Interfacial and emulsifying properties of Acacia senegal and Acacia seyal gum and their fractions

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Propriétés interfaciales et émulsifiantes de gommes d'Acacia *senegal*, Acacia *seyal* et de leurs fractions

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Symbols and Abbreviations	Explanation
AFM	Atomic force microscopy
AG	Acacia gum or gum arabic
AGP	Arabinogalactan-proteins
AGp	Arabinogalactan-peptide
A. *senegal*	Acacia *senegal*
A. *seyal*	Acacia *seyal*
BS	Backscattering
CC	Critical concentration
CI	Creaming index
$D_{3,2}$,	Surface average diameter or Sauter mean diameter
$D_{4,3}$	Volume mean diameter
E	Viscoelastic or dilatational modulus
E'	Elastic modulus
E''	Viscous modulus
GAGP	Gum arabic glycoprotein
GP	Glycoproteins
GPC	Gel permeation chromatography
GRAS	Generally Recognized As Safe
HIC	Hydrophobic interaction chromatography
IEC	Ion-Exchange Chromatography
LDPE	Low-density polyethylene
logP	Octanol/water partition coefficient
MALLS	Multi-angle laser light scattering
M_w	Average molar mass
M_w/M_n	Polydispersity index
RH	Relative humidity
R_H	Hydrodynamic radius
R_q	Root mean square of roughness value
RMSE	Root-mean-square-error
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy

Symbols and Abbreviations	Explanation
T	Transmission
WVP	Water vapor permeability
Γ	Surface load of the emulsifier at saturation
γ	Surface or interfacial tension
γ_{LV}	Interfacial tensions at liquid-vapor interface
γ_{SL}	Interfacial tensions at solid-liquid interface
γ_{SV}	Interfacial tensions at solid-vapor interface
γ^{p}	Polar component of surface tension
γ^{AB}	Polar component of surface tension
γ^{d}	Dispersive component of surface tension
γ^{LW}	Dispersive component of surface tension
γ^{+}	Electron acceptor parameter
γ^{-}	Electron donor parameter
δ	Phase angle
η	Intrinsic viscosity
θ	Contact angle
Π	Surface pressure

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General introduction

Chapter 1 – General introduction

Acacia gum (AG, E414), also named gum arabic, is a natural edible gummy exudate collected from *Acacia senegal* and *Acacia seyal* trees. The Acacia gum production is a protective mechanism of tree against insect and molds invasion and of healing of wounds (Clarke, Anderson, & Stone, 1979; Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Sanchez et al., 2018). Acacia gum is extensively used as food additive and an international definition was given by FAO/WHO during the 31st Codex Committee for Food Additive. This definition specifies that Acacia gum is "a dried exudation obtained from the trunks and branches of A. *senegal* (L) Willdenow or close species from Acacia such as A. *seyal* (leguminosae family)" (FAO, 1999). Although the difference in taxonomic series of these gums, i.e. Acacia *seyal* (A. *seyal*) is from *Gummiferae* and Acacia *senegal* (A. *senegal*) is from *Vulgares*, these two Acacia gums are composed of the same sugars, amino acids and salts components.

Acacia gums are found and harvested in arid regions of the sub-saharian belt, the so-called "gum belt of Africa", from Senegal to East Africa, beyond to Pakistan and India (Jales, 2018; Sanchez et al., 2018). Acacia trees are perfectly acclimatized to dry and desert areas and offer many advantages. Environmentally, they act as a barrier to desertification, it nourishes the soil by drawing nitrogen from the air and then transferring it to the soil. The main gum producing countries are Sudan, Chad, Nigeria covering 95% of global gum export market. Their main missions are to collect, dry, pack, and deliver the gums to a point of sale (Jales, 2018; Muller & Okoro, 2004). The major importing countries were India and France in 2014-2016 with 75% of world importation amount (Jales, 2018).

Since the ancient times, humans used Acacia gums for food, medicine and other non-food applications as an adhesive ingredient to bone technologies (Sanchez et al., 2018). At time of Ancient Egyptians, it was used as a pigment binder in paints and inks for making hieroglyphs and as adhesive to make flaxen wrappings for embalming mummies

(Verbeken, Dierckx, & Dewettinck, 2003; Whistler, 1993). By the Middle Age, Acacia gum was valued in Europe among scribes and illustrators for application of color. Between the 12th and 19th centuries, it was also used for pigments stabilization, in the composition of metallo-gallic ink, the most popular ink in Europe used by painters like Rembrandt. The modern industrial era has produced an explosion of manufacturing uses for Acacia gum. It is widely used as emulsifier, stabilizer, surface finishing agent, thickener and flavoring agent in food and non-food industries including pharmaceutical, printing, textile and cosmetic applications. It is used as a binder for color pigments in crayons, a coating for papers and a key ingredient in the microencapsulating process for the production of carbonless copy paper, laundry detergents, etc. In relation to its adhesive properties, it was deployed in matchsticks and in moisture-sensitive postage-stamp. In cosmetic industry, it is in the composition of face powder, masks, creams and lotions thanks to its adhesive and emulsifying properties. In food industry, Acacia gum is used for the production of beverages, confectionery, emulsions, flavor or colorant encapsulations, bakery products and brewing. As examples, Acacia gum allows a uniform distribution of essential oils and fats in aqueous solutions, the delay of sugar crystallization in confectioneries, icings, cake mixes, whipped creams. In beverages, it is especially used for the stabilization of essential oils and mixture of aroma compounds. In wine production, Acacia gum confers body, stabilizes the color and prevents polyphenols and proteins precipitation. The production of beverages and confectioneries are found to be the main applications of Acacia gum in food industries.

Acacia gum is becoming a highly strategic product with a huge growth of the demand, by 25%, over the last ten years. The gum is important for producing countries, especially for local people for whom it is often the only source of income, but as well for importing countries. Between an irregular production (dryness, politic instability of producing countries) and a growing demand of low cost natural gum, Acacia gum companies are faced to several challenges. The first challenge is to assure a high and constant quality of the product despite climatic fluctuations and processing variability (tapping, storage, transport, temperature control). The second one is to develop new

high-value added applications based on new knowledge on Acacia gum composition, structure and techno-functional properties and the development of new Acacia gum based products. For this reason, a research program, named the DIVA program, was created between the ALLAND&ROBERT Company (Port-Mort), a worldwide supplier of gums, and UMR IATE 1208 (Montpellier), a research unit dedicated to the engineering of agro-polymers and emerging technologies since 2012. Alland&Robert is a French company specialized in Acacia gum production since 1884 and worldwide recognized by the largest food groups. Their social and economic policies in France and in Africa cover many levels: to guarantee sustainable and quality raw materials, to ensure an ethical and clean production process, and to develop a sustainable partnership with social and safety practices and environmental and ethical compliance. Following this collaboration, the DIVA program is generally divided into fundamental and applications projects which are strongly interconnected. The different projects focused especially on the fractionation of A. *senegal* and A. *seyal* gums, the characterization of the composition, structural and physicochemical properties of Acacia gum macromolecules (whole Acacia gums and fractions), and the study of the interfacial and emulsifying properties of Acacia gum macromolecules (whole Acacia gums and fractions).

My PhD project is fully focused on the characterization of the interfacial and emulsifying properties of Acacia gum macromolecules (whole Acacia gums and its fractions). It is closely linked to the other topics mentioned earlier. Among them, the following subjects which are running in parallel or already fulfilled were crucial to the best accomplishment of my Ph.D.:

- Characterization of composition, structural and physicochemical properties of A. *senegal* and A. *seyal* gums

This subject aimed to review basic composition and structural properties of macromolecules from A. *senegal* and A. *seyal* gums in relation to their functional properties. The gums were especially characterized in terms of sugar, protein and

mineral composition and content, and degree of branching. This work was done during the prior Lopez Torrez Ph.D. project (Lopez Torrez, 2017).

- Fractionation/purification of Arabinogalactan-proteins (AGP) from Acacia *senegal* gum

The aims of this study are to isolate arabinogalactan-proteins (AGPs) from A. *senegal* gum according to their structural and physicochemical properties. The classical chromatographic methods were used including hydrophobic interaction chromatography (HIC) and Ion-Exchange Chromatography (IEC). These methods allowed to obtain isolated AGP fractions: three main fractions were isolated using HIC, i.e. HIC-F1, HIC-F2 and HIC-F3 while two major fractions were obtained using IEC, i.e. IEC-F1 and IEC-F2. After the isolation of these fractions, their biochemical composition, structural and physicochemical properties were characterized in-depth. This subject corresponds to the postdoctoral project of Apolinar Valiente.

- Physicochemical properties in solution of AGP from Acacia gums

The main objective of this project is to study the volumetric properties, partial specific volume and partial specific adiabatic compressibility; and hydrodynamic properties: dynamic viscosity, intrinsic viscosity, and hydrodynamic radius, of Acacia gums at 25°C and later at temperatures up to 70°C. A. *senegal*, A. *seyal* and the macromolecular fractions of A. *senegal*, i.e. HIC-F1, HIC-F2 and HIC-F3 from HIC and IEC-F1 and IEC-F2 from IEC fractionations were characterized. This work has been a part of the Ph.D. project of Mejia Tamayo (Mejia Tamayo, 2018).

In the framework of my PhD project, the main scientific objectives are:

(i) to establish the relationship between the biochemical composition and structural properties of A. *senegal* and A. *seyal* gums and their interfacial properties. For this purpose, the ability of Acacia gums and IEC-F1 fraction isolated from A. *senegal* dispersions to lower interfacial tension and to form interfacial film at hexadecane-water interface will be investigated and compared considering gum species and concentration. Despite the difference in biochemical

composition and structural properties between A *senegal* and A *seyal* gums, their interfacial properties will be compared as function of their protein content and the impact of their specific nature will be hypothesized. Moreover, by varying the gum concentration, not only the impact of protein content but also the high molar mass protein-rich AGPs content which is largely described as responsible of interfacial properties of Acacia gum will be assessed. The provision of different fractions of A *senegal* obtained by IEC purification will allow to confirm the major role of high molar mass protein-rich AGPs in interfacial properties of Acacia gums.

(ii) to evaluate the impact of dispersed phase nature on the A. *senegal* and A. *seyal* gums interfacial properties. Indeed, as the nature of the interfacial phase in contact with Acacia gums can impact their interfacial properties, the behavior of different compounds will be studied. Hexadecane was the first compound chosen for interfacial properties study of A. *senegal*, A. *seyal* and IEC-F1 because of abundant study on characterization of Acacia gums, HIC fractions and Acacia matured gums using this compound (Castellani, Al-Assaf, Axelos, Phillips, & Anton, 2010; Chanamai, Horn, & McClements, 2002; Dickinson, 1999; Dickinson, Murray, Stainsby, & Anderson, 1988). Special attention was given to other interfaces such as limonene and octanol, being different in their nature and polarity, in order to understand the effect of interfacial nature on the interfacial behavior of A. *senegal* and A. *seyal*. The new acquired knowledge about the interfacial properties of Acacia gums at each interface can allow us to better understand the emulsifying properties of Acacia gum.

(iii) to characterize the emulsions produced by A. *senegal*, A. *seyal* and a reconstituted A. *senegal* gum using well characterized fractions in order to relate their biochemical and structural properties to their interfacial properties. It is important to keep in mind that A. *senegal* is preferentially used to stabilize aroma compounds diluted emulsions in beverages with long storage, while A. *seyal* is used in emulsification step before aroma encapsulation, i.e. when the stability is not crucial. To achieve this objective, the selected dispersed oil phase was limonene with fixed concentration (5 wt%) because of its high content found in orange oil. The

stabilization of limonene emulsion alone is challenging due to its low viscosity. However, the use of limonene can offer the advantage to emphasize the difference in emulsifying behavior between A. *senegal* and A. *seyal* gums. In term of emulsification technology, microfluidisation was selected as it is adapted to the weak viscosity of Acacia gum dispersions allowing to investigate the effect of Acacia gum concentration variation. A high attention will be brought to the role of high molar mass protein rich AGPs to stabilize emulsions. Indeed, it is widely accepted that these AGPs were responsible of gum emulsifying properties (Alftrén, Peñarrieta, Bergenståhl, & Nilsson, 2012; Flindt, Al-Assaf, Phillips, & Williams, 2005; Nishino, Katayama, Sakata, Al-Assaf, & Phillips, 2012; Padala, Williams, & Phillips, 2009; Randall, Phillips, & Williams, 1988; Ray, Bird, Iacobucci, & Clark, 1995). The different approaches usually reported to demonstrate and understand the role of these AGPs consist to vary the gum concentration (Yao et al., 2013), to use different gums with different high molar mass AGPs and protein amount (Dickinson et al., 1988; Katayama et al., 2006), to use matured gums which contain more high molar mass protein-rich AGPs (Al-Assaf, Phillips, Aoki, & Sasaki, 2007; Aoki, Katayama, et al., 2007; Aoki, Al-Assaf, Katayama, & Phillips, 2007) and to study the emulsifying properties of gum degraded by proteolytic enzyme (Al-Assaf, Sakata, McKenna, Aoki, & Phillips, 2009; Flindt et al., 2005). Additionally, some authors reported the effect of gums constitutive fractions effect on the emulsifying properties and identified the most efficient fraction (Nishino et al., 2012; Ray et al., 1995). Moreover, another usually used method involves removing the adsorbed molecules to the droplet interface and characterizing these molecules (Buffo, Reineccius, & Oehlert, 2001; Katayama et al., 2006; McNamee, O'Riordan, & O'Sullivan, 2001; Mikkonen, Xu, Berton-Carabin, & Schroën, 2016; Nakauma et al., 2008; Randall et al., 1988; Xiang et al., 2015). According to the results obtained from the studies cited above, the important role of high molar mass protein rich fractions in Acacia gum emulsifying properties makes no doubt. However, the question is still asked "Are functional properties of Acacia gum simply the sum of functional properties of individual fractions or a positive synergism does exist?". In an attempt to answer this question, an innovative approach is proposed by mixing two

fractions isolated from A. *senegal* in order to control high molar mass protein-rich AGPs content in bulk and the total concentration of gum. The total concentration and the protein amount will be chosen according to general applications of Acacia gums. The two fractions are a high molar mass protein-rich fraction isolated from A. *senegal* using ion exchange chromatography and a low molar mass fraction isolated from the same gum using a traditional hydrophobic interaction chromatography. These fractions have been isolated and well-characterized in terms of biochemical composition and structural properties.

In parallel, the effect of Acacia gum specie will be investigated using A. *senegal* and A. *seyal*. Both gums are characterized by the difference of protein content, structural and physicochemical properties such as viscosity. On the other hand, other parameters of emulsification such as emulsification process and effect of glycerol addition will be also studied. The obtained knowledge could allow to better understand the effect of emulsifier nature on the physical characteristics of produced emulsions (globule size distribution, temporal particle stability caused by creaming, coalescence, Ostwald ripening) and within the context of developing a new gum with optimal emulsifying properties.

(iv) to characterize the surface structure and interfacial properties of A. *senegal* and A. *seyal* gums dried films and the relationship with water permeability and ability to trap aroma compounds. Indeed, Acacia gum is also widely used as coating agent with as major ambition to avoid fat oxidation. Currently, new trends are proposed to coat natural products as fresh fruits and fish (Ali, Maqbool, Ramachandran, & Alderson, 2010; Binsi et al., 2016; El-Anany, Hassan, & Rehab Ali, 2009; Jiang, Feng, Zheng, & Li, 2013; Maqbool, Ali, Alderson, Zahid, & Siddiqui, 2011). However, the in-depth knowledge about the surface properties is still not available as the water wettability. One explanation is the difficulty to form homogeneous film based on hyper branched polymer as Acacia gums without cracks. Therefore, the first step of this study will be to produce films without cracks on a model surface using spin coating technology. Then, the surface properties i.e. water wettability, surface free energy and

corresponding polar and dispersive components of Acacia gum films will be evaluated using contact angle measurements. Surface film structure will be characterized using microscopic observation. According to the gum structure and composition, the network formation of A. *senegal* and A. *seyal* at the interface of solid films will be discussed in regard to the determined surface properties. Moreover, the affinity between the films and different compounds varying in nature and polarity will be assessed to evaluate their potential impact on films functionalities as water permeability and aroma compounds retention. The new knowledge acquired in this section should allow to establish a relationship between interfacial properties of Acacia gums solid film and gum biochemical composition and structural conformation and therefore improve the use of Acacia gum as coating agent.

The better comprehension of the behavior of Acacia gums at different interfaces should allow to optimize their usage as emulsifier for beverages or aroma encapsulation and as coating agent for diverse products.

This thesis is divided into 5 chapters:

Chapter 1 mentions the context and objectives of the PhD project.

Chapter 2 presents an overview of the biochemical, physicochemical and structural properties of Acacia gums with a specific focus on the materials used in this project.

Chapter 3 contains the study of the effect of A. *senegal*, A. *seyal* and IEC-F1 and their concentrations on the lowering interfacial tension of hexadecane and the rheological properties of interfacial films. In the same chapter, the effect of oil drop nature on interfacial properties of Acacia gums was characterized.

Chapter 4 deals with the emulsifying properties of Acacia gums. The effect of biochemical composition and structural properties of Acacia gums on the formation of emulsion droplets and their stability was investigated. In order to control high molar mass protein-rich AGPs content in bulk and the total concentration, an innovative

approach by mixing two fractions isolated from A. *senegal* was used. In parallel, A. *seyal* and initial A. *senegal* were used to produce emulsions and their protein, sugar content and structural conformation effects on emulsifying properties were investigated.

Chapter 5 is focused on the properties of Acacia gum film surface. The film surface structure, water wettability and affinity between compounds with different polarity were highlighted. Moreover, functionalities of films as water permeability and aroma retention were related to these specific characteristics of gum films.

Chapter 6 presents the overall conclusion and perspectives.

Introduction générale

La gomme d'Acacia (AG, E414), également appelée gomme arabique, est un exsudat naturel comestible de gomme récolté sur les arbres Acacia *senegal* (A. *senegal*) et Acacia *seyal* (A. *seyal*). La production de gomme d'Acacia est un mécanisme de protection de l'arbre contre l'invasion des insectes et des moisissures et la cicatrisation des plaies (Clarke et al., 1979; Islam et al., 1997; Sanchez et al., 2018). La gomme d'Acacia est largement utilisée comme additif alimentaire et une définition internationale a été donnée par la FAO / OMS au cours du 31ème Comité du Codex pour les additifs alimentaires. Cette définition précise que la gomme d'Acacia est « une exsudation séchée obtenue à partir des troncs et des branches d'A. *senegal* (L) Willdenow ou d'espèces proches d'Acacia telles que A. *seyal* (famille des légumineuses) » (FAO, 1999). Bien qu'appartenant à des séries taxonomiques différentes, à savoir A. *seyal* appartient à l'espèce des *Gummiferae* et A. *senegal* à celles des *Vulgares*, ces deux gommes d'Acacia sont composées des mêmes sucres, acides aminés et sels.

Les gommes d'Acacia sont principalement récoltées dans les régions arides de la ceinture sub-saharienne, dite « ceinture de gomme d'Afrique », du Sénégal à l'Afrique de l'Est, au Pakistan et en Inde (Jales, 2018; Sanchez et al., 2018). Les arbres sont parfaitement acclimatés aux zones sèches et désertiques et leur présence offre de nombreux avantages. Sur le plan environnemental, ils agissent comme une barrière à la désertification, ils nourrissent le sol en utilisant l'azote de l'air puis en le transférant dans le sol. Les principaux pays producteurs de gomme sont le Soudan, le Tchad et le Nigeria, qui représentent 95% du marché mondial des exportations de gomme. Leurs principales missions sont de collecter, sécher, emballer et livrer les gommes à un point de vente (Jales, 2018; Muller & Okoro, 2004). Les principaux pays importateurs sont l'Inde et la France en 2014-2016, avec 75% du montant des importations mondiales (Jales, 2018).

Depuis les temps anciens, les humains utilisent les gommes d'Acacia pour l'alimentation, la médecine et d'autres applications non alimentaires comme par

exemple dans les technologies osseuses pour ses propriétés adhésives (Sanchez et al., 2018). À l'époque des anciens Égyptiens, la gomme était utilisée comme liant pigmentaire dans les peintures et les encres pour la fabrication de hiéroglyphes et comme adhésif pour fabriquer des emballages en lin pour l'embaumement des momies (Verbeken et al., 2003; Whistler, 1993). Au Moyen Age, la gomme d'Acacia était appréciée en Europe par les scribes et les illustrateurs pour l'application de la couleur. Entre le XIIe et le XIXe siècle, elle a également été utilisée pour la stabilisation des pigments, dans la composition de l'encre métallo-gallique, l'encre la plus populaire en Europe utilisée par des peintres tels que Rembrandt. L'ère industrielle moderne a engendré une explosion des utilisations manufacturières de la gomme d'Acacia. Elle est largement utilisée comme émulsifiant, stabilisant, agent de finition de surface, épaississant dans les industries alimentaires et non alimentaires, y compris les applications pharmaceutiques, textiles et cosmétiques ou dans l'imprimerie. Elle est intéressante de part ces propriétés comme liant pour les pigments de couleur dans les crayons, comme revêtement pour les papiers et comme ingrédient essentiel dans le procédé de micro-encapsulation pour la production de papier autocopiant, ou encore dans la fabrication des détergents pour lessive, etc. Ces propriétés adhésives, ont été largement employées pour les allumettes et les timbres-poste. Dans l'industrie cosmétique, on la retrouve dans la composition des poudres pour le visage, des masques, crèmes et lotions là aussi à cause de ces propriétés adhésives et émulsifiantes. Dans l'industrie alimentaire, la gomme d'Acacia est utilisée pour la production de boissons, de confiseries, d'émulsions, d'encapsulations de composés d'arômes ou de colorants, de produits de boulangerie et de brassage. À titre d'exemple, la gomme d'Acacia permet une distribution uniforme des huiles essentielles et des graisses dans des solutions aqueuses, mais aussi de retarder la cristallisation du sucre dans les confiseries, les glaçages, les mélanges à gâteaux, les crèmes fouettées. Dans les boissons, elle est spécialement utilisée pour la stabilisation des huiles essentielles et des mélanges de composés aromatiques. Dans la production de vins, la gomme d'Acacia confère du corps, stabilise la couleur et empêche la précipitation des polyphénols et des protéines. La production de boissons et de confiseries s'avère être la principale application de la

gomme d'Acacia dans les industries alimentaires.

La gomme d'Acacia est en train de devenir un produit hautement stratégique avec une forte croissance de la demande, d'environ 25% au cours des dix dernières années. La gomme est importante pour les pays producteurs, en particulier pour les populations locales, pour qui elle est souvent la seule source de revenus, mais aussi pour les pays importateurs. Entre une production irrégulière (sécheresse, instabilité politique des pays producteurs) et une demande croissante de gomme naturelle à faible coût, les entreprises de gomme d'Acacia sont confrontées à plusieurs défis. Le premier défi consiste à assurer une qualité élevée et constante du produit malgré les fluctuations climatiques et la variabilité du traitement (entaille, stockage, transport, contrôle de la température). Le second consiste à développer de nouvelles applications à haute valeur ajoutée basées sur de nouvelles connaissances de la composition, la structure et les propriétés techno-fonctionnelles de la gomme d'Acacia et sur le développement de nouveaux produits à base de gomme d'Acacia. Pour cette raison, un programme de recherche, baptisé DIVA, a été créé entre la société ALLAND & ROBERT (Port-Mort), fournisseur mondial de gommes, et l'UMR IATE 1208 (Montpellier), unité de recherche dédiée à l'ingénierie des agropolymères et des technologies émergentes depuis 2012. Alland & Robert est une société française spécialisée dans la production de gomme d'Acacia depuis 1884 et mondialement reconnue par les plus grands groupes alimentaires. Leurs politiques sociales et économiques en France et en Afrique couvrent plusieurs niveaux : garantir des matières premières durables et de qualité, garantir un processus de production éthique et propre et développer un partenariat durable avec des pratiques sociales et de sécurité éthique et en conformité avec le respect de l'environnement. Dans le cadre de cette collaboration, le programme DIVA est divisé en projets fondamentaux et d'applications fortement interconnectés. Les différents projets portent notamment sur le fractionnement des gommes d'Acacia *senegal* et d'Acacia *seyal*, la caractérisation de la composition, les propriétés structurales et physicochimiques des macromolécules de gomme d'Acacia (gommes d'Acacia entières et fractions) et l'étude des propriétés interfaciales et émulsifiantes des macromolécules

de gomme d'Acacia (gommes d'Acacia entières et fractions).

Mon projet de thèse est entièrement axé sur la caractérisation des propriétés interfaciales et émulsifiantes des macromolécules de gomme d'Acacia (gommes d'Acacia entières et leurs fractions). Il est étroitement lié aux autres sujets mentionnés précédemment. Parmi ceux-ci, les suivants, qui ont déjà été réalisés ou se déroulent en parallèle, ont joué un rôle crucial dans la réalisation de mon doctorat.

Il s'agit des projets portant sur :

- La caractérisation de la composition, des propriétés structurales et physico-chimiques des gommes d'A. *senegal* et A. *seyal.*

Ce sujet visait à examiner la composition de base et les propriétés structurales des macromolécules d'A. *senegal* et A. *seyal* en fonction de leurs propriétés fonctionnelles. Les gommes étaient spécialement caractérisées en termes de composition et de teneur en sucres, en protéines et en minéraux, et de degré de ramification. Ce travail a été effectué lors de la précédente thèse de doctorat de Lopez Torrez (Lopez Torrez, 2017).

- Le fractionnement/purification d'arabinogalactanes-protéines (AGP) à partir de gomme d'Acacia *senegal*

Les objectifs de cette étude sont d'isoler les arabinogalactanes-protéines (AGP) de la gomme A. *senegal* en fonction de leurs propriétés structurales et physico-chimiques. Des méthodes chromatographiques classiques ont été utilisées, notamment la chromatographie d'interaction hydrophobe (HIC) et la chromatographie par échange d'ions (IEC). Ces procédés ont permis d'obtenir des fractions d'AGP isolées : trois fractions principales ont été isolées en utilisant HIC, à savoir HIC-F1, HIC-F2 et HIC-F3, tandis que deux fractions majeures ont été obtenues en utilisant IEC, à savoir IEC-F1 et IEC-F2. Après l'isolement de ces fractions, leur composition biochimique, leurs propriétés structurales et physico-chimiques ont été caractérisées en profondeur. Ce sujet correspond au projet postdoctoral de Apolinar Valiente.

- Les propriétés physicochimiques en solution d'AGP des gommes d'Acacia

L'objectif principal de ce projet est d'étudier les propriétés volumétriques, le volume

spécifique partiel et la compressibilité adiabatique spécifique partielle; ainsi que les propriétés hydrodynamiques: viscosité dynamique, viscosité intrinsèque et rayon hydrodynamique des gommes d'Acacia à 25°C et jusqu'à 70°C. A. *senegal*, A. *seyal* et les fractions macromoléculaires d'A. *senegal*, à savoir HIC-F1, HIC-F2 et HIC-F3 issues de HIC et IEC-F1 et IEC-F2 issues du fractionnement par IEC ont été caractérisées. Ce travail fait partie du projet de doctorat de Mejia Tamayo (Mejia Tamayo, 2018).

Dans le cadre de mon projet de thèse, les principaux objectifs scientifiques sont :

(i) d'établir la relation entre la composition biochimique et les propriétés structurales des gommes d'A. *senegal* et d'A. *seyal* et leurs propriétés interfaciales. Pour y répondre, la capacité à réduire la tension interfaciale et à former un film à l'interface hexadécane-eau des gommes d'Acacia et de la fraction IEC-F1 isolée à partir d'A. *senegal* sera étudiée et comparée en tenant compte de l'espèce et les concentrations en en gomme. En dépit de la différence de composition biochimique et des propriétés structurales entre les gommes A. *senegal* et A. *seyal*, leurs propriétés interfaciales seront comparées et reportées en fonction de leur teneur en protéines et des hypothèses sur l'impact de leur nature spécifique seront émises. En outre, la variation de la concentration en gomme a un impact non seulement sur la teneur en protéines, mais également la teneur en AGP de masse molaire élevée et riches en protéines. Ces molécules largement décrites comme responsables des propriétés interfaciales de la gomme d'Acacia seront évaluées. La possibilité d'utiliser différentes fractions de *A. senegal* obtenues par purification permettra de confirmer le rôle majeur des AGP riches en protéines de masse molaire élevée dans les propriétés interfaciales des gommes d'Acacia.

(ii) d'évaluer l'impact de la nature de la phase dispersée sur les propriétés interfaciales des gommes A. *senegal* et A. *seyal*. En effet, la nature d'interface en contact avec les gommes d'Acacia pouvant influencer les propriétés interfaciales, le

comportement de différents composés sera étudié. L'hexadécane a été le premier composé choisi pour cette étude en raison de l'abondance d'études des propriétés interfaciales des gommes à l'interface de ce composé (Castellani et al., 2010; Chanamai et al., 2002; Dickinson, 1999; Dickinson et al., 1988). Une attention particulière a été accordée à d'autres interfaces, telles que le limonène et l'octanol, dont la nature et la polarité diffèrent afin de comprendre l'effet de la nature de l'interface sur le comportement de A. *senegal* et A. *seyal*. Les nouvelles connaissances acquises sur les propriétés interfaciales des gommes d'Acacia à chaque interface peuvent nous permettre de mieux comprendre les propriétés émulsifiantes de la gomme d'Acacia.

(iii) de caractériser les émulsions produites par A. *senegal*, A. *seyal* et à partir d'une gomme reconstituée en utilisant des fractions bien caractérisées d'A. *senegal*, ceci afin de relier leur aptitude à produire et à stabiliser des gommes à leurs propriétés biochimiques et structurales et à leurs propriétés interfaciales. Il est important de garder à l'esprit que A. *senegal* est préférentiellement utilisée pour stabiliser les composés aromatiques sous forme d'émulsions diluées dans les boissons à longue conservation, tandis que A. *seyal* est utilisée dans l'étape d'émulsification avant l'encapsulation des arômes, c'est-à-dire lorsque la stabilité n'est pas cruciale. Pour répondre à cet objectif, la phase d'huile dispersée choisie était le limonène à concentration fixe (5% en poids) en raison de sa teneur élevée dans l'huile d'orange. La stabilisation de l'émulsion de limonène seule est difficile en raison de sa faible viscosité. Cependant, l'utilisation du limonène peut offrir l'avantage de mettre en évidence plus clairement la différence de comportement émulsifiant entre les deux gommes. En termes de technologie d'émulsification, la microfluidisation a été choisie car elle est adaptée à la faible viscosité des dispersions de gomme d'Acacia, ce qui permet d'étudier l'effet de la concentration en gomme d'Acacia. Une grande attention sera portée au rôle des AGP de masse molaire élevée riches en protéines pour stabiliser l'émulsion. En effet, il est largement admis que ces AGP sont responsables des propriétés émulsifiantes des gommes (Alftrén et al., 2012; Flindt et al., 2005; Nishino et al., 2012; Padala et al., 2009; Randall et al., 1988; Ray

et al., 1995). Les différentes approches habituellement décrites pour démontrer et comprendre le rôle de ces AGP consistent :

- à faire varier la concentration de gomme (Yao et al., 2013),
- d'utiliser différentes gommes avec différentes quantité de protéines et donc d'AGP de masse molaire élevée (Dickinson et al., 1988; Katayama et al., 2006),
- d'utiliser des gommes matures qui contiennent plus de AGP riches en protéines et ayant des masse molaires élevées due à l'agrégation (Al-Assaf et al., 2007; Aoki, Katayama, et al., 2007; Aoki, Al-Assaf, et al., 2007)
- ou encore d'étudier les propriétés émulsifiantes de la gomme dégradée par l'enzyme protéolytique (Al-Assaf et al., 2009; Flindt et al., 2005).

De plus, certains auteurs ont rapporté l'effet des fractions constitutives des gommes sur les propriétés émulsifiantes (Nishino et al., 2012; Ray et al., 1995). De plus, une autre méthode habituellement utilisée consiste à récupérer les molécules adsorbées à l'interface des gouttelettes et à les caractériser (Buffo et al., 2001; Katayama et al., 2006; McNamee et al., 2001; Mikkonen et al., 2016; Nakauma et al., 2008; Randall et al., 1988; Xiang et al., 2015). D'après les résultats des études citées ci-dessus, le rôle prépondérant des fractions riches en protéines de masse molaire élevée dans les propriétés émulsifiantes de la gomme d'Acacia ne fait aucun doute. Cependant, une question reste toujours posée : « Les propriétés fonctionnelles de la gomme d'Acacia sont-elles simplement le résultat de la somme des propriétés des fractions individuelles ou une synergie positive existe-t-elle ? » En tentant de répondre à cette question, une approche innovante a été mis en place en mélangeant deux fractions isolées de A. *senega*l afin de contrôler la teneur en AGP riches en protéines de masse molaire élevée dans le milieu (bulk) et la concentration totale de gomme. La concentration totale et la quantité en protéines ont été choisies en fonction des applications générales des gommes d'Acacia. Les deux fractions sont une fraction riche en protéines de masse molaire élevée isolée de A. *senegal* en utilisant une chromatographie par échange d'ions et une fraction de faible masse molaire isolée à partir de la même gomme en utilisant une chromatographie d'interaction hydrophobe traditionnelle. Ces fractions ont été bien

caractérisées en termes de composition biochimique et de propriétés structurales.

En parallèle, l'effet de l'espèce de gomme d'Acacia sera étudié en utilisant A. *senegal* et A. *seyal*. En effet, les deux gommes sont caractérisées par la différence de teneur en protéines, de propriétés structurales et physico-chimiques telles que la viscosité. D'autre part, d'autres paramètres d'émulsification tels que le processus d'émulsification et l'effet de l'addition de glycérol seront également étudiés. Les connaissances obtenues pourraient permettre de mieux comprendre l'effet de la nature des émulsifiants sur les caractéristiques physiques des émulsions produites (distribution de la taille des globules, stabilité temporelle des particules, apparition des phénomènes de crémage, coalescence, maturation d'Ostwald) et d'envisager le développement d'une nouvelle gomme aux propriétés émulsifiantes optimales.

(iv) de caractériser la structure de la surface et les propriétés interfaciales de films séchés à base de gommes et la relation entre ces propriétés et la perméabilité à l'eau mais aussi la capacité à piéger des composés d'arômes.

En effet, la gomme d'Acacia est également largement utilisée comme agent de revêtement avec comme ambition majeure d'éviter l'oxydation des graisses. Actuellement, de nouvelles application sont envisagées comme l'enrobage des produits naturels comme les fruits frais et les poissons (Ali et al., 2010; Binsi et al., 2016; El-Anany et al., 2009; Jiang et al., 2013; Maqbool et al., 2011). Cependant, les connaissances approfondies sur les propriétés de surface ne sont toujours pas disponibles comme la mouillabilité de l'eau. Une explication est la difficulté de former un film homogène sans fissure à base de gomme d'Acacia qui est un polymère hyper ramifié. Par conséquent, la première étape de cette étude consistera à produire des films sans fissure sur une surface modèle en utilisant la technologie de revêtement par centrifugation (spin coating). Ensuite, les propriétés de surface, à savoir la mouillabilité de l'eau, l'énergie libre de surface et les composantes polaires et dispersives, des films de gomme d'Acacia seront évaluées en utilisant la mesure de l'angle de contact. La structure du film en surface sera caractérisée par une observation microscopique. En fonction de la structure et de la composition des gommes, la formation du réseau d'A.

senegal et d'A. *seyal* à l'interface des films solides sera discutée par rapport à des propriétés de surface déterminées. En outre, l'affinité entre les films et différents composés de nature et de polarité différentes sera évaluée pour estimer leur impact potentiel sur les fonctionnalités des films, telles que la perméabilité à l'eau et la rétention des composés d'arôme. Les nouvelles connaissances acquises dans cette section devraient permettre d'établir une relation entre la composition biochimique, la structuration du film solide et ses propriétés interfaciales et donc d'améliorer l'utilisation de la gomme d'Acacia en tant qu'agent de revêtement.

La meilleure compréhension du comportement des gommes d'Acacia à différentes interfaces devrait permettre d'optimiser leur utilisation comme émulsifiant pour des boissons ou l'encapsulation d'arômes et comme agent de revêtement pour divers produits.

Cette thèse est divisée en 5 chapitres :

Le chapitre 1 mentionne le contexte et les objectifs du projet de thèse.

Le chapitre 2 présente un aperçu des propriétés biochimiques, physicochimiques et structurelles des gommes d'Acacia, avec un focus spécifique sur les gommes et les fractions utilisées dans ce projet.

Le chapitre 3 étudie l'effet de A. *senegal*, A. *seyal* et IEC-F1 et leurs concentrations sur la diminution de la tension interfaciale de l'hexadécane et les propriétés rhéologiques du film interfacial. Dans le même chapitre, l'effet de la nature de la goutte sur les propriétés interfaciales des gommes d'Acacia a été caractérisé.

Le chapitre 4 traite des propriétés émulsifiantes des gommes d'Acacia. L'effet de la composition biochimique et des propriétés structurales des gommes d'Acacia sur la formation des émulsions, la taille des gouttelettes et leur stabilité a été étudié. Afin de contrôler la teneur en AGP riche en protéines de masse molaire élevée dans le milieu

(bulk) et la concentration totale, une approche innovante consistant à mélanger deux fractions isolées chez A. *senegal* est exposée. En parallèle, les gommes A. *seyal* et A. *senegal* ont été utilisées pour produire des émulsions. Par conséquent, l'effet de la teneur et composition en protéines et en sucres ainsi que la conformation structurale sur les propriétés émulsifiantes ont été étudiés.

Le chapitre 5 se concentre sur les propriétés de la surface de films de gomme d'Acacia. La structure de la surface des films, la mouillabilité de l'eau et l'affinité entre les composés de polarité différente ont été mises en évidence. De plus, les fonctionnalités des films comme la perméabilité à l'eau et la rétention en arômes seront reportées.

Le chapitre 6 clôture le document par une conclusion générale et des perspectives.

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Acacia gums

Chapter 2 – Acacia gums

This chapter presents a brief overview of Acacia *senegal* (A. *senegal*) and Acacia *seyal* (A. *seyal*) gums and their fractions obtained using chromatographic technics. The biochemical composition and the structural properties of A. *senegal* and A. *seyal* gums, and the fractions used in this Ph.D. project are also presented (Tables II.1 and II.2) and compared to literature.

1. A. *senegal* and A. *seyal* gums

Acacia gum macromolecules are highly glycosylated hydroxyproline-rich arabinogalactan-proteins (AGPs) belonging to the glycoprotein superfamily (Akiyama et al. 1984; Showalter 2001). Experimentally, AGPs are identified and defined by their ability to react with a chemical reagent: a phenylazoglycoside dye named Yariv reagent ((Yariv, Lis, & Katchalski, 1967; Yariv, Rapport, & Graf, 1962). AGPs are hyperbranched complex polysaccharide, neutral or slightly acidic, found as a mixture of calcium, magnesium, and potassium salt. Basically, AGPs are mainly composed of galactose, arabinose, rhamnose, glucuronic acid, proteins and minerals (D. M. W. Anderson & Stoddart, 1966; Idris, Williams, & Phillips, 1998; Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Lopez-Torrez, Nigen, Williams, Doco, & Sanchez, 2015; Menzies, Osman, Malik, & Baldwin, 1996; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006; Sanchez et al., 2018). Typically, A. *seyal* gum contains more arabinose than galactose sugar, while A. *senegal* gum is characterized by a higher content of galactose than arabinose (Idris et al., 1998; Lopez-Torrez et al., 2015; Nie et al., 2013b, 2013a; Sanchez et al., 2018). A. *senegal* gum is richer in protein than A. *seyal* gum (~1.5 to 3% vs. ~0.5 to 1%), but the relative distribution of amino acids between Acacia gum species remained quite similar (Islam et al., 1997). The proteinaceous part of Acacia gums is predominantly composed by hydroxylproline and serine with a

significant content of proline, threonine, histidine, and leucine amino acids (D. M. Anderson, Douglas, Morrison, & Wang, 1990; Lopez-Torrez et al., 2015; Mahendran, Williams, Phillips, Al-Assaf, & Baldwin, 2008; Mejia Tamayo et al., 2018; Randall, Phillips, & Williams, 1989). Both Acacia gum species contain also some minor components as traces of lipids, tannins, phenols and enzymes (D. M. W. Anderson, Bridgeman, Farquhar, & McNab, 1983; Kunkel, Seo, & Minten, 1997; Mhinzi, 2003a, 2003b). The biochemical composition and physicochemical properties of Acacia gums may vary depending on the geographical origin, age of the trees, climatic conditions and gum species (Idris et al., 1998; Islam et al., 1997; Lopez-Torrez et al., 2015).

AGPs consist of a hydroxyproline-rich core protein covalently linked to polysaccharide blocks rich in arabinose and galactose. The polysaccharide blocks exhibit a so-called type II arabinogalactan glycan structure with the polysaccharide backbone formed by 1,3-linked β-D-galactopyranosyl units. The side chains consist of two to five 1,3-linked β-D-galactopyranosyl units, joined to the main chain by 1,6-linkages. The main and side chains include units of α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl, and 4-O-methyl-β-D-glucuronopyranosyl, the latter two mostly as end-units (D. M. W. Anderson & Stoddart, 1966; Islam et al., 1997). The polysaccharide blocks of both Acacia gum species were organized into hyperbranched structure with a more branched polysaccharide architecture for A. *senegal* than for A. *seyal* (degree of branching of 78.2% and 59.2%, respectively) (Lopez-Torrez et al., 2015).

In term of structural parameters, the mean molar mass (M_w) of A. *senegal* is generally lower than that of A. *seyal,* while the polydispersity index (M_w/M_n) was higher for the former (Lopez-Torrez et al., 2015). This highlighted a higher proportion of high molar mass AGPs in A. *senegal* gum than in A. *seyal* gum. Generally, A. *seyal* gum is also characterized by a lower intrinsic viscosity (η) than A. *senegal* gum (Lopez-Torrez et al., 2015). The higher molar mass and lower intrinsic viscosity of A. *seyal* (lower hydrodynamic volume) reflects a higher compact structure of AGPs from this gum compared to those of A. *senegal* gum (Lopez-Torrez et al., 2015). This greater

compactness of A. *seyal* macromolecules could be explained by (i) the smaller charge density, thus less intra-chain electrostatic repulsion, (ii) the lower protein content, resulting in the less distance between polysaccharide blocks, and (iii) the presence of long flexible arabinosyl chains able to self-assemble and mutually interact through hydrogen bonds. According to the analysis of the conformation plots from SEC-MALLS experiments of A. *senegal* and A. *seyal* gums, it was advanced that the AGPs conformation from A. *seyal* varied from spheres to oblate ellipsoids whereas those from A. *senegal* varied from oblate ellipsoids to more anisotropic conformations, such as oblate and prolate ellipsoids (Gillis, Adams, Alzahrani, & Harding, 2016; Lopez-Torrez et al., 2015; Mahendran et al., 2008; Sanchez et al., 2008, 2018). Recently, the flexibility and hydration of both Acacia gums were studied through the characterization of their volumetric properties (Mejia Tamayo et al., 2018). Authors reported that A. *senegal* gum was more flexible and less hydrated than A. *seyal* gum according to the higher protein content and the greater value of partial specific volume of the former. This better molecular flexibility could be related to the better interfacial properties of A. *senegal* compared to A. *seyal*.

The biochemical compositions and structural properties of A. *senegal* (lots N° OF110676 and N° OF152413) and A. *seyal* (lots N° OF110724) gums used in this project are presented in Table II.1. The results are in accordance with those previously described. It can however be noted some slight differences between Acacia gums from a same specie, especially in their protein content. Concerning the A. *senegal* gum, the lot N° OF110676 is richer in protein than the lot N° OF152413 (27.0 $mg.g^{-1}$ vs. 21.5 $mg.g^{-1}$).

Table II.1: Biochemical compositions and structural parameters of A. *senegal* and A. *seyal* used in the PhD project. Adapted from *Lopez Torrez et al. 2015 and **Mejia Tamayo et al., 2018. na stands for non-available.

	A. *senegal** (lot N° OF110676)	A. *senegal*** (lot N° OF152413)	A. *seyal** (lots N° OF110724)
Total dry matter ($mg.g^{-1}$)	889.0±0.3	893.4±4.0	893.0±0.0
Moisture (%)	11.1	10.7	10.7
Sugar ($mg.g^{-1}$)[a]	940.0	944.4	950.0
Arabinose (%)	30.3±2.5	30.2±0.6	47.6±0.6
Galactose (%)	35.8±1.2	40.5±1.7	36.9±1.1
Rhamnose (%)	15.5±0.4	12.4±0.4	3.0±0.3
Glucuronic acid (%)	17.4±1.2	17.8±1.7	6.7±0.4
4-O-Me-Glucuronic acid (%)	1.0±0.1	1.0±0.1	5.8±0.6
Uronic acid/neutral sugar ratio	0.23	0.23	0.14
Protein ($mg.g^{-1}$)	27.0±0.0	21.5±0.9[b]	10.0±0.0
Mineral ($mg.g^{-1}$)	33.0±0.2	34.1±0.1	40.0±0.1
Average molar mass (M_w, $g.mol^{-1}$)	6.8×10^5	6.8×10^5	8.2×10^5
Polydispersity index (M_w/ M_n)	2.0	2.0	1.5
Branching degree (%)	78.2	78.0	59.2
Intrinsic viscosity ($mL.g^{-1}$)	22.8	29.8	16.5
Partial specific volume ($cm^3.g^{-1}$)	na	0.5842	na
Partial specific adiabatic compressibility($\times10^{11}$ $cm^3.g^{-1}.Pa^{-1}$)	na	-7.1	na

[a]Total content of sugars calculated from the difference of proteins and minerals from 1 000 $mg.g^{-1}$. [b]Protein content was measured using the Kjeldhal method.

