fractions of different molecular size: HAG1 < 2 kDa; 2 kDa < HAG2 < 10 kDa; and HAG3 > 10 kDa, obtained after enzymatic modification and membrane separation. Values given in parenthesis indicate the relative abundance of the type of bond for the particular monosaccharide (quantified from peak area in the glycosidic linkage chromatography profile).

<table>
<thead>
<tr>
<th>Monosaccharide</th>
<th>HAG1 % peak area</th>
<th>HAG2 % peak area</th>
<th>HAG3 % peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linkage type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Fucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-Fucp</td>
<td>-</td>
<td>14.4(80.4)</td>
<td>41.6(88.1)</td>
</tr>
<tr>
<td>1,3-Fucp</td>
<td>-</td>
<td>0.8(4.5)</td>
<td>1.2(2.6)</td>
</tr>
<tr>
<td>1,4-Fucp</td>
<td>-</td>
<td>0.7(3.9)</td>
<td>1.1(2.3)</td>
</tr>
<tr>
<td>1,2-Fucp</td>
<td>-</td>
<td>1.7(9.5)</td>
<td>2.8(5.9)</td>
</tr>
<tr>
<td>1,3,4-Fucp</td>
<td>-</td>
<td>0.3(1.7)</td>
<td>0.5(1.1)</td>
</tr>
<tr>
<td>t-Rhap</td>
<td>-</td>
<td>0.2(1.4)</td>
<td>-</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>1,2,4 Rhap</td>
<td>2.3(100)</td>
<td>6.9(48.6)</td>
</tr>
<tr>
<td>1,4 Rhap</td>
<td>-</td>
<td>7.1(50)</td>
<td>-</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>t-Araf</td>
<td>0.4(5.8)</td>
<td>0.8(53.3)</td>
</tr>
<tr>
<td>1,5 Araf</td>
<td>6.5(94.2)</td>
<td>0.7(46.7)</td>
<td>-</td>
</tr>
<tr>
<td>t-Galp</td>
<td>3.7(5.6)</td>
<td>3.7(20.2)</td>
<td>1.6(88.9)</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>1,4-Galp</td>
<td>58.8(88.7)</td>
<td>13.5(73.8)</td>
</tr>
<tr>
<td>1,6 Galp</td>
<td>-</td>
<td>0.8(4.4)</td>
<td>-</td>
</tr>
<tr>
<td>1,4,6 Galp</td>
<td>3.8(5.7)</td>
<td>0.3(1.6)</td>
<td>-</td>
</tr>
<tr>
<td>t-Glup</td>
<td>2.8(18.6)</td>
<td>2.6(40.6)</td>
<td>1.1(47.8)</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>1,4-Glup</td>
<td>8.5(56.3)</td>
<td>3.5(54.7)</td>
</tr>
<tr>
<td>1,2,4-Glup</td>
<td>3.8(25.1)</td>
<td>0.3(4.7)</td>
<td>-</td>
</tr>
<tr>
<td>t-Xylp</td>
<td>-</td>
<td>5.4(22.6)</td>
<td>7.4(15.8)</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>1,2,3-Xylp</td>
<td>-</td>
<td>18.5(77.4)</td>
</tr>
<tr>
<td>1,2,3-Xylp</td>
<td>-</td>
<td>-</td>
<td>1.2(2.5)</td>
</tr>
<tr>
<td>D-Galacturonic acid</td>
<td>t-GalAp</td>
<td>0.2(2.9)</td>
<td>2.8(24.6)</td>
</tr>
<tr>
<td>1,4-GalA</td>
<td>6.6(97.1)</td>
<td>8.5(74.6)</td>
<td>0.8(80)</td>
</tr>
<tr>
<td>1,4,6-GalA</td>
<td>-</td>
<td>0.1(0.9)</td>
<td>-</td>
</tr>
</tbody>
</table>
FIGURE 1
FIGURE 2
FIGURE 3

- B. longum 232
- B. infantis 233
- B. infantis 1497
- B. infantis 2238
- B. lactis
- L. acidophilus
- B. longum BI-05
- C. perfringens

Species: HAG1, HAG2, HAG3, Galactan, Cl. perfringens

Y-axis: 0 to 350
X-axis: HAG1, HAG2, HAG3, Galactan
Paper IV

Tragacanth gum: Functionality and new Prebiotics Potential
5. Tragacanth gum: Functionality and new Prebiotics Potential

This chapter is included review paper that explains mechanism of emulsion stabilization by gum tragacanth and explores the enzymatic production of different fractions of gum tragacanth and evaluates their prebiotic activity potential (Paper IV)

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Abstract
Gum tragacanth is a plant derived hydrocolloid that can increase viscosity and stabilize emulsions and which has a long history of use in food, pharma, and cosmetics. The gum is mainly produced in the Middle East, and permitted for food use in the US as well as in Europe (E-number E413). The gum is made up of heterogeneous hydrophilic polysaccharides, which contain various highly substituted pectin-like structural elements. Enzymatically produced low-molecular weight fractions of gum tragacanth exhibit potential prebiotic activity by promoting growth in vitro of Bifidobacterium longum subsp. infantis strains. These findings may lead to new uses of this gum as a starting point for enzymatic production of value-added prebiotic compounds for functional foods.

