Characterization of Two *Acacia* Gums and Their Fractions Using a Langmuir Film Balance

Marie-Laure Fauconnier,[†] Christophe Blecker,*[,‡] Joëlle Groyne,[†] Hary Razafindralambo,[‡,§] Emmanuel Vanzeveren,[†] Michel Marlier,[†] and Michel Paquot[§]

Unité de Chimie Générale et Organique, Unité de Technologie des Industries Agro-alimentaires, and Unité de Chimie Biologique Industrielle, Faculté Universitaire des Sciences Agronomiques de Gembloux, 2 Passage des Déportés, B-5030 Gembloux, Belgium

The mechanical properties of monolayers from two *Acacia* gums [*Acacia senegal* (L.) Willd. and *Acacia seyal* Del.] and their three fractions isolated by hydrophobic interaction chromatography were studied with a Langmuir film balance to obtain a more complete understanding of their action mode. The analysis of compression isotherms revealed that *A. senegal* gums globally exhibit better interfacial properties than *A. seyal* ones. The behavior of the whole gums appeared to be strongly influenced by their arabinogalactan−protein complex.

Keywords: *Gum arabic; monolayers; Langmuir film balance; Acacia senegal; Acacia seyal*

INTRODUCTION

Gum arabic is defined as an exudation from stems and branches of *Acacia senegal* (L.) Willd. or related species of *Acacia*; it is produced in particular climatic conditions (FAO, 1986). This gum is used for its functional properties (stabilizing and emulsifying) in several applications in the food industry (Islam et al., 1997), in the pharmaceutical industry, and also in painting (Snowden et al., 1987). Although gum arabic is widely used in these fields, its definite action mode is not completely elucidated.

Dickinson and co-workers (Dickinson et al., 1988, 1989, 1990, 1991a,b) published a series of papers related to the interfacial and emulsification properties of gum arabic. The ability of acacia gums to lower the interfacial tension during the early stages of adsorption was correlated with the emulsifying capacity and emulsion-stabilizing properties. It was also noted that only a small fraction of the gum had good film-forming properties but, when the concentration was sufficient to saturate the surface, a strong film was formed.

Chemical studies of *Acacia senegal* gum have demonstrated that it consists mainly of a highly complex polysaccharide of a branched β-(1,3)-linked galactose backbone with branches linked through the 1,6-positions, with arabinose, rhamnose, and uronic acids in ramified side chains (Osman et al., 1995). A proteinaceous material (∼2%) is included in this structure (Randall et al., 1988; Osman et al., 1993). Sugar composition, glucuronic acid content, protein content, and amino acid composition are reported in the literature [for a review, see Islam et al. (1997)].

Different techniques were used to fractionate gum from *Acacia senegal*, among which hydrophobic interaction chromatography gives three fractions: an arabinogalactan (AG; fraction 1), an arabinogalactan−protein complex (AGP; fraction 2), and glycoproteins (GP; fraction 3) (Randall et al., 1989; Williams et al., 1990; Osman et al., 1995). Physicochemical analysis undertaken on these fractions revealed that the AGP fraction could be implicated in the emulsifying and stabilizing properties of *A. senegal* gum. As a matter of fact, only the AGP molecules adsorb at the interface in an emulsion, whereas the other components have poor affinity for the interface and remain in the aqueous phase (Snowden et al., 1987; Dickinson et al., 1988, 1990; Randall et al., 1988).

The aim of the present study is to determine the mechanical properties of spread films from two acacia gums and their three isolated fractions, using a Langmuir film balance, to obtain a more complete understanding of their action mode.

MATERIALS AND METHODS

Chemicals. Water from a Milli-Q water purification system (Millipore S.A., Molsheim, France) was used exclusively. All reagents were of analytical grade; NaCl and KCl (purity >99.5%) were purchased from Sigma (St. Louis, MO), and 2-propanol was purchased from UCB (Leuven, Belgium).

Gums. *Acacia senegal* gum was atomized Sudanese gum, purchased from CNI (Rouen, France). *Acacia seyal* gum was atomized gum, also purchased from CNI.

Protein Content. The nitrogen content in the various samples was determined by semi-micro-Kjeldahl analysis using a Tecator Kjeltec auto 1030 analyzer (Höganäs, Sweden). The protein content was calculated using a conversion factor of 6.60 as proposed by Anderson (1986).

Hydrophobic Interaction Chromatography. A defined volume of a 10% (w/v) solution of *A. senegal* gum in water was applied to a phenyl-Sepharose CL-4B column (Pharmacia, Uppsala, Sweden) and eluted successively by 4.2 M NaCl, 2.0 M NaCl, and Milli-Q water. The flow rate was set to 0.5 mL/min. Absorbance was monitored at 214 nm. The same procedure was applied to the *A. seyal* gum.