2. Fraction of AGPs isolated from A. *senegal* gum

A. *senegal* gums are defined as a continuum of AGPs differing by their sugar, amino acid and mineral content and composition, sugar to amino acid ratio, polarity, number of charges, molar mass, size and shape (Islam et al., 1997; Mejia Tamayo et al., 2018; Randall et al., 1989; Renard et al., 2006). These AGPs can be separated according to their physicochemical properties using different chromatographic technics. In this PhD project, A. *senegal* gum is fractionated according to hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IEC).

The hydrophobic interaction chromatography (HIC) is the most used technique to separate AGPs from A. *senegal* gums (Randall et al. 1989; Renard et al. 2006). Using HIC, AGPs are separated according to their polarity. Usually, three fractions were obtained and traditionally named arabinogalactan-peptide (AGp), arabinogalactan-proteins (AGP) and glycoproteins (GP) according to their elution order and then their growing hydrophobic index. However, since all these fractions react to Yariv's reactant, they are all AGPs. Then, they will be more rigorously named HIC-F1 (AGp), HIC-F2 (AGP) and HIC-F3 (GP) in the order of elution to avoid any possible confusion. HIC-F1 is the most abundant fraction (85-92% of the whole gum) as compared to HIC-F2 (6-16% of the whole gum) and HIC-F3 (1-3% of the whole gum). The sugar composition was similar between the three fractions, with however a larger content of arabinose in HIC-F2 and HIC-F3 and a larger content of charged sugars in HIC-F1. HIC-F3 was the richest fraction in proteins with values around 10-40%, while the amount of proteins was around 8-10% and 1% for HIC-F2 and HIC-F1. These three HIC fractions differed also by their mean molar mass and high molar mass AGPs content (AGPs with M_w upper than 10^6 g.mol^{-1}). HIC-F1 was mainly composed of low molar mass AGPs, while HIC-F2 and HIC-F3 were richest in high molar mass AGPs. HIC-F1 can be considered as constituted by low molar mass AGPs poor in proteins. In contrary, HIC-F2 and HIC-F3 are mainly composed by high molar mass AGPs rich in proteins. The characterization of the volumetric properties of these three HIC fractions highlighted a

less hydrated and more flexible structure of HIC-F3, in contrast to a less flexible and more hydrated structure of HIC-F2, and especially HIC-F1 (Mejia-Tamayo et al. 2018).

In the DIVA research program, the Acacia *senegal* gum (lot N° OF110676) was also fractionated using HIC chromatography and the three eluted HIC fractions have been characterized. As HIC-F1 fraction is the only HIC fraction used in this PhD project, only its biochemical composition and structural parameters are presented and compared to those of HIC-F1 fraction obtained by Renard et al. (Table II.2) (Renard et al., 2006). The biochemical composition and structural parameters of these two HIC-F1 fractions were in accordance with slight differences. The HIC-F1 fraction obtained at UMR IATE is especially characterized by a lower protein content and a higher intrinsic viscosity in comparison to that obtained by Renard et al.

In this PhD project, we also used another AGPs fraction obtained with a different chromatography technique, the ionic exchange chromatography (IEC) (Apolinar-Valiente et al. 2018, submitted article). The fractionation of A. *senegal* gum (lot N° OF152413) using IEC allowed to obtain two fractions named IEC-F1 and IEC-F2. The biochemical composition and structural properties of IEC-F1, the only IEC fraction used in this PhD project, are presented in Table II.2. IEC-F1 is composed of the same sugar as HIC-F1. However, the proportions of galactose, arabinose and glucuronic acid are different. IEC-F1 contains a higher amount of arabinose, and a lower amount of galactose and glucuronic acid as compared to HIC-F1. The sugar composition of IEC-F1 is closer to that of HIC-F2 and HIC-F3 fractions than to HIC-F1. IEC-F1 is also characterized by a high protein content as compared to HIC-F1. Based on the amino acid composition of IEC-F1, it can be argued that this fraction corresponds to the mix HIC-F2 (30%) and HIC-F3 (70%) fractions. The AGPs of IEC-F1 presents a high molar mass with a value of $30x10^5$ $g.mol^{-1}$. Hence, IEC-F1 fraction is only composed of high molar protein-rich AGPs. As these AGPs are supposed to be crucial for the emulsifying properties of Acacia gums, this fraction appears to be suitable to characterize the emulsifying properties of Acacia gums and their involvement in this functional property.

Table II.2: Biochemical composition and structural parameters of fractions from A. *senegal* obtained using hydrophobic interaction chromatography (HIC) and ion exchange chromatography (IEC). Adapted from **Mejia Tamayo et al., 2018 *** Apolinar-Valiente et al. 2018. na stands for non-available values.

	HIC**			IEC***
	HIC-F1	HIC-F2	HIC-F3	IEC-F1
Yield (%)	na	na	na	4.2
Total dry matter ($mg.g^{-1}$)	921.6	926.2	921.9	na
Sugar ($mg.g^{-1}$)	961.3	918.3	813.0	882.1
Arabinose (%)	26.8	35.6	38.3	35.5
Galactose (%)	39.0	34.4	33.3	33.5
Rhamnose (%)	12.5	13.7	13.9	12.8
Glucuronic acid (%)	20.3	15.6	13.7	17.3
4-O-Me-Glucuronic acid (%)	1.4	0.6	0.7	1.0
Protein ($mg.g^{-1}$)	4.9±0.1[a]	63.1±1.2[a]	137.7±2.7[a]	114.9
Mineral ($mg.g^{-1}$)	30.5±1.1	19.3±1.1	49.3±2.6	3
Average molar mass (M_w, $g.mol^{-1}$)	3.5×10^5	15.0×10^5	16.0×10^5	30×10^5
Polydispersity index (M_w/M_n)	1.4	1.3	1.9	1.2
Intrinsic viscosity ($mL.g^{-1}$)	22.1	64.3	54.7	87.8

[a]Using the method of Kjeldahl.

3. Summary of Acacia gums and their fractions used in the different topic of this Ph.D. project

The crossing strategies used in the following chapters in order to investigate the effect of gums and different characteristics of compounds on interfacial and emulsifying properties of Acacia gums is summarized in the Table II.3.

Table II.3: Acacia gums, their fractions and different compounds investigated according to the objectives of this Ph.D. project presented in different color.

Compounds / Gums	Octane	Decane	Hexadecane	Hexanol	Octanol	Decanol	Linalool	Limonene
A. *senegal* (lot N° OF110676)								
A. *senegal* (lot N° OF152413)								
A. *seyal* (lot N° OF110724)								
IEC-F1								
HIC-F1+IEC-F1								

Chapter 3: Liquid-liquid interfacial properties:

To establish the relationship between the interfacial properties of Acacia gums dispersion and the gum specie, the concentration and the nature and polarity of interfaces.

Chapter 4: Emulsifying properties:

To investigate the emulsifying properties of Acacia gums, i.e. the emulsification ability and the stability, and to specify the role of high molar mass protein-rich AGPs usually described as responsible of these emulsifying properties.

Chapter 5: Solid-liquid interfacial properties:

To establish a relationship between the different film properties (surface structure, wettability, surface energy, affinity with different compounds) and the gum species, their biochemical composition and structural properties.

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Interfacial properties of Acacia *senegal* and Acacia *seyal* gums

Chapter 3 – Interfacial properties of Acacia *senegal* and Acacia *seyal* gums

This chapter focuses on the interfacial properties of Acacia gums dispersion taking into account the gum specie, the concentration and the nature and polarity of interfaces (air and varied liquids).

The major aim is to establish the relationship between the composition and structural properties of gums, and their interfacial properties such as the ability to decrease the interfacial tension and to form interfacial films at specific liquid/liquid interfaces through the measurement of interfacial tension but also the viscoelastic modulus. Indeed, the interfacial tension decrease depends on the amphiphilic characteristic and structure of the molecules, whereas the interfacial rheology is related to the capacity of interactions between adsorbed molecules and their structural stabilization (Benjamins, Lyklema, & Lucassen-Reynders, 2006). The second purpose is to determine the surface tension (liquid/air) of Acacia gums dispersions and to estimate the polar and dispersive components as a function of gum species. The knowledge of interfacial properties of Acacia gums should provide information about their emulsifying properties, i.e. their ability to form but also to stabilize emulsions.

For these purposes, A. *senegal* and A. *seyal* gums dispersion at different concentrations (between 0.05 and 5 wt%) were compared since they differ in their biochemical composition (particularly their protein content), in their AGPs distribution (especially their high molar mass protein-rich AGPs content) and in their structural properties. Furthermore, the interfacial properties of a specific fraction from A. *senegal* (IEC-F1) which was composed of high molar mass protein-rich AGPs were investigated in order to gain more information about the importance of this fraction which is currently described as having a major role in the interfacial properties of Acacia gums (Elmanan, Al-Assaf, Phillips, & Williams, 2008; Randall, Phillips, & Williams, 1988). A. *senegal*

and A. *seyal* gum dispersions at 5wt% were furthermore investigated at different liquid/liquid interfaces. Indeed, organic compounds with different chemical functions and polarity, i.e. hexadecane, limonene and octanol were considered. Hexadecane was chosen because it has been largely used in the characterization of Acacia gums interfacial properties (Castellani, Al-Assaf, Axelos, Phillips, & Anton, 2010; Dickinson, Galazka, & Anderson, 1991a, 1991b; Dickinson, Murray, Stainsby, & Anderson, 1988; Mahfoudhi et al., 2014; Mahfoudhi, Sessa, Ferrari, Hamdi, & Donsi, 2016). Limonene and octanol are aroma compounds characterized by different level of polarity and water solubility which could affect the interfacial properties of Acacia gums.

In order to acquire supplementary information about interfacial properties, the surface tension at the air interface of A. *senegal* and A. *seyal* dispersions at high concentration was determined and the polar and dispersive components calculated. This could contribute to explain the difference between the gums and to enhance the use of Acacia gums in food applications, e.g. as foam stabilizing agent.

As an introduction of this chapter, a quick point defining the terms and methods used in this chapter is shown.

I. Definition of terms and methods

Since we proposed to characterize both the surface and the interfacial tension, it is important to clearly define the terms and the methods used.

The term surface tension (γ) is used to characterize the interface air/liquid. It is an intrinsic property of a liquid, which is caused by the unbalanced forces of the liquid molecules at the contact of air and corresponds to the intermolecular forces to contract the surface of the droplet liquid for a determined volume. Indeed, surface tension is defined as the quantity of energy needed to increase the interfacial area in one unit of surface (Langevin, Delorme, & Cagna, 2004).

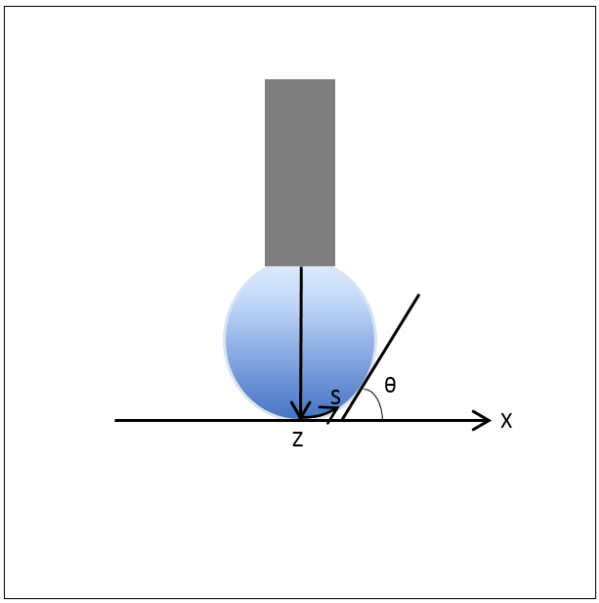
Interfacial tension is defined at any interface between two different media as liquid, solid or gas and their different combinations as the energy which must be expended to increase the size of the interface between the two adjacent phases which do not mix completely with one another. Then, surface tension is a derivation of interfacial tension, defined to a single liquid surface as water or Acacia gum suspensions when forces from the second surface is negligible or equal to zero (as for air). This explains why the two terms surface tension and interfacial tension at the air/liquid interface are used.

In this work we characterized:

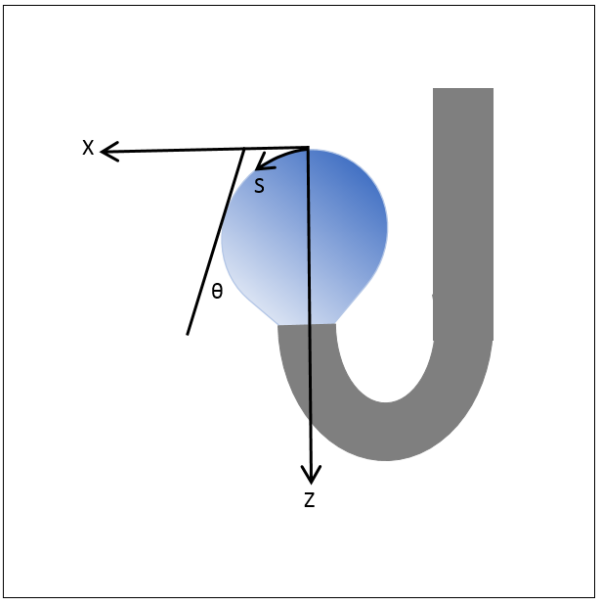
- the surface tension of A. *senegal* and A. *seyal* gums through pendant drop method (Figure III.1.A) and its dispersive and polar components using contact angle measurement or sessile drop method (Figure III.1.C).
- the interfacial tension of two immiscible liquids, i.e. Acacia gums dispersion and hexadecane, limonene, or octanol by the measurement of interfacial tension and viscoelastic modulus using rising drop method (Figure III.1.B).

Therefore, we proposed to succinctly describe the employed methods which are based on the same theory of axisymmetric drop shape analysis.

A.



B.



C.

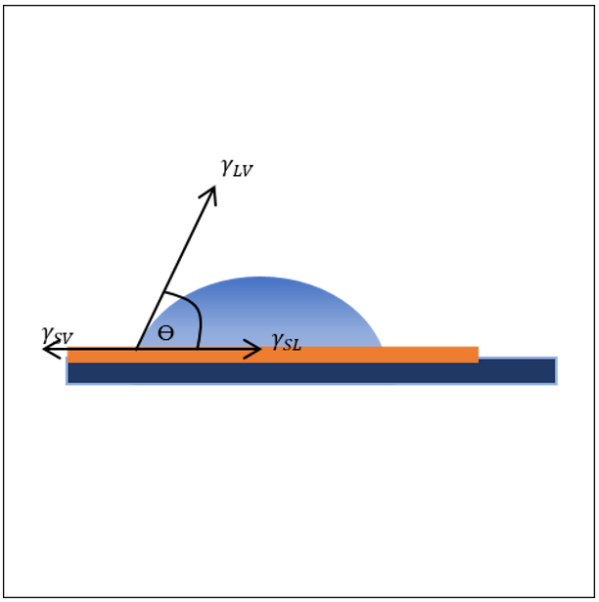


Figure III.1: Shape of droplet, pendant (A), rising (B) and sessile (C) drops, representing the forces at the apex for rising and pendant drops and at the triple point air-solid-liquid for sessile drop.

Pendant and rising drop methods:

The balance between gravitational and capillary forces of the liquid determines whether the rising (Figure III.1.B) or pendant (Figure III.1.A) drop could be formed. In both case, the shape of drop depends on the competition of two forces: surface tension and gravitation. The spherical shape results from the surface tension, i.e. the energy which tends to minimize the surface area while the curvature of drop interface changes in vertical direction due to the gravitational force. The degree of variation of spherical shape is related to the ratio between the drop weight (liquid density) and the surface tension. However, the theory of the measurement is the same for both drop types.

The analysis of axisymmetric drop shape was done using Young-Laplace Equation (Equation 1) which relates the Laplace pressure throughout the interface with the interfacial tension and the curvature of the interface (Berry, Neeson, Dagastine, Chan, & Tabor, 2015):

$$\Delta P = \gamma \left(\frac{1}{R1} + \frac{1}{R2}\right) \qquad (1)$$

where R_1 and R_2 represented the principal radii of curvature at the point at coordinate (X, Z), ΔP is the Laplace pressure corresponding to the pressure difference between inside and outside of the drop. According to Pascal's law, ΔP can be also expressed as:

$$\Delta P = P_{in} - P_{out} \qquad (2)$$

$$\Delta P = \Delta P_0 - \Delta \rho g Z \qquad (3)$$

where, ΔP_0 represented a reference pressure at $Z = 0$, g is the acceleration of gravity and $\Delta\rho = \rho_d - \rho$ with ρ_d and ρ the drop phase and continuous phase density, respectively.

Equation (1) can be expressed in terms of the cylindrical coordinates X and Z, together with the tangent angle θ, as shown in Figure III.1.A and III.1.B. Then, the Young–Laplace equation can be obtained as a coupled set of dimensionless differential equations in terms of the arc length S measured from the drop apex:

$$\frac{d\theta}{d\bar{S}} = 2 - Bo\,\bar{Z} - \frac{\sin\theta}{\bar{X}} \qquad (4)$$

$$\frac{d\bar{X}}{d\bar{S}} = \cos\theta \qquad (5)$$

$$\frac{d\bar{Z}}{d\bar{S}} = \sin\theta \qquad (6)$$

where the bar indicates dimensionless quantities scaled by the radius of curvature at the drop apex. All variables are shown in Figure III.1. In Equation (4), Bo corresponds to the Bond number (dimensionless):

$$Bo = \frac{\Delta\rho g R_0^2}{\gamma} \qquad (7)$$

The associated boundary conditions are: $\bar{X} = 0$, $\bar{Z} = 0$, $\theta = 0$ at $\bar{S} = 0$ and R_0 is the radius of curvature at the drop apex. Thus, the shape of the pendant drop depends on Bo. If the Bond number associated with a pendant drop can be determined together with the drop radius at the apex (R_0), the interfacial tension (γ) is then readily obtained from Equation (7). The interfacial tension of a liquid as water can evolve with time in the presence of surface active molecules or impurities. Therefore, the terms of "transient interfacial tension" and dynamic surface tension are usually used.

Sessile drop method:

Contact angle measurement is a simple and widely used method to estimate the solid surface free energy. This method is based on the measurement of the left and right angles formed at the solid-liquid-vapor interfaces of sessile drop which is deposited on solid surface (Figure III.1.C). The three forces interacting at the interface are interfacial tensions at liquid-vapor interface (γ_{LV}), solid-liquid interface (γ_{SL}) and solid-vapor interface (γ_{SV}). At equilibrium, the balance on the three phases is given by the equation of Young (Equation 8):

$$\gamma_{LV} \cdot \cos\theta = \gamma_{SV} - \gamma_{SL} \qquad (8)$$

The parameters γ_{LV} and $\cos\theta$ are measurable, but the values of of γ_{SV} and γ_{SL} are unknown and diverse approaches as Fowkes, Lifshitz-van der Waals/acid-base and Owens & Wendt (Karbowiak et al, 2006) allowing to estimated one of them using diverse liquids and system of equations can be applied. In this chapter, the method called "surface tension component approach" was used to estimate the polar and dispersive components of γ_{LV} for both Acacia gums considering that each surface energy is composed of different components (polar and dispersive) as proposed by Fowkes and using a specific support without polar component.

II. Publication 3 - Interfacial properties of Acacia gum at different liquid-liquid interfaces

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1. Introduction

An emulsion is defined as a thermodynamically unstable system constituted of two immiscible liquids. This system tends to break down during the storage. However, it can be stabilized by the addition of emulsifiers. The stabilization properties of emulsifier are variable and depend on their physicochemical and structural properties, and therefore their ability to adsorb and form a film at the interface.

Acacia gum is recognized as an efficient emulsifier due to its interfacial properties allowing not only to form a film by covering the surface of newly-formed emulsion droplets but also to stabilize them. Moreover, its high water solubility and the low viscosity of Acacia gum dispersions favor their industrial use in pharmaceutical and food industries, specifically for beverage industry. Acacia gum or gum arabic (AG, E414 EC) is "a dried exudate obtained from the stem and branches of Acacia *senegal* (L.) Willdenow or Acacia *seyal* (family Leguminosae)" (FAO, 1999). These two species differed in emulsifying properties: the former gives more stable emulsions than the latter in relation to their biochemical composition and structural properties (Flindt, Al-Assaf, Phillips, & Williams, 2005; Mansour & Hassan, 2016).

Both A. *senegal* and A. *seyal* gums are composed of highly glycosylated hydroxyproline-rich arabinogalactan-proteins (AGPs) belonging to the glycoprotein superfamily (Akiyama, Eda, & Kato, 1984). AGPs are mainly composed of sugars (D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid, and 4-O-methyl-D-glucuronic acid) with a small fraction of proteins (~ 0.8 – 3%) and minerals (~ 3 – 4%) (Lopez-Torrez, Nigen, Williams, Doco, & Sanchez, 2015; Street & Anderson, 1983). AGPs are organized into hyper-branched polysaccharide blocks covalently linked to the polypeptide backbone in serine- and hydroxyproline-rich domains (Lopez-Torrez et al., 2015; Mejia Tamayo et al., 2018). Depending on the gum specie and their origin (tree location, age, weather), the relative proportions of the constitutive sugars and protein, as well as the structural properties of AGPs, slightly varied. Typically, A. *senegal* is richer in protein than A. *seyal*, while the latter is richer in arabinose than the former (Biswas, Biswas, & Phillips,

2000; Gashua, Williams, & Baldwin, 2016; Lopez-Torrez et al., 2015). A. *senegal* gum is also more ramified and more flexible than A. *seyal* one suggesting a greater facility to cover the surface of droplet (Lopez-Torrez et al., 2015; Mejia Tamayo et al., 2018). Despite a greater molar mass, AGPs from A. *seyal* gum adopted a more compact conformation with a smaller hydrodynamic volume than the ones of A. *senegal* gum resulting in a less viscous dispersion (Lopez-Torrez et al., 2015) and potentially a faster diffusion ability to the interface.

Both gums are constituted by a continuum of AGPs differing by their sugar, amino acid and mineral content and composition, sugar to amino acid ratio, polarity, number of charges, molar mass, size and shape (Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Mejia Tamayo et al., 2018; Randall, Phillips, & Williams, 1989; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). These AGPs can be separated according to their physicochemical and structural properties using different chromatographic techniques. Using hydrophobic interaction chromatography (HIC), three main fractions were isolated from A. *senegal* as a function of their hydrophobicity.(Randall et al., 1989; Renard et al., 2006). HIC-F1, also called the arabinogalactan peptide fraction (AG), is the most abundant fraction (80-90% of total gum) as compared to HIC-F2, also called the arabinogalactan protein fraction (AGP, 9-10% of total gum) and HIC-F3, also called the glycoprotein fraction (GP, 1.3-2% of total gum). The sugar composition was similar between the three fractions, with however a larger content of arabinose in HIC-F2 and HIC-F3 and a larger content of charged sugars in HIC-F1. HIC-F3 was the richest fraction in proteins with values around 20-25%, while the amount of proteins was around 8-10% and 1% for HIC-F2 and HIC-F1. These three HIC fractions differed also by their mean molar mass and high molar mass (M_w) AGPs content (AGPs with M_w upper than $7.5x10^5$ $g.mol^{-1}$). HIC-F1 was mainly composed of low molar mass AGPs, while HIC-F2 and HIC-F3 were richest in high molar mass AGPs and supramolecular assemblies (M_w > 2-3 x 10^6 $g.mol^{-1}$) (Mejia Tamayo et al., 2018).

For A. *seyal*, the three main fractions are also isolated using HIC. The major fraction is constituted by AGPs containing low amount of protein. But the high molar masses

AGPs are present in lower extend in A. *seyal* than A. *senegal* (Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005). The distribution of protein in A. *senegal* differs from A. *seyal*: in the latter, protein location is not mainly found in the fraction historically named AGP (HIC-F2).

Recently, A. *senegal* gum was fractionated using ionic exchange chromatography (IEC) with the isolation of two fractions called IEC-F1 and IEC-F2 (Apolinar-Valiente et al. 2018). IEC-F1 (M_w = 3.0×10^6 g.mol^{-1}) corresponded to the high molar mass protein-rich AGPs from the classical HIC-F2 and HIC-F3 fractions, whereas IEC-F2 (M_w = 5.2×10^5 g.mol^{-1}) was mainly composed by HIC-F1 fraction. A high aggregation rate was observed for IEC-F1. The authors suggested that this could be related to the high value of arabinose/galactose (1.1) ratio, the low content of glucuronic acid group and the high amino acid content (115 mg.g^{-1}) in this fraction. The protein content, the high aggregation rate and the flexibility of AGPs from IEC-F1 were suitable to provide a greater interfacial and emulsifying properties to Acacia gum.

The lower emulsifying ability of A. *seyal* is generally attributed to its poor content in protein and high molar mass protein-rich AGPs but this affirmation needed to be confirmed.

The knowledge of interfacial properties of Acacia gums in relation to their biochemical composition and structural properties should provide useful information about emulsifying properties. Interfacial properties include both the ability to decrease interfacial tension at liquid-liquid interfaces and to stabilize these interfaces through the formation of interfacial film (Adamson & Gast, 1997). It was described that the adsorption of Acacia gum is a slow process and that long-time is needed to reach the equilibrium as described for proteins (Beverung, Radke, & Blanch, 1999; Bouyer et al., 2011; Dickinson et al., 1988; Erni et al., 2007). The interfacial properties of different Acacia gums specie were linked to nitrogen and protein content because of a relative good correlation between the protein content (varying between 0.6% to 47%) of Acacia gum samples and the interfacial activity (Dickinson et al., 1988). However, the same authors suggested that the variability in the emulsifying properties of the gum samples

depend also on the distribution of the protein between the low- and high-molecular weight fractions, and on the molecular accessibility of the protein/peptide to the adsorption at interface. Nowadays, it is widely accepted that the high molar mass protein-rich AGPs fractions mainly provide the interfacial properties of Acacia gum. Studying the interfacial tension of the three HIC fractions obtained from a matured A. *senegal* gum, Castellani et al. showed that GP (HIC-F3) fraction was more efficient to decrease the hexadecane interfacial tension with value at equilibrium of about 23 $mN.m^{-1}$ than AGP (HIC-F2) and AG (HIC-F1) fractions lowering interfacial tension value up to 30 $mN\ m^{-1}$ and 45 $mN\ m^{-1}$, respectively (Castellani et al., 2010). These authors also compared A. *senegal* and A. *seyal* and reported that there was no difference in the decrease of tension between both gums at the used concentration (0.05wt%).

In addition, dilatational rheology gives information about the orientation and interactions of molecules at the interfacial film and on the elastic and viscous nature of films (Sun et al., 2011). The rheological properties of films was often related to the stability of emulsions (Randall et al., 1989; Sun et al., 2011). It is generally known that Acacia gum interfacial films exhibit an elastic characteristic (Castellani et al., 2010; Erni et al., 2007; Sanchez et al., 2018; Vasile, Martinez, Pizones Ruiz-Henestrosa, Judis, & Mazzobre, 2016). Using HIC fractions obtained from a matured A. *senegal* gum, Castellani et al. showed that only AGP (HIC-F2) and GP (HIC-F3) were able to form interfacial film resulting in the change of dilatational modulus while no change was observed when AG (HIC-F1) was used indicating that this fraction poor in protein did not adsorb at the interface. Comparing A. *senegal* and A. *seyal* at 0.05wt%, Castellani et al. found that conventional A. *senegal* formed less elastic interfacial films than A. *seyal* after 15h at hexadecane-buffer interface. Contrary to Castellani et al., the comparison of interfacial rheology between A. *senegal* and A. *seyal* at limonene-buffer interface using Acacia gums at 3wt% demonstrated that A. *seyal* interfacial film showed interfacial viscosity while an elastic response was observed for A. *senegal* (Elmanan et al., 2008).

For the majority of interfacial experiments done so far, only one Acacia gum concentration was evaluated and experimented, the assessment of concentration effect should be instructive. In addition, interfacial properties of A. *senegal* was largely studied whereas only some studies were carried out on A. *seyal* and, as mentioned above, some confusing results were found (Castellani et al., 2010; Elmanan et al., 2008). Some scarce assessments studied the effect of oil nature on emulsion stability and interfacial properties of Acacia gums (Chanamai, Horn, & McClements, 2002; Dickinson et al., 1991b) demonstrating the need to consider the oil nature to better understand the behavior of the emulsifier.

The purpose of this study was to compare the liquid-liquid interfacial properties of Acacia gums dispersion. Interfacial tension kinetic and dilatational rheology of Acacia *senegal* and Acacia *seyal* with different protein content will be characterized using dynamic drop tensiometry. A range of gum concentrations (between 0.05 and 5%) was studied in order to easily discriminate the adsorption kinetic between both gums and to take into account the impact of real concentrations used for commercial flavor oil emulsions production. The combined influence of oil nature and emulsifier type on interfacial tension and dilatation rheology will be considered. Furthermore, the interfacial properties of the high molar mass protein-rich AGPs obtained from A. *senegal* using ion exchange chromatography will be investigated to confirm their preponderant role in this physicochemical properties of Acacia gums.

2. Materials and methods

2.1. Materials

Soluble powder of Acacia *senegal* (A. *senegal*, lot n° OF110676) and Acacia *seyal* (A. *seyal*, lot n° OF110724) were provided by ALLAND & ROBERT Company-Natural and organic gums (Port mort, France). Gums powders were purified by the same process including 4 steps: the dissolution, the elimination of insoluble matters, the pasteurization and the spray drying. The biochemical composition and structural

properties of these gums was previously characterized (Lopez-Torrez et al., 2015; Mejia Tamayo et al., 2018). Biochemically, A. *senegal* contained 829.0 $mg.g^{-1}$ of sugar, 27.0 $mg.g^{-1}$ of protein and 33.0 $mg.g^{-1}$ of mineral while A. *seyal* was composed by 843.0 $mg.g^{-1}$ of sugar, 10.0 $mg.g^{-1}$ of protein and 40.0 $mg.g^{-1}$ of mineral. The mean molar mass (M_w) of A. *senegal* and A. *seyal* gums was 6.8×10^5 and 7.1×10^5 $g.mol^{-1}$, respectively. The dry matter corresponded to 90% and 87% for A. *senegal* and A. *seyal*, respectively.

To investigate the impact of high molar mass protein-rich AGPs on the formation and characteristic of interfacial film hexadecane-buffer interface, A. *senegal* gum was fractionated by Ion Exchange Chromatography (IEC) according to Apolinar-Valiente et al. (Apolinar-Valiente et al. 2018, submitted article). The fraction obtained (named IEC-F1) contained only high molar mass protein-rich AGPs. It was characterized by a mean Mw of 30×10^5 $g.mol^{-1}$ and the following biochemical composition:, 100.1 $mg.g^{-1}$ of protein and 170.5 mg.g-^{1} of mineral based on total fraction humid mass.

The volatile organic compounds (hexadecane, limonene and octanol) and Florisil® (60-100 mesh) used for the purification of organic volatile compounds were purchased from Merck-Sigma Aldrich (Saint-Quentin Fallavier, France). The physicochemical characteristics of the organic volatile compounds are presented in Table III.5.

Acetate buffer at 10 mM (pH 5) was used to dissolve Acacia gums and prepared using anhydrous glacial acetic acid and sodium acetate trihydrate provided by Merck-Sigma Aldrich (Saint-Quentin Fallavier, France).

2.2. Purification of organic volatile compounds

Although the high purity of organic volatile compounds (99%), hexadecane and limonene needed a further purification. Two methods of purification were used in parallel for comparison purpose. The first purification method was done using Florisil® at the ratio of 2:1 (compound:Florisil) (Pérez-Mosqueda, Maldonado-Valderrama, Ramírez, Cabrerizo-Vílchez, & Muñoz, 2013). Then the mixture was stirred overnight

and subsequently filtered with glass wool in order to collect the pure compound. Another method was also used to purify compounds consisting to saturate the liquid with water by mixing them at the ratio of 1:3 (liquid-water) and agitating for 1 min. The mixture was left for 1h to separate organic and aqueous phases. Then, the organic phase was picked up and used for the measurements. As non-significant difference of interfacial tension of each compound was found between the two methods, they have been interchangeably used.

2.3. Preparation of Acacia gum dispersions

The dispersions of Acacia gums were prepared in 10 mM (pH 5.0) acetate buffer at different concentration (0.05, 0.5, 1, 5 and 20 wt% based on humid mass). The dispersions were stirred overnight at room temperature to ensure complete dissolution, and then centrifuged at 20 000 g for 30 minutes at 25°C to remove traces of insoluble matter.

2.4. Methods

2.4.1. Liquid-liquid interfacial properties measurement

Interfacial tension and viscoelasticity modulus at the oil-buffer interface were determined using a drop tensiometer (Tracker, IT concept, Longessaigne, France). A rising axisymmetric drop of volatile organic compounds was formed at the tip of U shaped needle connected with a syringe whose the formation and volume were controlled by a computer. The droplet volume of hexadecane, limonene and octanol was 10 µl, 15µL and 4µL, respectively. The drop was formed in a glass cuvette containing the gum dispersions at different concentrations. The syringe and cuvette were thermostatically-controlled at 25°C. The shape of the drop was monitored by a CCD camera and digitized. The digital images were recorded over time (for 24h in the case of hexadecane and 7h for octanol and limonene) and the interfacial tension was

determined through the Laplace equation (Equation 1) basing on the drop shape which resulted from the combination of surface tension and gravity effects.

Three characteristics parameters from the evolution of drop shape during time were obtained, i.e. area (A), volume (V) and interfacial tension (γ). The tensiometer efficiency was evaluated by measuring the interfacial tension of water in air at 25°C. A value of 71.3± 0.8 $mN.m^{-1}$ was found in agreement with literature value of 72 $mN.m^{-1}$ (Dickinson et al., 1988).

The interfacial tension of each purified organic volatile compound in Milli-Q water, i.e. in the absence of Acacia gums, was then determined after purification of the liquid. The experimental values of purified compound interfacial tension were found equal to 48.3±1.8, 27.8±0.6 and 8.4±0.4 $mN.m^{-1}$ for hexadecane, limonene and octanol, respectively. As the difference of these values compared to the values found in literature (Table III.5) remained slight, no further purification was performed.

Drop tensiometer also allows to investigate the dynamic behavior of the adsorbed layers, according to oscillating drop methodology which consists to apply sinusoidal oscillation of interface. The harmonic sinusoidal oscillation of drop volume resulting in droplet area deformation ($\Delta A/A$), was performed according to time (t) at 25°C with an amplitude of 0.1 ($\Delta V/V$) and an oscillation frequency (ω) of 0.1 s^{-1}. The oscillation was done for 5 repetitive cycles followed by 50 sec of pause period without oscillation before the beginning of another 5 oscillation cycles and so on. The measurement was carried out for 40h for hexadecane and 7h for octanol and limonene. It is possible to extract the interfacial tension during the "blank" periods where the interfacial area remained constant. These extracted data could be used to investigate the decrease of interfacial tension as a function of time.

The surface viscoelastic modulus (Equation (11)) and the phase angle (δ) were therefore derived from the change in interfacial tension γ (Equation (9)) resulting from the drop area fluctuation (Equation (10)):

$$\gamma = \gamma_0 \sin(\omega t + \delta) \quad (9)$$

$$A = A_0 \sin(\omega t) \quad (10)$$

$$E = A\,(\Delta\gamma)/\Delta A \quad (11)$$

The obtained dilatational viscoelasticity (E) is a complex quantity with real (E') and imaginary (E'') parts, the dilatational elasticity and dilatational viscosity, respectively (Ravera, Loglio, & Kovalchuk, 2010).

All interfacial experiments were carried out in triplicate. A new freshly drop was formed and interfacial tension measurements started once its settled volume was reached depending on volatile compound.

2.4.2. Modeling of data

Parameter estimation for Equations 12 and 13 was performed by using a non-linear fitting procedure from Matlab© (Matlab and statistics Toolbox Release 2015b, The MathWorks, Inc., Natick, Massachusetts, United States).

The first equation used for the fitting of the experimental data was a logistic curve model (Equation 12) as proposed by Castellani et al. (Castellani et al., 2010):

$$\gamma_t = \gamma_\infty + \frac{(\gamma_0 - \gamma_\infty)}{1 + (\frac{t}{t_{50}})^{SL_{50}}} \quad (12)$$

The physical parameters obtained by this model were: the interfacial tension at the free interface (γ_0), the interfacial tension at equilibrium (γ_∞), the time to reach the half of the total decrease of interfacial tension (t_{50}) and the decreasing rate or slope of the kinetic curve at the t_{50} point (SL_{50}).

Another estimation model has been used to characterize the properties of Acacia gum (Equation 3) (Mahfoudhi et al., 2014) :

$$\gamma = \gamma_f + (\gamma_1 - \gamma_f) \times \exp(-t/\tau_1) + (\gamma_2 - \gamma_f) \times \exp(-t/\tau_2) \quad (13)$$

where γ_f was the asymptotic interfacial tension for $t \to \infty$ and γ_1, γ_2, τ_1 and τ_2 corresponded to the dynamic parameters which described the decay kinetics of the interfacial tension overtime until an equilibrium value was reached. τ_1 represented the characteristic time of decay corresponding to the migration of the emulsifier to the oil-water interface, and τ_2 characterized the time decay involving the reorganization of the emulsifier at oil-water interface (Mahfoudhi et al., 2014).

Concerning parameter initialization for the estimation procedure, the parameters were set to different values taking into account the values previously estimated by Castellani et al and Mahfoudhi et al. (Castellani et al., 2010; Mahfoudhi et al., 2014).

3. Results and discussion

3.1. Interfacial properties of Acacia gum at the hexadecane-buffer interface

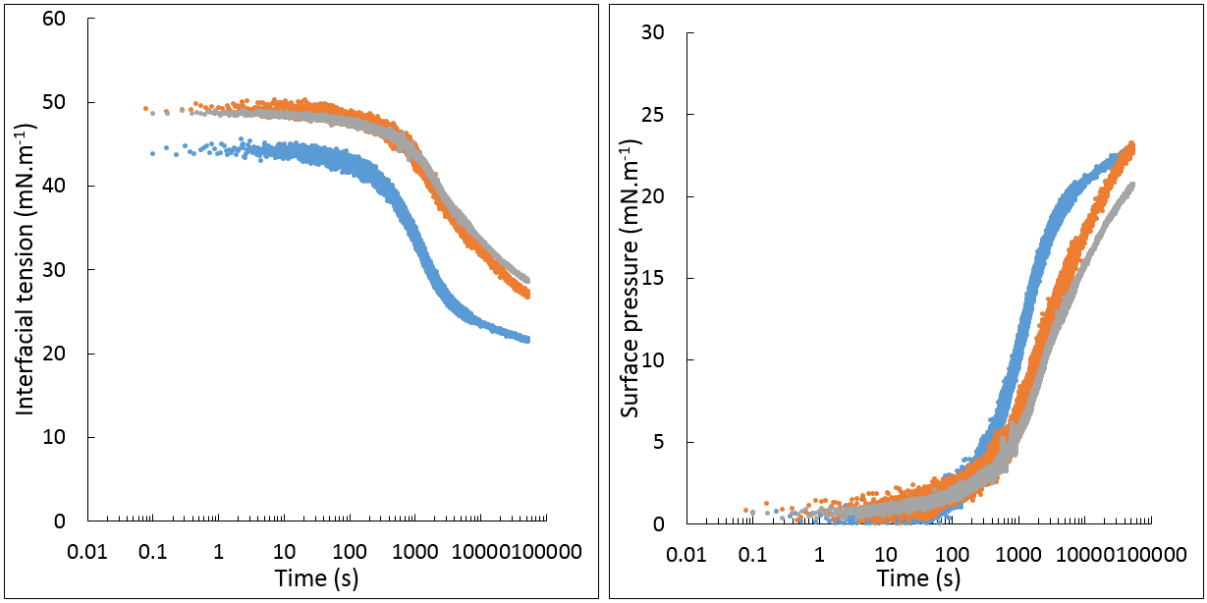
3.1.1. Effect of A. *senegal* and A. *seyal* gums concentrations on transient interfacial tension

The impact of Acacia gums on the transient interfacial tension (γ) of hexadecane was investigated by varying the specie (A. *senegal* and A. *seyal*) and the concentrations (0.05, 0.5, 1 and 5 wt%) of gum. The lowest concentration was selected to discriminate more severely between the two gums, since at these concentrations the most of gum is probably adsorbed at the interface. In contrast, the highest one (5 wt%) was in large excess to cover the drop interface, but it corresponded to the concentration applied for food emulsions and can give useful information of gums behavior during emulsification process. A kinetic time of 24h was chosen to be sufficiently long to achieve the equilibrium time as preconized for Acacia gums (Castellani et al., 2010). However, even

after 24h, the interfacial tension seemed to slightly evolve for all gum concentrations then the kinetic was prolonged to 40h for some experiments to reach the limiting values.

First, the repeatability of the method was studied because some variability between the different runs was observed which could be due to some variation in the real Acacia gum concentrations used and in the degree of hexadecane purity. Indeed, the concentration of gums can be slightly varied depending on the precision of weighting, the dilution step and the moisture content of the gums. In Figure III.2, the 3 repetitions of transient tension obtained with A. *senegal* at the theoretical concentrations of 0.05wt% and 5 wt% are reported. The poor repeatability of experiments at the weakest concentration was observed (Figure III.2.A) while appropriate repeatability was observed at 5 wt% (Figure III.2.C). For the three Acacia gum dispersions prepared at the theoretical concentration of 0.05 wt%, the real concentrations were determined by measuring the densities of the dispersions and equal to 0.043 (orange curve), 0.042 (grey curve) and 0.052 (blue curve) wt%, respectively. According to their close concentrations, similar kinetic was observed for the two first experiments (orange and gray curves), while the last (blue curve) strongly differed (Figure III.2.A). It is obvious that a slight difference in concentration can have a strong impact on the interfacial tension decrease and this especially at low concentration. The Figures III.2.A and III.2.C show also a difference in the initial value of interfacial tension with the lowest for the highest concentration. This difference can be explained by the effect of the highest Acacia gum concentration (5 wt%) causing the rapid decrease of initial interfacial tension.

A. B.



C. D.

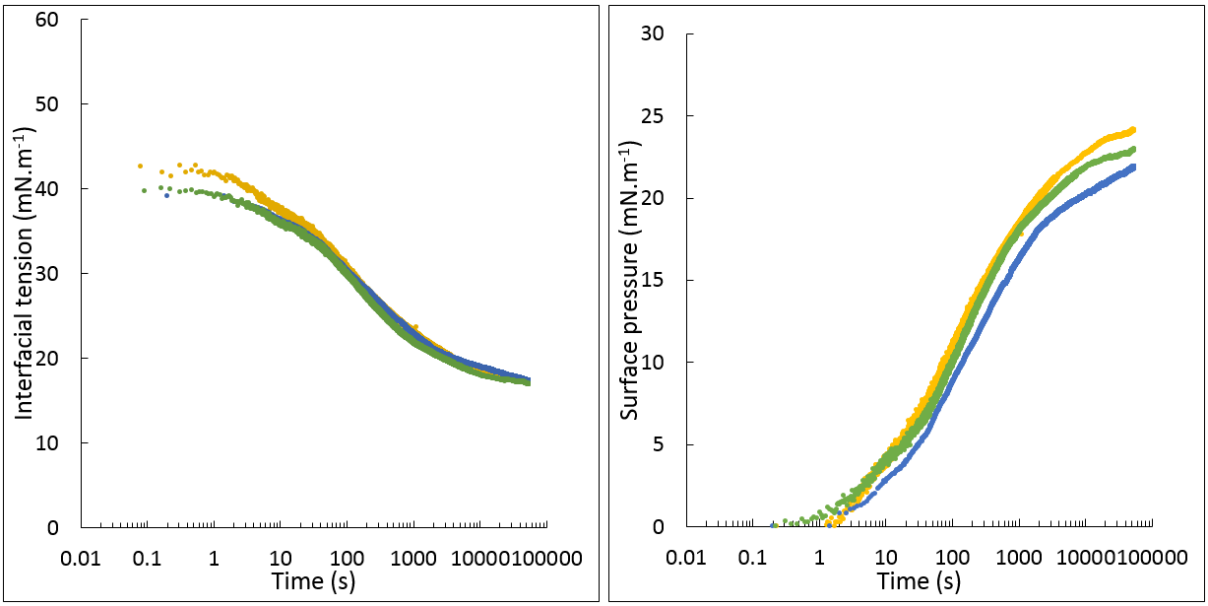


Figure III.2: Semi-logarithmic plots of interfacial tension (A and C) and surface pressure (B and D) of hexadecane in the presence of A. *senegal* at 0.05 wt%(A and B) and A. *senegal* at 5 wt% (C and D). Three replications were shown for each A. *senegal* gum concentration.

To override the variability of the initial tension, we have determined the dynamic surface pressure (Π). Indeed, the surface pressure corresponds to the change of

interfacial tension of the compound in contact with Acacia gum at time t_i compared to the initial interfacial tension value of the compound in water without emulsifiers or at t_0 (Beverung et al., 1999). In our case, the value of interfacial tension at time 0.06s was taken into account as initial value since it corresponded to the first measuring time. For gum dispersion at 0.05 wt% (Figure III.2.B), we assumed that the high variation observed in Figure III.2.A came from the difference in the concentration between the replications thus high A. *senegal* concentration rapidly decreased initial interfacial tension. Therefore, the variability between results were less pronounced when the data was reported in term of surface pressure.

For the Acacia gum concentration of 5 wt%, the effect of initial interfacial tension of hexadecane was more pronounced (Figure III.2.D). Despite the similar curve profile, the dynamic of surface pressure evidenced that the decrease of surface tension at equilibrium varied between 22 and 26 $mN.m^{-1}$ (Figure III.2.D). This difference could be due to the minor variation of concentration as previously explained or variation in hexadecane purity. Moreover, other intrinsic parameters of the apparatus can induce some variabilities in the analysis affecting the accuracy of the approach, e.g. temperature of the cell, the water evaporation, the great sensitivity of method which could be disturbed by a slight environmental vibration.