Keywords: Gum tragacanth, viscosity, prebiotic, Bifidogenic, enzymatic depolymerisation.

Introduction
The natural plant exudate gum tragacanth is obtained from the stem of the bush like plant “goat's-horn”, Astragalus species, and the gum is permitted for use in foods in both the US and in Europe. Gum tragacanth is made up of complex, highly branched, heterogeneous hydrophilic polysaccharides that contain L-arabinose, D-galactose, D-glucose, D-xylose, L-fucose, L-rhamnose, and D-galacturonic acid (1, 2). The gum saccharides form complexes with salts, notably salts with divalent cations, such as Ca$^{2+}$ salts (3). Tragacanthin is the water-soluble fraction, that confers high viscosity when suspended in water, and bassorin is the insoluble part which swells in water to form a gel (1). Tragacanthin appears to resemble pectin, and contains linear chains of galacturonic acid (probably 1,4-α-linked) rich in xylose with varying levels of fucose, the latter depending on the plant species from which the gum is obtained (4). Gum tragacanth rich in xylose and galacturonic acid may thus contain xylogalacturonan, whereas gum samples having high fucose, xylose, and galacturonic acid levels, may mainly contain fuco-xylogalacturonan, in addition to highly branched of arabinogalactan structures (1,5). Gum tragacanth has both emulsifying and stabilizing properties in emulsions, and is considered as being “bi-functional” by having both the capacity to facilitate emulsification as well as providing stabilization of the emulsion after its formation (6). The structure and composition of the gum are important issues in relation to emulsion stabilizing properties (4).

On the other hand, there is an increasing interest in the development and identification of new carbohydrates with prebiotic effects that selectively stimulate the growth or activity of certain beneficial intestinal bacteria (currently
lactobacilli and bifidiobacteria), which in turn may confer benefits upon well-being and health of the host (7). Due to its high viscosity inducing effects, it is not possible to include substantial levels, i.e. sufficient “dosages”, of gum tragacanth into foods to elicit a possible prebiotic effect. However, targeted enzymatic depolymerization has previously proven useful as a technique to decrease the viscosity, by making the lower molecule size of natural, highly viscose polysaccharides and hydrocolloids. Especially in combination with membrane separation, prebiotic compounds of different molecular size and structural composition can be obtained enzymatically from various natural, viscous polysaccharides (8-10).

Gum tragacanth could be another source of such production of prebiotics dietary fibers due to the unique sugar composition and structure encompassing both arabinogalactan and fucosylgalacturonan polymers. The presumed presence of \( \alpha_{1,2} \)-linked fucosyl moieties in the structure may confer unique bifidogenic properties just as it is known that terminal, fucosyl residues found in some human milk oligosaccharide structures (as \( \alpha_{1-2}, \alpha_{1-3}, \) and \( \alpha_{1-4} \) substitutions) support the growth of *Bifidobacterium longum* subsp. *infantis*, an organism considered as desirable in the infant-gut microflora (11). Exudates gums with arabinogalactan structure such as gum Arabic has for example been considered as a very well tolerated dietary fiber with bifidogenic properties believed to benefit intestinal health (12).

The objectives of this review are: Firstly, to examine the mechanism of emulsion stabilization by gum tragacanth, including an investigation of the correlation between gum structure and composition with stabilization of protein based emulsions. Secondly, to explore the enzymatic production of different fractions of gum tragacanth and evaluate their prebiotic activity potential.