π/*A* Isotherms (Film Balance). Compression isotherms (π/*A* diagrams) were established using a Langmuir film balance by LAUDA GmbH (Königshofen, Germany) at $T = 25$

* Author to whom correspondence should be addressed (telephone 0032/81622308; fax 0032/81601767; e-mail Blecker.c@fsagx.ac.be).

[†] Unité de Chimie Générale et Organique.

[‡] Unité de Technologie des Industries Agro-alimentaires.

[§] Unite de Chimie Biologique Industrielle.

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Table 1. Analytical Data of Hydrophobic Chromatography Fractions of *A. senegal* and *A. seyal* Gums

gum	fraction	eluent solution	*a* (%)	*b* (%)
A. senegal	whole gum		100	2.8
	fraction 1	4.2 M NaCl	88	2.3
	fraction 2	2.0 M NaCl	10	11.0
	fraction 3	water	2	14.0
A. seyal	whole gum		100	1.0
	fraction 1	4.2 M NaCl	95	1.4
	fraction 2	2.0 M NaCl	2	5.0
	fraction 3	water	3	1.3

[a] Proportion of the total weight. [b] Protein content.

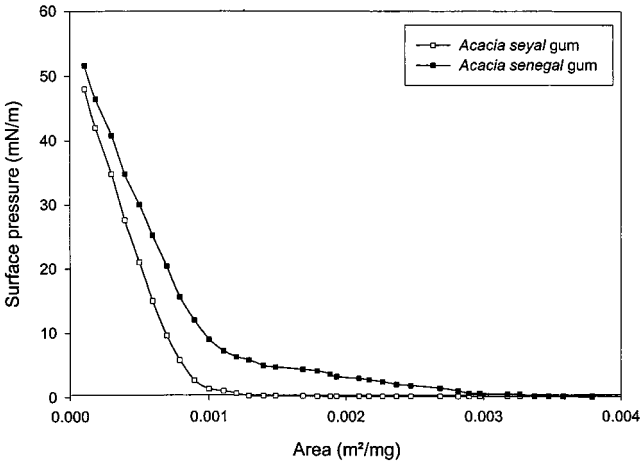

Figure 1. Compression isotherms for whole gums.

± 0.1 °C and at a constant compression rate of 92.7 cm^2/min. Subphase was 1 M KCl solution.

An adapted volume of a 10% (w/v) solution of sample (wholw gums or fractions) in 2-propanol/water (2:8, v/v) was spread using a 50 μL Hamilton microsyringe with its needle in the surface. The time elapsing between spreading and the start of compression was 1 h. Before each measurement, the purity of the subphase was checked by compressing the surface without any sample. The surface was considered as clean when the pressure reached upon maximal compression was <0.5 mN/m.

The limiting area (A_0) was determined by extrapolation at the intersection of the abscissa axis with the tangent of the π/A isotherm at $\pi = 2$ mN/m.

The film elasticity, $\epsilon = -A(d\pi/dA)$, was determined from the isotherm curve at the final part (at high pressures up to 20 mN/m), and the mean value was considered. Compression isotherm reproducibility was carefully checked by making at least three measurement sets for each measure. The variation coefficient for each parameter (A_0, ϵ) was <5%.

RESULTS

Three distinct fractions were isolated after fractionation of the *Acacia* gums using hydrophobic interaction chromatography. The relative proportion of each fraction and their protein content are given in Table 1. *A. senegal* gum exhibits a higher protein content than *A. seyal* gum. Among the different fractions collected, fraction 2 has a high protein content in both gums.

The mechanical properties of monolayers of the whole gums and of each fraction spread at the air−water interface were studied with a film balance. Compression isotherms corresponding to *A. senegal* and *A. seyal* whole gums are compared in Figure 1. The profiles of surface pressure against surface area per milligram of gum show a gradual increase in surface pressure when the film is compressed. However, it is interesting to note that this increase in surface pressure changes from one type of gum to the other. Isotherms obtained for *A. senegal* can be decomposed into two separate parts. At low compression level (low surface area), π increases regularly and weakly. At a surface coverage of ~0.001 m^2/mg and a surface pressure around 5 mN/m, a change of slope in the π/A curve suddenly appears; the surface pressure increases to a higher extent and reaches at the end of compression a value of 50.2 mN/m. The π/A curve for *A. seyal* does not exhibit such a discontinuity in its profile. Indeed, π remains close to the baseline (0 mN/m) until a surface coverage of 0.001 m^2/mg is achieved, where it begins to rise strongly in a regular way. At the end of compression, π reaches the value of 47.3 mN/m.