In order to increase the number of essays and to decrease the variability for a same concentration, the interfacial tension was also extracted from experiments carried out to characterize the viscoelastic modulus (when the droplet oscillation was stopped) and compared to the classic interfacial tension measurement. It is worth noting that the early state of interfacial tension (the first 50 s) could not be determined because the drop oscillation started immediately after the formation of the drop. Figure III.3 demonstrated that the interfacial tension kinetics obtained from the viscoelastic modulus measurements followed similar curves to that resulting of usual interfacial tension measurement. Moreover, the variability between data from two methods was not observed since the final values of interfacial was in the same magnitude, i.e. the range of values was 18.3-20.5 $mN.m^{-1}$ and 17.0-18.6 $mN.m^{-1}$ for gum concentration at

0.5 wt% and 5 wt%, respectively. Then, these curves will be taking into account in the determination of characteristic parameters in the following of the study. It is therefore important to keep in mind that when we are discussing the effect of gum concentration in the following part, we are talking about targeted values and we try to consider the impact of variability.

A. B.

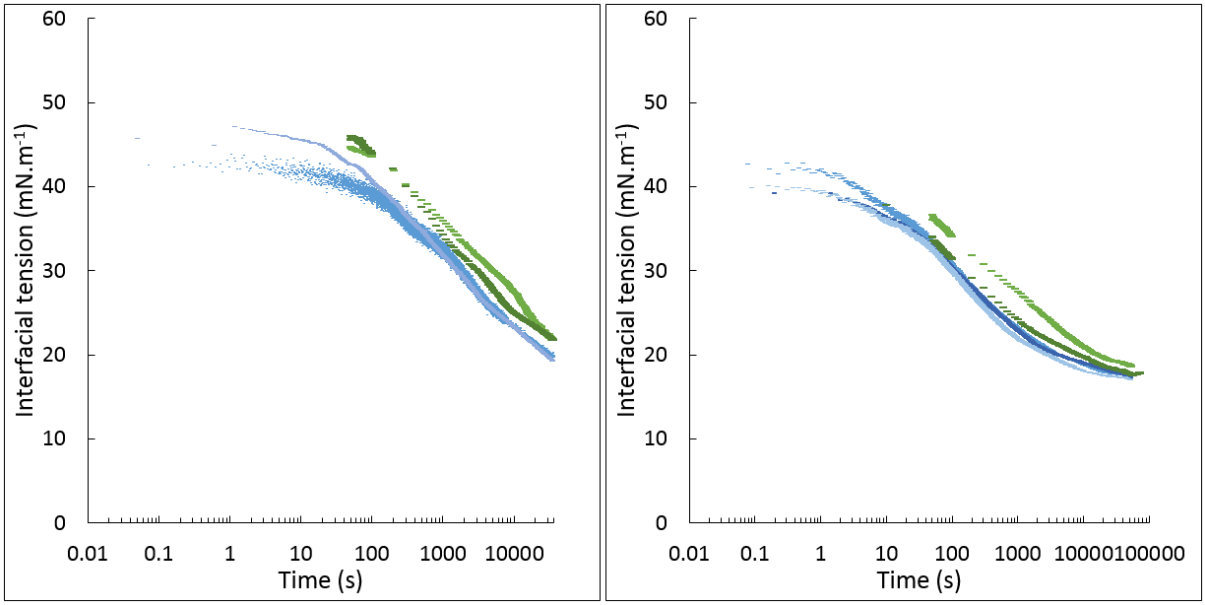


Figure III.3: Semi-logarithmic plots of interfacial tension profiles obtained from the measurement of static interfacial tension (blue tone) and viscoelastic modulus (green tone) and for A. *senegal* at 0.5 (A) and 5 wt% (B).

Another source of difficulty to interpret the effect of gums concentration on the ability of Acacia gum to lower interfacial tension is associated with the content of aggregates in dispersions. Indeed, the protein rich AGPs, and especially those from A. *senegal* gum, are able to self-associate and form reversible aggregates in the physicochemical conditions used (acetate buffer). The level of these aggregates seemed to depend on ionic strength (more aggregate in water than in buffer at 10mM) and gum concentration: lower level of aggregates was found for concentrations between 0.5 and

1 wt% compared to the lower (as 0.05 wt%) and higher (as 5 wt%) concentrations for A. *senegal* (personal data, Mejia Tamayo et al. 2018). At this date, the characterization of the nature, composition and content of these aggregates are incomplete, but it can be hypothesized that the increase of gum concentration allowed to increase the content of high molar mass protein-rich AGPs. However, their conformation, i.e. their aggregated form, could change for each concentration. Moreover, the amount of aggregates was higher for A. *senegal* than for A. *seyal*. This feature need to be considered when studying the effect of gum specie and concentration because their presence affected the interfacial tension as demonstrated in the case of matured gums (Castellani et al. 2010). Indeed, when maturated gums was compared to original ones, the former were more efficient to decrease the interfacial tension (Castellani et al, 2010). This ability was due to the increased content of high molar mass molecules rich in protein through the formation of aggregates induced by heat and Maillard reactions. However, if the aggregation during maturation process was complete and irreversible, in our case, the aggregation was reversible and the amount of aggregate was relatively low and concentration-dependent.

Regarding the concentrations with the lowest aggregates content (0.5 and 1 wt%), the effect of gum concentration was not obvious either for A. *senegal* and A. *seyal* since similar average values of interfacial tension at 15h were found for these two concentrations (Table III.1). This was certainly due to the weak difference between the two concentrations (2 times). Accordingly, the comparison between two concentrations (2 and 5%,) of A. *senegal* gum containing 1.37% of protein, i.e. on the fish oil–water interface showed only a slight difference in the tension decrease (Vasile et al., 2016). However, these authors found that the value of onset time which corresponded to the time to reduce the interfacial tension by 5% was 2 times higher for the weakest concentration. Then, this parameter which corresponds to the initial decrease of the tension seemed to evidence the concentration effect within a same gum. This assumption was confirmed regarding the other concentrations studied in this work. Indeed, the onset time decreased with the increase of concentrations for both gums

(Table III.1). The values exceeded 100s at 0.05 wt% and becoming null at 5 wt% in relation to the fast decrease of interfacial tension. The concentration effect was less pronounced comparing the interfacial tensions at 15h since similar final interfacial tensions were observed for the three highest concentrations for *A. senegal*. For A. *seyal*, the values of interfacial tension at 15h were close for the three lowest concentrations. It was obvious that for each gum, when a critical concentration was reached, no effect of gum concentration on pseudo-equilibrium interfacial tension occurred. Accordingly, the critical concentration may lie between 0.05 wt% and 0.5 wt% for A. *senegal* and between 1 wt% and 5 wt% A. *senegal*. A. *seyal* differ in their structure, composition but also protein content. It can be suggested that the critical concentration was, among other, a function of protein concentration independently to the specie of gums. The fastest initial decrease of interfacial tension, thus the highest interfacial diffusion, was reached when the protein concentration was superior to 0.01 wt% for both gums (Figure III.4). This protein content was reached for a concentration of A. *senegal* of 0.5 wt% and ≥ 1wt%. By consequence, for the high concentration (5 wt%) the effect of gum specie was not observed despite the higher protein content of A. *senegal* compared to A. *seyal*. This statement was in agreement with the observation of Dickinson et al. who observed a good correlation between the protein content and the interfacial tension. Additionally, authors remarked that other parameters such as distribution of the protein between the low- and high-molar mass AGPs, and the molecular accessibility of the protein/peptide to the adsorption at interface were implied in the efficiency of emulsifier (Dickinson et al., 1988). In the same vein, our results highlighted that the onset time decreased with the protein concentration (Figure III.4), but that this decrease was more pronounced for A. *seyal* than for A*. senegal* at equivalent protein concentration. This indicated that the protein concentration was not the only parameter to be considered. A. *seyal* was characterized by a more compact structure and lower viscosity than A. *senegal* and seems to diffuse faster than the latter for the same protein concentration. To better evidence the behavior of the two gums, experiments with low Acacia gum concentrations and using the same concentration of protein from A. *senegal* and A. *seyal* are needed to be done.

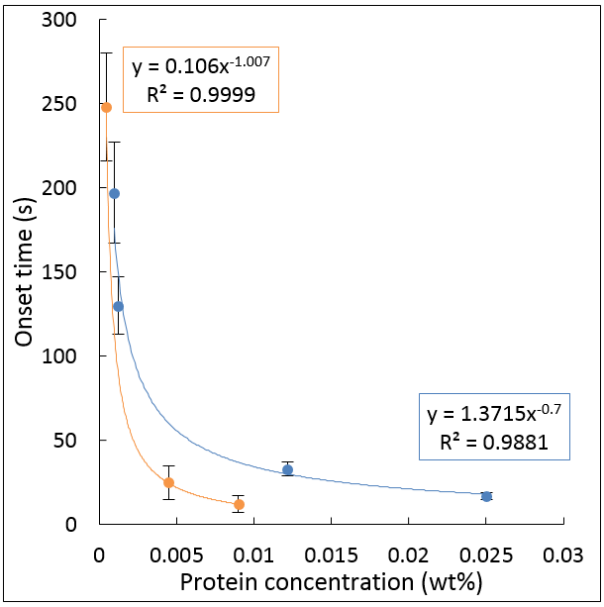


Figure III.4: Effect of protein concentration from A. *senegal* (blue) and A. *seyal* (orange) on the value of onset time. The fitting of data with power law was represented in solid line in the same color for each gum. The equations corresponding to the power law fitting were shown in the boxes.

In Table III.1, the onset times obtained for 0.05 wt% were weak comparing to the values reported by Castellani et al. for the same type of gums and at the same concentration (3308 s for A. *senegal* and 3808 s A. *seyal*) indicating that the gums used in our case faster diffused at the interface (Castellani et al., 2010). Moreover, the values of interfacial tension at 15h was found higher than those obtained in our work (39 and 38 $mN.m^{-1}$ for A. *senegal* and A. *seyal*, respectively). Castellani et al. prepared gum dispersions in ultra-pure water and not in acetate buffer at 10 mM with the final pH of 4.5 which could modify the charge of gums, the conformation of the high molar mass protein-rich AGPs and interfacial efficiency. Moreover the protein content of their gums was equal to 21 and 12 $mg.g^{-1}$ for A. *senegal* and A. *seyal* respectively compared to our values of 27 $mg.g^{-1}$ and 10 $mg.g^{-1}$. This suggested that the interfacial properties of Acacia gums were clearly not only depending on the protein content but also on structural and conformational parameters in interaction with the solvent nature.

Table III.1: Onset time of interfacial kinetic and the values of interfacial tension at 15h at hexadecane-buffer interface in the presence of different concentration of A. *senegal* and A. *seyal* gums. nd stands for non-determined.

Gum type	Gum concentration (wt%)	Protein concentration (wt%)	Onset time (s)	γ at 15h ($mN.m^{-1}$)
A. *senegal*	0.04	0.000972	197±30	27.8±1.1
	0.05	0.001215	130±17	21.6±1.9
	0.5	0.01215	33±4	18.2±1.6
	1	0.025	17±2	17.2±2.5
	5	0.1215	nd	17.3±0.6
A. *seyal*	0.05	0.00045	248±32	22.7±0.4
	0.5	0.0045	25±10	22.6±0.1
	1	0.0090	12±5	21.9±0.4
	5	0.045	nd	18.4±1.7

In the objective to better understand the dynamic of interfacial tension for each gum, several models were applied to point out the different successive mechanisms.

The entire data plotted in a semi-logarithmic scale of time as presented in Figure III.2.A, III.2.C and III.3 allowed to evidence the different regimes as described for proteins but also for Acacia gums (Beverung et al., 1999; Castellani et al., 2010). Indeed, the interfacial tension kinetic can be divided into three time regimes (Figure III.5.A). Regime I is the induction period or initial lag time characterized by minimal tension reduction. Its duration depends on the diffusion, quantity and interfacial affinity of emulsifiers. Regime II corresponds to a steep tension decline which is due to saturation, continuous rearrangement of interfacial molecules and initiation of gelation phenomenon. Regime III is attributed to slow relaxation of the adsorbed monolayer, continuous formation of gel-like network and possible build-up of multilayer. The duration of regime I (t_I) and the values of slope of regime II (S_{II}) and III (S_{III}) have been determined in order to compare quantitatively the effect of gums specie and concentration on the decrease of interfacial tension (Table III.2). This model was found adapted for low protein concentrations, i.e. 10 $mg.L^{-1}$ to describe the different

phenomena and to evidence the impact of structure and physicochemical properties (Beverung et al., 1999).

A. B.

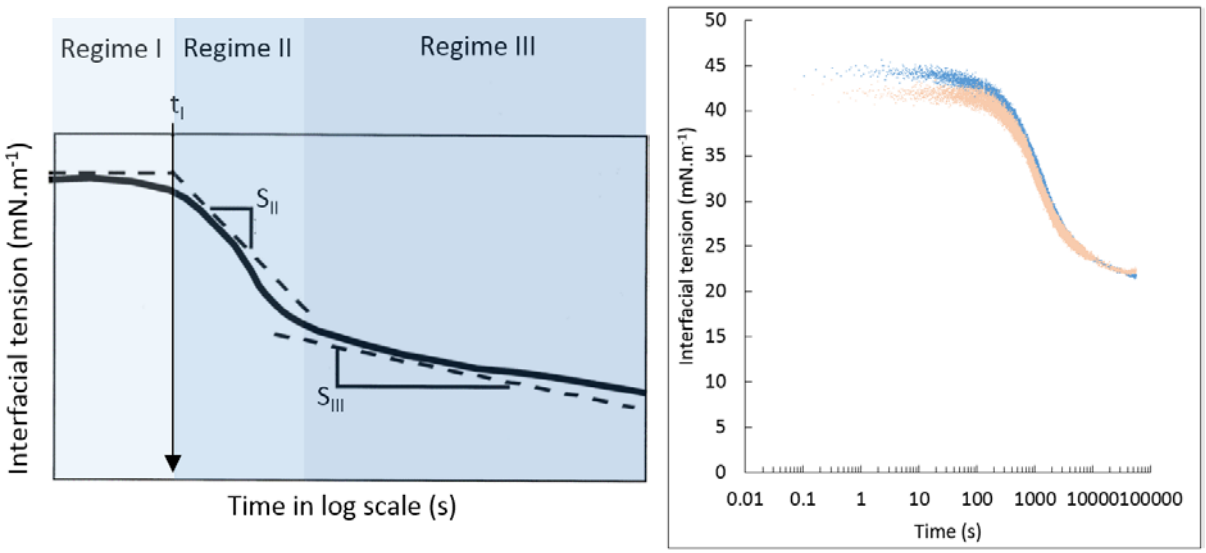


Figure III.5: Typical interfacial tension dynamic of protein adsorption at the oil-water interface (A). The parameters corresponding to duration of regime I (t_I), the slope of regime II (S_{II}) and III (S_{III}) were shown. (Adapted from Beverung et al., 1999). Semi-logarithmic plots of the interfacial tension of hexadecane in the presence of A. *senegal* (bleu curve) and A. *seyal* (beige curve) at 0.05 wt% (B).

For both gums at 0.05 wt%, i.e. low protein content, the three regimes were clearly identified (Figure III.5.B). The extend of induction period (with the interfacial tension lowering <10%) slightly varied between both gums being equal to 250 s and 320 s for A. *seyal* and A. *senegal*, respectively. This was in agreement with the difference in onset time. Both gums showed a similar profile with close value of slope for regime II (around -16.7 mN.m^{-1}.log(s)$^{-1}$). Regime III started at close values of tension and time (7000s) but the slope of this regime was weaker for A. *seyal* compared to A. *senegal*. The kinetic profiles indicated that for this gum concentration (i) A. *senegal* seemed to diffuse to interface faster than A. *seyal* (ii) the interface was saturated by A. *senegal* as well as by A. *seyal* after 24h (iii) slow conformation changes and rearrangement seemed to take place for both gums but seemed more restricted for A. *seyal*. As

highlighted for Acacia gums by Dickinson et al., the initial tension decrease was related to the ability and facility of protein part to adsorb and to surround the oil interface (Dickinson et al, 2003). This can occur when (i) the molecules have a low molar mass to rapidly diffuse, (ii) the protein part covalently linked to the polysaccharide is sufficiently accessible and (iii) the hydrophobic amino-acids are not buried. As A. *senegal* possesses a high content of protein-rich AGPs compared to A. *seyal* at this concentration, the initial adsorption was more rapid. Moreover, the saturation of the interface was favored by the greater flexibility of A. *senegal* AGPs which also authorized the relaxation. As mentioned earlier, A. *senegal* contains more aggregates than A. *seyal*, then the former could have a greater ability to lower interfacial tension. Different dynamic behaviors at the interface were also described for proteins depending on molar mass, conformation, surface hydrophobicity and bulk stability (Beverung et al., 1999). Beverung et al emphasized that the surface hydrophobicity and the bulk stability were directly related to the rate of the tension decline in the regime II. During the regime III, the surface hydrophobicity became less important, the conformational changes were promoted when protein was unstable in the bulk having a random coil structure (β-casein) compared to globular proteins as ovalbumin. It seemed difficult to compare the Acacia gums with pure proteins behaviors because are mixture of arabinogalactan proteins less and more aggregated. But we observed that the slope of regime II were similar for the both gums and differed for regime III. Accordingly, it can be suggested that the bulk stability was a key parameter which impact the kinetic. This needs to be verify.

For 5 wt% of gums, the extend of induction period was not observed for both gums (Table III.2). The transition between the three regimes were less marked at this gum concentration. This behavior was already described for globular protein as ovalbumin (Beverung et al., 1999). Moreover, the behavior of A. *seyal* seemed very similar to the one of A. *senegal.* It can be suggested that the regime III was rapidly reached because the content of high molar mass protein-rich AGPs was not limiting and that the

behavior to lower interfacial tension of A. *seyal* became similar to A. *senegal* independently to the conformational structure.

Table III.2: Kinetic parameters of interfacial tension corresponding to duration of regime I (t_I), the slope of regime II (S_{II}) and III (S_{III}) determined from plotting of interfacial kinetic on a semi-logarithmic scale for the adsorption of A. *senegal* and A. *seyal* at 0.05 and 5 wt% at hexadecane-buffer interface.

Gum type	Gum concentration (wt%)	Protein concentration (wt%)	t_I (s)	$-S_{II}$ ($mN.m^{-1}.log(s)^{-1}$)	$-S_{III}$ ($mN.m^{-1}.log(s)^{-1}$)
A. *senegal*	0.05	0.001215	250±29	16.0	3.2
	5	0.1215	nd	7.9	2.8
A. *seyal*	0.05	0.00045	320±35	17.5	2.4
	5	0.045	nd	5.9	2.7

The experimental data were also fitted by a logistic curve model (Equation 12) as proposed by Castellani et al. They found that this model allowed to connect the three described regimes by a sigmoidal decrease with a good correlation. For comparison purpose, we decided to report the parameters of the model only for gums at 0.05 wt% (Table III.3). Indeed, for the concentrations of 0.5, 1 and 5 wt%, because of the highly rapid diffusion phase and the lack of acquired data, a large variability in the values of t_{50} and SL_{50} was found. A good correlation between experimental data and estimated values was found with low value of RMSE (0.3 mNm^{-1}) for both gums at 0.05 wt%. As the lowest value of t_{50} corresponded to the highest capacity to decrease interfacial tension, the reported data indicated a lower capacity of A. *seyal* to lower interfacial tension compared to A. *senegal*. As evidenced for the onset time (Table III.1), the values of t_{50} found in this study was much lower than the value reported by Castellani et al. for A. *senegal* (t_{50} = 12 816 s) and A. *seyal* (t_{50} = 13 489 s). The value of SL_{50} indicates the capacity of emulsifier to form films which rapidly decreases interfacial tension, independently to the initial induction time (Castellani et al., 2010). The value of SL_{50} slightly differed between both gums indicating that each gums behaved in the

similar manner to lower interfacial tension at $t_{50.}$ The results were in agreement with the similitude of regime II slope previously mentioned. The value of SL_{50} found was in the same order of magnitude as the data of Castellani et al. indicating that the value of SL_{50} was less affected than t_{50} by the gum specie, origin, nature, composition and protein content. Finally, the values of γ_∞ were close for both gums suggesting that, at indefinite time, the lowering ability of interfacial tension was similar for both gums. According to these parameters, we could confirm that the behavior of both gums at 0.05 wt% especially differed by the initial diffusion-adsorption stage. In contrast, these assumptions were not obvious when 0.5 or 1 wt% were used (Table III.1).

Table III.3: Kinetic parameters from the fitting of the curves of interfacial tension with Equation 2 (model 1) and Equation 3 (model 2) for A. *senegal* and A. *seyal*. RMSE and SS stand for root mean square error and sum of the squared deviation, respectively.

	Model 1				Model 2					
	γ	t_{50}	SL_{50}	RMSE	γ_1	γ_2	τ_1	τ_2	γ_f	SS
A. *senegal*	22	1057	1.26	0.3251	40	26	1246	13651	21.6	4752
A. *seyal*	23	1254	1.6	0.3414	39	26	1203	14175	21.8	6180

Another estimation model (Equation 3) has been proposed by Mahfoudhi et al. to characterize the interfacial properties of Acacia gum (Mahfoudhi et al., 2014).

This equation allowed to determine, τ_1 which represented the time of decay corresponding to the migration of the emulsifier to the oil-water interface, and τ_2 the time decay involving the reorganization of the emulsifier at oil-water interface, respectively (Mahfoudhi et al., 2014). Thus, this model could be useful to better predict the gum behavior in the long term.

This model allowed only a good fitting with experimental data at low gum concentration (0.05 wt%). The value of γ_1, γ_2, τ_1 and τ_2 was in the same order of magnitude for A. *senegal* and A. *seyal* (Table III.3) and as described by Mahfoudhi et al the lower values of γ_1 and τ_1 than the one of γ_2 and τ_2 for each gum. The model was

not successful to clearly evidence the difference of interfacial ability of A. *senegal* and A. *seyal*. This should be due to that the estimation of parameters was done at the "long term" of measurement. This confirmed that, at this period, interfacial tension kinetic was not affected by gum specie as demonstrated by previous models.

To conclude this part, within a same gum, the selected concentrations were not adapted to precisely identify the impact of gum concentrations and to determine the critical concentration for which the lowering interfacial tension remained unchanged. However, the onset time values gave some useful indications and clearly established that the fast adsorption at the interface was dependent on the protein concentration but also to the gums specie. The lack of knowledge on the high molar mass protein-rich AGPs content of A. *seyal* and the difference of conformation forms of these AGPs in A. *senegal* depending on concentration and gums specie make difficult the comparison.

3.1.2. Effect of A. *senegal* and A. *seyal* gum concentration on viscoelastic modulus

Dilatational rheology was also studied to gain information about the viscoelastic properties of interfacial films. The evolution of viscoelastic (E), elastic (E') and viscous (E'') moduli was studied for A. *senegal* and A. *seyal* gums at the following concentrations 0.5, 1 and 5 wt%. The assessment of adsorption kinetic helps to determine the properties of interfacial films which was related to the stability of colloidal systems, such as oil-in-water emulsions (Dickinson, 2001, 2009; Wilde, Mackie, Husband, Gunning, & Morris, 2004, Vasile 2016). Indeed, the efficiency of Acacia gums to stabilize an emulsion was linked to the way the gum adsorbed onto interfaces and the final mechanical properties of the interfacial films (Shotton & Wibberley, 1961). As already mentioned for interfacial tension measurement, some variability within the repetitions can be observed and will be taken into account in the discussion. However, for each gum concentration, only one representing curve was reported.

The data acquired from the measurement of dilatational modulus for A. *senegal* at 0.5 wt% were shown in Figure III.6 as an example. Whatever the gums and concentrations,

the curves describing the dilatational modulus (E) and the elastic modulus (E') were close. Indeed, the variations of E' remained well above the viscous modulus (E''). Thus, the interfacial films clearly showed more elastic than viscous behavior in agreement with previous reported data for Acacia gum films (Bouyer et al., 2011; Castellani et al., 2010; Vasile et al., 2016). This was confirmed by the strong decrease of phase angle as observed in Figure III.6. Indeed, the phase angle (δ) is related to E'' and E' moduli through the following equation:

$$\tan\delta = \frac{E''}{E'} \qquad (14)$$

The lower the phase angle value, the less contribution of dilatational viscous characteristic to the dilatational viscoelastic modulus is. For both gums at all concentrations, the phase angle drastically decreased at the early stage (up to 20 000 s) and rapidly reached the value below 10° indicating a predominantly elastic interface.

The variation of E' and E'' with time but also with Acacia gum concentration for both gums are reported in Figure III.7. The increase of the two dilatational moduli with time indicated the built up of a molecular layer at the interface. For a given concentration, the maximum for the viscous modulus was reached before the one for the elastic modulus, showing that the interface became more and more elastic and structured with time as previously reported (Bouyer et al., 2011). The evolution of E' clearly depended on A. *senegal* concentration (Figure III.7.A). Indeed, for the highest gum concentration, the increase of E' started at the beginning of the experiment and the maximum value was reached earliest. The time that the maximal value was reached was ~2 200 s, 3 800 s, and 15 000 s for gum dispersions at 5, 1 and 0.5 wt%, respectively. For the lowest concentrations, a delay time was evidenced for the E' and E'' moduli and the increase of E'' was less pronounced than E'.

A. B.

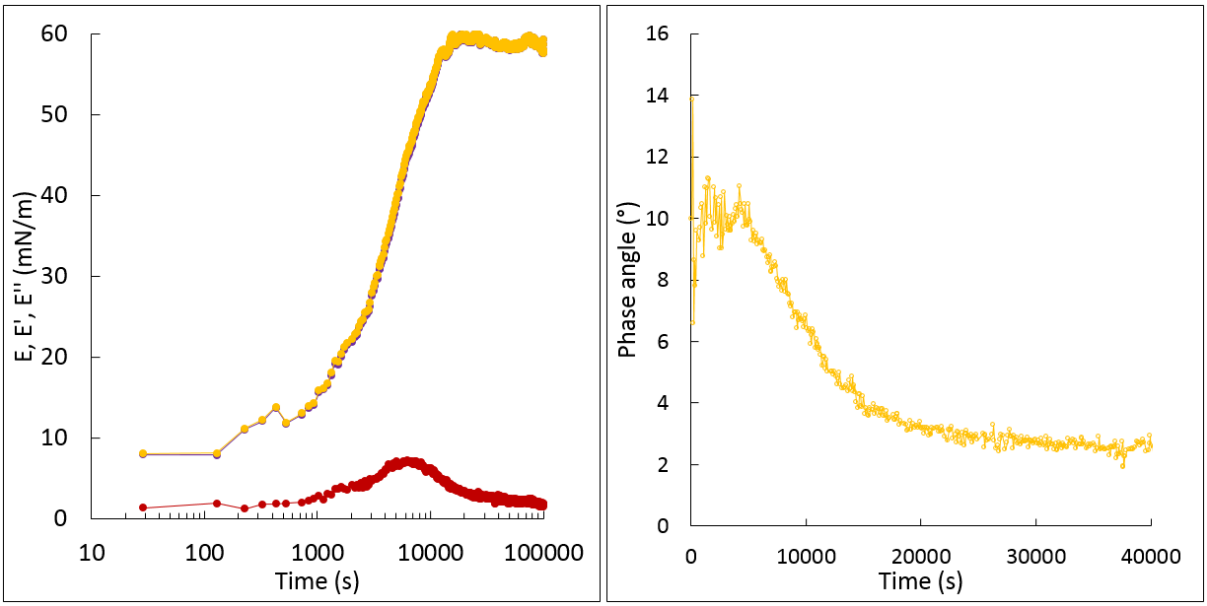


Figure III.6: Viscoelastic modulus (E, yellow), elastic (E', violet) and viscous (E'', red) moduli (A) and phase angle (B) of A. *senegal* 0.5wt% at the interface of hexadecane droplet. The scatter plots were linked by lines to guide the eyes.

It is admitted that Acacia gum is anchored by its hydrophobic proteinaceous part at the interface while the carbohydrate blocks extended into the bulk suspension (Dickinson, 2003; Fauconnier et al., 2000; Fincher & Stone, 1983). It was also demonstrated that among different samples of A. *senegal*, those having the most important part of high molar mass protein-rich AGPs (12%) showed the highest interfacial elasticity and that the elasticity collapsed with enzyme treatment inducing the decrease of high molar mass protein-rich AGPs (Elmanan et al., 2008). Therefore, the adsorption kinetic would be controlled by the diffusion of high molar mass protein-rich AGPs to the interface which would occupy a maximum of the free interface area. Then, depending on concentration and time, intermolecular interactions would take place between the carbohydrates block inducing a more compact conformation of the AGPs, the formation of multilayers and as a consequence an increase of the film elasticity as suggested by Bouyer et al. (Bouyer et al., 2011). The value of pH was another factor which can affect Acacia gum structural properties thus interfacial

properties. Castellani et al. reported that lowering the pH from 4.5 to 3.1 considerably improved the kinetic of Acacia gums at the hexadecane-water interface. At pH 3.1, an unorganized rapid adsorption occurred. Authors suggested that this may be caused by the neutralization of acidic moieties implying a smaller activation energy of interfacial adsorption than at pH 4.5.

A. B.

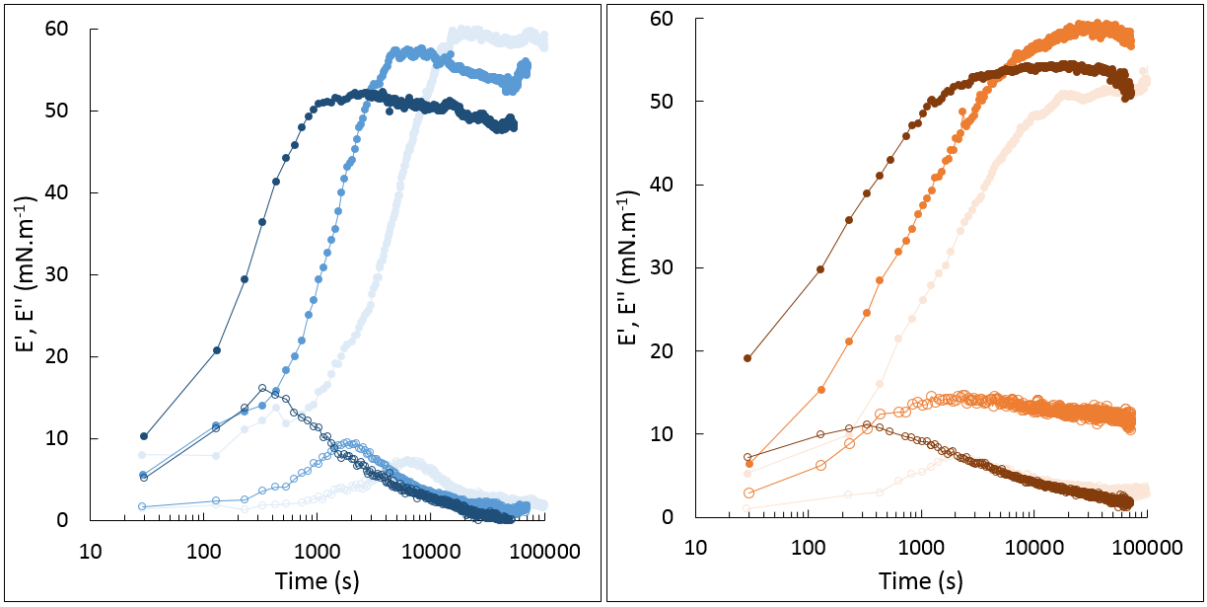


Figure III.7: Elastic (E', filled) and viscous (E'', empty) moduli for A. *senegal* (A) and A. *seyal* (B) at 0.5wt%, 1wt%, and 5wt%. The highest concentration was represented by the darkest color and the lowest concentration corresponded to lightest color. The scatter plots were linked by lines to guide the eyes.

The difference in maximum values of E' were observed as a function of concentration (from 50 to 60 $mN.m^{-1}$) with the lowest value found for the highest gum concentration. These results suggested that the high concentration favored the greater availability high molar mass protein-rich AGP to adsorb and to saturate to the interface but interfacial film was not optimally structured. The observed decrease of E' and loss of elasticity which was evidenced at 1 and 5% (Figure III.7.A) is consistent with a new rearrangement of the interface as described for proteins and Acacia gum (Beverung et al., 1999; Bouyer et al., 2011). High concentrations enhanced intermolecular

interactions with an increase of the thickness of the interfacial layers. Bouyer et al. studied the viscoelastic moduli of Acacia gum interfacial films at sweet almond oil-water interfaces and showed that the saturation was not reached with Acacia gum at 0.5% compared to 2.5% and that the maximum of E' for 0.5% was largely superior compared to the value obtained at 2.5% (Bouyer et al., 2011). They suggested that AGPs rearranged freely in their optimal conformation for the lowest concentration compare to the highest concentration (2.5%) for which the optimal interface organization is disturbed. In our case, it was clear that, AGPs needed more time to diffuse to interface (11h) and to form an elastic film when the concentration of A. *senegal* was equal to 0.5% but the saturation was evidenced since the maximum value of E' was reached and slightly decreased. This difference in behavior for the same concentration of gum but using sweet almond oil against hexadecane could be related to the nature of interface and also to the high molar mass protein-rich AGPs content of the A. *senegal* gums used. Bouyer at al. stipulated that the protein content was equal to 2.5% determined by Kjeldalh and that the average molar mass was about 3.5×10^5 g.mol^{-1} for the Acacia gum used (Bouyer et al., 2011). In our study the protein content determined using the same method was equal to 2.7% and an average molar mass of about 6.8×10^5 g.mol^{-1} was determined suggesting a higher content of high molar mass protein-rich AGPs. In agreement, any limiting long time decrease of viscoelastic modulus at hexadecane interface was reported by Castellani et al. using A. *senegal* at 0.05% but also with the matured gum which was richer in high molar mass protein-rich AGPs suggesting that the saturation was not reached because the amount of high molar mass protein-rich AGPs was too low for the concentration used (0.05 wt%). However, the increase rate of the elastic modulus was more pronounced for the matured compared to the not treated one. Moreover, they observed for A. *seyal* the same initial increase of viscoelastic modulus as for A. *senegal*. However, in a second phase while the elastic modulus of the latter increased weakly because the saturation level was reached, those of A. *seyal* strongly increased proving the efficiency of this gum to develop interactions over a long period of time. In our study, for a higher gum concentration than the one studied by Castellani et al., the increase of E' modulus seemed to be

slower for A. *seyal* than for A. *senegal*. For instance, the maximum values of E' for the highest concentration 5 wt% was reached at 12 000 s and 2 200 s for A. *seyal* and A. *senegal*, respectively (Figure III.7.A and III.7.B). However, the maximum value of E' for A. *seyal* was around (52 $mN.m^{-1}$) in the same order of magnitude of A. *senegal* for this concentration. In contrast, for the 1 wt% and 0.5 wt% of A. *seyal* dispersions, the maximal value of E' modulus was reached even after 40h suggesting that the gum continued to develop interfacial interactions. A slight decrease of E' was observed only for the highest concentration of A. *seyal* as described for A. *senegal* confirming that the saturation and successive rearrangement occurred when the high molar mass protein-rich AGPs content preferentially adsorbed at the interface were present in sufficient amount. For A. *seyal*, the behavior at interface could also depend on its more compact conformation and the weakest accessibility of the protein part which slowed down the increase of E' moduli (Flindt et al., 2005). Beverung et al. reported that protein molecules with a greater flexibility attainted sooner the equilibrium conformation (Beverung et al., 1999). In agreement with our observations, a more flexible structure of A. *senegal* compared to A. *seyal* was recently reported (Mejia Tamayo et al., 2018). In term of the viscous moduli value, as for A. *senegal,* the values of E" were always lower than the values of elastic moduli E'. The difference in maximum values of E" for the different concentration was less pronounced for A. *seyal* in contrast to A. *senegal*. Moreover, for all the concentrations, E" decreased with time but more rapidly than for A. *senegal,* proving that A. *seyal* is able to give a strong elastic films as A. *senegal* even if the high molar mass protein-rich AGPs content was lower. By contrast, Elemanan et al. have reported that for 3% of A. *seyal*, viscous modulus (G") was superior to elastic modulus (G') indicating a viscous surface rather than elastic at limonene interface using an interfacial rheometer (Elmanan et al., 2008). However, the A. *seyal* gum used for their experiments were characterized by lower content of protein (0.66%) compared to the gum used in this study (1%) confirming the crucial role of AGP in elasticity.

According to all these results, it can be concluded that both A. *senegal* and A. *seyal* allowed the formation of elastic films at hexadecane interface but that the biochemical

composition, the structural conformation of AGPs of the two gums and their concentrations affected the adsorption kinetic, the reorganization and the final composition of the films. As previously suggested, the interface rheological response of A. *senegal* gums at high concentration was reminiscent of the behavior of flexible globular proteins (Beverung et al., 1999; Elmanan et al., 2008; Erni et al., 2007). For A. *seyal*, its lower content in high molar mass protein-rich AGPs and its compact structure induced a slower formation and structuration of the films. A similar behavior could be observed for A. *senegal* at the lowest concentration.

3.1.3. Effect of IEC-F1 on transient interfacial tension and viscoelastic modulus

To highlight the role of a specific fraction and the role of global interfacial properties of the gum, the following part emphasize on the experiment of IEC-F1. The high molar mass protein-rich AGPs fraction (IEC-F1) was obtained by fractionating A. *senegal* gum using ion exchange chromatography. Its biochemical composition and structural properties were previously reported (Apolinar-Valiente et al. 2018, submitted article). It was composed of high molar mass protein-rich AGPs with a M_w of 3.0×10^6 g.mol^{-1} and the major population corresponded to 70% and 30% of the classic HIC-F2 of HIC-F3 fractions, respectively. The biochemical characterization of IEC-F1 showed a high ratio of arabinose/galactose (1.1) compared to A. *senegal* (0.77), a similar content of glucuronic acids but a very high amino acid content (˜100 mg.g^{-1}) against 21.5 mg.g^{-1} for A. *senegal*. These different elements suggested a variation in molecules aggregation which could involve changes in interfacial properties. Therefore, in this part, the interfacial properties of IEC-F1 were investigated since this fraction was suggested to play an important role in interfacial properties of A. *senegal* according to its composition and conformation.

The interfacial tension and viscous and elastic moduli measured for two concentrations of IEC-F1 and for A. *senegal* (5 wt%) are reported in Figure III.8. As this fraction contained a high amount of mineral salts ˜17% against 3% for A. *senegal*, this implies

that the gums concentration, i.e. in term of macromolecules, was nearest to 0.415 and 4.15 wt% than 0.5 and 5 wt% (the targeted concentrations).

A. B.

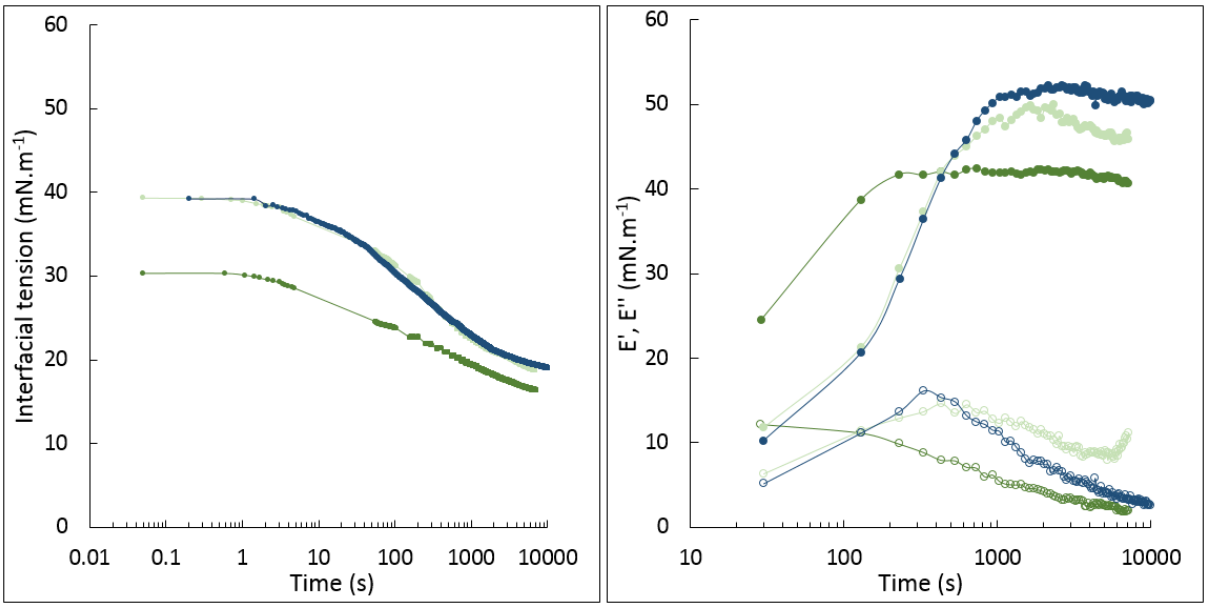


Figure III.8: Effect of IEC-F1 concentration (0.415 wt% (light green) and 4.15wt% (dark green)) and A. *senegal* at 5 wt% (dark blue) on interfacial tension of hexadecane plotted in a semi-logarithmic scale of time (A) and on viscoelastic modulus (E' (filled) and E'' (empty)) (B). The scatter plots were linked by lines to guide the eyes.

At the concentration of 4.15 wt%, the decrease of interfacial tension in the presence of IEC-F1 was pronounced since the first value of tension measured after 0.05 s was lower than those reported for the same time for raw A. *senegal* at the same concentration (Table III.4). For this reason, the time onset could not be determined. However, as evidenced by the Figure III.8 and the values of interfacial tension reported at different time in Table III.4, the initial decrease of interfacial tension depended on the concentration of IEC-F1: interfacial tension was faster lowered by 4.15 wt% than 0.415 wt% of IEC-F1. These results evidenced that the amount of AGPs was important to adsorb faster to interface and to lower interfacial tension.

It was also noted that the tension values obtained at selected times (60 s and 7 200 s) for the IEC-F1 fraction at 0.415wt% were similar to those found for A. *senegal* at 5wt% (Table III.4). The rate of high molar mass protein-rich AGPs in A. *senegal* was near *to* ~14%. The ratio of A. *senegal* and IEC-F1 concentrations (5/0.415) was equal to 12, indicating comparable level of high molar mass protein-rich AGPs in the two dispersions. This means that the ability of original gum to lower interfacial tension was well related to the preferential adsorption of these AGPs at the interface as previously suggested (Castellani et al., 2010; Fauconnier et al., 2000). Moreover, for the longest time of measurement (10h or 36 000 s), a value of 17 $mN.m^{-1}$ was reached using this low concentration (data not shown). The same value of interfacial tension was observed for the highest concentration of IEC-F1 but at a shorter time. In short, the final tension seemed to be independent on the concentration but the decrease rate was favored by the increase of concentration as in the case of original gum. It is worth noting that IEC-F1 contained high concentration of mineral salts due to the fractionation process and these mineral salts may affect the interfacial properties of this fraction.

Table III.4: Effect of A. *senegal* and IEC-F1 at 0.415 and 4.15wt% on the values of interfacial tension at 0.05s, 56s and 7200s (2h) at hexadecane-buffer interface.

	Low concentration		High concentration	
	A. *senegal* at 0.5wt%	IEC-F1 at 0.415wt%	A. *senegal* at 5wt%	IEC-F1 at 4.15wt%
Interfacial tension at 0.05 s ($mN.m^{-1}$)	44.1±2.2	41.2±3.0	40.5±1.8	30.5±0.2
Interfacial tension at 60 s ($mN.m^{-1}$)	41.2±1.7	34.1±1.7	32.3±0.5	24.7±0.4
Interfacial tension at 7200 s ($mN.m^{-1}$)	24.2±0.1	19.1±1.0	19.0±0.5	16.9±0.8

In term of dilatational interfacial rheology, the IEC-F1 fraction followed a similar behavior as the total gum with elastic modulus superior to viscous one (E' >> E''). The higher IEC-F1 concentration, the faster interfacial film formation was (Figure III.8.B). Using IEC-F1 at 0.415 wt%, elastic modulus increased with time up to 54

$mN.m^{-1}$ and then slightly decreased. For 4.15 wt% of IEC-F1, the elastic modulus reached faster the maximum E' modulus (19 min against 47 min for 0.415%wt) with a lower value (47 $mN.m^{-1}$) than IEC-F1 at 0.415 wt%. After reaching the maximum value, the E' modulus remained stable. As for interfacial tension measurement, the dilatational interfacial rheology observed for IEC-F1 at 0.415 wt% was very similar to those found for A. *senegal* at 5 wt% even if it was known that the content of high molar mass protein-rich AGPs was higher for A. *senegal* at 5wt% than for IEC-F1 at 0.415 wt%. The loss of elasticity for IEC-F1 at 0.415 wt% can be related to the rearrangement of the films according to the type and strength of saturation and freedom degree of adsorbed molecules and also to the changes in composition at the interface due to the presence of several type of AGP. Indeed, as specified, the IEC-F1 fraction corresponded to ~30% of HIC-F2 and 70% of HIC-F3 with the latter being more hydrophobic. Indeed, HIC-F2 contains around 6.3% of protein with mean molar masses of 1.4-1.8 × 10^6 $g.mol^{-1}$ while HIC-F3 have a highest content of proteins around ~13.7 % and molar masses ranging from 2.95×10^5 to 26.7×10^5 $g.mol^{-1}$ (Mejia Tamayo et al., 2018). The assessment of protein mixture behavior at the air-water interface or oil-water interface allowed Damodaran et al. to propose that the largest protein adsorbed first and when the surface became saturated, the smallest protein evinced the large protein and replaced them at the interface (Damodaran & Razumovsky, 2003). The authors indicated that this behavior was independent on the hydrophobic nature of the protein. It can be hypothesis that this competition phenomenon occurred also in the gum (Figure III.7.A) or fraction (Figure III.8.B) which are constituted by glycoproteins of different size. On the other hand, the competition for adsorption at the interface between GAGP of Acacia gum and β-casein, the interchange of molecules at the interface was demonstrated and it was proved that the thickness of adsorbed molecules of gum depended on the presence of the protein (Damodaran & Razumovsky, 2003).