**Functional properties, stability and application**

The use of gum tragacanth in foods has been approved as GRAS in the US for more than 50 years at a level of 0.2–1.3% and in Europe, where gum tragacanth has an E-number (E413) on the list of additives approved by the Scientific Committee for Food of the European Community (13, 14) . In spite of the availability of alternative materials, tragacanth gum has superior functional properties (viscosity and emulsification) compared to other gums and it is fairly stable over a wide pH range down to extremely acidic conditions at about pH 2 (15). Gum tragacanth is therefore used as the primary substitute in a number of applications and the gum has thus been used for many years as a stabilizer, thickener, emulsifier and suspending agent in food, pharmaceutical, and cosmetic products, as well as in technical applications based on its high viscosity at low concentration. The gum has unusually high stability to heat and acidity and exhibits effective emulsifying properties (6). Evaluation of the flow behavior of six species of Iranian gum
tragacanth dispersions confirmed very high viscosity inducing properties and has shown that gum tragacanth exhibits shear-thinning behavior in aqueous dispersion. Depending on the species of the plant origin of gum, the viscosity of 1.5% (weight/weight) solutions may typically range widely, e.g. reported from ~1.5 up to 35 Pa·s (1,4).

It also is pourable and has a creamy mouth feel and good flavor-release properties (16). Gum tragacanth is used in the food industry in citrus oil emulsions (17), salad dressings, condiments, sauces, bakery emulsions, oil and flavour emulsions, bakery fruit-based fillings and toppings (give a shiny, clear appearance and a creamy texture) (18), confectionery, soft drinks, jellies, desserts, ice creams (provide texture to the product), flavors, spices (15).

Mechanism of tragacanth gum stabilization

There have been different studies that have analyzed the stabilizing effect of gum tragacanth in different emulsion systems. Yokoyama, Srinivasan (19) have shown that the stabilizing effect of gum tragacanth was a result of the steric repulsion force and the stability can be controlled by changing pH. On the other hand, the ability of tragacanth gum to stabilize beverage emulsions could be due to residual surface activity in addition to enhancement of emulsion viscosity (20).

Considering the relation between gum tragacanth composition and emulsion stabilization, our own recent findings have shown a highly significant correlation between the creaming index and the methoxyl content of the soluble fraction in the gums. Also, a strong correlation was found between the total galacturonic acid content (in the full gum) and creaming index, indicating that it is not the galacturonic acid alone can stabilize the emulsions (4).

In summary, tragacanth gum stabilization of protein-emulsified emulsions is probably a result of two mechanisms: Firstly, formation of non-covalent protein–(gum) polysaccharide complexes via electrostatic interaction mainly by galacturonic acid content in the soluble tragacanthin part of the gum, and secondly, the viscosity increase induced by the insoluble fraction (bassorin) (Figure 1).

Potential prebiotic effect of tragacanth gum

The concept of prebiotics was introduced in 1995 by Gibson and Roberfoid as an alternative approach to the modulation of the gut microbiota mainly lactobacilli and bifidobacteria (7). From the new definition of prebiotic (updated in 2007) the criteria of a prebiotic is: “1- resistance to gastrointestinal acidity 2- fermented by intestinal microflora 3- selective stimulate of the growth or/activity of intestinal bacteria” (21). Until now, different prebiotic compounds have been produced for industrial application especially, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) and a main approach is via enzymatic processing (22-24). Hence, production of novel ingredients with potential prebiotic
activity from many different types of plant cell wall polysaccharides has been reported. Arabinogalacto-oligosaccharides can be made from soybeans by endo-galactanases, arabino-oligosaccharides can be made from sugar beet pulp by pectinases and other plant-cell wall degrading enzymes, rhamnogalacturonono-oligosaccharides can be made from apple by rhamnogalacturonases, arabinoxylo-oligosaccharides can be made from wheat by xylanases and galacturono-oligosaccharides can be made from polygalacturonic acid by endo-galacturonases (25-27). Also, differences in structure, such as changes in size and the presence of branch(s) and differences in the complexity of the substituent can cause significant changes in the prebiotic properties of oligosaccharides (28, 29).

Tragacanth gum is one of exudates gum that is naturally and commercially available and which enzymatically depolymerised exerts potential prebiotic effects by promoting growth of Bifidobacteria in single culture fermentations (Figure 2). In this study, gum tragacanth was hydrolysed by pectinolytic enzymes and purified into three fractions using membrane separation, which gave rise to production of different molecular size oligosaccharides (Figure 3). Interestingly, in addition to different molecular sizes also differences in composition and structures were achieved with this process. To assess the prebiotic potential of the products, different pure cultures of beneficial and pathogen bacteria were chosen. The results showed that, the beneficial bacteria, notably gut-related Bifidobacteria spp. grew significantly better on the small molecular size structures as compared on the larger gum tragacanth molecules. Smaller molecular size of plant polysaccharides such as potato galactan oligomers have previously been shown to support the growth of bifidobacteria better than high molecular weight polysaccharides (30). However, the high molecular weight fraction (with high fucose content) inhibited the growth of Cl. perfringens (ATCC 13124). L. acidophilus grew equally well on all the gum tragacanth fractions as well as on the galactan. In general, the growth of the putatively beneficial bifidobacteria including both B. longum subsp. Infantis and B. longum subsp. longum bacteria were significantly higher on gum tragacanth oligosaccharides than on galactan used as a control for established prebiotic activity (Figure 2).