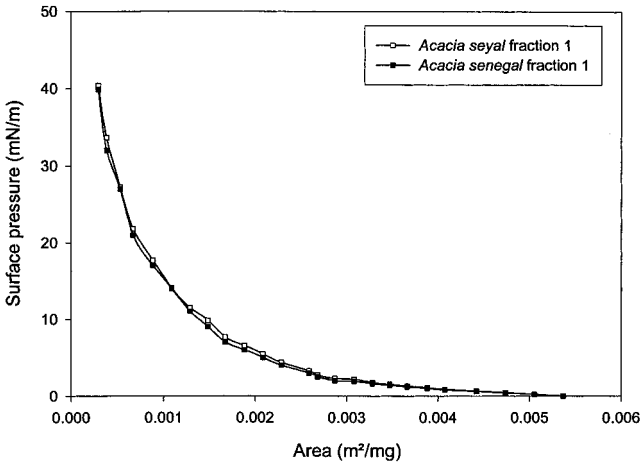

Figure 2. Compression isotherms for AG fractions.

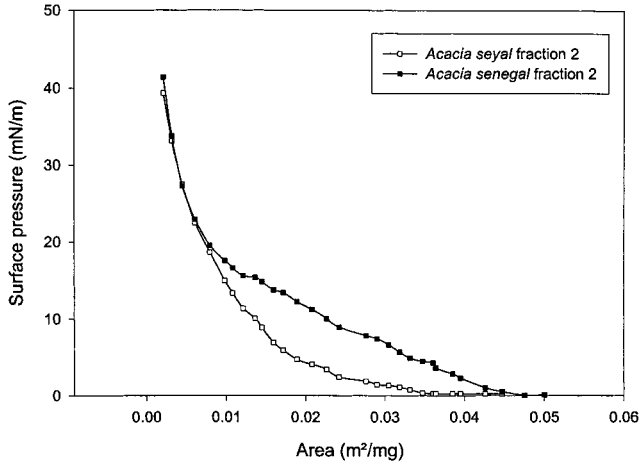

Figure 3. Compression isotherms for AGP fractions.

Compression isotherms for the fractions isolated from both gums by hydrophobic interaction chromatography are presented in Figures 2−4. Curves corresponding to fraction 1 of *A. senegal* and *A. seyal* gums can be considered as identical (Figure 2). When the film is compressed, π increases progressively to attain a value around 40 mN/m at the highest level of compression.

Qualitatively, compression isotherms of fractions 2 present a profile similar to the one of the corresponding whole gum (Figure 3). Therefore, the isotherm from fraction 2 of *A. senegal* gum exhibits a sharp inflection at a surface pressure of ~12 mN/m.

Results for fraction 3 indicate that, for the same surface area, fraction 3 from *A. senegal* gum develops a higher surface pressure than fraction 3 from *A. seyal*

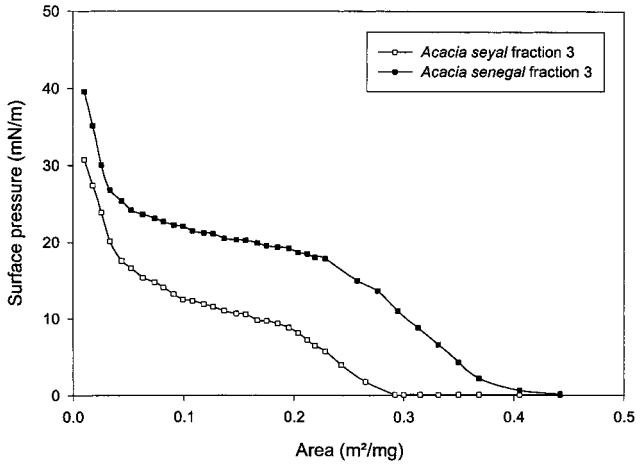

Figure 4. Compression isotherms for GP fractions.

Table 2. Limiting Area and Film Elasticity Extracted from Isotherm Curves of *A. senegal* and *A. seyal* Whole Gums and Fractions

gum	fraction	A_0 (m^2/mg)	ϵ (mN/m)
A. senegal	whole gum	3.22×10^{-3}	12.71
	fraction 1	5.01×10^{-3}	23.24
	fraction 2	5.44×10^{-2}	18.79
	fraction 3	4.37×10^{-1}	10.34
A. seyal	whole gum	1.26×10^{-3}	6.99
	fraction 1	5.01×10^{-3}	22.83
	fraction 2	3.64×10^{-2}	15.75
	fraction 3	2.91×10^{-1}	8.01

gum. In both π/A curves (Figure 4), a transition zone (low slope region) is observed from the expanded film to the condensed film.

To complement the isotherms' qualitative analysis, two quantitative parameters (A_0 and ϵ) were extracted from π/A curves. Values of limiting area (A_0) and film elasticity (ϵ) for each sample are given in Table 2.