3.2. Interfacial properties of 5wt% Acacia gum dispersion at the hexadecane-, limonene-, and octanol-buffer interface

The oil phase properties could strongly influence the behavior of emulsifier at interface resulting in the production of emulsions with more or less stability (Chanamai et al., 2002). Moreover, a previous study carried out in our laboratory with the same gums but at sunflower oil interface had shown difference in term of onset time, slope of regime depending on the concentration and the gum specie comparing to hexadecane (Lopez Torrez, 2017). Therefore, the physicochemical properties such as polarity, solubility, volatility of organic compounds are needed to be taken into account when the interfacial properties of an emulsifier were studied.

In this part, the interfacial properties of A. *senegal* and A. *seyal* at 5 wt% were determined for octanol and limonene and compared to those found previously for hexadecane. This concentration was chosen according to the concentrations used to produce limonene emulsion (Chapter 4). The three components are characterized by varied polarity and solubility in water (Table III.5). For comparison purpose, as the time of experiments was limited to 7h for limonene and octanol, the data of hexadecane were only reported up to this time.

Table III.5: Physicochemical characteristics of the used organic volatile compounds.

Compound formula	Chemical structure	Molecular weight (Da)*	Solubility in water at 25°C (mg.L^{-1})*	logP at 25°C*	Oil-water interfacial tension (mN.m^{-1})**	Oil-water interfacial tension (mN.m^{-1})***	Viscosity (mPa.s)
Hexadecane ($C_{16}H_{34}$)		226.4	0.0009	8.20	53.5 at 25°C[a]; 53.3 at 20°C [b]; 52 at 20°C[c]	48.3±1.8 at 25°C	3.454 at 20°C[d]
Limonene ($C_{10}H_{16}$)		136.2	13.8	4.57	27.2[g]	27.8±0.6 at 25°C	0.890 at 25°C[f]
Octanol ($C_8H_{18}O$)		130.2	540	3.00	8.52 at 20°C[b]	8.4±0.4 at 25°C	7.365 at 25°C[e]

From *ChemSpider.com, ** values found in the literature, *** experimental values, [a]Wu & Hornof, 1999, [b]Demond & Lindner, 1993, [c]Castellani et al., 2010, [d]Hardy, 1958, [e]Bhattacharjee & Roy, 2010, [f]Clará, Gómez Marigliano, Campos, & Sólimo, 2010, [g]Gregson, Rong, Sillick, & Parker, 2011.

3.2.1. Effect of A. *senegal* and A. *seyal* gums on transient interfacial tension

Figure III.9 shows the impact of volatile organic compound nature on the ability of A. *senegal* and A. *seyal* gums to lower interfacial tension. Both gums were able to decrease the interfacial tension of all compounds. The difference between the 3 compounds and the impact of gums were clearly obvious. A. *senegal* seemed to slightly lower more interfacial tension than A. *seyal* and affected the speed to decrease the tension by slowing down especially regarding limonene and octanol (Figure III.9). The characteristic diffusional lag time (phase I) which was not evidenced for hexadecane at the selected gum concentration was clearly observed for the two others compounds. It was more pronounced for octanol (between 150 and 200 s for A. *senegal* and A. *seyal*, respectively) than for limonene (between 10 and 20 s for A. *senegal* and A. *seyal*, respectively) and few dependent on the gum specie. For the same gum concentration at sunflower-water interface, the lag time was estimated around 22 s and 4 s for A. *senegal* and A. *seyal*, respectively, confirming the impact of interface nature and the slightly difference between the two gums (Lopez Torrez, 2017). However, the gum dispersions used in the study reported by Lopez Torrez was prepared in water while in our case gums were dissolved in acetate buffer and solvent can affect the conformation and aggregation of macromolecules.

For comparison purpose, the data were plotted in semi-log scale and the parameters of interfacial tension kinetic can be used according to Beverung et al. (Beverung et al., 1999). The decrease of interfacial tension in Regime II for limonene was less pronounced than hexadecane but more pronounced than for octanol (Figure III.9). For A. *senegal*, the slope of interfacial decrease in Regime II was –7.9, -6.5 and -1 $mN.m^{-1}.s^{-1}$ at the interface of hexadecane, limonene and octanol, respectively. On the other hand, for A. *seyal*, the value was –4.9, -4.1, –0.003 $mN.m^{-1}.s^{-1}$ at the interface of hexadecane, limonene and octanol, respectively. The values of interfacial tension after 7h can be classified as following from lowest to highest: octanol<limonene<hexadecane. This classification was also related to the initial values of the compounds interfacial tension in buffer. Indeed, the initial interfacial tension of each compound was strongly different

(Table III.5), then the surface pressure was calculated to facilitate the comparison (Figure III.10).

A. B.

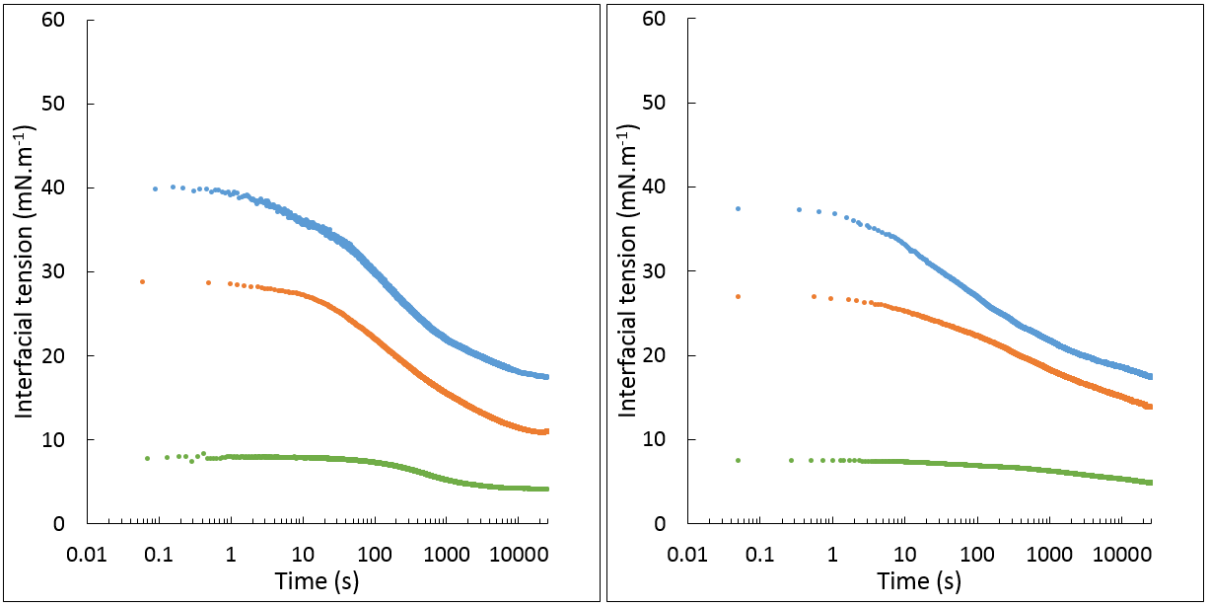


Figure III.9: Semi-logarithmic plots of the interfacial tension of hexadecane (blue), octanol (green) and limonene (orange) in the presence of A. *senegal* (A) and A. *seyal* (B) at 5wt%.

A less increase of surface pressure was observed for limonene compared to hexadecane. Indeed, the increase of surface pressure for hexadecane varied between 25 and 21 $mN.m^{-1}$ and for limonene between 17 and 14 $mN.m^{-1}$ for A. *senegal* and A. *seyal*, respectively. This showed the strong adsorption of both gums for the two compounds but with a highest affinity advantage for hexadecane and highlighted the most efficiency of A. s*enegal* as already specified. For octanol, the very low increase of surface pressure (between 3 and 4 $mN.m^{-1}$ depending on gums) suggested that both Acacia gums were not strongly absorbed at the interface.

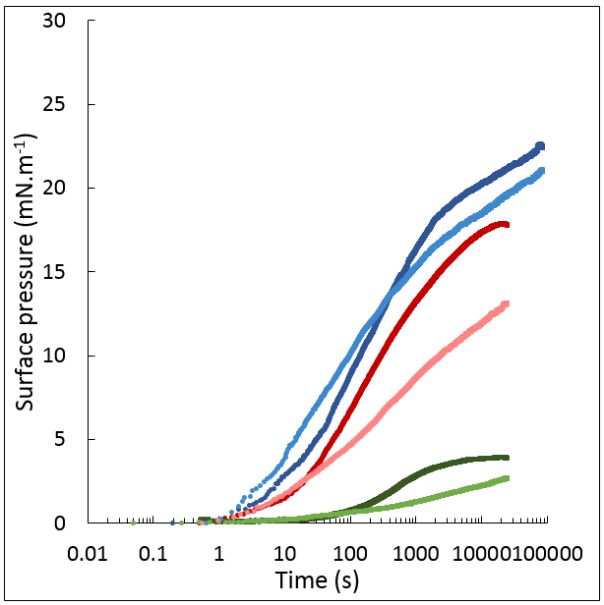


Figure III.10: Semi-logarithmic plots of the surface pressure of hexadecane (blue), limonene (red) and octanol (green) in the presence of A. *senegal* (dark tone color) and A. *seyal* (light tone color) at 5wt%.

Octanol has an amphiphilic characteristic inducing the reorientation of the molecule at the contact of aqueous solution as previously suggested for decanol, which has similar structure and interfacial tension in water (8.3 $mN.m^{-1}$) as octanol but a higher apolar characteristic (logP=4.57). The alcohol group could protrude into the bulk volume, favoring the formation of hydrogen bond with water molecules, and therefore the decrease of tension at the decanol-water interface (Chanamai et al., 2002). As suggested by Chanamai et al., when the interfacial tension of the compound fall below a certain value, i.e. around 8 $mN.m^{-1}$, the Acacia gums are not able to strongly adsorb at the interface. Moreover, this behavior was found independently of the concentrations tested. It can be suggested that, when Acacia gum was added, the interface was partially stabilized and a competition between the octanol molecules and the emulsifying molecules of gums occurred at the interface explaining the extended induction time. Therefore, the compounds with high polarity and water solubility should be used to emphasize the slight difference between gums which could be better observed. For long-time adsorption, the gums allowed to slightly decreased the absolute interfacial tension. The weak effect of A. *seyal* gum which is poorer in high molar mass

protein-rich AGPs content compared to A. *senegal* confirmed the preferential adsorption of these AGPs at the interface. For A. *senegal*, the presence of the three regimes was evidenced but the slope for Regime III was quasi null whereas for A. *seyal*, the Regime III was not still reach at the end of experiments. This confirmed that for octanol only the more hydrophobic molecules of the gums were adsorbed at the interface and conformational change or rearrangement were limited. Previous study on effect of A. *senegal* and A. *seyal* at 5 wt% on sunflower oil with the value of interfacial tension of ~30 mN.m-1 showed that A. *seyal* lowered interfacial tension faster than A. *senegal* indicating that the former diffused faster at the interface than the latter. For the long term, A. *senegal* allowed to slightly lower more interfacial tension than A. *seyal* with surface pressure of 10 and 8 $mN.m^{-1}$ for A. *senegal* and A. *seyal*, respectively (Lopez Torrez, 2017). The hydrophobicity of sunflower oil was not specified in the study but the logP value of 7.05 was found for linoleic acid which is the major compound of this oil (50-70wt%). Regarding this logP value which is slightly lower than hexadecane, it was clear that the ability of Acacia gum to lower interfacial tension was not only dependent on the polarity. It is obvious that sunflower was characterized by high viscosity. However, considering the 3 compounds studied which have weak viscosity ($<$ 7.4 mPa.s), the impact of both gums on the adsorption kinetic at the interface was clearly dependent on the physicochemical properties of oil phase such as polarity, i.e. the solubility in the bulk and the interfacial tension in water of the component. Indeed, the gums showed more difficulty to adsorb at the interface of the most polar components compared to the highest apolar components. For the mixture of several compounds as in the case of sunflower oil, the polarity of the major compound was not a sufficient parameter explaining the overall behavior of oil. This could be due to the intrinsic interaction between contained compounds within the oil.

Chanamai et al have also studied the effect of Acacia gum at the decane interface and have found a similar increase of surface pressure than for hexadecane (26 against 27 $mN.m^{-1}$) in relation to their similar interfacial tension in water. Decane is characterized by a higher value of logP (5.0) than decanol and a weak solubility (0.052 $mg.L^{-1}$ against

35 mg.L^{-1}) that explained the strong adsorption of gum compared to decanol (Chanamai et al., 2002). In our study limonene was characterized by intermediary properties between hexadecane and octanol (Table III.5) and it was obvious that both gums well adsorbed at the limonene interface but with more limited efficiency than observed for hexadecane. This can be attributed to its lower interfacial tension in water 28 mN.m^{-1} compared to hexadecane (48 mN.m^{-1}). The direct comparison of the behavior of limonene and decane interfaces was difficult since the gums specie, composition and concentration and solvent used by the cited authors were different of those used in our study. However, as the behavior of the two components was related to hexadecane, it can be suggested that the lack of efficiency of gums to lower interfacial tension of limonene was related to the stronger solubility (200 times) and in lower interfacial tension of limonene compared to decane. There is a need of knowledge of the component physicochemical properties involving in emulsion stability since it was proposed by Chanamai et al that the components with low polarity and low solubility will be more stable against Ostwald ripening and coalescence than the other. Polar and water-soluble components can diffuse in the bulk and favor the Ostwald ripening. Moreover, these compounds are characterized by low interfacial tension in water and emulsifiers as protein or Acacia gum could be underperforming to stabilize against coalescence. This assumption can be also tested observing the impact of the component polarity and interfacial tension in water on the dilatational rheology.

3.2.2. Effect of A. *senegal* and A. *seyal* gums on viscoelastic modulus

The dynamic of viscoelastic moduli was related to the compound nature but was also affected by the gum specie. As observed for hexadecane interface, the interfacial films of Acacia gum at the interface of octanol and limonene exhibited an elastic behavior since E' always followed the profile of E (data not shown) and was higher than E" (Figure III.11). Both viscous and elastic moduli initially increased as a function of time.

However, the highest elastic and viscous moduli were observed at the interface of hexadecane, followed by limonene and octanol. Moreover, the ratio between the maximum values of viscous modulus and elastic modulus was dependent on the compound. This ratio was higher for hexadecane than for limonene and octanol. The difference between the gums seemed less pronounced than between the aroma compounds.

A. B.

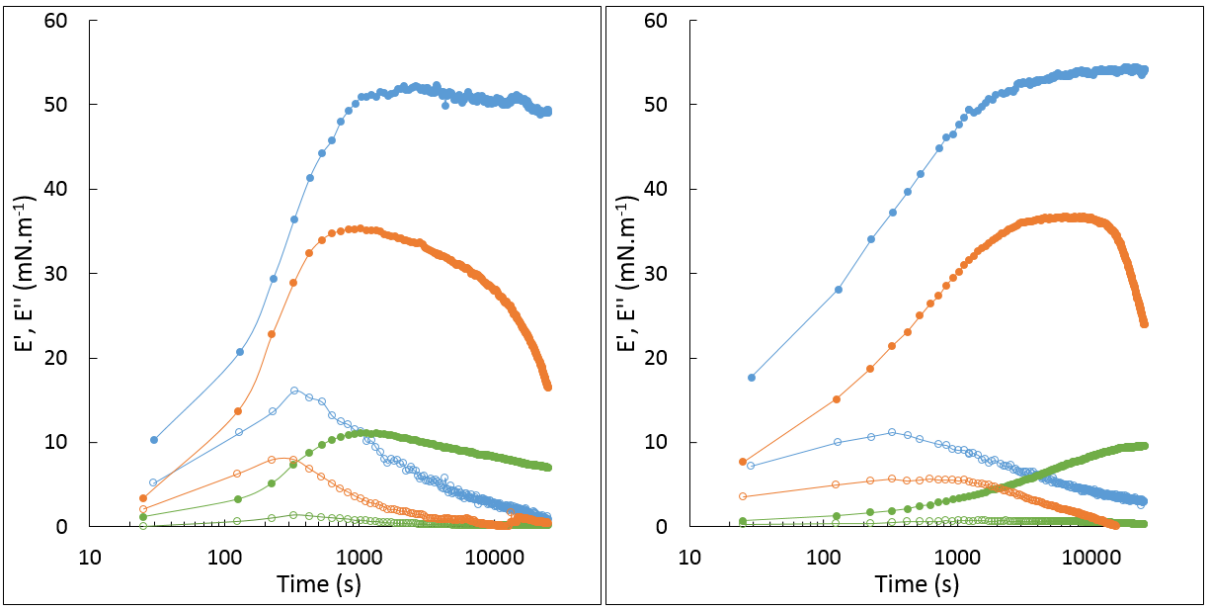


Figure III.11: Effect of volatile compound nature, hexadecane (blue), limonene(orange) and octanol (green), and gum specie, A. *senegal* (A) and A. *seyal* (B) on E' (filled) and E'' (empty) moduli. The scatter plots were linked by lines to guide the eyes.

For hexadecane and limonene, E'' increased and quickly reached its maximum then slowly decreased. For octanol, the variation of viscous modulus was only perceptible for A. *senegal* gums. For each gum, the rate of E' increase with time was in the same order of magnitude for limonene and hexadecane but was slower for octanol compounds. Moreover, the increase was faster for A. *senegal* compared to A. *seyal*. For A. *senegal*, after the maximum E' was reached, a slow decrease of E' at the interface of octanol and hexadecane was observed whereas the modulus drastically decreased for limonene.

This decrease suggested saturation of the interface and a rearrangement and/or desorption of the A. *senegal* interfacial films but also highlighted the impact of the nature of interface. The specific behavior of the gum at the limonene interface was also found for A. *seyal* while for octanol and hexadecane, the elastic moduli continue to slowly increase. It means that for the two gums, the elastic film formed at the limonene interface was the same nature and was affected by the same rearrangement but with a delay in time. Indeed, the dilatational modulus reached the maximum and decreased earlier for A. *senegal* than for A. *seyal*. As maximal values of E' and E" obtained for the three components with the two gums were close, it can be concluded that the behavior at the interface was also similar but it was delay for A. *seyal*. This delay in film formation as already mentioned could be due to the lower content and accessibility of high molar mass protein-rich AGPs in A. *seyal* compared to A. *senegal* (Flindt et al., 2005). Even if the process of interfacial film formation and the nature of interaction differed for A. *senegal* and A. *seyal*, the major factors impacting the interfacial film formation were the structure and nature of organic compound. The strong rearrangement observed at the limonene interface could be explained by its cyclic structure compared to the linear structure of hexadecane and octanol. The weak structural stability of film at limonene interface seemed to be related to the orientation of the molecules at the interface which can hinder the stabilization or the great capacity of limonene to diffuse into the bulk.

In conclusion, despite the highest interfacial tension at equilibrium of hexadecane (Figure III.9 and III.10), it seemed that the films at its interface exhibited the greatest elastic characteristic (Figure III.11). Therefore, the hexadecane emulsions formed by Acacia gums could probably have a good stability against coalescence compared to limonene and octanol. On the other hand, it could be hypothesis that the emulsions of octanol produced by Acacia gums could be more prone to coalescence. The production of octanol emulsions could be done to check this hypothesis.

III. Surface tension of Acacia gum dispersion

1. Introduction

As a surface active agent, Acacia gums can accumulate and form films also at liquid/air interface. This property is used in technological processes, e.g. food foam manufacture, but only some studies of the surface tension of Acacia gum dispersions were hitherto done (Cao, Zhang, Zhang, & Du, 2013; Castellani et al., 2010; Damodaran & Razumovsky, 2003). In this part, we would like to determine the surface tension of both suspension of A. *senegal* and A. *seyal* gums and to estimate the polar and dispersive components through an indirect method consisting to use a plate with a known surface free energy and the contact angle measurement. New knowledge of interfacial properties at liquid-air interface could contribute to explain the difference between the Acacia gums and to enhance the use of Acacia gums in food applications, e.g. as foam stabilizing agent. Moreover, this could give a supplementary information about surface conformation of Acacia gums between gum dispersion and gum semi-solid film (reported in Chapter 5).

2. Materials and methods

The surface tension measurement of Acacia gum dispersions (γ_{LV}) was done through the pendant droplet shape method using Digidrop goniometer (model ASE, GBX, Roman-sur-Isere, France) equipped with a video measuring system with a CCD camera. A droplet with a volume of 2 µL of Acacia gum dispersion at 20wt% was formed. The chosen gum concentration corresponded to the one used to form film of Acacia gums (reported in Chapter 5). The analysis of axisymmetric droplet shape was done following Young equation (Equation 8). Then, the surface tension of drop-forming liquid was determined as already explained above using an image analysis software Windrop++v1 (GBX, Roman-sur-Isere, France).

In addition, the contact angle (θ) of Acacia gum dispersions on a dispersive coating SFE-DS plate was determined by depositing 2µL of gum dispersion on the plate. The

measurement of contact angle was done on the left and right sides of the drop and averaged. At least 10 measurements were carried out for each sample in ambient condition in similar temperature and relative humidity (RH), i.e. 25±2 °C and 25±5%RH.

Combining the surface tension of gum dispersion (γ_{LV}), the contact angle and the properties of dispersive coating SFE-DS plate which has a surface free energy of 13.5 ± 0.5 mN.m^{-1} with zero polarity (<0.1 mN.m^{-1}), the dispersive (γ_{LV}^{d}) and polar (γ_{LV}^{p}) components of surface tension of the gum solutions were calculated using the equations of Fowkes (Equation 15 and 18).

According to Fowkes (Fowkes 1968), the surface tension (γ) can be described by the sum of a dispersive component γ^{d} and a polar component (γ^{p}) which are attributed to the polar and non polar interactions:

$$\gamma = \gamma^{d} + \gamma^{p} \qquad (15)$$

The mathematical model proposed by Fowkes to calculate the surface energy takes into account only the dispersive interaction for the study of interfacial energy between water (W) and saturated hydrocarbons (H) without polar components. The interactions between the two phases involve only the apolar contribution of surface energy. Thus, if only the dispersive components were available between two phases, the surface energy corresponds to the geometric mean of the dispersive components of the surface tension of each phase:

$$\gamma_{WH} = \gamma_{WV} + \gamma_{HV} - 2\sqrt{\gamma_{WV}^{d} \cdot \gamma_{HV}^{d}} \qquad (16)$$

where, γ_{WH} corresponds to interfacial energy between water and saturated hydrocarbons and γ_{WV} and γ_{HV} are surface energy of water and hydrocarbons, respectively. γ_{WV}^{d} and γ_{HV}^{d} represent the dispersive components of water and hydrocarbons phases.

In the case of solid-liquid interface, this equation becomes:

$$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2\sqrt{\gamma_{SV}^{d} \cdot \gamma_{LV}^{d}} \qquad (17)$$

where, γ_{SL} corresponds to interfacial energy between solid and liquid drop and γ_{SV} and γ_{LV} are the surface energy of solid and liquid, respectively. γ_{SV}^{d} and γ_{LV}^{d} represent the dispersive components of solid and liquid, respectively.

Taking into account dispersion forces and Young's equation, Fowkes proposed the following Equation (18):

$$\gamma_{LV} \cdot (\cos\theta + 1) = -2\sqrt{\gamma_{SV}^{d}} \cdot \sqrt{\gamma_{LV}^{d}} \qquad (18)$$

This approach needs only a single contact angle measurement of a liquid with a known γ_{LV}^{d} on the solid surface in order to estimate γ_{SV}^{d}. However, this approach is true only when only dispersive forces act in at least one of two phases.

3. Results and discussion

As expected the surface tension of gums were inferior to the value of water (i.e. 72 $mN.m^{-1}$). proving that the Acacia gums were able to decrease the surface tension of water (Table III.6). A value of 67 $mN.m^{-1}$ was also reported for a solution of Acacia

gums at 2% (Bergenstahl, Fogler, & Stenius, 1986). The net reduction compared to water was about 5 $mN.m^{-1}$. This was in agreement with reduction found by Damodoran et al. for gum arabic glycoprotein (GAGP) at above 4wt% at the saturated monolayer coverage (Damodaran & Razumovsky, 2003). GAGP corresponded to the first fraction isolated from crude Acacia gum by gel permeation chromatography and represented about 10% of total mass with 90% carbohydrate and 10% protein content. The decrease of surface tension of Acacia gums was weak compared to other hydrocolloids as β-casein for which the reduction was about 18 $mN.m^{-1}$ measured in the same condition as for GAGP (Damodaran & Razumovsky, 2003). These results were consistent since the interfacial properties of protein are known to be superior to Acacia gums.

Table III.6: Contact angle (θ) of film forming solution at 20wt% on dispersive plate and its surface tension (γ_{LV}) and dispersive (γ_{LV}^{d}) and polar (γ_{LV}^{p}) components determined using Equation of Fowkes (Equation 18) and Owens and Wendt (Equation 19).

Gum type	θ (°)	γ_{LV} ($mN.m^{-1}$)	γ_{LV}^{d}($mN.m^{-1}$)	γ_{LV}^{p}($mN.m^{-1}$)
A. *senegal*	136.4±3.7	67.8±2.8	9.3	58.5
A. *seyal*	132.2±4.1	67.8±3.6	6.5	61.3

The effect of gum specie on surface tension was not significantly observed. This meant that the value of surface tension was not affected by the difference of biochemical composition and structural properties. It may be because the concentration used was too high and the effect of different parameters was compensated by the concentration of gum.

The results of contact angle measurement are reported in Table III.6. First, regarding the high contact angles values of both gum dispersions on the apolar SFE-DS plate, the low affinity between the gums and the dispersive coating was obvious and in agreement with the major hydrophilic characteristic of gums. An ANOVA analysis showed that the values were similar for the two gums suspensions.

Both gums contained polar and dispersive components which was in agreement with the amphiphilic characteristic of gums. However, the polar components of surface tension were higher than dispersive components confirming the preponderant hydrophilic characteristic of Acacia gum. On the other hand, the dispersive part was not negligible representing 6.5% of the value of surface tension for A. *seyal* and 9.3% for A. *senegal*. As expected, the slightly higher hydrophobic characteristic of the A *senegal* gum compared to A. *seyal* was in agreement with a higher content of protein and by consequence of hydrophobic amino acids, a lower content of arabinose and charged glucuronic acids compared to A. *seyal*. These results were in agreement with the outcome previously found by Mejia-Tamayo et al (Mejia Tamayo et al., 2018). A further study measuring the impact of concentration on the surface tension at the liquid-air interface is needed in order to better understand whether this difference in dispersive component could be detected at lower gum concentrations using this method.

IV. Major outcome

In this chapter, we showed the impact of gum concentration and specie, high molar mass protein-rich AGPs and oil nature on the interfacial properties of Acacia gum including the ability to lower interfacial tension and to form stabilizing interfacial films. The main results are described in the following table:

Interfacial tension kinetic: impact of gums and oil
• At 0.05wt%, A. *senegal* allowed to lower interfacial tension faster than A. *seyal*. This could be due to (i) higher protein concentration (ii) greater content of high molar mass protein-rich AGPs and aggregate form (iii) greater structural flexibility. • For a given protein concentration, A. *seyal* seemed to diffuse faster to the interface than A. *senegal*. This could be due to the more compact structure of the former. • The interfacial properties of IEC-F1 confirmed the major role of high molar mass protein-rich AGPs in interfacial properties of A. *senegal* since transient interfacial tension was similar for the IEC-F1 at 0.415wt% and A. *senegal* at 5wt%. • Independently of gum composition and structural properties, the higher gum concentration, the faster interfacial tension decrease at the early stage of measurement • The higher initial interfacial tension and the lower water solubility of compounds, the faster Acacia gums diffuse at the interface. • The difference between two gums in the ability to lower interfacial tension was more highlighted at octanol interface because of the higher competition at the interface between Acacia gum molecules and soluble.
Rheology of interfacial films: impact of gums and oil
• Elastic films were formed by all gums at all concentrations. • The interfacial films formation was faster for A. *senegal* than A. *seyal* due to the higher content of high molar mass protein-rich AGPs. • The dilatational interfacial rheology was very similar for IEC-F1 at 0.415 wt% and A. *senegal* at 5 wt% in relation to the content and role of high molar mass protein-rich AGPs in interfacial properties of Acacia gums. • Independently of gum composition and structural properties, the higher gum concentration, the faster maximum value of viscoelastic modulus was reached

- At all concentrations of A. *senegal*, at 5wt% of A. *seyal* and 0.415wt% of IEC-F1, the value of viscoelastic modulus decreased after the maximum value was reached due to the interfacial saturation and new rearrangement of interfacial molecules.
- For A. *seyal* at 0.5wt% and 1 wt%, the interfacial saturation was not reached due to the low content of high molar mass protein-rich AGPs, the more compact molecules and the weak accessibility of protein part of this gum.
- For IEC-F1 at 5wt%, no decrease was observed after the maximum value of elastic modulus was reached. This could be due to the interfacial saturation occurring without new arrangement of interfacial molecules.
- The higher interfacial tension and the lower water solubility of compounds, the higher value of maximum viscoelastic modulus.
- The value of viscoelastic modulus drastically decreased after the maximum value was reached at the interface of limonene. This could be due to the stronger rearrangement at the limonene interface which can be explained by its cyclic structure compared to the linear structure of hexadecane and octanol.
- For all compounds, the viscoelastic modulus of A. *senegal* interfacial film reached the maximum value before the one of A. *seyal* films due to the higher content and accessibility of high molar mass protein rich macromolecules in A. *senegal*.

Surface tension of Acacia gum dispersion

- A. *senegal* and A. *seyal* at 20wt% were similarly able to decrease surface tension of water.
- Both gum dispersions showed polar and dispersive components in agreement with their amphiphilic characteristic with the value of polar component higher than dispersive one.
- A. *senegal* showed a higher value of dispersive component than A. *seyal* due to a lower content of arabinose, charged glucuronic acids and especially a higher protein content and by consequence a greater amount of hydrophobic amino acids compared to A. *seyal*.

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Emulsifying properties of Acacia gums and involvement of high molar mass protein-rich AGPs

Chapter 4 – Emulsifying properties of Acacia gums and involvement of high molar mass protein-rich AGPs

In the previous chapter, taking into account all the results, A. *senegal* showed greater interfacial properties than A. *seyal* allowing the faster formation of films at the interface. The behavior of both gums at the interface depended not only on the protein content but also on the amount of high molar mass protein-rich macromolecules and on the structural conformation such as the molecular compactness, the accessibility of protein part and the molecular flexibility. The data obtained from the comparison of interfacial properties between IEC-F1, a high molar mass molecules protein-rich fraction, and original A. *senegal* confirmed the important role of these macromolecules in interfacial properties of A. *senegal* gums. The interfacial properties were clearly affected by oil phase nature and the choice of dispersed phase can impact the emulsion formation and stabilization. The understanding of the fundamental forces that drive Acacia gum to interfaces and the role of high molar mass protein-rich macromolecules in the process that lead to tension reduction is essential to anticipate the mechanism of the films formation and their properties at interfaces in order to stabilize emulsions.

The aims of this chapter are to study the emulsifying properties of Acacia gums, i.e. the emulsification ability and the stability, and to specify the role of high molar mass protein-rich AGPs usually described as responsible of these emulsifying properties. Therefore, a special focus on the impact of these AGPs on emulsifying properties was made through an innovative approach consisting to formulate reconstituted gums from two purified fractions which have been previously characterized in term of biochemical composition and structural properties. In parallel, the assessment of the emulsifying properties of A. *senegal* and A *seyal* was also done since these gum species differ by their biochemical composition and structural properties, but also by their high molar

mass protein rich AGPs content. Thus, the effect of gums specie, protein and high molar mass protein-rich AGPs concentration will be addressed.

The global objective is to establish the relationship between the functional properties and the biochemical composition, structural and physicochemical properties such as interfacial properties and viscosity of natural gums and reconstituted gums.

To reach these objectives, the emulsification process was standardized using microfluidisation as high energy emulsification technology. As dispersed phase, limonene (5 wt%) was selected. Despite its low viscosity which can be a drawback for stability study, it was demonstrated that the difference in interfacial properties between both gums was emphasized compared to more apolar compounds as hexadecane.

The parameters analyzed were the droplet size to estimate the emulsifying ability, and the creaming index and delay time to estimate the emulsion colloidal stability.

This chapter includes:

- A first part which evidences the effect of microfluidisation process parameters on the emulsifying ability of A. *senegal* and A. *seyal* gums.
- A second part in the form of an article which reports the major objectives described above: effect of A. *senegal* and A. *seyal* gum concentrations and high molar mass protein rich AGPs on the emulsifying properties. This article is going to be submitted to "Food chemistry".
- A third part which reports some complementary studies as the effect of strong addition of glycerol on the size and colloidal stability of emulsions and the calculation of the surface load of droplets.

I. Preliminary study - Effect of process parameters on limonene emulsion droplet size using Acacia gums as emulsifier

The most commonly high-energy emulsification techniques are rotor-stator, ultrasound and high-pressure systems such as valve homogenizer and microfluidizer (Jafari, He, & Bhandari, 2007a; Schultz, Wagner, Urban, & Ulrich, 2004).

A preliminary study has demonstrated that ultrasound technology was not adapted to the preparation of limonene emulsions with Acacia gums. Indeed, the combined presence of limonene and gums promoted the damage of the probe and the formation of aggregates. We have turned to microfluidisation which is able to produce emulsions with small droplet size from a large material range and has been already tested with Acacia gum (Zhang, Peppard, & Reineccius, 2015). Moreover, microfluidisation was shown to be more efficient to produce narrow droplet size distribution than ultrasonication for limonene emulsions stabilized by the combination of maltodextrin and surface-active biopolymer (Hi-cap) (Jafari, He, & Bhandari, 2007b).

A high pressure is used in microfluidisation system in order to guide the flow stream through micro-channels toward the interaction chamber where droplet collision occurs along with cavitation and shear allowing to obtain fine emulsion (Maa & Hsu, 1999).

The process parameters influence the final droplet size, and as an evidence, the controlled increase of microfluidizer pressure and number of passes is a determining factor (Jafari, He, & Bhandari, 2006; Zhang et al., 2015). When microfluidisation energy increased over moderate pressure (400-600 bar), an "over-processing" phenomenon, i.e. the droplet re-coalescence occurred (Jafari et al., 2007a).

Therefore, in this section, the effect of microfluidizer pressure and number of passes were firstly studied in order to define adapted parameters for the production of limonene emulsions using A. *senegal* and A. *seyal* gums

The limonene/Acacia gum emulsions were prepared at a fixed concentration of limonene (5 wt%) and a defined concentration for each gum using a microfluidizer at

room temperature (~25°C). The emulsification pressure was varied from 190 to 1310 bar and the number of passes from 1 to 5. The concentrations of Acacia gums at 10 wt% and 20 wt% for A. *senegal* and A. *seyal*, have been selected in regard to the emulsification ability of the two gum species towards limonene (data reported in the following article).

As expected and described in literature (Jafari et al., 2007a; Zhang et al., 2015), the increase of pressure from 190 to 1310 bar using 1 pass induced a decrease of the droplet size ($D_{4,3}$) for emulsions prepared with both Acacia gums (Figure IV.1). Whatever the pressure, the droplet size was inferior to 1 µm for A. *senegal* and never reached this values for A. *seyal*. Moreover, the droplet size decrease was more pronounced for A. *senegal* (31%) than A. *seyal* (21%). Similar decrease was reported for 10 wt% corn oil emulsion stabilized by 5 wt% of A. *senegal* gum: the droplet size decrease reaching 20% (from 0.82 to 0.65 µm) when the pressure of microfluidisation increased from 620 to 1300 bar and with 3 passes (Bai, Huan, Li, & McClements, 2017).

In microfluidisation process, the droplet disruption occurs when the local stresses on droplet become greater than the retaining forces for a sufficiently long time. The droplet size depends on the applied energy density (E_v) as the following equation:

$$D_{4,3} = C.E_v^{-b} \qquad (1)$$

where b is a constant affected by the flow condition in the dispersing volume, C is a constant depending on the efficiency of droplet disruption and E_v is the ratio of the power input on the volume flow rate of emulsions (Stang, Schuchmann, & Schubert, 2001). Since the residence time in microfluidic interaction chamber was short and the input volume of emulsions was small, the energy density corresponded therefore to the pressure input (Jafari et al., 2007a).

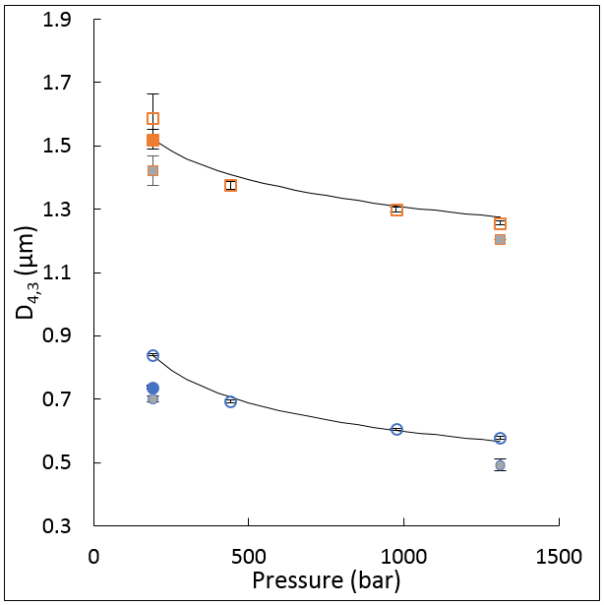

Figure IV.1: Effect of pressure and passes number (1 pass (empty), 3 passes (filled) and 5 passes (gray)) on volume mean diameter $D_{4,3}$ of 5 wt% limonene emulsions stabilized by 10 wt% A. *senegal* (○) and 20 wt% A. *seyal* (□) at around 25°C. The estimated variation of $D_{4,3}$ using equation 1 was represented in line for 1 pass.

Our results showed that the decrease of $D_{4,3}$ as a function of microfluidisation pressure followed a power law with the sum of squared deviations <0.01 and b values of -0.19 and -0.12 for emulsions stabilized by A. *senegal* and A. *seyal*, respectively. The b value was higher for emulsions prepared with A. *senegal* than A. *seyal* which could be related to the difference of concentration used (10 and 20 wt% respectively) and thus viscosity between both gums. Indeed, the former was characterized by two times lower value than the latter in relation to the concentrations used. Bai et al. reported a b value of -0.34 for emulsions of corn oil at 10 wt% produced by 5 wt% A. *senegal* containing 3.2% of protein (Bai et al., 2017). The authors observed difference in the value of b depending on the emulsifier type and highlighted that their values were lower (in absolute value) than previously reported for microfluidisation process (generally between |0.6| and |0.8|). The same trend was reported by Jafari et al. using maltodextrin and modified starch or by Qian et al. using β-lactoglobulin (Jafari et al., 2007a; Qian & McClements, 2011). This could be explained by the nature of emulsifier, i.e. biopolymer which

adsorbs slowly in the case of large molecule retarding the breakup of droplet or favoring the re-coalescence (Bai et al., 2017). The higher absolute value of b found for emulsions produced by A. *senegal* compared to A. *seyal* suggested that microfluidisation treatment is more efficient for A. *senegal*. Our results confirm this as it was observed that the droplet size of coarse emulsions produced before microfluidisation by rotor-stator for A. *senegal* at 10wt% and A. *seyal* at 20wt% was 7.19 ± 0.51 µm and 3.95 ± 0.24 µm, respectively.

Thus, it is important to note that, depending on the homogenizing system, the physicochemical properties and structural conformation of gums were the main factors determining the emulsion droplet size.

A way to increase the process time and favor the droplet disruption is to increase the number of passes. Therefore, it was varied between 1 and 5 at the lowest and highest pressure (Figure IV.1). For emulsions produced with A. *senegal*, a $D_{4,3}$ decrease was observed but was more pronounced at 190 (16%) than 1310 bar (12%). Similar impact was found for emulsions prepared with A. *seyal*, the effect of the number of passes being greater at 190 bar (10%) than at 1310 bar (4%). At the high pressure, the time process increased and could induce a re-coalescence of droplet explaining the weak effect observed for both gums as described by Jafari et al. for modified starch (Jafari et al., 2007a). According to our results, the emulsification pressure showed a higher impact than the number of passes on $D_{4,3}$ decrease. This greater effect of pressure on droplet was previously reported for 5% of Miglyol oil emulsions stabilized by 10% of Acacia gum (Zhang et al., 2015). Comparing the two gums, the droplet size of emulsions produced by A. *seyal* was always higher than those produced by A. *senegal* whatever the process conditions in relation with the poorer emulsification ability of the former. Moreover, the impact of pressure and number of passes was always more pronounced on A. *senegal* than A. *seyal*. Nevertheless, in the remaining experiments, the moderate microfluidisation conditions with one pass at 440 bar was used to study the effect of gum type and amount of high molar mass protein-rich AGPs on limonene emulsions.

II. Publication 2 – Emulsifying properties of Acacia gums: Impact of Acacia gum species, A. *senegal* vs A. *seyal*, and high molar mass protein-rich AGPs content from A. *senegal*.

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Abstract

The emulsifying properties of A. *senegal* and A. *seyal* were compared using limonene (5wt%) as dispersed phase and the microfluidisation technique. The concentration of each gum was varied and the ability to produce stable emulsions was measured through the droplet size and the delay time and creaming index. A. *senegal* suspensions allowed to produced emulsions with smaller droplet size and greater stability during storage than A. *seyal*. These results were related to the biochemical composition (protein and sugar contents) and structural conformation (molecular compactness, flexibility, accessibility of protein moiety) of each gum. The increase of apparent viscosity of Acacia gum dispersions had weak effect on droplet size but strongly impact the emulsion stability. To evidence the importance of high molar mass protein-rich AGPs in the emulsifying properties, reconstituted gums with varied amount of characterized fraction from A. *senegal* rich in these AGPs (0.9 to 29%) were tested at different concentrations (4 to 19.7%). The results showed that to produce emulsions with small droplet size and high emulsion stability, the high molar mass protein-rich AGPs fraction needed to be combined with high total concentration. As for the same protein content, the reconstituted gum allowed a greater emulsion stability compared to original A. *senegal* gum, it can thus be hypothesized that the aggregated form of the AGP plays a major role. This work allowed to anticipate the composition and structural conformation of formulated gums and to adapt it to targeted applications.

Keywords: Acacia gum, AGPs aggregation, emulsifying properties, emulsions, microfluidics, limonene

1. Introduction

Emulsions are extensively used in food, pharmaceutical, cosmetic and petroleum industries (Khan et al., 2011; McClements, 1999; Müller, Petersen, Hommoss, & Pardeike, 2007; Schramm, 1992). Emulsions are generally characterized by two phases or more which are immiscible with each other. They are classified into two types: oil-in-water emulsions (O/W) with the oil droplets dispersed in an aqueous continuous phase, and water-in-oil emulsions (W/O) with the water droplets dispersed in an oil phase (McClements, 1999). In aroma field, the most common type is O/W emulsions that are mainly used after dilution for beverages flavoring or as a prior step in the encapsulation process. The most used and effective emulsifier for aroma compound emulsions is Acacia gum (AG, E414 EC) in relation to its ability to decrease interfacial tension, to form a protective film around emulsion droplet and to stabilize the liquid-liquid interface through steric and electrostatic repulsions and hydration forces (Castellani, Guibert, et al., 2010; Lopez-Torrez, Nigen, Williams, Doco, & Sanchez, 2015; Sanchez et al., 2018). This ability to stabilize the emulsion is crucial to avoid the break down and migration of droplet with time (Padala, Williams, & Phillips, 2009; Zhang et al., 2015; Jafari et al., 2007a) which induce coalescence, creaming or sedimentation but also flocculation and Ostwald ripening (Dalgleish, 1997; Rousseau, 2000). The stability is dependent on the nature, structural conformation, concentration and viscosity of dispersing phase and the inter solubility between dispersed and dispersing phases. In contrast to other hydrocolloids, Acacia gum is characterized by high water solubility, low viscosity at concentrated gum dispersions and good surface properties (Eric Dickinson, 2009; McNamee, O'Riorda, & O'Sullivan, 1998). These properties can be related to the structure of Acacia gum which is constituted by different arabinogalactan proteins (AGP) more or less represented with different molar masses (between 2.8×10^5 and 2.5×10^6 g mol^{-1}) and protein content (0.35 to 12.7%) (Akiyama, Eda, & Kato, 1984; Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Mejia Tamayo et al., 2018; Randall, Phillips, & Williams, 1989; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). The chemical composition, structural and

physicochemical properties also vary according to the origin of gums. This is relevant to the two authorized gums, i.e. A. *senegal* and A. *seyal*, the former being richer in protein (2.7% of protein) than the latter (1% of protein) (Lopez-Torrez et al., 2015). Moreover, A. *seyal* gums have a less branched structure but a higher mean molar mass and compactness than A. *senegal* ones. (Lopez-Torrez et al., 2015).

By hydrophobic interaction chromatography (HIC), Acacia gum is separated into three main fractions historically named arabinogalactan-peptide (AG or HIC-F1), arabinogalactan-protein (AGP or HIC-F2) and glycoprotein (GP or HIC-F3) which differ especially by their molar masses and protein content. The three main fractions are also present in A. *seyal* and as for A. *senegal*, the major component is the arabinogalactan fraction with low protein content. But in A. *seyal* the high molar mass AGP fraction is present in lower extent than in A. *senegal* (Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005). Moreover, a low molar mass protein-rich component was found (fraction 4) in A. *seyal* which does not appear to be present in A. *senegal*. The distribution of protein is different in A. *senegal* and in A. *seyal*: in the latter, protein location is not mainly found in high molar mass fraction. Using ionic exchange chromatography, A. *senegal* is separated into two fractions called IEC-F1 and IEC-F2 (Apolinar-Valiente et al. 2018). The characterization of IEC-F1 using size exclusion chromatography combined with laser light scattering (SEC-MALLS) showed that AGPs from IEC-F1 (M_w = 3.0×10^6 g.mol^{-1}) corresponded to the high molar mass protein-rich AGPs from the classical HIC-F2 and HIC-F3 fractions, whereas IEC-F2 (M_w = 5.2×10^5 g.mol^{-1}) was mainly formed by HIC-F1 fraction. For IEC-F1, a high aggregation was observed which could be due to the high value of ratio arabinose/galactose (1.1), the low content of glucuronic acid group and the high amino acid content (115 mg.g^{-1}). Authors suggested that the high aggregation of IEC-F1 and its flexibility could greatly improve emulsifying properties of Acacia gum.