**Conclusion**

- Tragacanth gum is permitted for food use and can stabilize different types of emulsion formulations, but is notably efficient as a viscosity enhancer and stabilizer in acidic solutions
- Different tragacanth gum samples obtained from different species of Astragalus have different composition, and produce different levels of soluble and insoluble gum fractions
- Emulsion stabilization of the gum is related to the gum composition and structure, and mainly galacturonic acid content and degree of esterification are important
• low molecular size oligosaccharides produced enzymatically has higher potential prebiotic activity than longer chain gum saccharides

• Tragacanth gum can be a new source for development of innovative functional foods with health claims

**Acknowledgement.** The authors would like to acknowledge Tarbiat Modares University and the Ministry of Science, Research and Technology of Iran for their financial support.
References


Figure 1. The scheme of stabilization mechanism of protein-emulsified (WPI indicating whey protein isolate) emulsions by Gum Tragacanth.
Figure 2. Differential growth of bacterial strains on enzyme catalyzed pectolytic enzyme degradation products from gum tragacanth against potato galactan used as control. Growth responses for the 4 substrates is shown for a substrate concentration 10 g/L for all bacterial strains. Data are given as average values of 3 growth assay replicates and shown ± S.D.
Figure 3. Schematic flow sheet for the enzymatic hydrolysis process of tragacanth gum and membrane separation steps

Tragacanth Gum Solution

Pectin Lyase Polygalacturonas

Membrane separation
Retentate >10KDa
Membrane separation
Permeate <10KDa

Prebiotic Activity test
Retentate 2-10KDa
Permeate <2 KDa
6. Conclusion

The carbohydrate compositional and methoxyl and acetyl contents of six different species of *Astragalus* to acquire different gum tragacanth were examined. This work is prerequisite to assess effect of structural composition on mechanisms of effect of this type of gums for application in different area mainly food application. Also, production of new products with functional properties was achieved via enzymatic modification.

The hypothesis of paper I and III were to get knowledge of physicochemical properties of different species of *Astragalus* and find relationship between the gum composition and stabilization of emulsion. The results presented that the sugar-compositional analysis of gum tragacanth from different species of *Astragalus* by HPAEC-PAD were different values for each of the sugar components (arabinose, xylose, galactose, glucose, fucose, galacturonic acid and traces of rhamnose); therefore, the sugar composition of *Astragalus* gum exudates is strongly species-dependent and the functional properties of the gums are greatly influenced by their sugar compositions. Galacturonic acid was high in the soluble part of all species whereas L-fucose and partially xylose was major in insoluble fraction. The species with high fucose, may likely have high xylose and galacturonic acid content, and vice versa, whereas gums having high arabinose content may also contain high galactose levels, and vice versa.

The results in paper I showed that positive correlation between the methoxyl content of the soluble part of the gums content and creaming index indicate that methoxyl groups may have properties that play a role in the emulsification properties of tragacanth gums. Also, galacturonic acid of gum tragacanth samples from different *Astragalus* species was correlated with creaming index. Hence, particularly good emulsion stabilization properties will occur if gum tragacanth is divided into a soluble and an insoluble gum fraction, and the soluble fraction is rich in fucose and high in methoxyl content. Evaluating of the correlation between acetyl content of the gum tragacanth samples and the creaming index shown no significant relationship could be established.

In all samples prepared from gum tragacanth, the apparent viscosity decreased with increasing shear rate, indicating a shear-thinning nature of tragacanth gum in solution.

In general as we discussed in paper IV, the factors for determining how tragacanth gums work to stabilize emulsions were may hydrophobic bonding between tragacanth gum and WPI emulsified emulsion particles with increasing viscosity of emulsion, but apparently also the composition, notably the total galacturonic acid content in the gum, the amount of methoxyl groups and probably also fucose in the solubilized part.

Consequently, our finding confirmed that hypothesis that all exudates tragacanth gum had different composition and functional properties and any research carried out on gum tragacanth or any industrial application of this gum without respect to the plant species will lead to misleading results.

Nowadays, producing ingredient with health beneficial or food ingredient (Functional foods) has been interested and demanded. Thus, finding and making new source with high stability in heating and acidic condition and lower viscosity to enable the supply of higher dosages of the gum saccharides for food application is essential. The results from
composition of different gum shown that *A. gossypinus* has specific monosaccharide composition mainly high level of fucose (Paper I and III).