DISCUSSION

Two *Acacia* gums from different origins were selected in this study because of their distinct functionalities. Indeed, *A. senegal* gums are more efficient than *A. seyal* gums in emulsifying essential oils (Dickinson et al., 1990).

Fractionation of these gums showed that they had elution profiles which consisted of three distinct parts, respectively, fractions 1, 2, and 3. The literature refers to these three fractions as, respectively, arabinogalactan (AG), arabinogalactan−protein complex (AGP), and glycoproteins (GP). If both gums (*A. senegal* and *A. seyal*) had similar elution profiles, differences were observed in the proportions and in the protein content of each fraction between the two gums.

Each fraction from *A. senegal* contained more proteins than the corresponding fraction from *A. seyal*. Moreover, *A. senegal* gum comprised a higher ratio of AGP than *A. seyal*. It is worth remembering that AGP and protein contents are thought to be important in the process of emulsification (Anderson and Weiping, 1991; Ray et al., 1995).

A Langmuir film balance was used to characterize the monolayers of the whole gums and their fractions. The mechanical properties of surface-active material at the air−water interface were evaluated by compression. For each compression isotherm, the abscissa was expressed in m^2/mg (surface area divided by the amount of material spread at the subphase surface), which allowed us to compare the intrinsic properties of each sample.

Because AG from both gums led to the same compression isotherms, it appeared that the shape of π/A curves of whole gums was mainly influenced by the properties of their AGP and GP fractions. In particular, the inflection AGP curve from *A. senegal* was also observed in the *A. senegal* whole gum curve. This slope modification in the isotherm profile probably results from a change in the film organization at the interface during compression.

The curve for AGP from *A. seyal* did not exhibit the first phase of slow increase in surface pressure. Therefore, π only increased in one regular phase (no inflection in the diagram). The different behavior between AGP of *A. senegal* and *A. seyal* is probably due to the difference in composition between these two *Acacia* species (sugar and amino acids composition) (Islam et al., 1997).

All A_0 and ϵ values for *A. senegal* (whole gum and fractions) were higher than values for *A. seyal*.

A_0 indicates monolayer expansion. *A. senegal* films are thus more expanded than *A. seyal* ones. When A_0 values of the three fractions of the same gum are compared, it also appears that the film expansion extent at the interface follows the order GP > AGP > AG. Glycoproteins are unfolded at the air−water interface and occupy a large surface area per milligram. Concerning the arabinogalactan−protein complex, it can be assumed that the more hydrophobic polypeptide chain of this fraction adsorbs at the interface, whereas the hydrophilic carbohydrate attached to the chain protrudes out into the solution, providing a strong steric barrier (Islam et al., 1997). The arabinogalactan fraction leads to the smallest value of A_0. This weak expansion can be attributed to the short hydrodynamic radius of this fraction (9.2 nm) (Randall et al., 1989).

ϵ is related to the change in surface pressure per unit change in film interfacial area. Therefore, the elasticity of a film measures its resistance to change in film area (Graham and Phillips, 1980).

In comparison to *A. seyal* gum, *A. senegal* gum forms more elastic films, which therefore exhibit a better resistance to mechanical disturbance. This property is required to obtain good foam and emulsion stability (Damodaran, 1994). Because gum arabic monolayers show no clear collapse behavior, the surface pressure at the end of compression (maximal surface pressure) can be used as a stability indicator, similarly to the collapse pressure. As the elasticity, the film stability is notably higher in the case of *A. senegal* gum. Moreover, the maximal surface pressure of whole gum is higher than the corresponding parameter for each fraction. This could arise from a synergy among the three fractions resulting in whole gum behavior or from water-insoluble cross-linked monosaccharides present in the whole gum but eliminated by chromatographic separation (thus absent in each fraction). However, the fact that reconstituted gum from the three isolated fractions exhibits the same interfacial properties as the initial gum (results not shown) defends the hypothesis of synergy between AG, AGP, and GP.

Beyond the protein content and the emulsifying properties, mechanical properties of the monolayers can be used to discriminate *Acacia* gum species. Moreover,

our results suggest that compression isotherms could be useful to understand quality variations of *A. senegal* gums.

CONCLUSIONS

In conclusion, the film balance studies at the air−water interface actually provide a useful method for evaluating the mechanical gum arabic properties and for studying the influence of arabinogalactan, arabinogalactan−protein complex, and glycoproteins on the whole gum monolayers. *A. senegal* gum globally exhibits better interfacial properties than *A. seyal* gum. In addition to its proportion in the gum (quantitative aspect), intrinsic properties of the AGP fraction (qualitative aspect) contribute to the interfacial behavior of the whole gum.

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