In this study, we are focusing on the impact of structural and composition of natural Acacia gums and high molar mass protein-rich AGPs on their surface activities in emulsifying process. Regarding the most studied gum, A. *senegal*, the interfacial model

called 'wattle blossom model' was proposed to explain the action of gums: it suggested that the more hydrophobic protein chain anchors at the interface and the protruding hydrophilic carbohydrate blocks attached to proteinaceous chain provide a strong steric barrier against flocculation and coalescence (Islam et al., 1997). It is generally accepted that the surface properties are provided by the protein-rich high molar mass AGPs (Nishino, Katayama, Sakata, Al-Assaf, & Phillips, 2012; Randall, Phillips, & Williams, 1988, 1989). The consequence of the hydrolysis of A. *senegal* using a mixture of proteases was the loss of emulsifying properties according to the significant decrease of protein-rich high molar mass AGPs (Chikamai, Banks, Anderson, & Weiping, 1996). On the other hand, in order to increase the amount of high molar mass AGPs (i.e. aggregates), the production of 'SUPER GUM^{TM}' was done using controlled Maillard reaction on A. *senegal*. The average molar mass of Acacia gum increased from about 4.2 x 10^5 g.mol^{-1} to about 20 x 10^5 g.mol^{-1} suggesting that the maturation process induced the aggregation of AGPs (Al-Assaf, Phillips, Aoki, & Sasaki, 2007). The use of 'SUPER GUM^{TM}' allowed to produce emulsions characterized by small droplet size and better stability (Al-Assaf et al., 2007). This reinforced the assumption that emulsifying properties of gums were greatly influenced by the high molar mass protein-rich AGPs (Al-Assaf et al., 2007; Aoki et al., 2007; Castellani, Guibert, et al., 2010; Xiang et al., 2015). The efficiency of different gum fractions of A. *senegal* to produce and stabilize citrus oil emulsions was studied and the comparison was done on equal nitrogen content (Ray, Bird, Iacobucci, & Clark, 1995). The fraction corresponding to 10.7% of total gum, containing AGPs with mean molar mass of 6.5 x 10^6 g.mol^{-1} and a 0.91% of nitrogen content equivalent to 6% of protein produced emulsions with similar droplet size as the whole gum with 2.2% protein. The use of other fractions, with low mean molar mass of 0.034 x 10^5 g.mol^{-1} and 2.1% protein, allowed to produce lower emulsion droplet size. However, this fraction induced a high instability of emulsion. This study has demonstrated that for comparable protein content, the fractions can produce either good or poor emulsions depending on their composition.

The lowest emulsifying properties of A. *seyal* is generally reported to be linked with its lowest protein content. In addition, the compact conformation of A. *seyal* makes difficult the accessibility of the protein backbone to the interface. Indeed, the high molar mass protein-rich AGPs of A. *seyal* were difficultly hydrolyzed by protease (Flindt, Al-Assaf, Phillips, & Williams, 2005).

Although it was clear that protein content alone was not enough to predict the efficiency of Acacia gum (Dickinson 1991) and that the high molar mass protein-rich AGPs played a crucial role, there are still some confusions. Indeed, Padala et al. reported that protein rich AGPs of Acacia gum adsorbed onto limonene oil droplets with no significant molar mass dependence (Padala et al., 2009). Aggregation process induced by gums maturation was indirectly assumed to play an important role in emulsifying properties of Acacia gum. However, the effect of AGP aggregates naturally present in gums on the emulsifying properties is not clearly demonstrated yet.

The purpose of this study was to understand the effect of natural high molar mass protein-rich AGPs content on the formation and stability of limonene based emulsification. To reach this objective, reconstituted gums with controlled high molar mass protein-rich AGPs concentrations were formulated by mixing two selected fractions: one containing exclusively high molar mass AGPs rich in protein and the other mainly low molar mass AGPs poor in protein. This approach allows to overcome the natural variability of gums and to clearly define the impact of each type of AGPs. In a first stage, the emulsifying properties of A. *seyal* and A. *senegal* have been compared varying the concentration but bearing in mind that the difference between both gums was not limited to their protein and high molar mass protein-rich AGPs content. The conditions of emulsification were selected to easily evidence both emulsifier ability and destabilization phenomena. Pure limonene was used as dispersed phase and moderated microfluidization conditions were applied.

2. Materials and methods

2.1. Materials

Acacia *seyal* (A. *seyal*, lot n° OF110724) and Acacia *senegal* (A. *senegal*, lot n° OF152413) gum powders were provided by ALLAND & ROBERT Company-Natural and organic gums (Port mort, France). The same purification process with elimination of insoluble materials, pasteurization and spray drying were applied on both gums. Their biochemical composition was previously characterized showing a higher protein content in A. *senegal* (2.2%) than A. *seyal* (0.7%) (Lopez-Torrez et al., 2015; Mejia Tamayo et al., 2018).

The reconstituted gums were formulated by mixing two selected fractions of A. *senegal* gum at different rates: the first called HIC-F1 obtained via Hydrophobic Interaction Chromatography (HIC) according to the classical fractionation method (Randall et al., 1989; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006) and the second named IEC-F1 obtained by Ion Exchange Chromatography (IEC) recently characterized (Apolinar-Valiente et al. 2018, submitted article). HIC-F1 showing a low protein content (0.4% of protein) is mainly composed by low molar mass AGPs while IEC-F1 contains only high molar mass protein-rich (11.5% of protein) AGPs (Mejia Tamayo et al., 2018; Apolinar-Valiente et al. 2018). The composition and structural properties of these two fractions, A. *senegal* and A. *seyal* are presented in Supplementary Table IV.1.

Acetate buffer (10 mM, pH 5) was prepared with anhydrous glacial acetic acid and sodium acetate trihydrate powder provided by Merck KGaA (Darmstadt, Germany) and Fluka-Sigma Aldrich (Saint-Quentin Fallavier, France), respectively.

Limonene (97% of purity) used as oil phase for emulsion was purchased from Fluka-Sigma Aldrich (Saint-Quentin Fallavier, France). Glycerol (99% purity) used to adjust the viscosity of gums dispersions was provided by Acros Organics (Ilkirch, France).

2.2. Preparation of the dispersions of Acacia gum and the mixture of fractions

The Acacia gums and fractions powders were dispersed in 10 mM acetate buffer (pH 5) and stirred during 8h at room temperature to ensure the total hydration. The concentrations of gums were based on wet weight of powder and varied between 0.5 and 20 wt% for A. *senegal*, and between 5 and 25 wt% for A *seyal*. The moisture content of gum was 12% and 10% for A. *senegal* and A. *seyal*, respectively. Before use, all gum dispersions were centrifuged at 5 000 rpm and 25°C during 20 minutes to eliminate insoluble matters.

The influence of apparent viscosity of the Acacia gum dispersions on the formation and stability of limonene emulsions was investigated by adding glycerol to A. *seyal* dispersions. For this, 8 wt% of glycerol was added to 20 wt% A. *seyal* dispersion to reach the comparable viscosity of A. *senegal* dispersion at 20 wt%.

The reconstituted A. *senegal* gums were formulated according to an experimental design and resulted from the mixing of HIC-F1 and IEC-F1 fractions: the total concentration varied between 1.3 to 19.7 wt% with IEC-F1 content ranging from 0.9 to 29.1 wt% of the total concentration.

2.3. Methods

2.3.1. Apparent viscosity of Acacia gum dispersions

The apparent viscosity of Acacia gum dispersions without and with glycerol was measured at 25°C using a rotating stress controlled rheometer (RheoCompass MCR 702, Anton Paar, Les Ulis, France) equipped with a sanded cylindrical geometry (cup diameter: 22mm; bob diameter 19.997 mm). The apparent viscosity of samples was measured at increasing shear rate from 0.1 to 1000 s^{-1}. For all dispersions, the flow curves showed a Newtonian behavior with a plateau at high shear rate (data not shown). The apparent viscosity obtained at 100 s^{-1} was used to compare samples.

2.3.2. Preparation of limonene emulsions

The oil in water emulsions were prepared by adding 5 wt% of limonene to 95 wt% of Acacia gum dispersions (original or reconstituted). Coarse emulsions were preliminarily prepared using rotor/stator homogenizer (Silverson L4RT, Evry, France) equipped with a square hole high shear screen stator at 7500 rpm for 5 min at room temperature (~25°C). Afterwards, the coarse emulsions were homogenized using a microfluidizer with a F12Y diamond interaction chamber (LM20, Microfluidics Corporation, MA, USA) using a pressure of 440 bars and 1 pass. In order to control the temperature during the emulsification step, the outlet coil was immersed in a water bath maintained at 25°C. All emulsions were prepared in triplicate.

2.3.3. Emulsion droplet size measurements

The mean droplet diameter and distribution of emulsions were determined by laser light scattering using a Mastersizer 2000 (Malvern Instrument, Orsay, France) with an obscuration of ~10%. Refractive index of 1.33 for water and 1.47 for limonene were used. For all emulsions, three cycles of measurements were performed 10 minutes after the emulsification step. The mean droplet diameter was expressed as the volume mean diameter ($D_{4,3}$):

$$D_{4,3} = \sum n_i d_i^4 / \sum n_i d_i^3$$

where n_i is the number of droplets of diameter d_i.

2.3.4. Emulsion stability measurements

The colloidal stability of emulsions was monitored using a vertical scan analyzer Turbiscan Tower (Formulaction, Toulouse, France) equipped with a pulsed near infrared light source (λ = 880 nm) and two synchronous detectors, a transmission (T) and a backscattering (BS) detectors. 15 ml emulsion sample (equivalent to ~3.5 cm

height) were loaded into cylindrical glass tubes 15 minutes after emulsification and scanned throughout its entire height. The transmittance and backscattering were recorded every 1 min 50 sec during the first 24h before recording one measurement at days 2, 3, 7, 15, 24 and 30 during the storage of emulsions at 25°C. Each sample was analyzed in duplicate. The Turbiscan allows to monitor the emulsion stability in time and space and to characterize the emulsion instability phenomena such as creaming, sedimentation and coalescence.

In order to compare all samples, the creaming index was calculated using the following equation:

$$\text{Creaming index (\%)} = \text{Hs/Ht} * 100$$

Where Hs is the height of the serum layer determined at the apex of ΔBS at the bottom of sample and Ht is the total height of emulsion. The backscattering profiles were plotted in delta mode in order to see the variation of the intensity of BS in comparison to the first measurement.

The time for which the destabilization of emulsions reached 10% called "delay time" was evaluated. It corresponds to the time when the backscattering at bottom zone decreases from 10% of its height (Castel, Rubiolo, & Carrara, 2017).

2.3.5. Experimental design

Response surface methodology was used to evaluate the effect of two independent variables on the droplet size and the stability of emulsions. The first independent variable was the amount of IEC-F1 (x_1, 0.9 – 29.1 % of total concentration) and the second was the total concentration of the A. *senegal* reconstituted gums resulting from the mixing of fractions HIC-F1 and IEC-F1 (x_2, 1.3 – 19.7 wt%). The experiments were planned using central composite design (CCD) according to a 2^2 factorial plan with star points, central point. In Table IV.2, the coded and uncoded independent variables were listed. Eight experimental setting and four central points were carried out randomly. The repeatability of the emulsification method was estimated by repeating

the center points (4 times). The experimental design and data analysis were generated using Statistica version 10 (Paris, France). A second-order polynomial equation was constructed to estimate the responses using the following equation:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2$$

where y is the estimated dependent variable, b_0 a constant, b_i the linear effect coefficient, b_{ii} the quadratic effect coefficient, b_{ij} the interaction coefficient for the regression equation, x_1, x_2 the independent variables (Montgomery, 2011). The adequacy of the models was evaluated by lack of fit, coefficient of determination (R^2) and adjusted R^2 (Adj-R^2) analysis.

3. Results and discussion

3.1. Effect of concentration and gum type on emulsion droplet size and stability

The emulsification property of Acacia gums was studied by producing emulsions with limonene (5 wt%) and A. *senegal* (0.5 to 20 wt%) and A. *seyal* (5 to 25 wt%) gums using a microfluidizer operating at 440 bar and 1 pass.

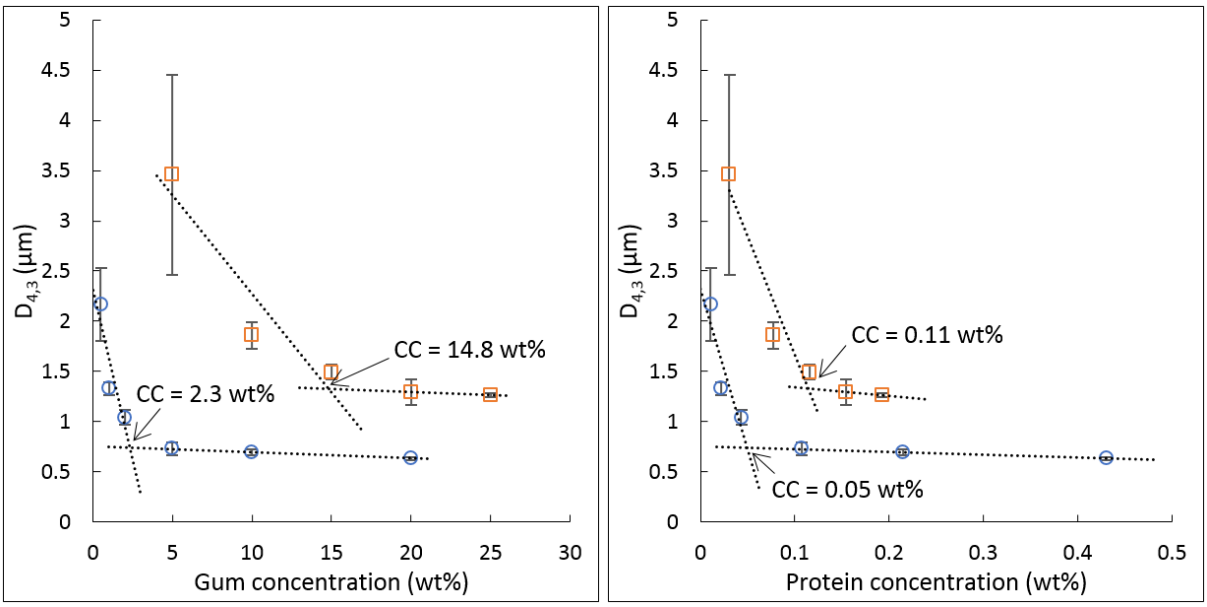
All emulsions, even those produced at the lowest concentrations of Acacia gums, showed a monomodal droplet size distribution with an index of polydispersity close to 1 (data not shown). As expected, the $D_{4,3}$ decreased with the increase of Acacia gum concentration for both gum emulsions (Figure IV.2.A). Two phases can be distinguished in the evolution of $D_{4,3}$ according to the concentration with a strong decrease of $D_{4,3}$ before reaching a quasi-constant value. For the emulsions produced with A. *senegal*, the $D_{4,3}$ decreased from 2.2 µm to 0.7 µm (i.e. decrease of 68%) for concentration ranging from 1 to 5 wt% before reaching a constant $D_{4,3}$ value around 0.65 µm for the

upper concentrations. For the emulsions stabilized by A. *seyal*, $D_{4,3}$ strongly decreased from 3.5 µm to 1.5 µm (i.e. decrease of 57%) for concentrations ranging from 5 wt% to 20 wt% before remaining unchanged ($D_{4,3}$ ~ 1.3 µm) for the upper concentrations. As previously stated for emulsions produced with different emulsifiers using microfluidizer, the two phases are related to different operating parameters: (i) for the first phase characterized by the strong $D_{4,3}$ decrease, the major parameter is the initial emulsifier concentration which is needed to cover the newly formed droplets and prevent their coalescence (ii) whereas for the second phase, the major parameter acting on the droplet size is the energy input provided by microfluidisation (Bai et al., 2017; Charoen et al., 2011; Tcholakova, Denkov, & Banner, 2004; Tcholakova, Denkov, Sidzhakova, Ivanov, & Campbell, 2003). This last hypothesis was clearly confirmed by complementary essays which showed the decrease of $D_{4,3}$ with the pressure increase for emulsions prepared with Acacia gum at concentrations of 10 wt% for A. *senegal* and 20 wt% for A. *seyal* (data not shown).

The Acacia gum concentration between these two phases can be viewed as a critical concentration that was approximated to 2.3 wt% for A. *senegal* and 14.8 wt% for A. *seyal* gum (Figure IV.2.A). It was previously observed that the critical concentration varied depending on both emulsifier properties (surface activity, molar mass, structural configuration) and disperse phase properties (nature, polarity and viscosity) (Bai et al., 2017; Charoen et al., 2011; Ozturk, Argin, Ozilgen, & McClements, 2015). It was found largely inferior, i.e 0.1% for emulsions of Acacia gum using rice bran oil (5%) as dispersed phase and stronger operating conditions (642 bar with 3 passes) and without specification on the composition of Acacia gum that could influence the formation of oil droplets (Charoen et al., 2011). For emulsions of 10 wt% corn oil obtained at 900 bar and 3 passes, a concentration of 3 wt% of A. *senegal* demarked the two regions (Bai et al., 2017). The critical concentrations found in our study were close or higher compared to literature, that can be explained by (i) the unfavorable process condition used (440 bar and 1 pass), (ii) the weak viscosity of limonene compared to oil (0.89 mPa.s versus 50 mPa.s, respectively), (iii) the ratio dispersed phase and gum (iv) the

biochemical composition of Acacia gums, and especially the protein content, that could differ.

A. B.



C. D.

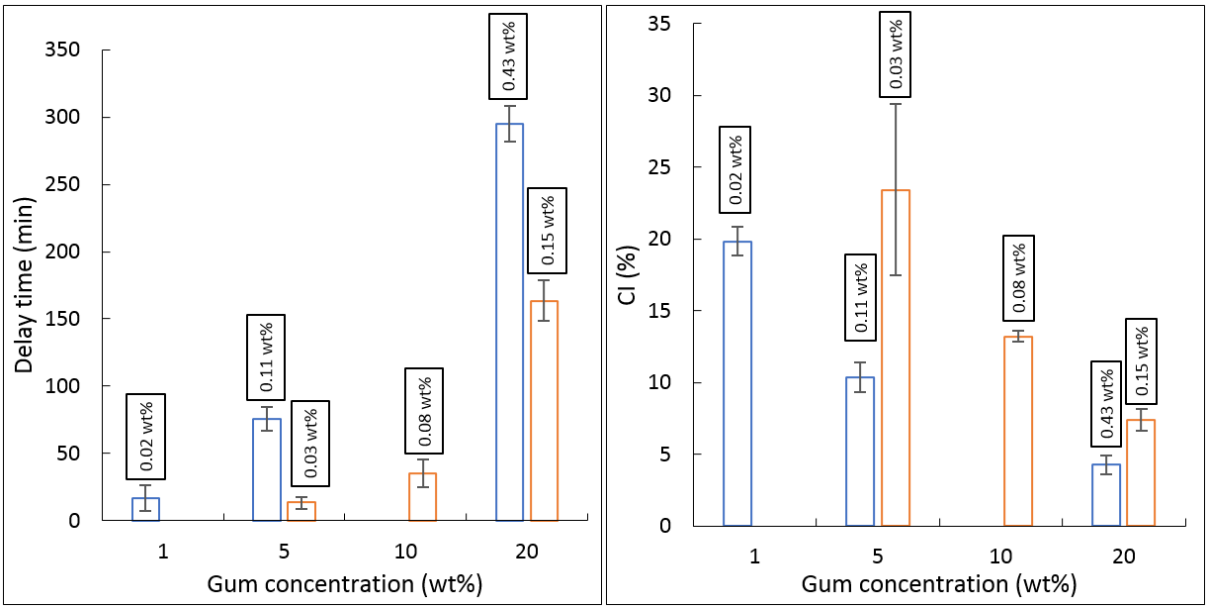


Figure IV.2: Effect of A. *senegal* (blue) and A. *seyal* (orange) and protein concentration on the characteristics of 5 wt% limonene emulsions. The emulsion droplet size was expressed as volume mean diameter ($D_{4,3}$) (A and B) and the critical concentrations (CC) of gum and protein were estimated. The emulsion colloidal stability of emulsions was described by the delay time (C) at 10% of destabilization of emulsions and the creaming index (CI) at 24h of storage (D). Boxes on top of the histogram denotes the protein content within each emulsion (C and D).

It was especially showed that the viscosity of the disperse phase is crucial for the emulsification efficiency. The droplet breakage tended to be more difficult in microfluidizer device when the oil viscosity is too high because the time in the disruption zone is insufficient to the droplet deformation (Qian & McClements, 2011; Schultz et al., 2004; Walstra, 1993). In contrast when the viscosity is too low as for limonene, it can be suggested that the residence time is sufficiently long to favor the deformation and disruption but the risk of re-coalescence could increase (Qian & McClements, 2011).

Whatever the Acacia gum concentration, the $D_{4,3}$ of emulsions produced by A. *senegal* were always lower than the ones produced by A. *seyal*. It is well demonstrated that the surface activity properties of Acacia gums are closely related to their protein-rich AGPs that preferentially adsorbed at interface (Castellani, Al-Assaf, Axelos, Phillips, & Anton, 2010; E. Dickinson, Murray, Stainsby, & Anderson, 1988; Randall et al., 1988; Ray et al., 1995). Dickinson et al. also evidenced a good correlation between the Acacia gum protein content and its emulsifying properties but presumed that other parameters influenced too (E. Dickinson, 2003; E. Dickinson et al., 1988). By reporting the droplet size as a function of the calculated protein rate (Figure IV.2.B), the estimated critical concentration of protein was higher for A. *seyal* (0.11 wt%) than for A. *senegal* (0.05wt%). Hence the protein content was not sufficient to explain the differences observed between the surface activity properties of both Acacia gums. Some differences in the polarity and conformation between the AGPs of both gums could also be advanced. Indeed, the two gums differed by their polarity both in solution or on the surface of structured film, A. *senegal* being characterized by a more hydrophobic surface than A. *seyal* (Aphibanthammakit et al., 2018; Mejia Tamayo et al., 2018). Moreover, the high molar mass protein-rich AGPs from A. *senegal* gum adopted a more extended and flexible conformation than those of A. *seyal* (Lopez-Torrez et al., 2015; Mejia Tamayo et al., 2018). Therefore, the protein backbone of AGPs from A. *senegal* was found to be more accessible to protease, and then to the bulk environment, than that of A. *seyal* (Flindt et al., 2005). Recently, it was reported that the critical

concentration of protein decreased with increasingly exposed hydrophobicity due to an increase of the adsorption rate (Delahaije, Gruppen, Giuseppin, & Wierenga, 2015). This behavior is in well accordance with the lower critical concentration found for A. *senegal* than for A. *seyal*. Moreover, AGPs were not only composed by a protein core but also by some carbohydrate blocks covalently linked to the protein backbone. The carbohydrate blocks contained especially some arabinose and glucuronic acids in various amount according to the Acacia gum type. A. *seyal* gum is richer in arabinose and poorer in glucuronic acid as compared to A. *senegal* gum. As the arabinose promotes intra and intermolecular hydrogen bonding (Chalikian, 1998) and the glucuronic acid provides negative charge, their relative quantity in both gums impacted the aggregate form of AGPs in suspension but also the flocculation/coalescence phenomena between droplets during emulsification process. Then the bigger droplet size obtained with A. *seyal* could also be explained by an increase of flocculation/coalescence between droplets in relation to the higher arabinose and lower glucuronic acid contents. It could be also hypothesized that the higher presence of aggregated AGPs in A. *senegal* promoted the emulsifying activity.

In addition to the study of droplet size after emulsification, the colloidal stability of emulsions was investigated by measuring the changes in backscattering (BS) intensity with time at 25°C using a Turbiscan Tower. BS intensity is closely related to the number and diameter of droplets in emulsions. The ΔBS according to time of some emulsions (i.e. emulsions produced with 1, 5 and 20 wt% of A. *senegal* and 5, 10 and 20 wt% of A. *seyal*) are shown in supplementary data (Supplementary figure IV.1). Whatever the concentrations of both Acacia gums used, all emulsions were unstable following the same behavior. Initially, BS in the middle of the samples decreased suggesting an increase in droplet sizes due to flocculation and/or coalescence phenomena. With time, the decrease of BS in the bottom of emulsions was more pronounced and accompanied by its increase in the top of the samples. Hence, the former phenomenon reflected a clarification process while the BS increase in the top of emulsion corresponded to the increase of droplet concentration due to creaming. The

influence of both gums (variety and concentration) on the destabilization of limonene – Acacia gum emulsions were further studied and compared by determining the delay time (Figure IV.2.C) and calculating the creaming index (CI) at 24 hours of storage at 25°C (Figure IV.2.D). The increase of Acacia gum concentration improved the stability of emulsions by both delaying the instability mechanism and reducing the creaming index (CI). The delay time increased from 17 to 295 minutes and 14 to 164 minutes for concentrations ranging from 1 to 20 wt% for A. *senegal* and 5 to 20 wt% for A. *seyal* (Figure IV.2.C). In the same way, the CI values decreased from 19.9 to 4.2 % and 23.4 to 7.4 % for concentrations ranging from 1 to 20 wt% for A. *senegal* and 5 to 20 wt% for A. *seyal* (Figure IV.2.D). As expected for a same concentration of gums, the delay time for A. *senegal* was always higher than for A. *seyal* and the CI was always lower highlighting the best colloidal stability properties of A. *senegal* gum. These results can be related to several parameters. The bigger size of droplets combined to the low viscosity of A. *seyal* dispersion favoring the move of droplets could intensify the creaming rate compared to the emulsions produced by A. *senegal*. Moreover, A. *seyal* contains more arabinose groups (Supplementary Table IV.1) which are implied in hydrogen bounding and could induce new liaisons between droplets favoring the emulsion instability. Moreover, the difference in stability between emulsion produced by both gums could be amplified by the higher uronic acid content over neutral sugars of A. *senegal* (ratio of 0.23) compared to A. *seyal* (ratio of 0.16) (Mejia Tamayo et al., 2018). Then the highest negative charge of A. *senegal* carbohydrate part could favor the electrical stability of emulsion through electrostatic repulsions compared to A. *seyal*. Moreover, although the similar effect of two gums on lowering interfacial tension, A. *senegal* gum reached faster the maximum value of viscoelastic modulus than A. *seyal*. This indicated a greater ability to form a protecting film (Aphibanthammakit PhD thesis Chapter 3, 2018). Therefore, this could also explain the weaker flocculation/coalescence observed during the storage of emulsion with A. *senegal* gum. The value of delay time remained weak compared to the value of 168 h found for an emulsion prepared with 20 wt% Acacia gum and corn oil (10 wt%) but obtained by

ultrasound (Castel et al., 2017). In the same manner, the CI was lower than the value observed in this study for limonene emulsion produced by A. *senegal*. It is obvious that the disruption process of droplet was different giving initial droplet size of corn oil (546 nm) lower than the one found in our conditions suggesting a link between stability and droplet size. However, the polarity and viscosity of the two oil phases strongly differ, corn oil being more viscous (52.3 mPa.s at 23.9°C for corn oil against 0.89 mPa.s for limonene at 25°C) and more apolar than limonene which could induce the greater quality of emulsions (Clará, Gómez Marigliano, Campos, & Sólimo, 2010; Noureddini, Teoh, & Clements, 1992).

As already discussed, the two gums differed in viscosity, i.e. A. *senegal* being more viscous than A. *seyal*. According to the Stoke's law, the creaming rate depends on the droplet size but also on the viscosity of the continuous phase. By increasing the viscosity, the droplet velocity decreases and the stability of emulsion against gravitational separation increases (McClements, 1999; Risch & Reineccius, 1988; Walstra, 1993). To confirm the importance of gum viscosity on colloidal stability, the viscosity of A. *seyal* solution at 20 wt% was adjusted to that of A. *senegal* at 20 wt% by the addition of glycerol (8 wt%) before the preparation of limonene-gum emulsion and the characterization of its droplet size and colloidal stability. While the droplet size was unchanged compared to the emulsion produced by A. *seyal* without glycerol, the colloidal stability was improved in presence of glycerol (Table IV.1). Indeed, the delay time and CI of emulsion prepared with A. *seyal* containing glycerol were respectively around 2 times higher and 1.7 times lower than those of emulsions prepared with only A. *seyal*. Moreover, the colloidal stability was found to be similar to that of emulsions prepared with A. *senegal* gum at 20 wt% presenting the same apparent viscosity. From this result, it could be concluded that at the high concentration of Acacia gum, the quantity of AGPs and protein was in sufficient amount to cover the surface of droplet and the increase of viscosity of continuous phase did not affect the droplet size but it played a crucial role in the colloidal stability of emulsions. The increase of bulk viscosity is known to favor the stabilization by steric effect (Jin et al.,

2017). An increase of the stability of orange oil emulsion has also been observed by adding glycerol to Acacia gum (Mirhosseini, Tan, & Taherian, 2008). In this case, the glycerol addition induced pH changes and increase of the negatively charged ζ-potential and the repulsive forces. In addition to viscosity increase, the stabilization effect of glycerol could be also explained by its contribution to the charge of the A. *seyal* suspension. It is worth noting that desionised water was used to prepare gum dispersion in the study reported by Mirhosseini et al. while we used acetate buffer at 10 mM and pH 5. In their case, the pH increased from 4.0 to 4.4 and the absolute value of ζ-potential from 25.6 to 28.9 mV with the glycerol addition of 1.5 wt%. Therefore, as our suspension was prepared in buffer, the change of ζ-potential should be less pronounced than the one observed by Mirhosseini et al. To confirm this hypothesis, the effect of glycerol on pH and ζ-potential need to be investigated.

Table IV.1: Volume mean diameter ($D_{4,3}$) and stability expressed in delay time and CI of 5 wt% limonene emulsions as a function of apparent viscosity of Acacia gum aqueous phase.

Aqueous phase	Glycerol concentration (wt%)	Apparent viscosity at 100 s^{-1} (mPa.s)	$D_{4,3}$ (µm)	Delay time (min)	CI (%)
A. *seyal* at 20 wt%	0	29.4±1.2	1.30±0.13	164±15	7.4±0.7
A. *seyal* at 20 wt%	8	39.6±0.6	1.34±0.005	339±84	4.4±0.1
A. *senegal* at 20 wt%	0	41.0±0.4	0.66±0.03	295±13	4.3±0.7

In short, the better emulsifying properties of A. *senegal* in terms of droplet size and colloidal stability was confirmed. The increase of concentrations and by consequence of the protein content of A. *seyal* even going beyond the critical concentration of A. *senegal* showed low effect suggesting that the structural conformation of AGPs in A. *senegal* gums is a major factor defining emulsion droplet size. The assessment of Acacia gums dilatational rheology at 25°C using 5wt% of gums showed that A. *senegal* was able to reach faster the maximum value of viscoelastic modulus at limonene surface compared to A. *seyal* (Aphibanthammakit PhD thesis Chapter 3, 2018). Therefore, the smaller emulsion droplet size produced by A. *senegal* could be *inter alia* in relation to its greater interfacial properties than A. *seyal*.

3.2. Effect of high molar masses protein-rich AGPs on emulsion droplet size and stability

The high molar mass protein-rich AGPs are generally accepted to be mainly responsible of the emulsifying properties of Acacia gum. The analysis of emulsion continuous phase using gel permeation chromatography (GPC) showed that only a small quantity of AGPs which corresponded to the high molar mass protein-rich AGPs were adsorbed to the oil droplet interface (Flindt et al., 2005; Randall et al., 1988). Furthermore, a linear relationship between emulsion stability and both molar mass of A. *senegal* gum and high molar mass AGPs content was previously reported (Nishino et al., 2012). However, these studies indirectly determined the relationship between the quantity of AGPs and the emulsifying properties of Acacia gums through the desorption of interfacial molecules which could induce error during experimentation (Katayama et al., 2006; Mikkonen, Xu, Berton-Carabin, & Schroën, 2016; Randall et al., 1988). Additionally, when the comparison of high molar mass protein-rich AGPs content was done, Acacia gums from different origins (batches) were used with as a consequence potential differences in their biochemical and structural properties, and therefore in their emulsifying properties (Nishino et al., 2012).

Herein the role of high molar mass protein-rich AGPs content and conformation in emulsifying properties of A. *senegal* gum was investigated using a reconstituted gum formed by mixing two fractions (IEC-F1 and HIC-F1) obtained from the same batch of Acacia *senegal* gum. These two fractions differed totally by their protein and high molar mass protein-rich AGPs contents. HIC-F1 was characterized by a low protein content (0.45%) and a mean molar mass of 3.5×10^5 g.mol^{-1} ; while, IEC-F1 that contained only high molar mass protein-rich AGPs presented a protein content of 11.5% and a mean molar mass of 3.0×10^6 g.mol^{-1}. Moreover, this fraction is rich in aggregates. Indeed, it was reported by dynamic light scattering measurements that IEC-F1 presents three molecular populations of AGPs with R_H around 17, 75 and 250 nm confirming the aggregation behavior of high molar mass protein-rich AGPs (Apolinar-Valiente et al., 2018, submitted article). By varying the proportion and the amount of these two

fractions in the reconstituted gum, it was possible to control and modify both the protein and high molar mass protein-rich AGPs content of this newly formed gum.

Table IV.2: Table of ANOVA for the experimental variables as a linear, quadratic and interaction terms of each response variable and corresponding coefficients for the predictive models.

Source	$D_{4,3}$ (µm)		Delay time (min)		CI (%)	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
b_0	1.960*	<0.0001	96.610	0.0701	7.036*	0.0033
Linear						
b_1	-0.045*	<0.0001	-8.206	0.0628	0.107	0.2076
b_2	-0.125*	<0.0001	-0.917	0.8515	-0.453*	0.0233
Quadratic						
b_{11}	0.0006*	0.00029	0.102	0.2723	-0.0027	0.2337
b_{22}	0.0033*	<0.0001	0.657*	0.03506	0.01516*	0.037
Interaction						
b_{12}	0.0012*	0.0003	1.200*	0.00388	-0.005	0.1015
Lack of fit		0.00036*		0.5178		0.4261
R^2	0.9307		0.995		0.931	
Adj-R^2	0.873		0.990		0.874	

b_0 : a constant. b_i: the estimated linear coefficient of the quadratic polynomial equations. b_{ii}: the estimated quadratic coefficient of the quadratic polynomial equations. b_{ij}: the estimated interactive coefficient of the quadratic polynomial equations. 1: FA content; 2: total concentration of gum. * stands for a significant value.

To avoid numerous experiments by changing one parameter while the others are kept constant and as the amount of original matter was limited, a response surface methodology was used to study the effect of IEC-F1 content (0.9 – 29.1 % of total concentration) and total concentration (IEC-F1 and HIC-F1 mixture varying between 1.3 – 19.7 wt%) on droplet size ($D_{4,3}$) and emulsion colloidal stability (delay time and CI). The randomized runs of the experiments and experimental responses were presented in Supplementary table IV.2. The second-order polynomial response surface model was carried out on the values of each measured variable (Supplementary table IV.2). Analysis of variance (ANOVA) was used in order to determine the statistical significance of regression coefficients and for the fitting of the model. Table IV.2 showed the estimated coefficients of regression of the model for the response variables and the

corresponding coefficients of determination (R^2) and adj-R^2. A significant lack of fit (p-Value < 0.05) indicates the failure of the model representing the experimental data and the response predictor is discarded (Koocheki, Taherian, Razavi, & Bostan, 2009). If the response surface model fitted for the response variable, it indicated that the variable was assessed as a function of linear, quadratic and interactions effects of IEC-F1 content and fraction mixture content. As specified by Montgomery (2001), a model is well fitted to the experimental data if it presents a significant regression and a non-significant lack of fit (Montgomery (2001)).

Despite a relative high coefficient of determination (R^2) and adjusted R^2 (adj-R^2), a significant lack of fit (p value < 0.05) was observed for the $D_{4,3}$ variable indicating that the proposed model is not adapted to predict the experimental values as illustrated by the graphic comparison between predicted and experimental value (Supplementary figure IV.2). As the coefficients of regression for each variable were characterized by significant p-value, it was not possible to propose a reduced model. In order to achieve a more adequate model, complementary experiments inducing emulsions with larger droplet size are specifically needed. Additionally, it can be expected that the variability of $D_{4,3}$ was higher when large droplet was obtained, i.e. for low content of IEC-F1 and low total concentration. Indeed, if the repeatability of $D_{4,3}$ was good (variation coefficient of 1.22%), it has been determined for the central points (15% of x_1 and 10.5 wt% of x_2) and reflected only the variation for small droplet size.

By contrast, the two others variables used to characterize the stability (CI and delay time) were well predicted by the regressions models with a non-significant lack of fit (p value < 0.05) and high R^2 and adj-R^2 values (Table IV.2). Despite the non-significant p-value for some variables, they were kept in the model. The adequacy of the polynomial regression model was confirmed since the experimental results and predicted values were in good agreement (Supplementary figure IV.2). The model allowed to identify the major effect of each independent variable on the stability of 5 wt% limonene emulsions.

Regarding the regression coefficients of the model of delay time (Table IV.2), the only significant p-values were found for quadratic effect of total concentration and interaction effect. This latter was the major effect on delay time. In contrast, the ANOVA study of regression coefficients of CI indicated that linear and quadratic effects of total concentration were significant and that the major effect was induced by the linear effect. This highlighted the importance of total concentration for both stability parameters. However, the delay time showing the fast loss of colloidal stability was affected by protein concentration through the interaction effect while for CI measured after 24h of storage, it was the total concentration which played the major role on variation values.

In order to evidence the relationship between IEC-F1 content and total fraction concentration in the mixture on the different selected variables ($D_{4,3}$, delay time and CI), the experimental values are represented in Figure IV.3 using the easiest optimization technique called "one variable at a time" or using the surface response (Figure IV.4). Figure IV.3.A clearly showed that $D_{4,3}$ decreased with the increase of IEC-F1 content, i.e. the high molar mass protein-rich AGPs content. Moreover, the effect of IEC-F1 content on $D_{4,3}$ was greater in the emulsions with the lowest total concentration, i.e. for total concentration of 4 wt% the reduction of droplet size was around 49 % compared to total concentration of 17 wt% with 22% decrease of $D_{4,3}$. This result demonstrated the combined effect between IEC-F1 content and total gum concentration on droplet size. High IEC-F1 content was especially useful to decrease the emulsion droplet size when the emulsions are characterized by low Acacia gum concentrations. This confirmed that high molar mass protein-rich AGPs adsorbed preferentially on droplet surface allowing to stabilize the newly formed droplet and preventing against re-coalescence during emulsification process. This could be due to the high protein content and the greater molecular flexibility of AGPs of IEC-F1 which favored their location at the droplet interface (Apolinar-Valiente et al., 2018, submitted article).

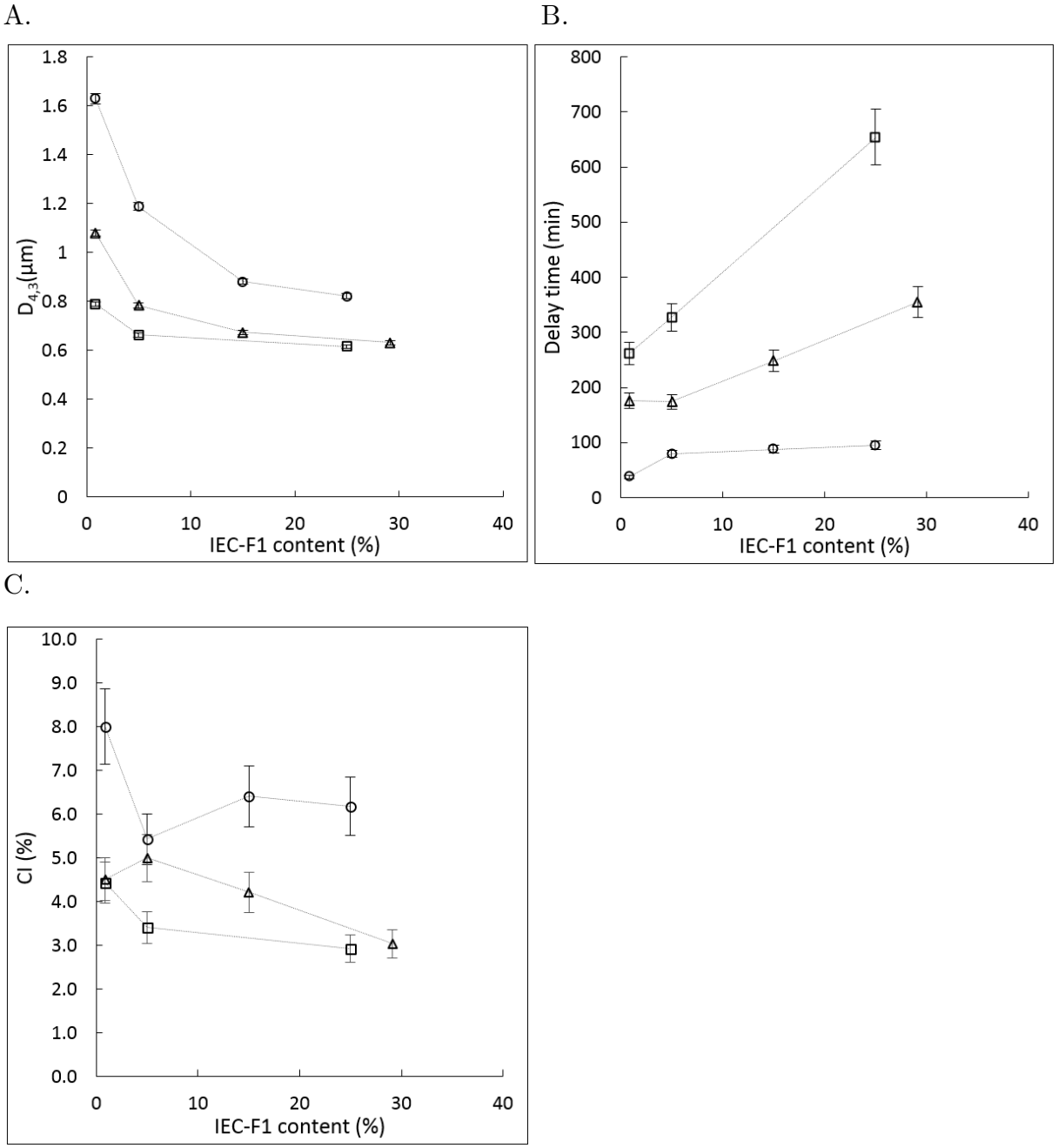


Figure IV.3: Effect of IEC-F1 content and concentration of reconstituted gums (4 wt% (○), 10.5 wt%(Δ) and 17wt% (□)) on volume mean diameter $D_{4,3}$ (A), delay time (B) and CI (C) of 5wt% limonene emulsions. The lines were drawn to guide the eyes.

The delay time was affected by total concentration and IEC-F1 content. At high total concentration, the increase of delay time was more pronounced (Figure IV.3.B). The slight decrease of total concentration, for example from 22 to 15 wt%, needed to be compensated by a strong increase of IEC-F1 content, from 15 to 35% to obtain the

same delay time (Figure IV.4.A). This compensation was less pronounced at the high total concentration. For a given total concentration, the CI slightly varied with the IEC-F1 content (Figure IV.3.C). However, the increase of total concentration was prevalent for the decrease of CI. To decrease the CI, a sufficient content of IEC-F1 is needed ($>$ 20%) but even if the content of IEC-F1 is high, the total concentration should be enough ($>$10 wt%) to avoid strong variation of CI (Figure IV.4.B). These results showed that to avoid coalescence of droplets at relative long time (weak CI) a sufficient concentration of gum should be used. This result confirmed the crucial role of bulk viscosity but also to electro-steric repulsions. These latter were favored by the presence of high molar mass protein-rich AGPs from Acacia gum which contained higher amount of uronic acids (18.3% of whole sugar) as reported by Apolinar Valiente et al. (Apolinar Valiente et al. 2018 submitted article). Moreover, it was shown that IEC-F1 was able to adsorb at the interface of hexadecane allowing to decrease interfacial tension and to form elastic interfacial film (Aphibanthammakit PhD thesis Chapter 3, 2018).

The effect of AGPs on emulsifying properties of Acacia gum appeared to be directly linked to the protein or nitrogen content (Anderson & Weiping, 1991; E. Dickinson et al., 1988; Ray et al., 1995). To confirm this assumption, the protein and high molar mass protein-rich AGPs contents of emulsions were calculated using the proportion and biochemical composition of each fractions in reconstituted A. *senegal* gums, but also in initial A. *senegal* gums using the biochemical composition mentioned in Supplementary Table IV.1. The impact of protein and high molar mass protein-rich AGPs concentration on the emulsifying properties of gums is summarized in Figure IV.5.