The next hypothesis was to firstly, examine possibility of producing different molecular size of gum tragacanth and secondly, evaluate prebiotic effect of these fractions. So, our finding shown that enzymatic process integrated with membrane separation can produce different molecular size of gum tragacanth with different carbohydrate profiles (paper II). Small molecular size mainly composed of arabinose and galactose whereas high molecular size was high fucose and xylose.

Linkage analysis of each fraction had good information to elucidate structure of gum tragacanth in more detail. In HAG1 and HAG3, the frequency of the 1,4-linked galactose bonds was dominating; we presume that these bonds are due to the presence of β-1,4-linked galactan structural elements. The dominance of terminally linked fucose and (1,β)-2linked xylose, as well as terminally linked xylose in HAG3 with the high galacturonic acid content underscored that HAG3 contained the typical fuco-xylo-galacturonan structural elements of gum tragacanth. These findings confirmed that it is possible to produce low viscosity of tragacanth gum with different composition for some application that viscosity is problem.

The glycosidic bonds, the molecular size, as well as the monosaccharide composition of the prebiotic carbohydrates thus define the effect and the eventual relative increase in beneficial bacteria, including certain *Bifidobacterium* and *Lactobacillus* species. Higher molecular size inhibited growth of pathogenic clostridia and growth better on the *lactobacillus* spp. whereas, lower molecular size grew better than higher size on *bifidobacteria* spp. Also, The linkage analysis confirmed the dominance of terminally linked fucose and (1,β)-2-linked xylose in HAG3 structure and this similarity with human milk oligosaccharides that contain 2-Fucoylated lactose can be one of the source of producing of human milk oligosaccharides. Thus our illustrations confirmed potential bioactivity of gum tragacanth that contained some unique substituted saccharides, notably the highly substituted fuco-xylo-galacturonan and the arabinogalactan structures after depolymerization especially in lower molecular weight. Although, higher molecular weight may be use as antimicrobial ingredient because of inhibition of clostridia.

In summary, the data are a first step indicating that gum tragacanth may be a source of functional poly- and oligosaccharides that may exert beneficial bioactivity as prebiotics. The eventual application of enzymatically hydrolyzed gum tragacanth oligomers in foods would be a new, value-added application of a classic food gum.

### 6.1 Future perspectives

Compositional and physicochemical properties information was established and will use in food industrial application based on interaction and effect on the formulation. Although, there is need more information about how interaction of arabinogalactan and xylogalactronan in the gum tragacanth structure. The distribution pattern of the methoxyl groups on the galacturonic acid backbone, i.e. whether in blocks or randomly distributed, plays a crucial role. Also, chains of de-methoxylated galacturonic acids may exist in the soluble fraction of gum tragacanth, which would help explain why the methoxylation degree varied so widely among the soluble gum tragacanth fractions. It may well be that a random, but high degree of substitution, will act to stabilize emulsions (and not cause gelation) as observed for enzymatically modified pectin.
Knowledge about the structural make up of tragacanth gum is important for tailoring of enzymatic process. So, development of hydrolyzing methods using mono active enzyme and produce of new functional properties of gum is one of the goals in the future work. Also, a physicochemical property of high fraction (HAG3) of gum tragacanth is needed to find application in industry.

At present work we assessed potential prebiotics in pure culture so; there is a severe lack of knowledge about the specific carbohydrate structural effects and about the interactions among different “probiotic” and pathogenic strains \textit{in vivo}. Any data on this would provide a significantly improved foundation for tailoring of carbohydrate structures for gut health. Therefore, examination of different fraction of gum tragacanth in human gut flora is needed to establishment of fractions as prebiotic compound.

At present, prebiotic carbohydrates purification represents the most expensive operation in food manufacturing involving these types of compounds, accounting for around 90\% of costs of food production. Current commercial processes for carbohydrate's purification are usually based on chromatographic processes involving ion-exchange resins or activated charcoal. Also, membrane processes, such as nanofiltration, seem to show potential for large scale carbohydrate mixtures separation. Therefore, purification of oligosaccharide using new economical technique such as supercritical fluid extraction is important issue to examine effect of specific oligomers with different DP on prebiotic effects.

Evaluation of antimicrobial properties of six species is another interesting area mainly to extend shelf life of food products with natural ingredient.

Also, the variety in composition is one of the approaches that could be modified via enzymatic process and test prebiotic effect. Other application in formulation of nano-emulsions or nano-liposomes is need to explore.

Modification of larger fraction of tragacanth gum (HAG3) with special enzyme such as xylogalacturonan hydrolysis and evaluation of bioactivity of different compound is interesting mainly in prebiotic area.
7. References


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