A. B.

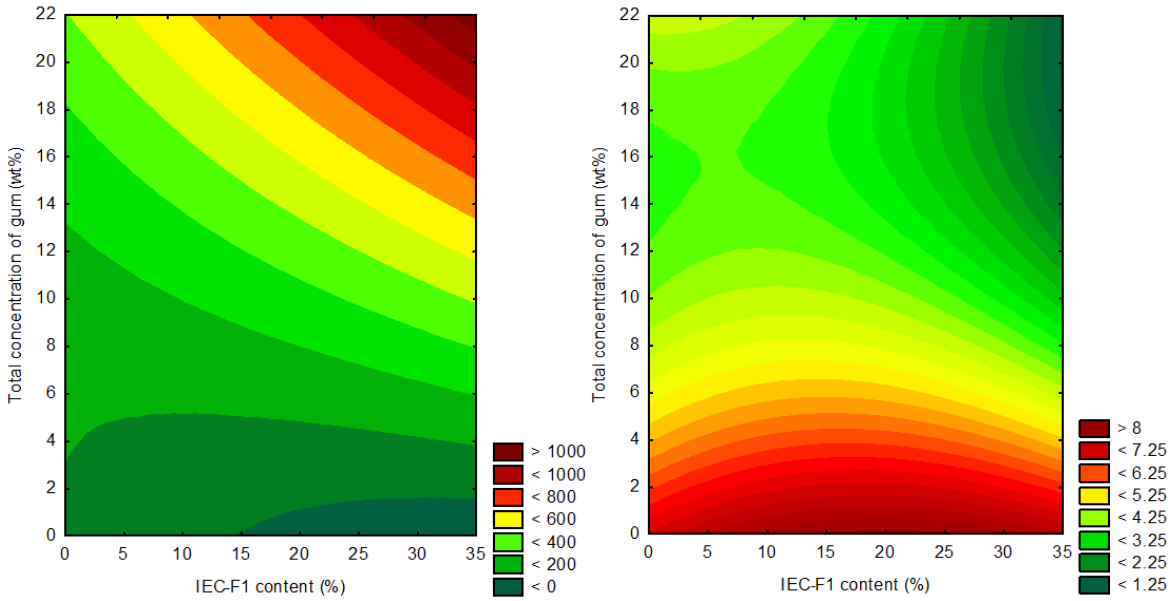
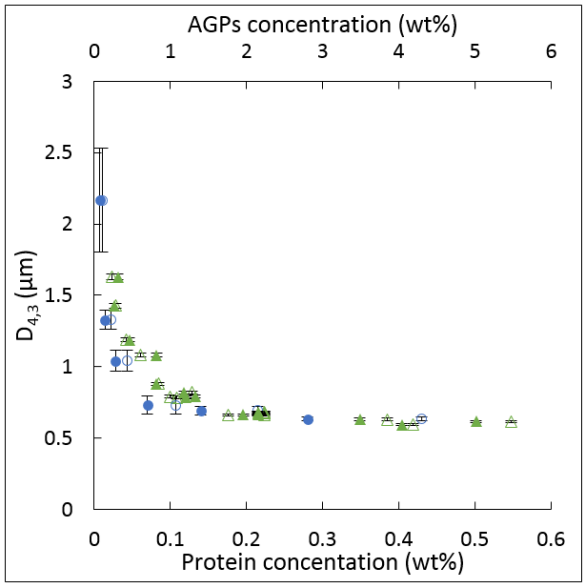


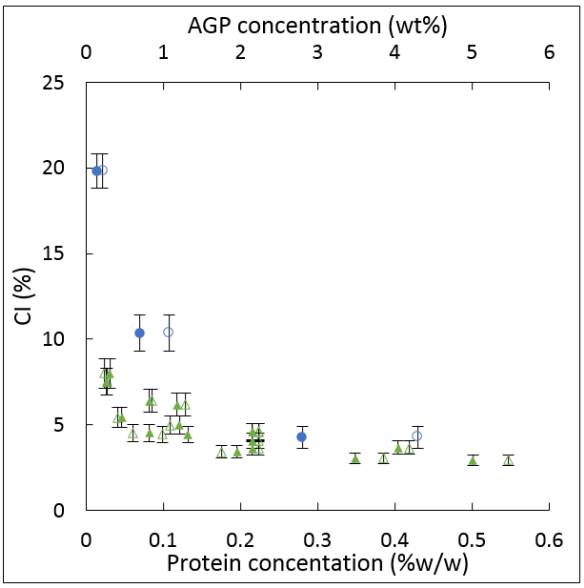
Figure IV.4: Response surface effect of IEC-F1 content and total concentration on delay time (A) and CI (B) of 5 wt% limonene emulsions produced by reconstituted gums.

$D_{4,3}$ drastically decreased up to protein concentration of 0.15 wt% for all emulsions. It means that an increase of protein content above 0.15 wt% or 2 wt% of high molar mass protein-rich AGPs did not allow a further decrease of $D_{4,3}$. This protein or high molar mass protein-rich AGPs contents were determined for both initial and reconstituted A. *senegal* gum. This estimated critical protein concentration was in agreement with the one found for emulsions produced with proteins (Delahaije et al., 2015). As similar droplet size was observed between emulsions produced by reconstituted and initial gums, the reconstitution of gums using fractions is therefore a good approach to understand the emulsifying properties. Randall et al showed that the minimal droplet size was reached using 12% of Acacia gum which corresponded to 0.227 wt% of protein in solution. Our results were in agreement with these data. They also calculated that only 30% of total protein were adsorbed on droplet surface (Randall et al., 1988).

A.



B.



C.

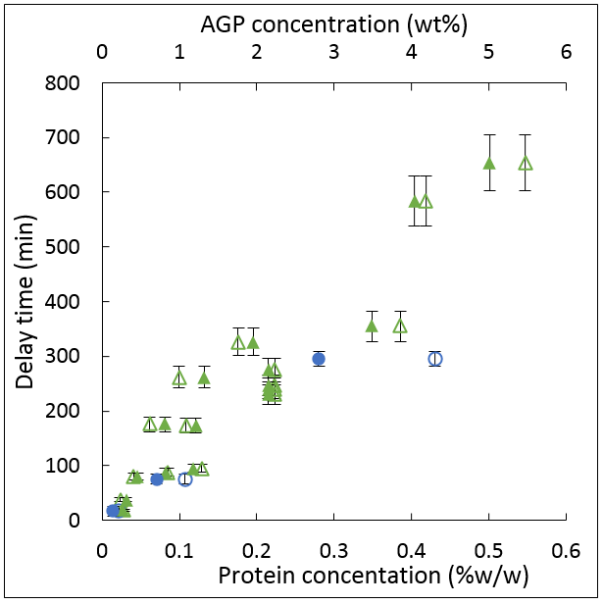


Figure IV.5: Effect of protein (empty) and AGPs (filled) concentration from initial (circular symbol) and reconstituted A. *senegal* gums (triangle symbol) on volume mean diameter D4,3 (A) and stability expressed in CI (B) and delay time (C) for 5wt% limonene emulsions.

When initial A. *senegal* was used, CI strongly decreased with the protein concentration increase. For reconstituted gums, the CIs were already weak at low protein concentration and slightly decreased with the protein concentration (Figure IV.5.C). For the same protein content of 0.1 wt%, CI was two times higher for the emulsion

produced with initial A. *senegal* than reconstituted gum. For the delay time, the same difference of behavior between the initial and reconstituted gum was observed. The delay time stopped to increase with the protein concentration (above around 0.2 wt%) when the initial A. *senegal* gum was used. This showed that the reconstituted gum was more efficient to stabilize emulsions. Several explanations could be suggested such as the difference in interfacial film properties, the viscosity of system, the greater flexibility of IEC-F1 and the presence of aggregated AGPs. The interfacial rheological films properties of IEC-F1 were investigated compared to the ones of A. *senegal* (Aphibanthammakit PhD thesis Chapter 3, 2018) and it was observed that IEC-F1 at 0.415 wt% was able to form interfacial films with a similar rheological characteristic as A. *senegal* at 5 wt% and if the concentration of IEC-F1 was increased, the interfacial properties increased too in contrary to the behavior of A. *senegal*. Although the minerals content was not the same as for the fraction used in this chapter, the similar ability of IEC-F1 to form interfacial film could be an argument to explain the highest efficacy of reconstituted A. *senegal*. Moreover, the higher viscosity of IEC-F1 dispersions compared to A. *senegal* ones at a same concentration could also describe the difference of behavior. Indeed, this difference in viscosity was due to the higher content of high molar mass AGPs in IEC-F1 that were characterized by a higher intrinsic viscosity (Apolinar-Valiente et al., 2018, submitted article). Apolinar-Valiente et al. reported that IEC-F1 was a more flexible molecule compared to A. *senegal* (Apolinar-Valiente et al., 2018, submitted article). This greater flexibility could induce greater interfacial properties of IEC-F1 thus better emulsifying properties. Castellani et al. reported a good correlation between the emulsifying properties of matured gum rich in aggregates and its ability to lower interfacial tension and to form interfacial film (Castellani, Al-Assaf, et al., 2010). This was in agreement with the conformation of macromolecules constitutive of the fractions. Accordingly, Xiang et al. suggested that the stabilization of the conjugated linoleic acid (CLA) interface droplet was due to the aggregation and rearrangement of protein conformation of AGP rather than the selectively adsorption of AGP of high molar mass (Xiang et al., 2015). Moreover, this

aggregation increased with Acacia gum concentration. In our case, it could be suggested that more AGP aggregation occurred when reconstituted gum was used at high concentration favoring the emulsion stabilization. It was also possible that the ζ-potential of the reconstituted gums differed from those of initial gums for a given protein content. A high absolute value of ζ-potential (>25 mV) is indicative of a deflocculated emulsions (Mirhosseini et al., 2008) thus a greater emulsion stability than a low absolute value of ζ-potential (<25 mV) which indicates flocculated emulsions. To confirm this hypothesis, ζ-potential of emulsions produced by reconstituted gum should be measured. However, this hypothesis can be already presumed based on the higher glucuronic acid content of reconstituted gum compared to initial gum (Supplementary Table IV.1).

It is worth noting that, in our case, the results were specific for the limonene emulsions produced at the selected conditions of emulsification (pressure and passes number). The change in emulsification parameters such as oil phase type and concentration, addition of weight agent, pH and emulsification technique can influence the emulsifying properties of Acacia gum (E. Dickinson, Galazka, & Anderson, 1991).

4. Conclusion

In this study, the role of high molar mass protein-rich AGPs in emulsifying properties of Acacia gum was investigated by two ways (i) comparing A. *senegal* and A. *seyal* gums which differed in the content of the specific AGPs and protein but also in their sugar composition and structural conformation, and (ii) varying the amount of high molar mass protein-rich AGPs in reconstituted gums.

Comparing the two gum species (A. *senegal* and A. *seyal*), for all range of concentrations, the $D_{4,3}$ of limonene emulsions produced with A. *senegal* were always lower than the ones produced with A. *seyal*. Accordingly, the critical concentration was lower for A. *senegal* compared to A *seyal* but surprisingly not in terms of protein content. For all conditions, the flocculation and/or coalescence revealed by creaming

phenomena affected the emulsion stability. At the same concentration of gums, the colloidal stability was greater (higher delay time and lower CI) for emulsions produced by A. *senegal* than the ones produced by A. *seyal*. These results suggested that the biochemical composition (protein and sugar content) and structural properties (molecular compactness, flexibility, accessibility of protein moiety, aggregates content) and relative polarity of the two gums affected their emulsifying properties. A. *senegal*, having a more hydrophobic character, a higher protein and glucuronic acid content, a greater molecular flexibility leading to high accessibility of protein moiety is characterized by better emulsifying properties than A. *seyal*. This latter possesses a greater arabinose content which could cause intra and intermolecular hydrogen bonding leading to flocculation/coalescence of droplet during process and storage. The differences in viscosity of both gums impacted especially the emulsion stability as confirmed by the addition of glycerol to emulsions produced by A. *seyal*.

Regarding the reconstituted gums, the results highlighted the combined effect between protein content and total gum concentration on the capacity of low droplet size formation by Acacia gum. Both response surface and "variable at a time" representations confirmed the importance of protein content and total concentration for a short term stability of emulsions (Delay time) while the total gum concentration seemed to play a major role in the long term stability of emulsions (CI). This study emphasized the importance of structural conformation of the high molar masses protein-rich AGPs. As the rate of the aggregated forms was higher in water suspensions compared to acetate buffer (pH 5, 10 mM) suspensions, it can be anticipated that the emulsifying properties will be better by the selection of good solvent but also of the concentration.

Acknowledgements

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Supplementary data

Supplementary table IV.1: Biochemical composition of A. *senegal*, A. *seyal*, fractions isolated by HIC (HIC-F1) and IEC (IEC-F1) from A. *senegal* in dry basis (mean ± standard deviation).

Component ($mg.g^{-1}$)	A. *senegal**	HIC-F1*	IEC-F1**	A. *seyal**
Total sugars[a]	944.4	965.46	861.1	978.6
Galactose	382.5	374.9	296.9	334.5
Arabinose	285.2	257.6	314.7	474.3
Rhamnose	117.1	120.2	113.5	31.3
Glucuronic acid	168.1	195.1	153.3	75.3
4-O-me-glucuronic acid	9.4	13.5	8.9	62.6
Proteins	21.5	4.04	114.9	7.1
Minerals	34.1	30.5	24.0	14.3
Molar mass (M_w, $g.mol^{-1}$)	6.8×10^5	3.5×10^5	30×10^5	7.1×10^5
Mw $> 7.5 \times 10^5$ $g.mol^{-1}$ (%)	14	7	97	20

* adapted from Mejia Tamayo et al., 2018.

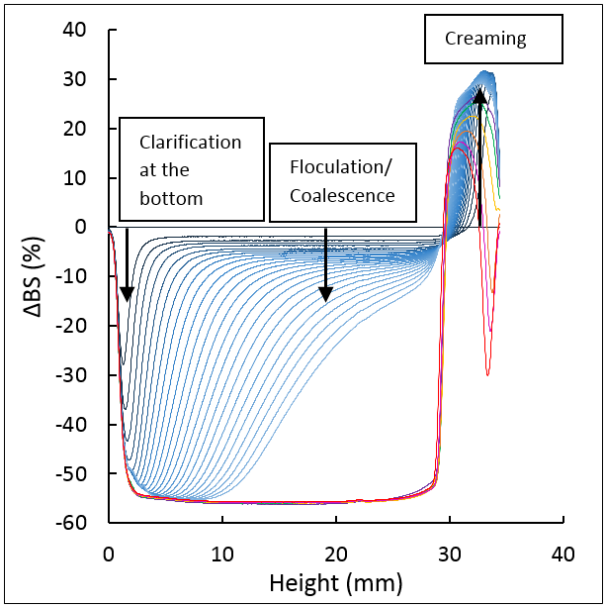
** adapted from Apolinar-Valiente et al., 2018.

Supplementary table IV.2: Matrix of the central composite design of two variables in units along with the experimental response.

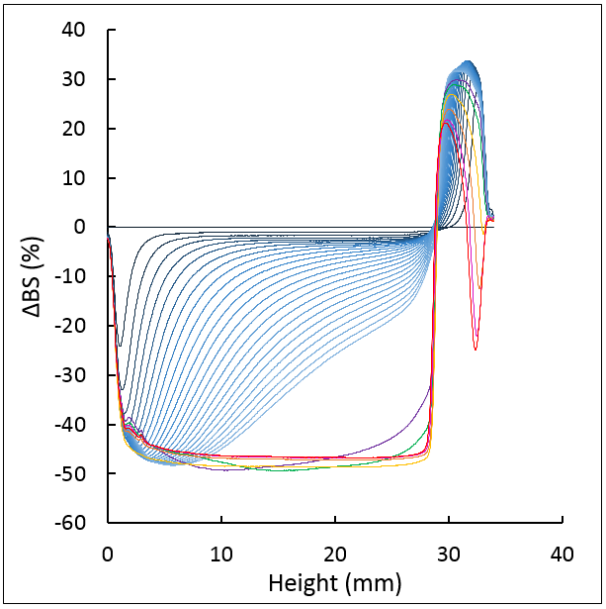
Treatment runs	IEC-F1 content (%)	Total concentration of gum fractions mixture (wt%)	Response variable		
			$D_{4,3}$ (µm)	CI at 24h (%)	Delay time (min)
1	5	4	1.188	5.425	80
2	5	17	0.663	3.408	327
3	25	4	0.821	6.176	95
4	25	17	0.615	2.922	654
5 (C)	15	10.5	0.672	4.059	241
6 (C)	15	10.5	0.677	3.633	275
7*	0.9	10.5	1.080	4.510	176
8*	29.1	10.5	0.631	3.036	355
9*	15	1.3	1.429	7.516	18
10*	15	19.7	0.594	3.659	584
11(C)	15	10.5	0.662	4.551	247
12(C)	15	10.5	0.681	4.585	230

C, center point; *, star point (axial).

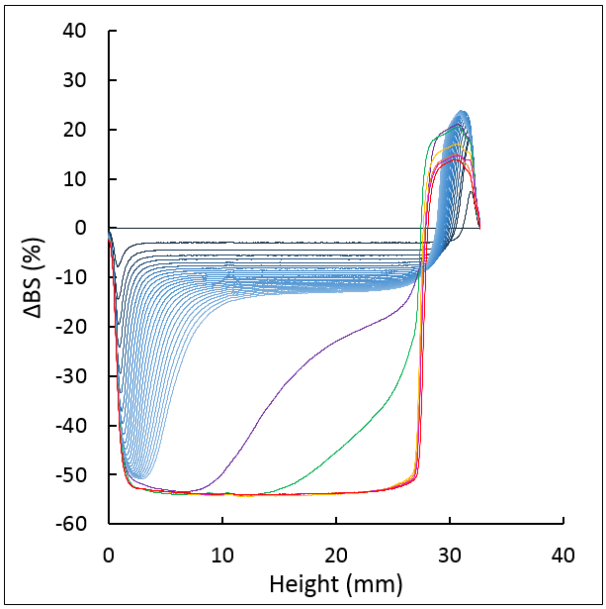
A.


Creaming
Clarification at the bottom
Floculation/ Coalescence
ΔBS (%)
Height (mm)

D.


ΔBS (%)
Height (mm)

B.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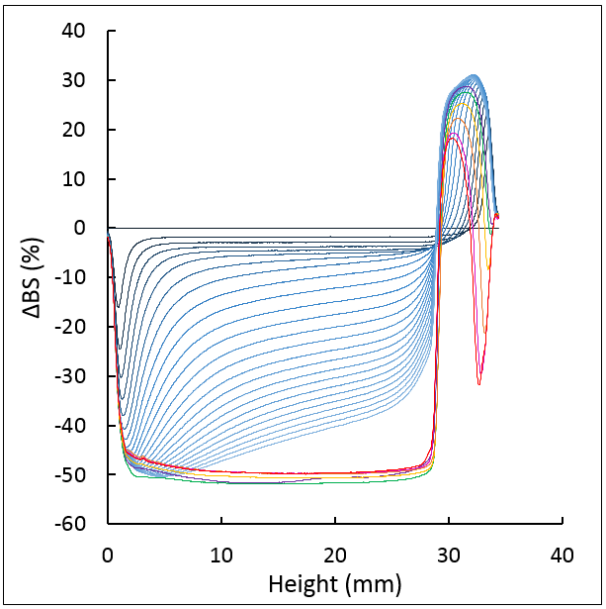
ΔBS (%)
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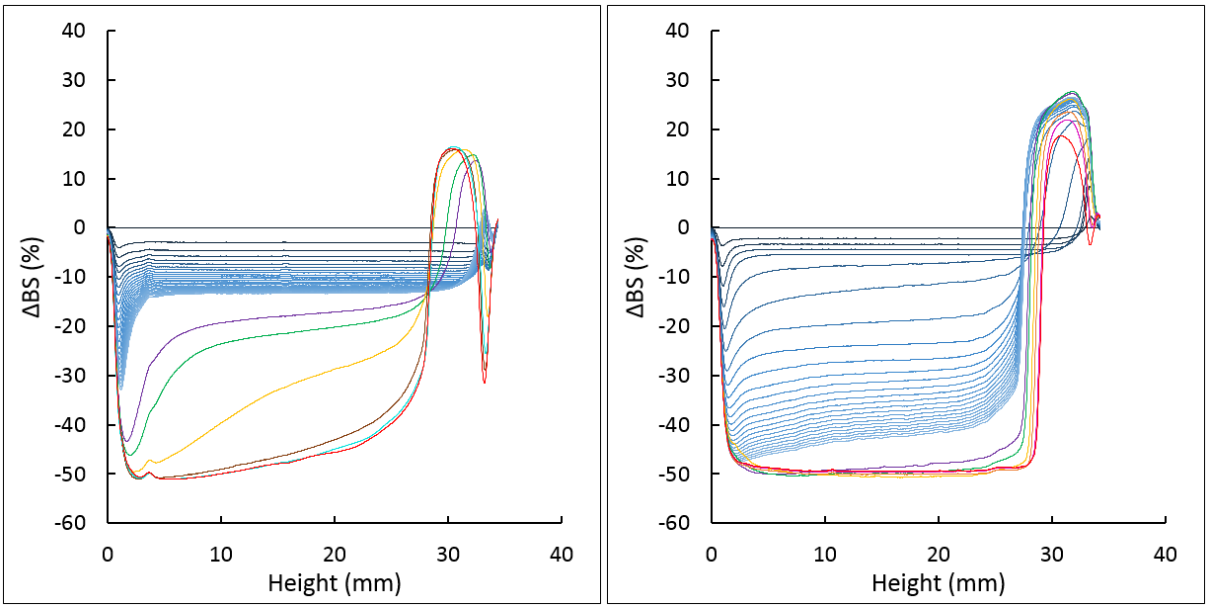
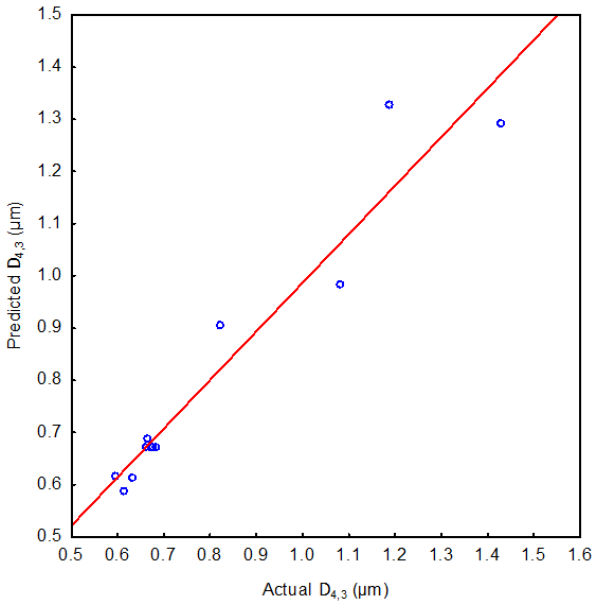


Supplementary figure IV.1: Changes of delta backscattering intensity with time of limonene emulsions stabilized by A. *senegal* at 1 wt% (A), 5 wt% (B) and 20 wt% (C) and A. *seyal* at 5 wt% (D), 10 wt% (E) and 20 wt% (F). The measurements were carried out 15 min after emulsification (black) at 25°C for every 1h during the first 24h (blue) and then one measurement was done at 2 (purple), 3 (green), 7 (yellow), 15 (brown), 24 (cyan) and 30 (red) days of storage.

A.



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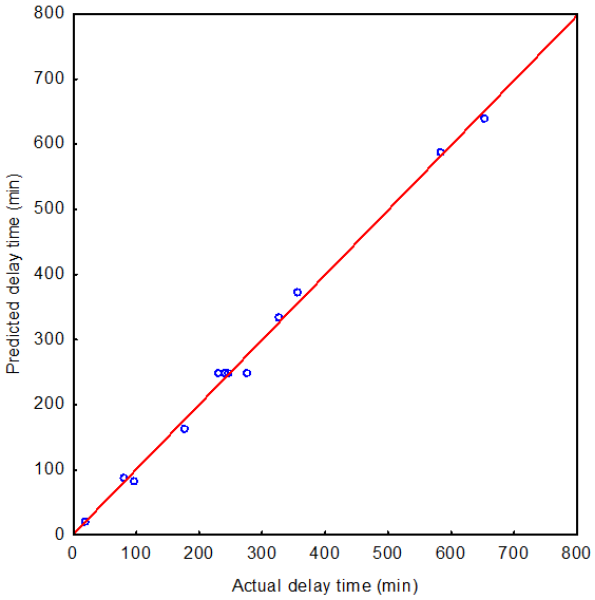

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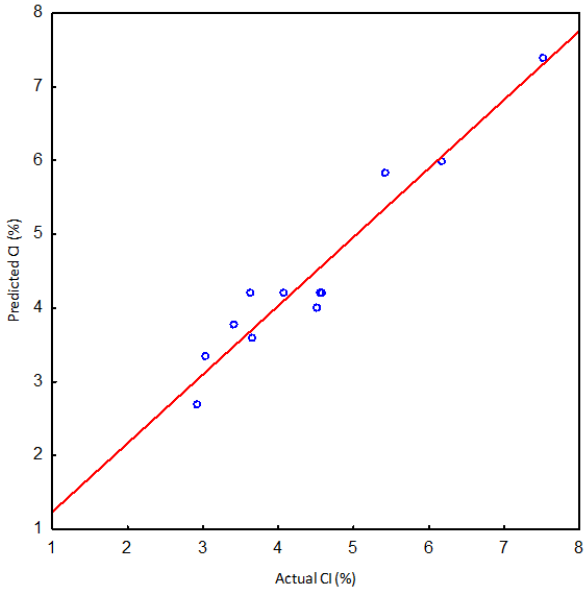


Supplementary figure IV.2: Predicted and actual values of $D_{4,3}$ (A), delay time (B) and CI (C) of 5 wt% limonene emulsions produced by reconstituted gums.

III. Complementary studies

1. Effect of glycerol addition on emulsion droplet size and stability

As previously demonstrated, the addition of glycerol to increase the viscosity did not impact the droplet size but the stability of emulsion. The addition of glycerol was relatively low (8%) compared to the other solvent, i.e water which represented 67 % of the suspension, but it is possible that its presence affected the composition of the oil and dispersive phase in relation to their relative solubility. In this complementary study, the glycerol was added to an A. *seyal* dispersion at 5 wt% to reach the apparent viscosity of A. *seyal* dispersion at 20 wt%. It needed to supplement with 60 wt% of glycerol which became the major solvent compared to water (only 30 wt%). Then, the concentration and viscosity of dispersed phase was kept constant (5 wt%) whereas the continuous phase is varied in viscosity but also in composition. It is important to note that the viscosity of the dispersed phases were in the optimum range defined for high pressure system, i.e. between 1 and 200 mPa (Schultz et al., 2004).

According to the respective solubility of limonene in water and in glycerol-water, the ratio of dispersed phase, i.e. limonene, to dispersive phase was not similar. The stability of emulsions could increase with glycerol addition, because a part of limonene was solubilized in glycerol contained in the aqueous bulk. Indeed, with a log P (octanol/water partition) of -1.76, glycerol is considered as a less polar compound than water but as a polar compound compared to limonene. This means that in the suspensions containing glycerol, the limonene could be better solubilized.

Therefore, the prior estimation of miscibility level or the interaction between glycerol and limonene compared to the one between water and limonene was done using the Hansen solubility parameters and calculating the $\Delta\delta$ using the following equation (here given for glycerol (G) and limonene as solute (L)):

$$\Delta\delta_T{}^2 = (\delta_{DG} - \delta_{DL})^2 + (\delta_{PG} - \delta_{PL})^2 + (\delta_{HG} - \delta_{HL})^2$$

where δ_D, δ_P and δ_H are the Hansen solubility parameters for the dispersive, polar and hydrogen bonding interactions. δ_T corresponds to the overall Hildebrand solubility parameter. The same equation was used for evaluate the interaction between water and limonene.

The values of Hansen solubility parameters for glycerol, limonene and water were reported in Table IV.3.

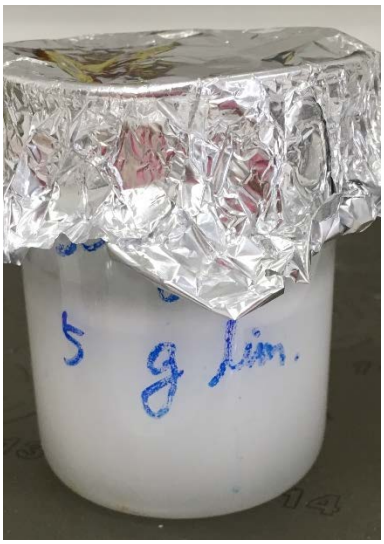
Table IV.3: Hansen solubility parameter values of limonene, glycerol and water.

Substance	δ_D,	δ_P	δ_H	δ_T	Refs.
Glycerol	17.4	12.1	29.3	36.16	Hansen, 2007
Limonene	18.0	1.0	1.0	18.1	Auras, Harte, & Selke, 2006
Water	15.5	16.0	42.3	47.8	Hansen, 2007

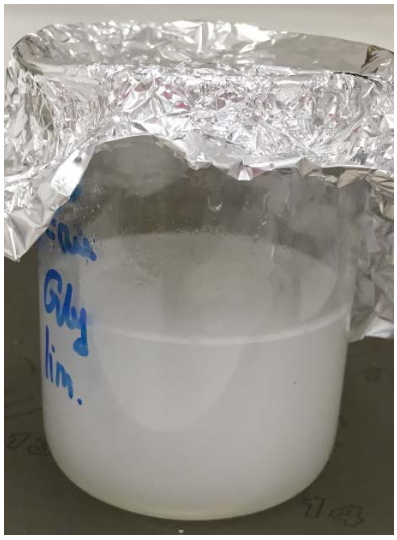
The lower the $\Delta\delta_T$, the better is the affinity between the solvent and the solutes assuming the "like dissolves like" rule. Comparing both $\Delta\delta_T$, equal to 44.01 for water and to 30.40 for glycerol, limonene has clearly a higher affinity for glycerol than for water thus the presence of glycerol at high concentration has to affect dispersed phase.

The solubility of limonene in glycerol and the ability of glycerol to stabilise emulsion droplet without emulsifier was also experimentally checked by homogenizing 5wt% of limonene with water at 90wt% or 60wt% of glycerol and 30wt% of water using rotor/stator homogenizer at 7500 rpm for 5 min and their stability was monitored. For the mixture containing glycerol, water and glycerol were prior blended using magnetic stirrer before adding limonene.

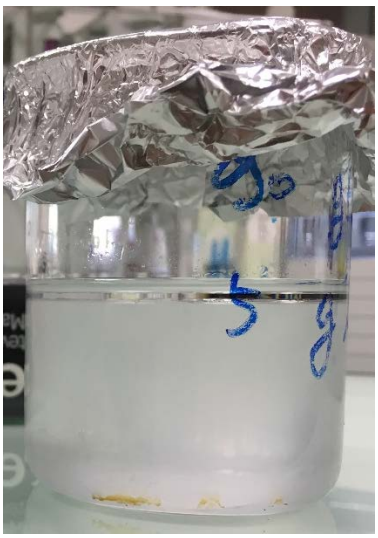
A.1



B.1



A.2



B.2

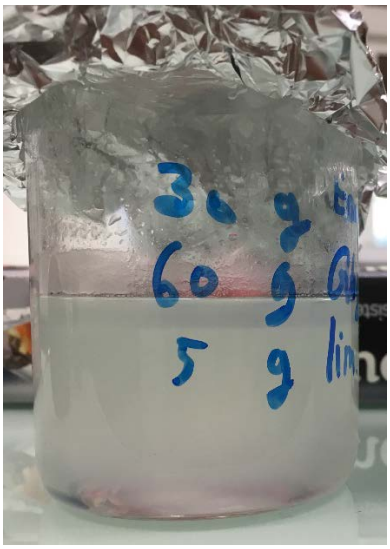


Figure IV.6: Macroscopic observation of mixture 5wt%limonene-90wt% water (A) and 5 wt% limonene-30wt% water-60wt% glycerol (B) 8 min after homogenization (1) and one day after storage at room temperature (2).

Figure IV.6.A and IV.6.B showed that both glycerol and water allowed the production of limonene emulsion. The turbidity of the emulsion without glycerol seemed to be higher than the emulsions with glycerol. This result was in agreement with the data obtained by BS measurement. After 1 day of storage at room temperature (~25°C) a

shading ring limonene/water was clearly observed in case of emulsions without glycerol whereas for emulsions with glycerol addition, it allowed to delay the phase separation. These results confirmed that the drastic greater stability of emulsion with high glycerol content was due to the solubility of limonene in glycerol and we did not produce the same emulsion when glycerol was present or absent.

Comparing the two emulsions produced by the dispersion of A. *seyal* at 5 wt% containing or not glycerol, a strong decrease of $D_{4,3}$ was observed (35%) with glycerol addition (Table IV.4). However, the droplet size remained higher than the one of emulsion produced using 20 wt% of A. *seyal*. This result suggested that the apparent viscosity increase of continuous phase allowed to decrease the emulsion droplet size by restricting the movement of droplet and therefore frequency collision preventing re-coalescence (Khouryieh, Puli, Williams, & Aramouni, 2015; Qian & McClements, 2011). As highlighted by Qian et al. who have also added glycerol to increase the viscosity of continuous phase, the effect of glycerol addition is dependent on the emulsifier nature (Qian & McClements, 2011). These authors showed an appreciable decrease of droplet size with the increase of glycerol concentration with 2 wt% SDS whereas with 2 wt% β-lactoglobulin, the change was limited. From the droplet size decrease, the authors deducted that the shear forces are strongly implied in the disruption of the droplet during the microfluidization process and suggested that the difference between emulsifiers can be related to the slowest adsorption to the droplet surface of β-lactoglobulin which did not allow to avoid the re-coalescence. A. *seyal* at 5wt% and β-lactoglobulin at 2wt% dispersions are both characterized by a Newtonian behavior and low viscosity (Garti & Reichman, 1993) but their sensitivity to glycerol addition differed. It is worth noting that the increase of β-lactoglobulin concentration (from 1 to 10 wt%) did not allow to decrease emulsion droplet size (Qian & McClements, 2011) and we can assume that, at the tested concentration (2 wt%), the lowering of droplet size by β-lactoglobulin was already optimal. Then, the effect of glycerol addition was null. By contrast, for A. *seyal*, the estimated critical concentration was 14.8 wt % (as demonstrated earlier in the 'to submit' article). This indicated that for the

concentration of 5wt%, the decrease of emulsion droplet size is possible induced by glycerol. This confirmed that the effect of glycerol addition on emulsion droplet size particularly depended on nature and emulsifier concentration too.

Table IV.4: Volume mean diameter ($D_{4,3}$) and stability expressed in delay time and CI of 5 wt% limonene emulsions as a function of apparent viscosity of Acacia gum aqueous phase.

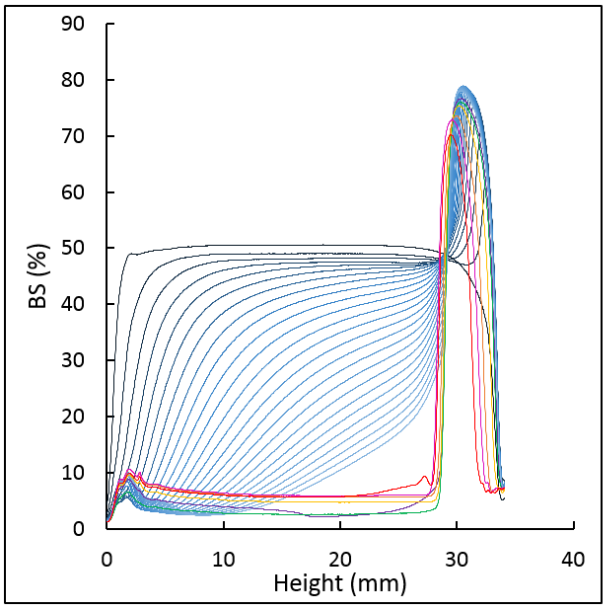
Aqueous phase	Glycerol concentration (wt%)	Apparent viscosity at 100 s^{-1} (mPa.s)	$D_{4,3}$ (µm)	Delay time (min)	CI (%)
A. *seyal* at 5 wt%	0	2.4±0.0	3.46±1.00	13.5±4.4	23.4±6.0
A. *seyal* at 5 wt%	60	29.4±1.2	2.23±0.004	674.3±41.1	9.1±0.5
A. *seyal* at 20 wt%	0	29.4±1.2	1.30±0.13	163.5±15.1	7.4±0.7

Other weighting agents such as ester gum (Zhang et al., 2015) or PEG (Wooster, Golding, & Sanguansri, 2008) were added to the emulsion formulation in order to improve the viscosity of the continuous phase. The consequence is an enhanced disruption of droplet thus a decrease of droplet size as described for glycerol. These authors reported that there is an optimal ratio of disperse/continuous phase viscosity allowing the formation of the smallest droplet size. According to them, for turbulent shears as to colloid mills, this ratio is optimum between the range of 0.1 and 5. The most important disruption mechanism of droplet in microfluidisation process results from the combination of laminar extension flow at the inlet of interaction chamber and the turbulent flow in the interaction chamber (Schultz et al., 2004). These last authors affirmed that the viscosity ratio has little influence because the oil droplet disruption mainly occurred in turbulent flow. In our case, due to the low viscosity value of limonene (0.923 mPa.s^{-1}) and A. *seyal* gum, the values of η_d/η_c were very low for the three A. *seyal* emulsions varying between 0.35 and 0.03. The strong decrease of the ratio when the viscosity increased by addition of glycerol or by increase of gum concentration ratio could justify the greater droplet disruption. It can be also suggested that for the specified limonene/Acacia gum emulsions in regard to the weak viscosity

of Acacia gum and limonene, the optimum range ratio would be defined by weaker values than those proposed.

However, even though the apparent viscosity increase impacted the droplet size, this effect is limited and suggested that a certain amount of A. *seyal* gum molecules having the required emulsifying properties were necessary to obtain low droplet size. By contrast concerning the emulsion stability, the glycerol addition improved greatly delay time comparing to the emulsions produced by A. *seyal* at 5 or 20 wt%. The decrease of CI value was less pronounced and the value remained similar to those established for A. *seyal* at 20 wt% (Table IV.4). It is important to note that the initial BS of emulsions produced by A. *seya*l at 5wt% with 60 wt% of glycerol showed the lowest value (Figure IV.7.B). Since BS intensity is a function of the volume of dispersed phase (number of droplets) and the particle size (Mengual, Meunier, Cayré, Puech, & Snabre, 1999), this means that these emulsions contained less droplets than the one containing only gums. The major destabilization process was always creaming but less coalescence/flocculation was detected during the first 24h compared to emulsions without glycerol (Figure IV.7.A and IV.7.C). This enhancement of emulsion stability could be due to (i) partial solubility of limonene in glycerol and (ii) the specific formation of new hydrogen bonds between hydroxyl groups of glycerol and A. *seyal* gum as previously suggested for film formation (Aphibanthammakit et al., 2018). The new created network can induce a thicker film at the interface or restricted movements of the droplets preventing flocculation and recoalescence and favored stabilization by steric repulsion. As already stated, an increase of the stability of orange oil emulsion has been observed by adding glycerol to Acacia gum (Mirhosseini et al., 2008). Indeed, the addition of a sufficient amount of glycerol (1.5 wt%) allowed to increase significantly the pH value (9%) and the negatively charged ζ-potential (10%) thus the repulsive forces. Authors explained these changes by the presence of the negatively charged side group (-OH) on the glycerol and therefore the increase of pH value.

A.



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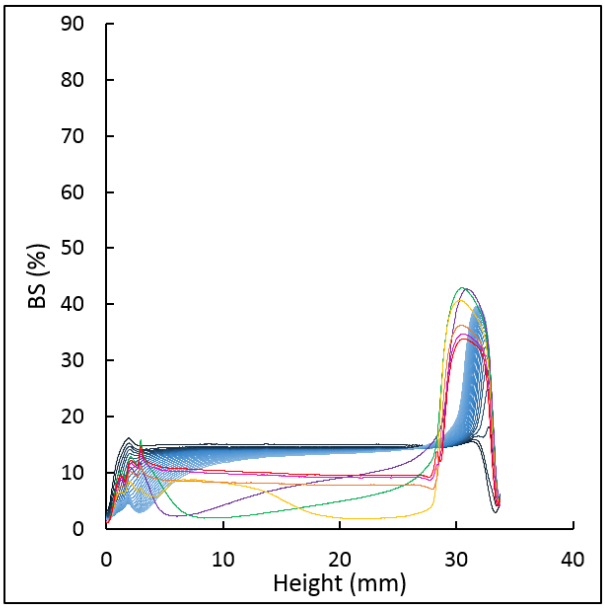

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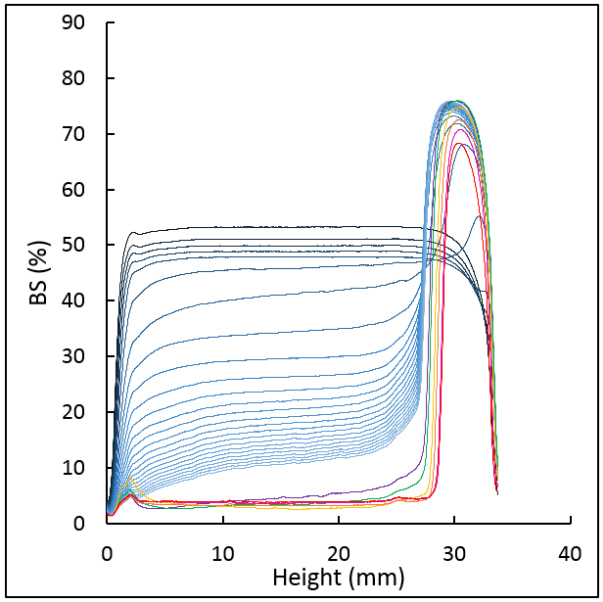

Figure IV.7: Changes of backscattering intensity with time of limonene emulsions stabilized by A. *seyal* at 5 wt% (A), by A. *seyal* at 5 wt% with 60 wt% of glycerol (B) and by A. *seyal* at 20 wt% with 8 wt% of glycerol (C). The measurements were carried out 15 min after emulsification (black) at 25°C for every 1min 50 during the first 24h (blue) and then one measurement was done at 2 (purple), 3 (green), 7 (yellow), 15 (brown), 24 (cyan) and 30 (red) days of storage.

The addition of solvent or the presence of other aroma compounds which can help to partially solubilize limonene in water appear as a good solution to stabilize limonene emulsion. This also confirms that the formulation (limonene-Acacia gum-buffer) used to produce emulsion in this study are not fully optimal for the production of long-term stable emulsions.

2. Calculation of surface load concentration

The surface load of the emulsifier at saturation (Γ) is an indicator to better understand the formation process of natural emulsifier-stabilized emulsions. This parameter allowed to estimate the required amount of emulsifier to form emulsion. Indeed, it corresponded to the mass of surfactant needed to cover a unit area of droplet surface. It is expressed in $mg.m^{-2}$. Two different methods, named method 1 and method 2, have been used to estimate the value of surface load on droplet using Acacia gums as emulsifier:

(i) method 1 - by using an equation involving some final emulsions parameters:

$$\Gamma = (D_{3,2} \times Cs)/(6 \times \phi) \qquad (1)$$

where $D_{3,2}$, Cs and ϕ corresponded to the surface average diameter or Sauter mean diameter (m), the concentration of emulsifier in the emulsions ($kg.m^{-3}$) and the volume fraction of dispersed phase, respectively (Bai et al., 2017; McClements, 2007).

(ii) method 2 - by measuring the Acacia gum content in the cream and/or serum layers. First, the two layers were separated by centrifugation. The non-adsorbed molecules in the serum layer can be directly analyzed using SEC-MALLS. To characterize the adsorbed molecules, SDS had to be added to the cream phase. Indeed, surfactant SDS allowed to displace the proteinaceous molecules from the interfaces. These molecules were then separated and analyzed using SEC-MALLS (Katayama et al., 2006;

Mikkonen et al., 2016; Nakauma et al., 2008). Using this method 2, the value of surface load can be estimated based on the Equation 1. Other parameters can be also calculated as the recovered mass (%) and the concentration of adsorbed and non-adsorbed molecules ($mg.mL^{-1}$) (Katayama et al., 2006).

Although the estimation of surface load for both methods was based on the same equation (Equation 1), these methods differ in the way to determine the concentration of adsorbed-molecules. Indeed, method 1 supposed that the total emulsion droplet surface was rapidly covered by all the emulsifier present in the emulsions, i.e. Cs is equivalent to the adsorbed concentration., while, for method 2, the concentration of adsorbed molecules was indirectly experimentally determined. Generally, the use of the method 1 was only for the comparison purpose between different emulsifiers. This method rarely represents the reality when large molecule as Acacia gums and other polysaccharides were used (Bai et al., 2017). However, as suggested by these authors, the estimation of the surface load was useful to comparison purpose. The results obtained by different authors with these two methods by using Acacia gum as emulsifier and different dispersed phase are presented in Table IV.5. The highest value of surface load (31 $mg.m^{-2}$) was found using the method 1 probably because all molecules of emulsifiers were assumed to adsorb to interfaces. For the method 2, the value of surface load drastically varied, i.e. between 0.761 and 14 $mg.m^{-2}$. This variation should be dependent on, among others, the considered molecular types and probably the nature of oil phase. To authors' knowledge, there are not any study which compares the two methods of surface load estimation.

Table IV.5: Values of surface load of Acacia gum droplet estimated by different methods and for varied dispersed phases. AG stands for Acacia gums.

Emulsion formulation	Measurements	Γ (mg.m^{-2})	Refs.
10 wt% corn oil with 90wt% aqueous phase (0.1 – 10 wt% AG)	Method 1	**31**	Bai et al., 2017
20 wt% sweet orange oil with 0.25-25 wt% AG	Method 2	4 by AG and 1.2 - 1.6 by proteineaceous component	Randall et al., 1988
6.5% orange oil with 20.0% AG, 0.1%sodium benzoate and 73.4% of deionized water	Method 2	4.67 - 14.00 depending on gum composition	Buffo, Reineccius, & Oehlert, 2001
Refined soy oil/AG ratio varying between 0.25 and 5.0	Method 2	0.761- and 0.853 depending on oil/AG ratio with non-linear relationship between Γ and oil/AG ratio	McNamee et al., 2001
15 wt% oil phase (d-limonene and medium chain triglyceride with 50% of weighting agent) with 5-25 wt% AG	Method 2	7.22 for 25% AG with low molecular weight and 7.04 for 25% AG with high molecular weight	Katayama et al., 2006
15 wt% medium chain triglyceride with 1.0 – 10% AG containing 2.25 wt% of protein	Method 2	6	Nakauma et al., 2008
15 wt% conjugated linoleic acid with 85wt% aqueous phase (2.5 to 15.0 wt% AG)	Method 2	The maximal value ~25 for 5 wt% AG	Xiang et al., 2015
1 or 5 wt% Rapeseed oil with 1 wt% AG	Method 2	2.5	Mikkonen et al., 2016

In this study we have evaluated the surface load of the emulsifier at saturation using the method 1. To calculate the surface load, we have considered the droplet size for (i) the lowest emulsifier concentrations up to the critical concentrations, then making the average of surface load for all considered concentrations (limited concentrations) or (ii) the critical concentrations (Table IV.6). As expected, the surface load of A. *senegal* was lower than the one of A. *seyal*: about 11 times considering the limited concentrations and the critical concentrations confirming the relevant interfacial properties of A. *senegal* compared to *A seyal*. The surface load at saturation of A. *senegal* was found equal to 31 $mg.m^{-2}$ (using the same type of calculation as us) for emulsions of 10 wt% corn oil (Bai et al., 2017). This value was in the same order of magnitude as our result. The slight difference can be explained by the variation of density of the oil phase which plays an important role in the surface load evaluation. Indeed, the density of limonene is relatively weak with a value 0.841 kg/m^3 (https://pubchem.ncbi.nlm.nih.gov consulted on the 11th of April 2018) while the density of corn oil was 0.92 kg/m^3 (Noureddini et al., 1992). Moreover, this difference could be due to the gum origin. The high value of surface load evaluated for A. *seyal* suggested that the adsorbed concentration was largely different of the emulsifier concentration in solution and that the molecules having emulsifying properties as AGP were sparsely accessible.

Table IV.6: The values of surface load (Γ) calculated as a function of gum concentration or protein concentration using Equation 1 for emulsions produced by A. *senegal*, A. *seyal* and reconstituted A. *senegal*.

	A. *senegal*			A. *seyal*		
	Limited concentration	Critical concentration	Protein content	Limited concentration	Critical concentration	Protein content
Γ ($mg.m^{-2}$)	57	63	1.35	647	701	4.98

Assuming that the protein part is preferentially adsorbed to the interface, we have estimated the surface load taking into account the protein content of each gum at the critical concentration. In this case, the surface load was equal to 1.35 $mg.m^{-2}$ for A. *senegal* and 4.98 $mg.m^{-2}$ for A. *seyal* and was in agreement with literature (Table IV.5). However, the hypothesis that only the protein part adsorbed at the interface was discarded and the estimation of surface load from the content of high molar mass protein-rich AGPs could be more relevant. This calculation can be done for A. *senegal* and the obtained values (8.8 $mg.m^{-2}$) for the critical concentration was in the same order of magnitude as with method 2 for weak Acacia gum concentration (Table IV.5). This indicated that method 2 could be the most relevant for the estimation of surface load. Unfortunately, this information about high molar mass protein-rich AGPs was not available for A. *seyal* gums.

The calculation of surface load as a function of high molar mass protein-rich AGPs was also done for the reconstituted A. *senegal* gum. For comparison purpose, we decided to use similar AGP concentrations from reconstituted A. *senegal* (0.31 wt%) and from original A. *senegal* (0.32 wt%) for the calculation. The surface load was estimated equal to 18.9 $mg.m^{-2}$ against 8.8 $mg.m^{-2}$ which is 53% higher than the one of initial A. *senegal*. These deviations of surface load value were related to the higher value of $D_{3,2}$ of emulsion produced by reconstituted A. *senegal* (1.54 µm against 0.69 µm for reconstituted and initial A. *senegal*, respectively) but also to the slight difference between the concentration of AGPs from reconstituted and initial A. *senegal*. To verify the preponderant role of droplet size, another AGP concentration was chosen with similar $D_{3,2}$ and minimal deviation between AGP concentration from initial and reconstituted A. *senegal*, i.e. 14 wt% and 13.2wt% of AGP respectively. We found that the value of surface load for initial and reconstituted A. *senegal* was 33.5 $mg.m^{-2}$ and 35.06 $mg.m^{-2}$, respectively. Again, the difference in surface load value (4%) corresponded to the slight difference in AGP concentration between gums (5%) confirming the role of total concentration to obtain the smallest droplet size. These

results confirmed that the reconstitution of gums is a good approach to characterize and understand the emulsifying properties of A. *senegal*.

The capacity of an emulsifier to form emulsions is related to its interfacial properties at oil-water but also air-water interfaces. Despite the quantitative differences in interfacial behavior at these interfaces, a similar general trend of adsorbed layer can be observed (E. Dickinson, 1999; Ganzelves, 2007). Damodaran et al., have studied the adsorption isotherm of one component isolated from Acacia gum through gel permeation chromatography, at the air-water interface. This component named GAGP which represent 10% of gum is a hydroxyproline–rich glycoprotein with ˜90% carbohydrate and 10 % protein (Damodaran & Razumovsky, 2003) and having a molar mass of 2.2 to 5.6×10^5 $g.mol^{-1}$ (Churms, Merrifield, & Stephen, 1983; Qi, Fong, & Lamport, 1991). Taking into account the protein content and molar mass, this fraction was similar to IEC-F1. Authors reported that the saturated monolayer coverage at the air-water interface was reached above 400 $mg.L^{-1}$ (˜0.04 wt%) of bulk concentration with a surface concentration of 250 $mg.m^{-2}$. Authors pointed out that the surface concentration decreased up to 32 $mg.m^{-2}$ for 39 $mg.L^{-1}$ of bulk concentration. This last result was in agreement with the estimated surface load using method 1. Indeed, the value of surface load decreased with bulk concentration. Using a competitive adsorption experiments, authors demonstrated that despite the high value of surface concentration, the formed GAGP interfacial films were able to inhibit the penetration of β-casein even at unsaturated monolayer (at the bulk concentration of 39 $mg.L^{-1}$). They concluded that this was due to the thermodynamic incompatibility through steric repulsive interactions between protruding polysaccharide chains of GAGP and the flexible β-casein from the bulk causing the inability of β-casein to mix with or dissolve in the GAGP films. This competition phenomenon between molecules can also occur within entire gums because they contain several molecules rich in proteins which can compete to the surface and hinder their mutual adsorption. As specified, this study was done at air-water interface and further studies are needed to confirm the last hypothesis by

measuring the interfacial properties of different fractions mixtures at the oil-water interface and comparing their emulsifying properties.

Finally, the method 2 used to estimate and characterize adsorbed interfacial molecules needs to be carried out to confirm the estimated results using method 1. Other method could be also done, such as competitive adsorption experiments, allowing to characterize in-depth interfacial films.

IV. Major outcome

In this chapter, through an innovative approach we demonstrated that the emulsifying properties of Acacia gum depended on several factors: (i) functional synergism between high molar mass protein-rich AGPs content and total gum concentration (ii) gum concentration for a given gum specie (A. *senegal* and A. *seyal*) and (iii) emulsification process using microfluidizer. The main results are described in the following table:

Gum characteristics	
Reconstituted A. *senegal* gum	• The short term emulsion stability mainly depended on the functional synergism between the amount of high molar mass protein-rich AGPs and the total gum concentration. • The long term emulsion stability was mostly related to the total gum concentration. • Reconstituted gums allowed a better stability than initial A. *senegal* in relation to a greater content of aggregated AGPs of IEC-F1 and resulting in higher molecular flexibility and greater interfacial properties but also to the higher bulk viscosity. • Innovative and appropriated approach allowing to better understand the role of high molar mass protein-rich AGPs in emulsifying properties of A. *senegal*.
Gum concentration	• For A. *senegal* and A. *seyal*, the increase of gum concentration decreased emulsion droplet size and enhanced emulsion stability. • The critical concentrations to reach the smallest droplet size in the process conditions used were 6.4 times lower for A. *senegal* (~2.3 wt%) than for A. *seyal* (~14.8 wt%).
Gum specie	• At the same gum concentration, the emulsion droplet size was lower using A. *senegal* than A. *seyal* in relation to the greater high molar mass protein-rich AGPs and protein content of the former.

	• The better stability of emulsion produced by A. *senegal* compared to A. *seyal* could be due to: (i) the smallest emulsion droplet size of emulsion produced by A. *senegal*, (ii) the higher apparent viscosity of A. *senegal* bulk, (iii) the higher uronic acid content of A. *senegal* allowing a greater electrostatic repulsion compared to A. *seyal* and (iv) the higher content of arabinose of A. *seyal* favouring new liaison between droplets.
Process parameters	
Emulsification process	• The decrease of droplets size of emulsion produced by A. *senegal* was greater than the one of emulsion produced by A. *seyal*.
Addition of glycerol	• The addition of glycerol allowed to increase the viscosity of aqueous phase and to enhance the stability of emulsion while droplet size was weakly decreased.

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Surface properties of Acacia *senegal* vs Acacia *seyal* films and impact on specific functionalities

Chapter 5 – Surface properties of Acacia *senegal* vs Acacia *seyal* films and impact on specific functionalities

The previous chapters reported the relationship between the interfacial (liquid/liquid) and emulsifying properties of Acacia gums, depending on their biochemical composition, structural properties and concentration, and the properties of interfaces (oil nature and type of emulsifier). Moreover, the assessment of the surface tension of Acacia gum dispersions at air/water interface allowed to obtain supplementary information about the behavior of Acacia gums. The results confirmed that, for the high concentration of gum used (20 wt%), A. *senegal* and A. *seyal* allowed to decreased the surface tension of water showing both polar and dispersive components in agreement with their amphiphilic properties. A. *senegal* gum was characterized by a higher dispersive component than A. *seyal* gum in relation with its biochemical (higher protein and lower arabinose contents) and structural (higher flexibility) properties.

Acacia gum dispersions at high concentration are not only used in the form of liquid. As a film forming colloid, Acacia gums are also largely used in the form of film with low water content. In confectionery it is used to isolate the centers in dragee. Indeed, the centers, e.g. chocolate, almond, nuts, can be coated with gum dispersion to prevent fat oxidation and the migration of fat through the sugar coating. The Acacia gum based films are also used in the coating of fruit and fish allow to extend their self-life (Ali, Maqbool, Alderson, & Zahid, 2013; Binsi et al., 2016; El-Anany, Hassan, & Rehab Ali, 2009; Jiang, Feng, Zheng, & Li, 2013; Maqbool, Ali, Alderson, Zahid, & Siddiqui, 2011). The understanding of fundamental interfacial properties of Acacia gum films such as film surface structure, the behavior of contact angle between liquids with different

physicochemical properties and gum films is crucial to obtain Acacia gum films with optimal characteristics.

In this chapter, the surface structure and characteristics of films obtained with both A. *seyal* and A. *senegal* gums were investigated. The experiments and conclusions were reported in a published article discussing:

- the film formation process: supports and glycerol addition
- the surface structure characterized by SEM and AFM
- the surface properties evaluated by contact angle measurement.

The wettability and the free surface energy of films were determined as the affinity of the films for different organic volatile compounds on gum films. Functionalities as water vapor permeability and ability to retain aroma compounds have been investigated.

The objectives were to establish a relationship between the different film properties and the gums nature and biochemical composition.

I. Publication 1 – Surface properties of Acacia *senegal* vs Acacia *seyal* films and impact on specific functionalities

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Abstract

The microstructure and surface properties of spin coated films were studied to determine the structuration of Acacia gum films depending on gum type and composition. The difference between A. *seyal* and A. *senegal* films was clearly evidenced by surface morphology (SEM and AFM) and through the contact angle measurement: A. *senegal* films having a smoother and more hydrophobic surface ($\theta = 62°$) than A. *seyal* films ($\theta = 42°$) characterized by aggregated structuration. The film hydrophobicity increased with glycerol addition for both gum films (A. *senegal*, $\theta = 68°$ and A. *seyal*, $\theta = 50°$). This could be due to hydrogen-bonding between hydroxyl groups of plasticizer and polar groups of Acacia gums favoring their reduction on films surface. Both gum films behave as dual polar surface showing high disperse component of free energy compared to the polar component. Both gums showed strong affinity for apolar compounds ($\theta < 20°$). The overall results indicated that the structuration of films depended on the protein content and accessibility. Similar surface properties were found with self-supported films: A. *seyal* cast films being still more hydrophilic than A. *senegal* ones, demonstrating that the former provides a more favorable environment for water interaction than the latter. The specific interactions pointed for each gum films with water and apolar compounds were reflected in functionality such as water vapor permeability and efficiency to retain limonene and linalool. The knowledge of these properties is recommended to design specific coatings anticipating water loss of the coated product and to evaluate antimicrobial efficiency when active agents as aroma compounds are incorporated in the film.

Keywords: Acacia gum; edible films; surface properties; water vapor permeability; aroma compounds; emulsion based films.

1. Introduction

Over few decades, films of biopolymers as polysaccharides, proteins or blends are used in diverse applications. They have been developed to supply the demand for new materials for medical and pharmaceutical uses, targeting tissue and cell lesion therapy thanks to their non-toxic characteristics, biocompatibility and biodegradability (Silva, Ducheyne, & Reis, 2007). In packaging domain, coated papers, self-supported films or edible coating formed directly onto food surface were developed to reduce or replace the synthetic polymers (Miller & Krochta, 1997; Nieto, 2009). They function as relative high barrier to water vapor, gases such as O_2, CO_2 or ethylene and aroma compounds and help to limit the degradation reactions, to improve the quality and to extent the shelf life of foodstuffs. Biopolymer films or coating are also used as carriers of active compounds to protect them and to control their delivery (Arfa, Preziosi-Belloy, Chalier, & Gontard, 2007; Hambleton, Debeaufort, Bonnotte, & Voilley, 2009; Hashemi Gahruie, Ziaee, Eskandari, & Hosseini, 2017). For this purpose, biopolymers have to possess film-forming properties and the ability to retain the active compounds through specific interactions and emulsifying properties. Acacia gum is one of these biopolymers.

Acacia gum also called gum arabic (AG, E414 EC) is a natural arabinogalactan-protein (AGP) type polysaccharide with a low protein content (<3%). Acacia gum is defined as "a dried exudate obtained from the stem and branches of Acacia *senegal* (L.) Willdenow or Acacia *seyal* (family Leguminosae)" (FAO, 1999). Acacia gum is described as a continuum of molecular species which distinguishes by their protein to sugar ratio, molar mass and charge density (Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006). The polysaccharide main chain backbone is formed of 1,3-linked β-D-galactopyranosyl units with numerous side chains. Side chains have units of α-L-arabinofuranosyl, α-L-rhamnopyranosyl and β-D-glucuronopyranosyl and 4-O-methyl-β-D-glucuronopyranosyl acid that are mostly located at the end units (Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Verbeken, Dierckx, & Dewettinck, 2003). Depending on the botanic type, the proportion in the different types of sugars differs: A. *seyal* contains

greater proportion of L-arabinose relative to D-galactose (ratio 1.6) than A. *senegal* (ratio 1). Moreover, A. *seyal* gum is characterized by significantly more 4-O-methyl-D-glucuronic acid, but less L-rhamnose and unsubstituted D-glucuronic acid than A. *senegal* one (Phillips & Williams, 2009). A. *senegal* and A. *seyal* differs also by their protein content, the last being poorer than the former. In terms of structure, an important point is that the average molar mass of A. *seyal* is greater than A. *senegal* despite a smaller hydrodynamic volume (Lopez-Torrez, Nigen, Williams, Doco, & Sanchez, 2015). A. *seyal* molecules are then more compact than A. *senegal* ones and give less viscous dispersions. The interfacial properties of Acacia gum dispersions have been largely described (Sanchez et al., 2017). Acacia gum is able to decrease the interfacial tension at gas-water, liquid-liquid or solid-liquid interfaces and to stabilize interface through electrostatic and steric repulsion and hydration forces (Castellani, Al-Assaf, Axelos, Phillips, & Anton, 2010; Lopez-Torrez et al., 2015; Sanchez et al., 2017). These properties are related in part to the polarity and structure of the gum as demonstrated by the comparison between conventional A. *senegal* and matured A. *senegal* gum. Indeed the maturation process consisting to increase the molar mass of in protein-rich high macromolecules resulted in a better ability to lower the interfacial tension (Castellani, Al-Assaf, et al., 2010). As edible ingredient, Acacia gum is largely used for emulsification and encapsulation of food products (Buffo, Reineccius, & Oehlert, 2001; Kim & Morr, 1996; McNamee, O'Riorda, & O'Sullivan, 1998) but less for coating or film applications. Indeed, films of Acacia gums lack of strength after casting and drying. Acacia gums are polymers with high degree of branching, A. *senegal* having the highest degree of branching (Lopez-Torrez et al., 2015). This high degree of branching could prevent the intermolecular interactions and induce less cohesive films than those obtained with linear polymers (Nieto, 2009).

However, some examples of coating applications showed that Acacia gum is successful to protect food against oxidation, to maintain the quality and to enhance the shelf-life of banana, papaya, mushroom, tomato, Anna apple and Indian mackerel (Ali, Maqbool, Ramachandran, & Alderson, 2010; Binsi et al., 2016; El-Anany, Hassan, & Rehab Ali,

2009; Jiang, Feng, Zheng, & Li, 2013; Maqbool et al., 2011). The efficiency of Acacia gum enriched with garlic oil and cinnamon as active coating was also demonstrated to preserve fish and meat against microbial degradation and oxidation (Rakshit & Ramalingam, 2013).

Understanding surface properties of films such as roughness, water wettability, and free surface energy allow to establish relationship between the chemical composition and properties of the developed films and to improve the material applications and the coating–food compatibility (Basiak, Lenart, & Debeaufort, 2016).Then the knowledge of surface water wettability is needed to select biopolymers with enhancing adhesiveness, cohesiveness for better spreading of the coating on a solid surface. Moreover, the controlled tuning of wettability may expand the applications domain of these materials (Farris et al., 2011). Contact angle measurement is the most basic method to determine the wettability of a surface by a liquid and the surface tension parameters (Basiak et al., 2016; Farris et al., 2011; Karbowiak, Debeaufort, Champion, & Voilley, 2006). It consists of measuring the angle formed at the three-phase contact line where liquid, gas and solid phases intersect. The lower the contact angle value, the more the affinity between the liquid and solid surface. Contact angle between film surface and water droplet is usually used to evaluate the film surface hydrophobicity which is an important indication to control moisture transfer (Kokoszka, Debeaufort, Hambleton, Lenart, & Voilley, 2010). Contact angle measurement was also used to determine the wettability of hydrophobic surface films by different compounds as alkanes (Fox & Zisman, 1950; Neumann, Haage, & Renzow, 1971). Surface formed with polar molecules or domains as found in Acacia gum or proteins are regarded as hydrophilic as an intrinsic property whereas the hydrophilicity can be modulated by the presence of other chemical groups. Moreover, the characterization of the behavior at the surface and wetting dynamics, i.e. physicochemical phenomena as spreading, absorption, swelling involved at the solid/liquid interface results in information about the structure and self-organization of the matrix exposed to air or support

(Bialopiotrowicz & Jańczuk, 2002; Farris et al., 2011; Kalin & Polajnar, 2013; Karbowiak, Debeaufort, & Voilley, 2006).

The objectives of this study were to compare the surface organization of Acacia *senegal* and Acacia *seyal* gum films depending on their composition and supposed conformations in aqueous medium and to establish the relationship between these structuration and specific functionalities of self-supported films. To reach these objectives, the microstructure of spin-coated films was first characterized by SEM and AFM. The surface properties, i.e. water wettability, free surface energy and affinity between films and organic volatile compounds, were investigated using contact angle measurements. Then, the water vapor permeability and aroma retention of self-supported films were studied and related to the surface properties. The final goal is to design active edible coatings with different wetting property compatible with varied fruits or vegetables allowing limiting water loss, to retain aroma compounds as antimicrobial agent and by consequence to increase shelf-life.

2. Materials and methods

2.1. Materials

Acacia *seyal* (A. *seyal*, lot n° OF110724) and Acacia *senegal* (A. *senegal*, lot n° OF110676) gum powders were provided by ALLAND & ROBERT Company-Natural and organic gums (Port mort, France). Both gums were obtained by the same purification process with elimination of insoluble materials, pasteurization and spray drying. Their biochemical composition were previously characterized and are reported in Table V.1 (Lopez-Torrez et al., 2015). Acetate buffer at 10 mM, pH 5 used to dissolve gums was prepared with anhydrous glacial acetic acid and sodium acetate trihydrate provided by Merck KGaA (Darmstadt, Germany) and Fluka- Sigma Aldrich (Saint-Quentin Fallavier, France), respectively.

Table V.1: Biochemical composition of Acacia *senegal* and Acacia *seyal* gums in dry basis (mean ± standard deviation) (adapted from Lopez et al., 2015).

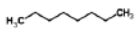
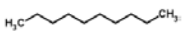
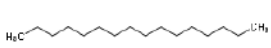
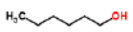
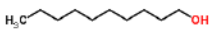
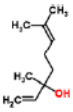
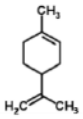
Component ($mg.g^{-1}$)	A. *senegal*	A. *seyal*
Total dry matter	889.0±0.27	893.0±0.02
Total sugars[a]	829.0±0.52	843.0±0.13
Galactose[b]	296.8±9.9	311.1±8.9
Arabinose[b]	251.2±20.7	401.3±5.1
Rhamnose[b]	128.5±2.9	25.3±2.5
Glucuronic acid[b]	144.2±9.5	56.5±3.4
4-O-me-Glucuronic acid[b]	8.3±0.4	48.9±4.6
Proteins	27.0±0.01	10.0±0.04
Minerals	33.0±0.24	40.0±0.07

[a] total content of sugar was calculated by the difference of proteins and minerals from total dry basis.

[b] sugar composition was determined by GC-MS.

Glycerol (GLY) (99% purity and density 1.265) used as plasticizer was provided by Acros Organics (Ilkirch, France). Methanol, sulfuric acid and hydrochloric acid used for cleaning the glass plates were provided by VWR chemicals (Fontenay-sous-Bois, France). The volatile organic compounds (octane, decane, hexadecane, hexanol, octanol, decanol, linalool and limonene at 99% of purity) and the liquids of reference used to determine the surface tension of gum films, diiodomethane and ethylene glycol, were from Fluka-Sigma Aldrich (Saint-Quentin Fallavier, France). Physicochemical characteristics of volatile organic compounds were reported in Table V.2.

Table V.2: Physicochemical characteristics of the used organic volatile compounds.

Compound formula	Chemical structure*	Molecular weight (Da)*	logP at 25°C*	Vapor pressure at 25°C ($mm.Hg^{-1}$)**	Solubility in water at 25°C ($mg.L^{-1}$)*
Octane C_8H_{18}		114.229	4.27	14.157	0.66
Decane $C_{10}H_{22}$		142.282	5.01	1.430	0.052
Hexadecane $C_{16}H_{34}$		226.441	8.20	0.005	0.0009
Hexanol $C_6H_{14}O$		102.175	2.03	0.947	5900-6260
Octanol $C_8H_{18}O$		130.228	3.00	0.079	540
Decanol $C_{10}H_{22}O$		158.281	4.57	0.0085	37
Linalool $C_{10}H_{18}O$		154.249	2.97	0.016	1590
Limonene $C_{10}H_{16}$		136.234	4.57	1.541	13.8

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2.2. Methods

2.2.1. Preparation of Acacia gum film-forming dispersions

Film-forming dispersions were prepared by dispersing 20 g of Acacia gums (wet weight) in 70 g of acetate buffer (at pH 5, 10 mM). Different amount of glycerol was afterwards added (i.e. 0, 10, 15 and 20 wt% of wet weight of gum) and the mass of the suspension was adjusted to 100 g with buffer. The suspension was left overnight at room temperature under stirring to ensure complete dissolution. Gum solutions were centrifuged at 20 000 g for 30 minutes at 25°C to remove traces of insoluble matter.

2.2.2. Preparation of Acacia gum based films

2.2.2.1. Spin coated films

Spin coating consists in applying a thin film evenly across the surface of a substrate by casting a solution while it is rotating at high speed. It is a dynamic application with fast drying due to solvent evaporation (Hall, Underhill, & Torkelson, 1998). 800 µL of gum dispersions without or with glycerol at the concentration determined for cast films were deposited by SPIN 150 spinner (SDS Europe, Vourles, France) on two different supports one hydrophilic and the other hydrophobic: glass (RS, France) and Low Density Polyethylene (LPDE) blades for comparison purpose with cast films.

The glass blades were previously cleaned by immersion in a solution of methanol and hydrochloric acid (1:1, v/v) for 30 minutes and then overnight in a bath of sulfuric acid. The LDPE blades were cleaned with ethanol. All blades were extensively rinsed in mili-Q water after cleaning and used rapidly. In order to form a uniform film, the following conditions were used: speed of 1500 rpm for 300 seconds with an acceleration of 100 $rpm.s^{-1}$ at a controlled temperature (25 °C). The spreading and drying of gum dispersions have been performed by the evaporation of water on the support at high speed rotation. Before the film surface characterization, the spin coated films on blades were stored for 24 h in a desiccator at 25 ±2 °C and 52±1 %RH (relative humidity) maintained by using saturated solution of magnesium nitrate.

2.2.2.2. Cast films

When producing Acacia gum film by casting, adding a plasticizer and selecting a convenient support should be considered to optimize their macroscopic aspect, their mechanical properties (flexibility) and to be able to peel off the film. Therefore, different glycerol contents (0, 5, 10, 15, 20 wt% of gum solubilized in buffer) were tried and two types of support were tested: glass as hydrophilic surface and LDPE as hydrophobic material. For both gums, LDPE petri dishes have been selected as support and the optimal addition of glycerol depended on gum characteristics: 20 % for A. *seyal* and 15

% for A. *senegal*. For A. *senegal*, higher amount of glycerol than 15 % induced sticky films and decreased handle ability whereas A. *seyal* films containing 15 % of glycerol could not peel off in a whole piece.

In case where an aroma compound was added at 5 wt%, the dispersion was additionally emulsified with rotor/stator L4RT homogenizer (Silverson, Evry, France) equipped with a square hole high shear screen stator at 7500 rpm for 5 min at room temperature.

The size distribution of the aroma droplets in emulsions was determined by laser light scattering using a Mastersizer 2000 (Malvern Instruments, Orsay, France). Refractive indices of 1.33 for water, 1.473 for limonene and 1.462 for linalool were used. For all emulsions, three cycles of measurements were performed at ambient temperature and 15 minutes after the emulsification process. The mean droplet diameter was expressed as the volume mean diameter ($D_{4,3}$):

$$D_{4,3} = \sum n_i d_i^4 / \sum n_i d_i^3 \qquad (1)$$

where n_i is the number of droplets of diameter d_i.

All of the films were slowly dried for 24 h at 25 °C and 60%RH in a constant climate chamber (HPP260, Memmert, Schwabach, Germany). Then, the films were peeled off and stored at controlled temperature (25±2 °C) and RH (52±1%) before their characterization.

2.2.3. Microstructure characterization of films

2.2.3.1. Thickness measurement

The mean thickness of the spin coated and casted films was measured at 5 random positions with an electronic digital micrometer (Lab-Kits, Lab Instruments & facility, Xiangtan, China) with a range from 0 to 25 mm and graduation of 0.001 mm.

2.2.3.2. Scanning electron microscopy (SEM)

The microstructure of the spin coated films deposited on glass and LDPE supports and casted films was observed using a scanning electron microscope S-4500 (Hitachi, Verrières-le-Buisson, France) at a magnification of ×4800 and using an accelerating voltage of 10 kV. Film samples were mounted on stub with double sided carbon tape. The edge and the film surface were coated with silver and platinum, respectively, before SEM analysis. For the spin coated films, blades were cut up and directly mounted on stub.

2.2.3.3. Atomic force microscopy (AFM)

To observe the film surfaces at nanoscale level, an atomic force microscope (dimension 5, Bruker, Santa Barbara, US) equipped with a cantilever (super sharp silicon AFM probes) and piloted by a nanoscope 5 controller was used. The operation mode used was repulsive tapping at a scanning rate of 152-748 kHz with a constant force of 50 $N.m^{-1}$. The surface areas were scanned at a range from 9 to 100 μm^2 operating under air and at room temperature. The 2D, 3D images and root mean square of roughness value (R_q) were obtained using software Gwyddion 2.49 released 2017-08-15. This parameter represents the standard deviation of the distribution of surface heights allowing to describe the surface roughness by statistical method. This parameter is very sensitive to large deviation from the mean line. The values of R_q were calculated by the following equation:

$$Rq = \sqrt{\frac{1}{l}\int_0^l \{y(x)\}^2 \, dx} \qquad (2)$$

where y(x) described the surface profile over the length (l) in function of height (y) and position (x) (Gadelmawla, Koura, Maksoud, Elewa, & Soliman, 2002).

2.2.4. Contact angle measurement and characterization of film surface properties

Surface hydrophobicity and wettability of films were evaluated from static contact angle by the sessile drop method using a Digidrop goniometer (model ASE, GBX, Roman-sur-Isere, France) equipped with a video measuring system with a CCD camera and with an image analysis software Windrop++v1 (GBX) for data acquisition. A droplet (2 µL) of water was deposited on the film surface with a precision syringe. According to the so-called pick-up procedure when the contact between liquid and solid is made, the measurements of contact angle between the width of the drop and the tangent at the drop boundary were realized on both sides and averaged. At least 3 measurements were carried out for each sample in ambient condition in similar temperature and RH (25±2 °C and 25±5%RH).

Acquired data were the contact angle (θ), the droplet volume (V) and the droplet width in contact with the solid surface (L).

In dynamic mode, three kinetic parameters can be calculated by monitoring the variation of contact angle, droplet volume and droplet width for 10 s using a software-assisted image-processing procedure: the velocity of angle variation, the droplet absorption and the spreading rate of droplet. The velocity of angle variation ($\Delta\theta$) as a function of time was obtained according to the following equation:

$$\Delta\theta\ (°.s^{-1}) = (\theta_{t_f} - \theta_{t_0})/(t_f - t_0) \quad (3)$$

where θ_{t_f} is the contact angle (°) at the final time t_f (s), θ_{t_0} is the contact angle at the initial time t_0 (s).

Absorption (ΔV) and spreading (ΔL) rate of drops were calculated from the drop volume and drop width kinetic, respectively, using the following equations:

$$\Delta V\ (\%) = (V_{t_f} - V_{t_0})/V_{t_0} \times 100 \quad (4)$$

$$\Delta L\ (\%) = (L_{t_f} - L_{t_0})/L_{t_0} \times 100 \quad (5)$$

where V_{t_f} is the volume (µl) of droplet at the final time t_f (s), V_{t_0} is the volume (µL) at t_0 (s), L_{t_f} is the width (mm) of droplet at the final time t_f (s), and L_{t_0} is the drop width (mm) at t_0 (s).

In addition, the temporal variation of experimental water contact angles was fitted using the equation (6) proposed by Farris et al. (Farris et al., 2011):

$$\theta(t) = \theta_{t_0} \exp(kt^n) \quad (6)$$

where k should be related to the $\Delta\theta$ velocity and n to the process resulting to the contact angle evolution. In theory, n = 0 and n = 1 indicate pure absorption and pure spreading phenomena respectively. Parameter estimation for Equation 6 was performed by using a non-linear fitting procedure from Matlab© (Matlab and statistics Toolbox Release 2015b, The MathWorks, Inc., Natick, Massachusetts, United States). Estimated values for k and n were obtained by 2 ways: (1) fixing the value of θ_{t_0} and (2) estimating additionally the parameter θ_{t_0}. Concerning parameter initialization for the estimation

procedure, n was set to different values, in order to avoid local minimum, while θ_{t_0}, when estimated, was set to the first experimental measurement. Parameter k was pre-estimated by interpolating experimental data around t = 1 s which allow to rewrite equation (6) as:

$$k = \ln(\theta(1)/\theta_{t_0}) \qquad (7)$$

The film surface tension or surface free energy (γ, in $mN.m^{-1}$) and polar and dispersive components were calculated using the acid-base approach (equation (9)) based on the Young's equation (equation (8)) (Karbowiak, Debeaufort, & Voilley, 2006; van Oss, Ju, Chaudhury, & Good, 1989) :

$$\gamma_{LV} \times \cos\theta_{t_0} = \gamma_{SV} - \gamma_{SL} \qquad (8)$$

where γ_{LV}, γ_{SV} and γ_{SL} are the surface tensions of the liquid-vapor, solid-vapor and solid-liquid interfaces.

The acid-based method was developed to express total surface tension (equation (9)) in term of a dispersive component or Lifshitz-van der Waals component (γ^{LW}) and a polar component or Lewis acid-base component (γ^{AB}):

$$\gamma = \gamma^{LW} + \gamma^{AB} \qquad (9)$$

Van Oss et al. (van Oss et al., 1989) proposed to determine the solid surface tension by using three reference liquids and the equation (10). This equation takes into account

the change of surface free energy and considering the dispersive and polar components and the γ^- and γ^+parameters of polar component:

$$\gamma_{LV}(1+\cos\theta_{t_0}) = 2\left(\left(\gamma_{SV}^{LW}\gamma_{LV}^{LW}\right)^{1/2} + (\gamma_{SV}^{+}\gamma_{LV}^{-})^{1/2} + (\gamma_{SV}^{-}\gamma_{LV}^{+})^{1/2}\right) \quad (10)$$

Therefore, three reference polar and apolar liquids, milliQ water (18.2 MΩ), ethylene glycol and diiodomethane for which γ^{LW}, γ^+ and $\gamma^-$are known, were used to determine the surface tension of Acacia gum films.

2.2.5. Affinity of Acacia gum films for organic volatile compounds

Contact angle measurements were used to evaluate the affinity between A. *senegal* and A. *seyal* films and the organic volatile compounds described in Table V.2. A droplet (2 µL) of the selected compound was deposited on the spin coated films (glass support) and the variation with time of contact angle was monitored for 0.5 s since the total wettability was reached quickly for all compounds. The contact angle values at equilibrium and the absorption (ΔV) and spreading (ΔL) variation of drop were determined.

2.2.6. Water vapor permeability

Water vapor permeability at 25°C of the casted films was determined using a standardized gravimetric method (AFNOR NF H00-030, 1974). Disks of film samples were placed between two Teflon-based rings on the top of glass cells containing distilled water to provide a relative humidity of 100 %. Cells were put in a desiccator containing silica gel and placed in a ventilated chamber at 25°C. Tests were repeated 5 times for each sample. The theoretical RH gradient was 100% but was controlled by a relative humidity probe and the measured gradient was used for WVP calculation using the following equation (11):

$$WVP = (\Delta m \times e)/(\Delta t \times A \times \Delta p) \quad (11)$$

where $\Delta m/\Delta t$ is the weight loss per unit of time ($g.s^{-1}$), A is the film area exposed to moisture transfer (9.08×10^{-4} m^2), e is the film average thickness (m) and Δp is the measured water vapor pressure difference between the two sides of the film (Pa).

2.2.7. Extraction of aroma compounds from emulsion based films

The following extraction procedure was used to quantify the residual amount of limonene and linalool in films after drying. Pieces of films (0.1 g) were immersed in 20ml water and n-pentane mixture (50:50 v/v). 100 µL of an internal standard solution (10 $g.L^{-1}$ of 2-heptanol in absolute ethanol) were added, and the mixture was shaken for 16h under magnetic agitation (500 min^{-1}). The solutions were then frozen to easily separate the water phase from the organic phase containing limonene or linalool and 2-heptanol. The removed organic phase was dried over ammonium sulfate ($(NH_4)_2SO_4$) and analyzed by gas chromatography. The analysis was carried out on a CP-3800 Gas chromatography (Varian, Les Ulis, France) equipped with a J&W scientific DB-5 column (30 m x 0.320 mm i.d. x 0.25 µm of thickness and a flame ionization detector (FID); hydrogen, 30 $mL.min^{-1}$; air; 300 $mL.min^{-1}$; nitrogen; 30 $mL.min^{-1}$). Hydrogen was used as carrier gas with a flow rate of 1.5$ml.min^{-1}$. The oven temperature was programmed to rise from 40 to 150 °C at 6 °$C.min^{-1}$, then at 15 °$C.min^{-1}$ to 250 °C, and held at 250 °C for 10 min. Injector and detector temperatures were adjusted at 250 °C and 300 °C respectively. Injections were done in split mode with a 1:20 ratio.

Quantifications of limonene and linalool were performed using the internal standard, 2-heptanol. For these two components the response coefficients relative to this internal standard (k) were determined as 0.74±0.01 and 0.57±0.03, respectively. The extractions were done in triplicate.

2.2.8. Statistical analysis

The one-way analysis of variance was performed with Statistica version 10 (France) to detect significant differences between and within the groups of results. The signification level used was 0.05.

3. Results and discussion

Two techniques namely spin coating and casting, were used in this study to make the different Acacia gum films. Spin coating was carried out to obtain thin films (around 2±1 µm) with smooth surface and to investigate accurate surface properties without risk of variation due to roughness. Casting allows obtaining thicker self-supported films after removing from the support and was performed to measure functionalities as the water vapor permeability and aroma retention. Thus the thickness of cast films (180±21 µm) was 100 times higher than those of spin coated films.

3.1. Microstructural characterization and surface properties of spin coated films

In this part, the microstructure and the surface properties of spin coated films were investigated. Thus, the surface self-organization of the two gums, A. *senegal* and A. *seyal*, in relation to their composition was established.

3.1.1. Film microstructure

A rough surface can impact the measured value of contact angle value (Yuan & Lee, 2013) and by consequence the properties of films. The microstructure of A*. seyal* and A*. senegal* spin coated films without glycerol and with 20 % and 15 % glycerol, respectively, was first analyzed by SEM and AFM. The effect of support type, gum type and glycerol addition were studied.

The morphology of spin coated films was not affected by the nature of the support (data not shown), therefore only the films coated on glass support were presented in Figure V.1 and V.2. The observation of microstructure by SEM and AFM showed that Acacia gum film surface was smooth and compatible with contact angle measurement since the root mean square of roughness value (Rq) was weak (< 2 nm), i.e. representing less of 0.1% to the film thickness. However, the film surface morphology was clearly depending on the nature of the gum. The surface of A. *senegal* films studied by SEM (Figure V.1) appeared smooth and homogeneous with some aggregated particles distributed on the surface whereas A. *seyal* films showed an irregular surface with a

repetitive organization as numerous large particle were uniformly distributed onto the surface. Additionally, the complex topography from AFM (Figure V.2) confirmed the more heterogeneous morphology of A. *seyal* film surfaces. A clear difference between the root mean square of roughness value (Rq) was observed which is equal to 0.71 nm and 1.95 nm for A. *senegal* and A. *seyal,* respectively. In agreement with our study, Castellani et al. (Castellani, Gaillard, et al., 2010) observed by AFM that A. *senegal* dried films obtained by depositing a solution at 1 $\mu g.mL^{-1}$ on mica plate at 28 $mN.m^{-1}$ of surface pressure and applying the Langmuir-Blodgett method to transfer the films, showed a smooth surface with some aggregated particles inducing some protrusion.

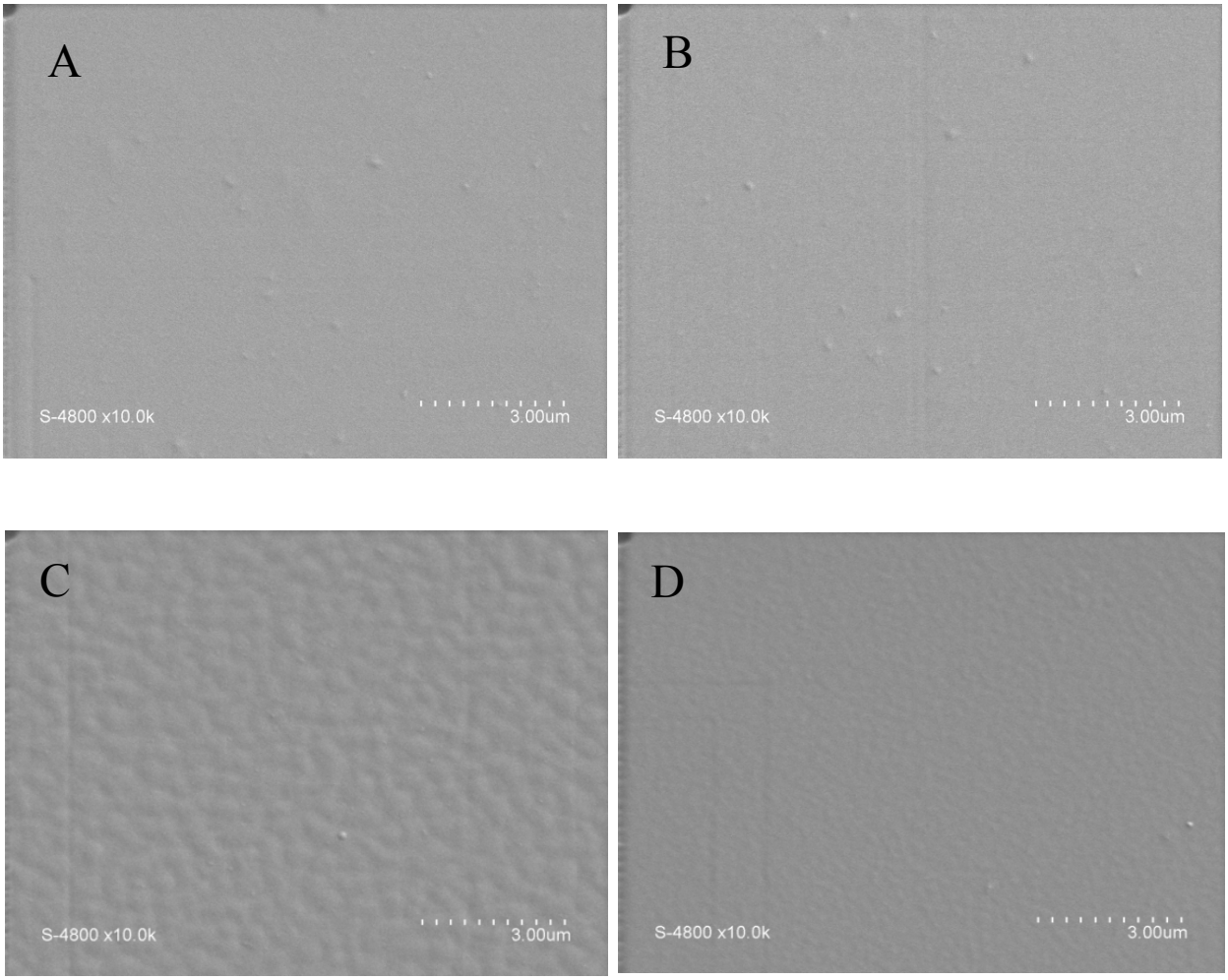

Figure V.1: SEM micrographs (x10.0k) of a spin coated film of A. *senegal* without glycerol (A), A. *senegal* with 15 wt% of glycerol (B), A. *seyal* without glycerol (C) and A. *seyal* with 20 wt% of glycerol (D) deposited on glass support.

The higher surface roughness of A. *seyal* films compared to A. *senegal* ones could be due to differences in their chemical composition and structural property. While composed with the same core structure of 1,3-linked β-D-galactopyranose chain substituted in position 6 by side chains, the sugar blocks of A. *seyal* gum presents the lowest degree of branching (~60% branching vs 80% branching for A. *senegal*) with short side branches (Lopez-Torrez et al., 2015). Moreover, A. *seyal* is richer in arabinose than A. *senegal*, this sugar favoring intra and intermolecular hydrogen bonding (Chalikian, 1998) and then probably playing a key role in its compact conformation. Recently, it was confirmed that A. *seyal* has higher hydration ability than A. *senegal* (Mejia Tamayo et al., 2018).

It can be hypothesized that in contact with air and in concentrated medium, the macromolecular conformation and polarity characteristics of A. *seyal* induced their aggregation. Despite the higher polydispersity of A. *senegal* ($M_w/M_n = 2$) compared to A *seyal* ($M_w/M_n = 1.5$), the former produced a more homogeneous film surface (Lopez-Torrez et al., 2015; Sanchez et al., 2017).

A. *senegal* is also identified by a higher protein amount (Table V.1) than A. *seyal* with especially a higher proportion of protein-rich AGPs. Moreover, the protein fraction from these protein-rich AGPs was found to be more accessible to enzyme (protease), and then to the external environment, for A. *senegal* suspension than for A. *seyal* ones (Flindt, Al-Assaf, Phillips, & Williams, 2005). It can be hypothesized that to decrease the surface tension the process differed for A. *senegal* and A. *seyal*. In the case of the later, a clustering or aggregation of AGPs mediated by strong macromolecules interactions implying hydroxyl groups in hydrogen bond can be suggested whereas in the case of the former the solvent-accessible proteins part can move preferentially to the interface with air and recover all the surface explaining the homogeneity of A. *senegal* surface films. Aggregation of polysaccharide chain was already observed with cellulose ester ultrathin films in order to avoid an unfavorable interaction with air (Kosaka, Kawano, Salvadori, & Petri, 2005). At liquid-liquid interface, the structural

'wattle blossom' model is proposed to explain the adsorption of protein-rich AGPs of A. *senegal*: the protein part anchors at the oil-water interface and the repelling polar sugar blocks attached to this chain provide a steric barrier over the interface (Dickinson, 2003). Similar model could explain the structuration of A. *senegal* film.

The effect of glycerol addition on film surface smoothness was also investigated. The compatibility between Acacia gum and glycerol as plasticizer was demonstrated since there was no phase separation with glycerol addition as observed by SEM and AFM in contrast to alginate-based films (Hambleton et al., 2009). SEM showed that A. *senegal* films surface was apparently not strongly affected by the addition of glycerol. In contrast, for A. *seyal* films, glycerol seemed act by decreasing the aggregated particles size and inducing more homogeneous and smooth film surface. Rq determined from AFM measurement confirmed these results. Indeed, Rq of A. *senegal* films with 15 % of glycerol remained constant with a value of 0.74 nm (as compared to 0.71 nm without) whereas Rq decreased under 1.69 nm for A. *seyal* films with 20 % of glycerol (compare to 1.95 nm without). Then the slight decrease of the A. *seyal* films roughness could be induced by intra- and/or intermolecular interaction changes due to new interactions, and especially hydrogen bonding between water, glycerol and A. *seyal* molecules. Indeed, glycerol is a protic solvent which each molecule has three donor and three acceptor sites favorable to the establishment of hydrogen bonding with water, Acacia gum molecules and glycerol molecules. In water-glycerol mixed solvent containing dextran, a branched polysaccharide, it was shown that the hydrogen bonding sites were more available than in pure water allowing its better solvation and expansion with an increase of the coil radius in this mixed solvent (Antoniou & Alexandridis, 2010). Similar effect can be suggested for A. *seyal gum* with the establishment of hydrogen bonding between glycerol and hydroxyl group of sugars from A. *seyal* gum molecules allowing a better solvation of these molecules at the interface, and then preventing their aggregation.

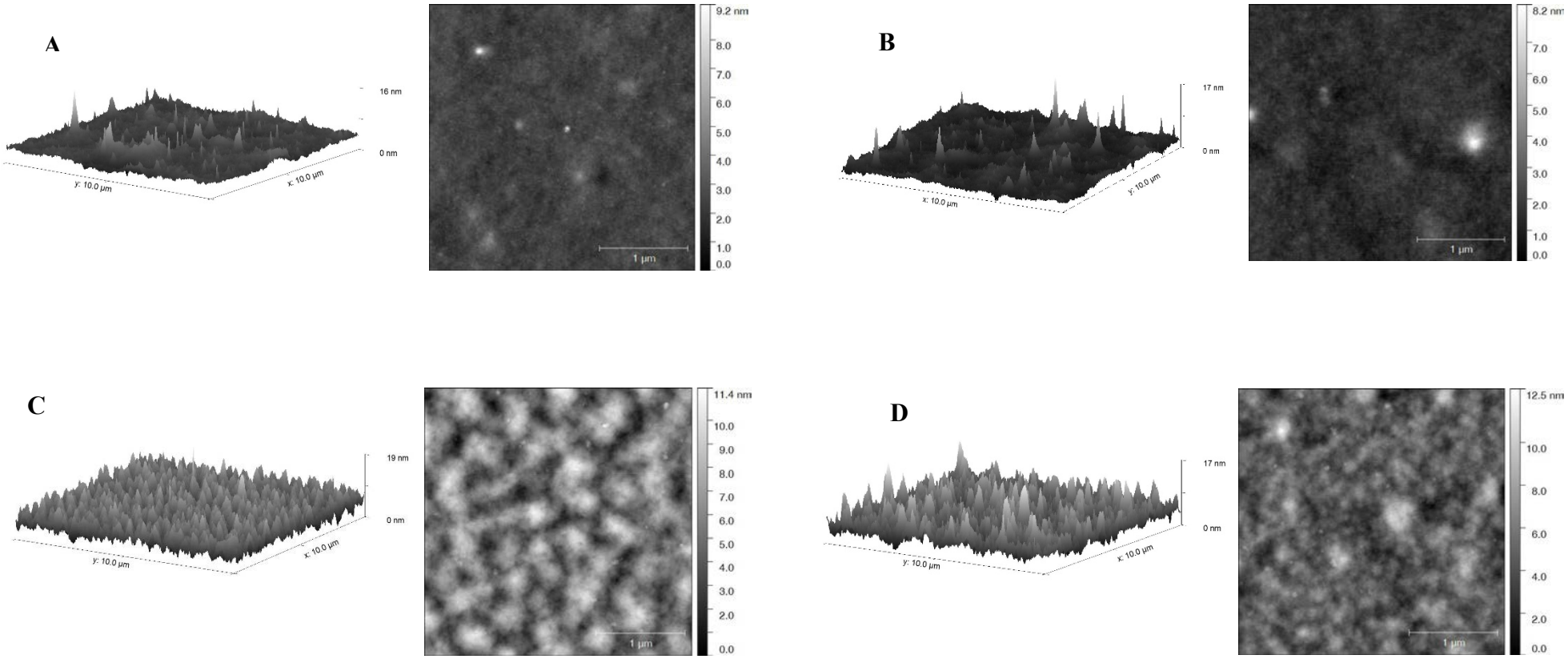

Figure V.2: AFM 3D (10x10 μm^2) and 2D images (3x3 μm^2) of A. *senegal* film without glycerol (A), A. *senegal* film with 15% glycerol (B), A. *seyal* film without glycerol (C) and A. *seyal* film with 20% glycerol (D) on glass support.

This structural morphology study has revealed that the surface of Acacia films was smooth and clearly compatible with contact angle measurements. However, difference in surface properties between both gums films was obvious.

3.1.2. Surface properties of films

3.1.2.1. Water contact angle measurement

The value of contact angle θ with water is a current parameter used to measure the degree of hydrophobicity of a material (Karbowiak, Debeaufort, & Voilley, 2006). As defined by Vogler (Vogler, 1998), the relative "hydrophilic" term was applied to surfaces exhibiting a water contact angle not exceeding 65 degrees ($\theta<65°$) and "hydrophobic" to surfaces exhibiting a $\theta\geq65°$, based on the appearance and disappearance of long-range hydrophobic interactions. Basically, hydrophobic surfaces are less water wettable than hydrophilic ones.

In Table V.3, the contact angles of spin coated films are reported as a function of support (glass or LDPE blade), gum types and glycerol addition. The contact angle values were immediately measured after droplet deposition (θt_0 value). Whatever the film composition, there was no significant effect of the support nature on the contact angle values. This confirmed the SEM observation that the structuration of gum films was similar on the hydrophobic (LDPE) and hydrophilic (glass) surface. The impact of support on contact angle values was not generally described in literature but, for the present study, the nature of support was assessed to find the convenient support for dried films to be peeled intact (Karbowiak, Debeaufort, Champion, et al., 2006). The only study reporting support effect concerned β-casein films for which a difference of structuration depending on support hydrophobicity was observed with change in wettability (Nylander & Tiberg, 1999).

A. *seyal* films with θ around 40° displayed hydrophilic surface whereas the contact angle values (around 60°) of A. *senegal* films were close to the limit 65° value, suggesting that surfaces were equally hydrophobic and hydrophilic (Vogler, 1998). Then, A. *seyal* films showed significantly lower θ value than A. *senegal* confirming that the last films were

more hydrophobic in relation to its less strong moisturizing ability (Mejia Tamayo et al., 2018). This variation can be related to the differences in the protein content of the two gums, the establishment of specific intramolecular bond and resulting structuration at the surface of the films. Moreover, the difference between the two gum films was in agreement with the specific structural morphologies observed by SEM and AFM.

Comparing to values reported in the literature, the θ values found for Acacia gum films were in the range of the values reported for polysaccharides and proteins films varying from hydrophilic (θ =32°) to hydrophobic surface (θ = 106°) (supplementary data). Whereas some polysaccharides films were reported as highly hydrophobic such as those made with Kafiran or chitosan, others were more hydrophilic such as starch films. However, protein based films were generally characterized by hydrophobic surface properties with θ≥65°.

As suggested by microstructural observations and the underlying assumptions, protein-rich AGPs from A. *senegal* gum could be mainly located at the air/solid interface with a structuration depending on the protein content and its accessibility. The protein adsorption at the air/water interface or to various kind of surfaces has been intensively studied (Damodaran, 2004; Vogler, 2012). This last author promoted the idea that protein adsorption at the solid/liquid interface is not fundamentally different than adsorption to the air/liquid interfaces in the case of hydrophobic solid surface. The level of surface hydrophilicity is the result to water displacement and change in water-protein interaction. Then, the hydrophobic part of protein is found at the surface. However, the water displacement is function of different parameters as protein concentration, protein molecular size, protein conformation (Damodaran, 2004) and adsorbent surface energy (water wettability) (Vogler, 2012).

The comparison of adsorption characteristics at air-water interface between β-casein and a glycoprotein obtained from crude gum after gel filtration purification has shown that the affinity of the last for interfaces was weaker than that of proteins (Damodaran & Razumovsky, 2003). The purified glycoprotein was a hydroxylproline-rich

glycoprotein (GAGP) with ~90% carbohydrate and 10% protein content and molecular weight between 2.2×10^6 - 5.6×10^6 $g.mol^{-1}$. The monolayer coverage of the surface was estimated and depended on the way the GAGP lies at the interface (flat or by cross-sectional area).

As demonstrated for apricot gums, the contact angle values slightly differed with gum origin and strongly changed with concentration (5 and 20%): increasing by instance from 37.7° to 50.6° with the concentration increase (Chichoyan, 2015). However, the film surfaces were always characterized by hydrophilic surface in contrast to our results. The author suggested the impact of composition to explain differences between varieties, i.e. the high hydrophilic films will be rich in uronic acids. The impact of concentration can be correlated to the water content and the reorganization of gum molecules at the surface. The fact that both Acacia gums are constituted by a continuum of AGPs with different protein content more and less accessible makes difficult to predict their behavior at interfaces. The higher content of sugar units correlated with a low protein content of A. *seyal* and the hydrophilic surface of films argued to the presence of carbohydrate part of AGPs at the surface whereas the protein part may cover the surface for A. *senegal* films. As glycerol affected microstructure, its addition in film-forming solution can also induce changes in surface properties and help to understand the structuration of films.

Table V.3: Water contact angle (θ) of Acacia gum spin coated films deposited on glass and LDPE supports. Kinetic parameters from water contact angle measurements: Velocity of contact angle variation during 10sec ($-\Delta\theta$) in °.s^{-1}; water droplet width (ΔL) and volume ($-\Delta V$) variation expressed in %. Main parameters obtained from fitting equation 4 to experimental results describing contact angle evolution rate (k), physicochemical phenomena involving in kinetic process (n) and root mean square error (RMSE).

		Parameters derived from contact angle measurement for spin coated films				Estimated parameters for spin coated films		
		θ at t_0 (°)	$-\Delta\theta$ (°.s^{-1})	ΔL(%)	$-\Delta V$(%)	-k	n	RMSE (°)
Glass support	A. *senegal* film without glycerol	62.2^{a}±0.3	2.6^{a}±0.1	23.9	4.5	0.119^{a}±0.007	0.63±0.01	1.20±0.06
	A. *senegal* film with 15 wt% of glycerol	68.3^{b}±1.2	4.0^{b}±0.4	44	4	0.165^{b}±0.008	0.73±0.13	1.31±0.35
	A. *seyal* film without glycerol	42.4^{c}±0.1	1.6^{c}±0.02	20	2	0.121^{a}±0.000	0.57±0.01	0.86±0.10
	A. *seyal* film with 20 wt% of glycerol	50.8^{d}±1.5	3.0^{d}±0.2	38	7	0.305^{c}±0.013	0.52±0.04	1.55±0.25
LDPE support	A. *senegal* film without glycerol	60.3^{a}±1.2	2.1^{e}±0.2	19	3	0.075^{d}±0.005	0.79±0.04	0.61±0.19
	A. *senegal* film with 15 wt% of glycerol	69.5^{b}±1.4	3.7^{b}±0.2	37	7	0.131^{a}±0.007	0.76±0.01	1.15±0.06
	A. *seyal* film without glycerol	41.7^{c}±0.5	1.4^{c}±0.1	15	6	0.066^{d,e}±0.001	0.72±0.03	0.44±0.07
	A. *seyal* film with 20 wt% of glycerol	49.2^{d}±0.8	2.8^{a,d}±0.2	32	8	0.184^{b}±0.003	0.65±0.01	0.83±0.03

Roman superscripts denote statistically significant differences in each column.

3.1.2.2. Effect of glycerol on water contact angle value

Similar behavior for both gums was observed with the addition of glycerol with an increase of the contact angle value and then of the film surface hydrophobicity (Table V.3). The increase was stronger for A. *seyal* films, the most hydrophilic polymer, than for A. *senegal*. This suggested a new surface organization in the presence of glycerol that is reflected by the increasingly smooth surface observed by SEM and AFM, especially for A. *seyal* gum films.

According to literature, the effect of glycerol seems dependent on the hydrophobicity of films. For instance, the addition of glycerol to an iota-carrageenan based film, characterized by a hydrophobic surface ($\theta = 88.3 \pm 4.0°$), tended to increase the surface hydrophilicity ($\theta = 44.1 \pm 3.8°$) (Karbowiak, Debeaufort, Champion, et al., 2006). Authors suggested that the structure and composition of the film surface was changed by the addition of glycerol, inducing either a reduction of polymer–polymer interactions or an exposition of glycerol molecules at the surface of the film. Similar decrease of the water contact angle was reported for varied other hydrocolloids films (Supplementary table V.1) (Ahmadi, Kalbasi-Ashtari, Oromiehie, Yarmand, & Jahandideh, 2012; Ghasemlou, Khodaiyan, Oromiehie, & Yarmand, 2011; Jouki, Khazaei, Ghasemlou, & Hadinezhad, 2013; Seyedi, Koocheki, Mohebbi, & Zahedi, 2014). The only exception found in literature was for sage seed gum films, characterized by hydrophilic surfaces, since the contact angle value increased with glycerol concentration, as observed for Acacia gums. Sage seed gum is a galactomannan containing 28 to 32% of uronic acid but also a protein part which represents on average 2%, i.e. between A. *seyal* (1%) and A. *senegal* (2.7 %). The films of sage seed gum can be considered as hydrophilic ($\theta < 65°$) as for A. *seyal* films (Razavi, Cui, Guo, & Ding, 2014; Razavi, Mohammad Amini, & Zahedi, 2015). The authors explained the increase of hydrophobicity by the formation of hydrogen bond between the hydroxyl group of plasticizer and the hydrophilic group of the gums chain (carboxyl and hydroxyl group) reducing the available hydroxyl groups at the film surface. Then, the level of establishment of hydrogen bonding

between glycerol and hydroxyl group of Acacia gums is function of the sugar content and could (i) relatively decrease the available hydroxyl groups at the film surface and consequently the hydrophilic contribution, and (ii) induce conformational changes of Acacia gum molecules favoring the richness at the surface of hydrophobic residues such as acetyl group of 4-O-Me-Glucuronic acid (specifically for A. *seyal*) and of hydrophobic amino acids such as alanine, isoleucine, leucine, glycine, phenylalanine and valine. These amino acids are present in both gums but they represented 6.56 $mg.g^{-1}$ for A. s*enegal* against 2.28 $mg.g^{-1}$ for A. *seyal* (Lopez-Torrez et al., 2015).

3.1.2.3. Contact angle evolution

Due to the hydrophilic nature of biopolymer films, the true water contact angle at equilibrium is unattainable and evolution of drops can occur due to absorption, spreading, swelling and evaporation (Colivet & Carvalho, 2017; Farris et al., 2011). Then, the kinetic changes of contact angle value have been studied and fitted by the model proposed by Farris et al. (Farris et al., 2011). In Table V.3, the evolution of contact angle and the variation of water droplet volume and width during the first 10 s are reported. The increase of the droplet width in contact with film surface is correlated with the area variation. It indicates the spreading phenomena whereas the decrease of the droplet volume corresponds to the absorption phenomena. The evaporation was considered as negligible and swelling did not occur.

Water contact angle diminished with time demonstrating the affinity of water for the both films. However, the contact angle evolution was faster for A. *senegal* than A. *seyal* films in relation to the higher hydrophobicity of the former. In the same way, the addition of glycerol favoring surface hydrophobicity increased the contact angle velocity for both gums. For all samples, the increase of droplet width (expressed in %) was higher than the volume evolution, suggesting that the changes of contact angle were mainly due to spreading. Comparing to other biopolymers, the contact angle evolution for Acacia gum films was close to the one of pectin and pullulan films, which was also

due to spreading phenomena. For other biopolymers such as chitosan and gelatin films, both absorption and spreading occurred (Farris et al., 2011).

Additionally, the variation of water contact angles was fitted using the equation (4) which allowed to obtain "k" and "n" parameters. Estimated values for k and n were obtained by 2 ways: (1) fixing the value of θ_{t_0} and (2) estimating additionally the parameter θ_{t_0}. The last procedure was used since the estimated θ_{t_0} values were in agreement with the experimental values. Fitting the contact angle evolution shows a good correlation between the estimated values and experimental ones for all samples as proved by the RMSE which is always inferior to the standard deviation. According to Farris et al. (Farris et al., 2011), "k" is related to the velocity of contact angle variation and the value of n provided indication about the evolution process, with n = 0 and 1 representing total absorption and total spreading process, respectively. k values were well correlated to the experimental data in term of the glycerol effect but not to the gum type that had no effect on contact angle evolution. For all samples, the values of n were close but slightly higher than 0.5 which indicated that absorption and spreading phenomena occurred during the contact angle evolution, with however a slight prevalence of spreading phenomenon. The model proposed by Farris et al. allowed to confirm the affinity of gums films for water but failed to predict precisely the evolution of contact angle and the predominant phenomenon. This might be due to the short time used to follow the evolution of the contact angle and deduced the value of model parameters.

3.1.2.4. Free surface energy

Using three liquids of reference (water, ethylene glycol and diiodomethane) and applying the method of van Oss, the surface tension (γ) of films without glycerol was evaluated. Additionally, the surface thermodynamic properties were characterized by the dispersive (γ^{LW}) components of surface tension and polar (γ^{AB}) but also by electron donor (γ^-) and acceptor (γ^+) parameters of the polar component. When a film contains both γ^- and γ^+ parameters at appreciable levels, it will be defined as "bipolar" whereas a

surface is considered as monopolar when one kind or the other (γ^- or γ^+ parameters) dominates (van Oss, Chaudhury, & Good, 1987). The surface tension (Table V.4) appeared higher for A. *seyal* films (46.8 $mN.m^{-1}$) than for A. *senegal* films (40.5 $mN.m^{-1}$). This confirmed that the chemical structure of film at the surface differed. The surface energy of Acacia gum films was in the same order of magnitude (12 to 60 $mN.m^{-1}$) than the values found for other polysaccharides and protein films (Supplementary table V.1). Both films of Acacia gums were characterized by polar and dispersive components and behaved as polymers with dual polarity. However, the polar component was remarkably lower than the dispersive component for both gums (Table V.4).

Table V.4: Contact angle (θ) of diiodomethane and ethylene glycol for Acacia gum spin coated films. Surface tension (γ), disperse (γ^{LW}), and polar (γ^{AB}) components, electron-donor (γ^-) and electron-acceptor (γ^+) parameters of the polar component, in $mN.m^{-1}$, calculated according to the method of van Oss.

	θ diiodomethane (°)	θ ethylene glycol (°)	γ	γ^{LW}	γ^{AB}	γ^-	γ^+
A. *senegal* film	$40.9^a \pm 0.7$	$44.1^a \pm 0.5$	$40.7^a \pm 0.5$	$39.2^a \pm 0.4$	$1.5^a \pm 0.1$	$21.3^a \pm 0.6$	0.0 ± 0.0
A. *seyal* film	$45.0^b \pm 0.3$	$51.6^b \pm 1.1$	$46.5^b \pm 1.3$	$37.0^b \pm 0.2$	$9.5^b \pm 1.2$	$56.8^b \pm 1.1$	0.4 ± 0.1

Roman superscripts denote statistically significant differences in each column.

In agreement with Acacia gum films, gelatin (Farris et al., 2011), whey proteins or wheat starch films (Basiak et al., 2016) showed polar and dispersive components of surface tension. The latter was predominant meaning that these films have more affinity for apolar compounds but are able to interact with polar compounds such as water. Pectin, pullulan and chitosan (Farris et al., 2011) films showed polar component value (γ^{AB}) equal to zero suggesting that these polysaccharides films behaved

preferentially as apolar surface. Moreover, the polar component of A. *senegal* (1.3 $mN.m^{-1}$) was significantly lower than that of A. *seyal* (9.7 $mN.m^{-1}$) according to its higher hydrophobicity. The value of surface tension of an arabinogalactan (without protein part) having molar mass of 8×10^5 $g.mol^{-1}$ was reported equal to 50.2 $mN.m^{-1}$ and its parameters were consistent with the results obtained with A. *seyal* films with $\gamma^{LW} = 37.6$ $mN.m^{-1}$, $\gamma^{AB} = 12.6$ $mN.m^{-1}$ (van Oss, 1994). For both gums but also for the arabinogalactan mentioned before ($\gamma^- = 53.1$ $mN.m^{-1}$ and $\gamma^+ = 0.75$ $mN.m^{-1}$), the electron donor (γ^-) represented the major contribution of the polar component (γ^{AB}) but differed between the two gums: the higher value was observed for A. *seyal.* For Van Oss, Chaudhury, and Good (van Oss et al., 1987), all polysaccharides are monopolar bases and the value of γ^- exceeded 28 $mN.m^{-1}$. It was the case for A. *seyal* but not for A. *senegal*, confirming as previously suggested that the dominant polysaccharide part was preferentially exposed at the surface of A. *seyal* gum films. Among the polysaccharide studied by Farris et al., only pullulan had the value of γ^- exceeded 28 $mN.m^{-1}$ ($\gamma^- = 35.6$ $mN.m^{-1}$). The authors justified this by the fact that pullulan possessed a strong monopolar electron donor characteristic in relation to the high content of hydroxyl groups. The behavior of A. *seyal* seemed to be more similar to arabinogalactan or pullulan in agreement with the high content of arabinose and the flexibility of the backbone which allowed to water to interact promptly (Farris et al., 2011). The hydration properties of monosaccharides and affinity of water strongly depend on the number of hydroxyl group (pentose against hexose for example) but also on the relative position, the proximity, of other polar groups (Chalikian, 1998). Moreover, the value of γ^+ for A. *seyal* is slightly superior to zero unlike A. *senegal* indicating that some electron acceptor groups could be present at the A. *seyal* film surface such as carboxyl function present in glucuronic acids or amino acids. However, A. *seyal* was poorer in glucuronic acids and amino acid than A. *senegal.* The dual polarity seemed less pronounce for A. *senegal* than A. *seyal* films that could be explained by (i) the ose compositions less rich in arabinose, (ii) the absence of COO^- group at the surface in the relation to the value of γ^+ equal to zero and (iii) the higher

content of apolar amino acids in relation to the higher content of protein-rich AGPs at the surface as previously stated.

3.1.3. Affinity of volatile organic compounds for Acacia gum films as determined by contact angle measurement

For a better understanding of the surface organization, the wetting of Acacia gums films by apolar compounds was studied.

The contact angle values of different organic compounds chosen (Table V.2) in order to investigate the effect of polarity and chemical function were measured. As the compounds are volatile at atmospheric pressure, the effect of evaporation was first studied by measuring the volume of droplet formed on the tip of needle in the air for 5 min. The volume variation was found negligible for all tested compounds excepted for octane which evaporated at a rate of 0.017 µL.s^{-1}. For this compound, the evolution of contact angle with time could not be evaluated.

The contact angle values between the different volatile compounds and both Acacia gum films were weak, being all <20°, showing the high affinity between Acacia gum films and hydrophobic compounds and confirming the dual polarity of the Acacia gum films (Figure V.3). Whereas it is generally admitted that small contact angles below 20° cannot be accurately measured due to the difficulty to assign a tangent line when the droplet profile is almost flat (Yuan & Lee, 2013), the values were repeatable and there was a graduation in the affinity with the compounds polarity as demonstrated for linear alcohols and alkanes (Figure V.3). This effect of alkanes polarity on contact angle values were also described for an apolar PTFE (polytetrafluorethylene) film (Fox & Zisman, 1950; Neumann et al., 1971). Additionally, the chemical nature of the compound impacted the contact angle values since the values for alkanes were lower than those of alcohols for the similar logP value. Thus, there was a better affinity between Acacia gum films and alkanes than alcohols. The increase of contact angle values for alkanes with decrease polarity showed non-linear relationship but the affinity

is always high even if the hydrophobicity of the compound is high. This non-linearity was not observed for alcohols because the number of values were insufficient to conclude.

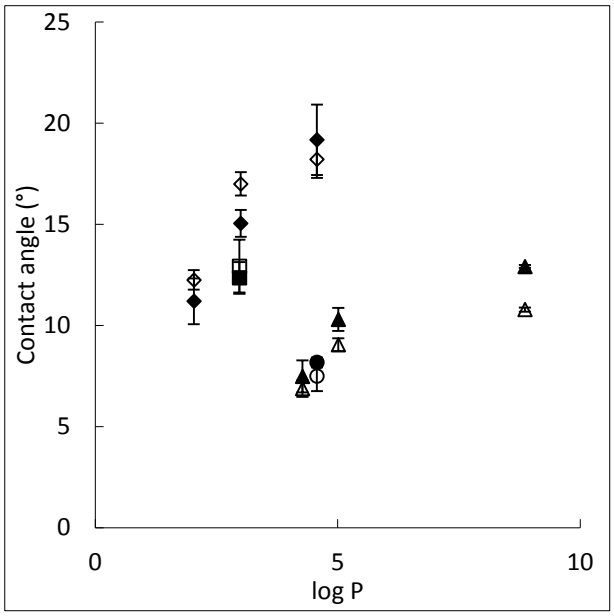

Figure V.3: Contact angle values of alcohols (◇, ◆), alkanes (▲, ▵), linalool (□, ■), limonene (○, ●) for films of A. *senegal* (black) and A. *seyal* (white) gums as a function of hydrophobicity of components (expressed by logP).

The contact angle for a terpene (limonene) and a terpenyl alcohol (linalool) was also measured for both Acacia gum films (Figure V.3). Limonene is a more apolar compound than linalool and showed the lower contact angle value as observed for alkanes and alcohols. Linalool characterized by a linear structure and limonene by a cyclic structure are C10 compounds and showed similar affinity that decanol and decane, respectively. However, it seemed that the cyclic molecule had slightly more affinity for Acacia gum films than the linear one. The values of contact angle found using diiodomethane (Table V.4) which is also an apolar compound (logP = 2.48), were higher than those measured for other organic compounds with a similar polarity. These results demonstrated that contact angle values depended not only on the polarity and structure but also on chemical nature of the compound.

Despite the difference in surface tension and value of polar and dispersive components between A. *seyal* and A. *senegal* gum films, the contact angle values were not significantly affected by the nature of gum excepted for decanol characterized by a logP of 4.57. For this compound, A. *senegal* film showed a greater affinity. It can be hypothesized that decanol possesses a sufficient long chain to interact with A. *senegal* gum films with its two extremities: one (OH) with a polar part and the other (CH_3) with an apolar part of the surface. For A. *seyal*, the interaction with decanol will be limited to one extremity (OH) explaining the lower affinity.

Leelaphiwat et al. (Leelaphiwat et al., 2012) had studied the affinity between eucalyptol (an oxide monoterpene with logP of 2.74 (source: chemspider.com)) and polymeric packaging materials with different hydrophobicity by contact angle measurement and found that the affinity was stronger for polyethylene (θ=9°) and polypropylene (θ=11°), the most hydrophobic materials. However, the affinity remained high, with contact angle of 14°, for the average polar polymers as polyamide and polyethylene terephthalate. Moreover, the results showed that contact angle value followed the same trends as the solubility coefficient of eucalyptol for the polymers.

The contact angle evolution was also monitored and for a same compound family, the velocity increased with polarity (Table V.5). Depending on the compound, the evolution was similar for both Acacia gum films or more pronounced for A. *senegal* gum films (decane, hexadecane and limonene). In the same way, depending on the compound and the gum, the evolution was due to more or less to absorption and spreading. For the A. *seyal* gum films, droplet width and volume moved in the same way for the majority of the compounds, then the contact angle evolution might be due to both spreading and absorption. However, for hexanol and limonene spreading was dominating. For A. *senegal* gum films, spreading and absorption were implicated in the same manner for hexanol and linalool the most polar components, spreading was preponderant for octanol and decanol and absorption clearly dominated for decane,

hexadecane and limonene, the most apolar compounds. Then it seems that for this gum film, the dominant phenomenon depended on the polarity of compounds.

In the case of Acacia gum films, the laws describing the affinity between films and compounds were complex and not due only to the surface properties of films or only to the physicochemical properties of organic compounds. Moreover, even if the affinity for all apolar organic compounds was strong, the affinity for water was not negligible (especially for A. *seyal*). These results could be related to the specific structure and composition with dual polarity of Acacia gum films allowing affinity for both polar compound (water) and apolar compounds. As said above, both Acacia gums contain hydroxyproline-rich glycoproteins known to bind hydrophobic ligands such as tannins and phenolics in plants (Kieliszewski & Lamport, 1994). Then the strong affinity for apolar compounds suggested the presence of these molecules at the surface of *A. senegal* films with the protein part exposed to the surface as previously suggested and reported also by the 'wattle blossom' model at liquid-liquid interface. Protein-rich AGPs are present in greater extend in A. *senegal* than in A. *seyal* but the protein part is differently distributed between A. *senegal* and A. *seyal* (Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005). Moreover, for A. *seyal* gum the protein can difficulty cover the interface for steric reason. Considering the overall results, it can be argued that the structuration of A. *seyal* film differed implying inter and intramolecular hydrogen bonding of polysaccharides and allowing to expose the most hydrophobic part of the molecules at the surface.

Table V.5 : Parameters derived from volatile organic compounds contact angle measurements for Acacia gum films on glass support: Velocity of contact angle variation during 10sec ($-\Delta\theta$) in °.s^{-1}; water droplet width (ΔL) and volume ($-\Delta V$) variation expressed in %.

	$-\Delta\theta$ (°.s^{-1})		ΔL (%)		$-\Delta V$ (%)	
	A. *senegal*	A. *seyal*	A. *senegal*	A. *seyal*	A. *senegal*	A. *seyal*
Octane	ND	ND	ND	ND	ND	ND
Decane	$0.95^{a}\pm0.01$	$0.71^{a}\pm0.05$	11	13	29	12
Hexadecane	$0.54^{b}\pm0.04$	$0.44^{b}\pm0.02$	6	8	9	9
Hexanol	$0.49^{b}\pm0.05$	$0.55^{a,b,c}\pm0.07$	8	11	9	7
Octanol	$0.39^{b,c}\pm0.05$	$0.44^{b,c}\pm0.04$	7	6	3	8
Decanol	$0.30^{c}\pm0.13$	$0.37^{b}\pm0.06$	5	5	1	7
Linalool	$0.48^{b,c}\pm0.02$	$0.47^{b,c}\pm0.08$	8	6	8	7
Limonene	$0.71^{d}\pm0.05$	$0.62^{a,c}\pm0.06$	9	13	17	10

ND: not determined. Roman superscripts denote statistically significant differences in each column.

3.2. Properties of cast films

The purpose of this part was to study the microstructure and functionalities of Acacia gum cast films as water vapor permeability and aroma retention efficiency and to establish relationship between these properties and the hydrophilicity/hydrophobicity of film surface.

3.2.1. Film microstructure

The microstructure of cast films made with glycerol was observed by SEM. The upper surface of film (in contact with air during casting) was smooth for both gums (supplementary data). For A. *seyal* gum films, some hollows were observed which could be due to the air bubble break during drying step, but at this scale the homogeneity of film was obvious. These observations were in agreement with the favorable impact of glycerol on films homogeneity previously described for spin coated films.

3.2.2. Water contact angle

In parallel to the analysis of spin coated films and for comparison purposes, water contact angle for casted films containing glycerol was measured. The contact angle values were 74.3±1.4 and 47.4±1.9 for A. *senegal* and A. *seyal*, respectively (Table V.6). The contact angle value of A. *senegal* was then higher than the one on A. *seyal* cast films, as found for spin-coated films. Even if cast A. *seyal* films showed similar ϑ than that for spin-coated films, θ was significantly higher (69.5 against 74.3) for A. *senegal* cast films and the films became more hydrophobic. As the moisture content of spin coated and cast films were similar (~10%), this difference could be the result of a different structural rearrangement during drying process. Indeed, the drying is fast for spin coating while for casting the drying was due to slow solvent evaporation. It can be suggested that in parallel to the water evaporation during slow drying, the protein-rich AGPs which are present in A. *senegal* moved preferentially to air-solid interface. For A. *seyal*, the intramolecular network should rapidly be established and maintained whatever the drying process. As the value of contact angle discriminated the surface

properties of both films, one (A. *seyal* films) behaving as hydrophilic surface and the other (A. *senegal* films) rather as hydrophobic surface, water vapor permeability is expected to be different between the two films.

3.2.3. Water vapor permeability (WVP)

The water barrier properties of A. *senegal* and A. *seyal* cast films with glycerol were measured. The water vapor permeability of both Acacia gum films was high with an order of magnitude of 10^{-11} mol.m^{-1}.s^{-1}.Pa^{-1}. However, A. *senegal* gum films showed lower water vapor permeability than *A. seyal* gum films (Table V.6). These differences were consistent with the value of contact angle showing the greater hydrophobicity of A. *senegal* gum films than A. *seyal* ones.

Table V.6: Contact angle of cast films, thickness and water permeability.

Films	Water contact angle (°)	Thickness (µm)	WVP (10^{-11} mol.m^{-1}.s^{-1}.Pa^{-1})
A. *senegal*	74.3±1.4	180±0.021	2.05±0.15
A. *seyal*	47.4±1.9	180±0.021	3.01±0.15

It is well known that hydrophilic polysaccharide and protein based films possess high water vapor permeability (Fernandes Nassar, Dombre, Gastaldi, Touchaleaume, & Chalier, 2018; Ghasemlou et al., 2011; Jouki et al., 2013; Razavi et al., 2015; Sharma & Singh, 2016; Wagh, Pushpadass, Emerald, & Nath, 2014; Zhang & Whistler, 2004; Zhang, Zhao, & Shi, 2016). The WVP of different polysaccharides and proteins films varied from 0.07 to 14x10^{-11} mol.m^{-1}.s^{-1}.Pa^{-1} depending on the nature of the polymer, the RH gradient and the content of glycerol (Supplementary table V.2). Compared to other gums, the WVP of Acacia gum films was in the same order of magnitude that the films of cress seed gum prepared with glycerol at 25% (1.17x10^{-11} mol.m^{-1}.s^{-1}.Pa^{-1}) (Jouki et al., 2013), but higher than sage seed gum prepared with glycerol at 40% (0.24x10^{-11} mol.m^{-1}.s^{-1}.Pa^{-1}) (Razavi et al., 2015). Despite the protein content of sage seed gum that is in the range of Acacia gums, the barrier properties of Acacia gum

films are closer to those of cress seed gum films which did not contain proteins (Karazhiyan et al., 2011). It remained difficult to compare these films with Acacia gum films as the differences observed could be due to the composition and structure of those gums but also to the glycerol content that varied between 15% and 40% according to the gum films.

It was shown for Acacia gum films that the addition of glycerol induced more homogenous films. However, it is important to highlight that the glycerol content in both films are different and that glycerol can modify WVP. It has been reported that the permeability increased with the addition of glycerol as plasticizer to the cress seed gum, kafiran, gellan, casein, and whey protein isolate based films (Ghasemlou et al., 2011; Jouki et al., 2013; Kokoszka et al., 2010; Wagh et al., 2014; Yang & Paulson, 2000). Authors explained the increase of WVP with the addition of glycerol by (1) the increase of free volume in the matrix due to the reorganization of network (2) the adsorption of water molecule that could be promoted by the presence of hydrophilic glycerol molecule and (3) the formation of glycerol cluster at high concentration of glycerol. For Acacia gum films which contain AGPs with different protein content, the high WVP could be explained by the reduction of intermolecular force between the macromolecules occurring due to the addition of glycerol. For films made with gum Cordia, an anionic polysaccharide covalently bound to protein, the WVP increased with the increase of glycerol (Haq, Hasnain, & Azam, 2014). It was shown by FTIR analyzes that the interaction with glycerol and the nature of hydrogen bonding changed with the concentration. Glycerol can be bound by its hydroxyl group to water, polymer and other molecules of glycerol. Moreover, hydrogen bonding between the polymer hydroxyl groups occurred. At low concentration of plasticizer as in the case of Acacia gum films, the hydroxyl groups of the polymer preferentially interacted with glycerol inducing the solvation effect of plasticizer.

Additionally, the higher percentage of glycerol in A. *seyal* films could partially explain the greater permeability of A. *seyal* films. In the goal to confirm this hypothesis, films

of A. *senegal* containing 20 wt% of glycerol were performed and the water vapor permeability assessed, a value of 2.75±0.12 x 10^{-11} mol.m^{-1}.s^{-1}.Pa^{-1} was found indicating that the glycerol contributed to the increase of WVP. It can be concluded that the higher rate of glycerol induced the lower barrier properties of A. *seyal* gum films compared to A. *senegal* gum films. However, the water affinity of each gum could play a role in the difference of behavior as already stated.

3.2.4. Aroma compound retention of films

The limonene and linalool emulsion based films were formed in order to measure the aroma retention efficiency by Acacia gum films and to make a relationship between aroma level maintained in the emulsion based film and the results of contact angle measurements for these two compounds.

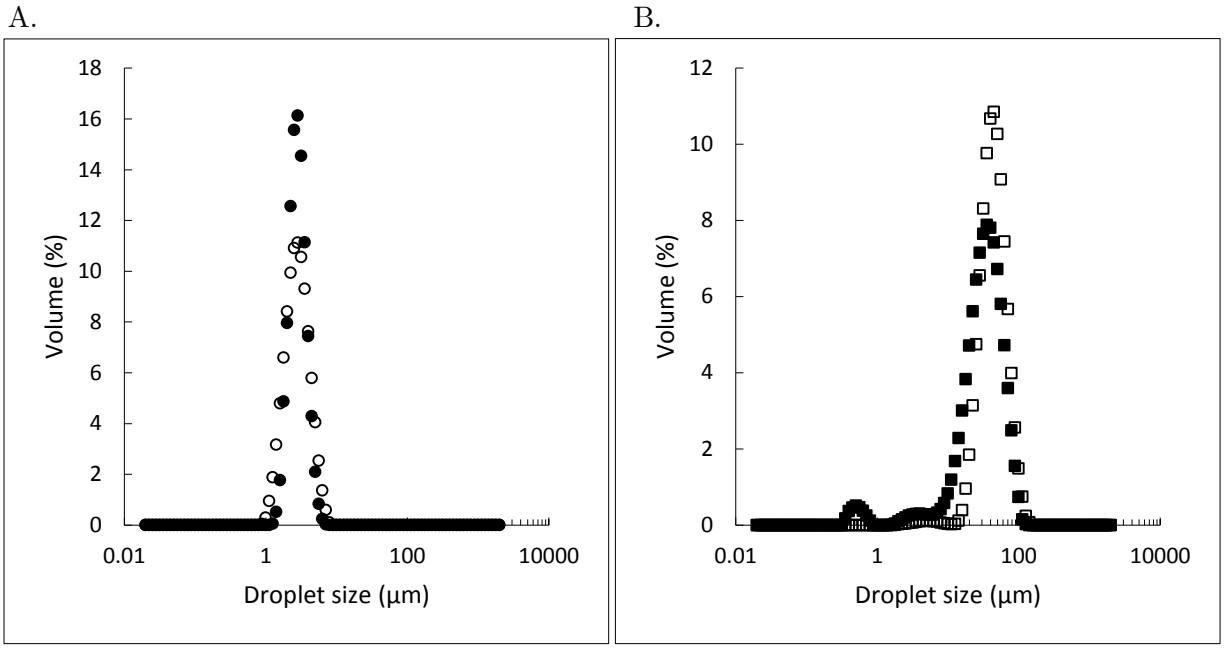

Figure V.4: Droplet size distribution of limonene (A) and linalool (B) emulsions stabilized by A. *senegal* (black) and A. *seyal* (white) gums.

First of all, the aroma emulsions were formed and the emulsion droplet size was measured since the size and homogeneity of droplet distribution can affect important film characteristics (Debeaufort & Voilley, 1995; Mchugh & Krochta, 1994). The limonene emulsions produced by both gums showed a monomodal particle size distribution (Figure V.4.A) with a $D_{4,3}$ around 3 µm whereas the linalool emulsion exhibited a multimodal distribution and higher average droplet size (Figure V.4.B). Moreover, for this compound, the nature of gum impacted the size: A. *senegal* allowing to obtain smaller droplet size (around 38 µm) than A. *seyal* (around 50 µm).

Acacia gums are well known for their emulsifying properties (Dickinson, Murray, Stainsby, & Anderson, 1988). It could be noted that the emulsification process was not especially efficient with linalool since the droplet size was very high. As the concentration of gums and the process conditions were the same, we can assume that the droplet size was dependent on the dispersed phase. The formation of smaller limonene droplet size compared to linalool could result of combination between two properties: (1) the polarity of the two components; linalool being more polar and soluble in aqueous phase than limonene could diffuse fast in the water phase inducing flocculation and (2) the affinity of component for gums; linalool having a smaller affinity for gums as demonstrated by contact angle measurement. The effect of oil type on emulsion droplet size was also reported for cinnamon or ginger essential oil incorporated sodium caseinate-based films or for basil and thyme essential oils incorporated in chitosan based films specially for emulsions produced by a milder emulsification process (Atarés, Bonilla, & Chiralt, 2010; Bonilla, Atarés, Vargas, & Chiralt, 2012). The authors suggested that the smaller droplet size of thyme oil could be due to the greater surfactant activity of thyme oil amphiphilic components.

The fact that A. *senegal* allowed to produce smaller droplet size for linalool than A. *seyal* is due to its higher emulsifying properties in relation to its higher protein-rich AGPs and greater viscosity (Table V.1 and values given in paragraph 3.1). In contrast, the gum type is less impacting for limonene. This result could be related with the

behavior noted when contact angle evolution was followed, i.e. whereas absorption dominated for limonene and A. *senegal* gum films, absorption was counterbalanced by spreading which is usually the fastest phenomenon for A. *seyal* gum films.

After the drying process, aroma emulsion based films were also characterized. The macroscopic observation showed that films with limonene had a homogeneous surface in relation with a good repartition of limonene. As expected, the films containing linalool showed heterogeneous aspect with a more transparent border and a light-tight center because of the phase separation of film-forming emulsions during film drying (data not shown). Similar results of destabilized film-forming solution due to creaming during drying process were reported with hexanal and limonene emulsions formed by iota-carrageenan (Fabra, Chambin, Voilley, Gay, & Debeaufort, 2012). Among emulsions characteristics playing important role in emulsion stability, it is admitted that the emulsion stability is partly related to the emulsion droplet size (Chanamai & McClements, 2000) thereby during the slow drying process of film, linalool emulsion destabilization was favored resulting in the heterogeneous aspect of linalool films. The stability of emulsions could be improved by reducing the emulsion droplet size by using secondary homogenization and high pressure homogenizer such as microfluidizer which will allow to increase the homogeneous repartition of aroma compound in films (Bonilla et al., 2012).

In term of aroma retention efficiency by Acacia gum films, the results showed that Acacia gum based films had the capability to retain limonene and linalool (Table V.7). As expected, limonene was more retained than linalool by both Acacia gum films despite its 10 times higher volatility compared to linalool (Table V.2). The effect of gum type observed for linalool emulsions was not found again for the retention of aroma by films but it can be assumed that the variability between samples were high due to the bad repartition of linalool.

In conclusion, the higher retention of limonene compared to linalool by Acacia gum films was consistent with the results of contact angle showing that limonene had more

affinity for Acacia gum films than linalool. This strong correlation could be useful in the fast prediction of aroma compound retention parameter and the selection of active agent for the design of antimicrobial Acacia coating. Indeed, numerous aroma compounds such as limonene and linalool are GRAS (Generally Recognized As Safe) compounds and exhibit antimicrobial properties against various microorganism strains (Espina, Gelaw, de Lamo-Castellví, Pagán, & García-Gonzalo, 2013; Suppakul, Miltz, Sonneveld, & Bigger, 2003).

Table V.7: Limonene and linalool retention by A. *senegal* and A. *seyal* gum films (in $mg_{aroma}.g^{-1}_{dry\ film}$ and %).

Aroma compound	Acacia gum types	mg $_{aroma}.g^{-1}$ $_{dry\ film}$	% of Retention of aroma compounds
Limonene	A. *senegal*	141.9±10.4	59.4
		157.6±11.1	66.0
	A. *seyal*	158.7±11.2	67.6
		126.5±8.9	53.9
Linalool	A. *senegal*	88.8±17.1	38.0
		85.6±16.5	36.6
	A. *seyal*	77.5±15.0	33.4
		88.6±16.9	38.2

4. Conclusion

The contact angle method is a powerful tool to investigate the surface properties of biopolymer films with respect to water but also to other apolar compounds. The difference of structural surface organization observed by AFM between A. *seyal* and A. *senegal* spin coated films was clearly confirmed through their wettability and free surface energy: A. s*eyal* gum films being more hydrophilic and having higher free surface

energy and polar component than A. *senegal* gum films. The protein part and the apolar groups of A. *senegal* would be concentrated at the interface giving homogeneous surface whereas A. *seyal* gum films were characterized by an aggregation phenomenon due to the richness of sugar units at the surface and strong inter and intra molecules interactions. Furthermore, the addition of glycerol affected the film surface properties by increasing their hydrophobicity due to new formed interactions between glycerol and gum molecules. The amphiphilic properties of Acacia gums were supported by the low value of contact angle of apolar compounds on Acacia gum films demonstrating high affinity for these compounds. The difference between the two gums is only expressed by variations of contact angles with time evidencing that absorption counterbalanced by spreading for the most apolar compounds depend on gum type and their emulsifying properties.

In the case of cast films, they exhibited same wetting properties as spin coated films even if the difference of film microstructure was not observed by SEM. A good correlation between water contact angle value and the WVP depending on gum type was established. Moreover, the higher retention of limonene compared to the retention of linalool was in agreement with the greater affinity of limonene with Acacia gum spin coated films measured by contact angle technique.

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Supplementary table V.1: Water contact angle and surface energy of different polysaccharide and protein films. NA stands for non-available value.

		Glycerol (%)	θ water (°)	γ (mN.m^{-1})	Refs.
Polysaccharides	Starch	0	43	60.28	Basiak *et al.*, 2016.
	Pullulan	0	30.53	44.72	Farris *et al.*, 2011.
	Chitosan	0	103.70	27.8	Silva *et al.*, 2007.
		0	91.53	46.21	Farris *et al.*, 2011.
		0	78-82	NA	Suyatma *et al.*, 2005.
		20	70	NA	Suyatma *et al.*, 2005.
	Carrageenans	0	88.30	NA	Karbowiak *et al.*, 2006.
		23	44.10	NA	Karbowiak *et al.*, 2006.
	Kafiran	0	106.41	NA	Ghasemlou *et al.*, 2011.
		35	95.44	NA	Ghasemlou *et al.*, 2011.
	Psyllium hydrocolloid (PH)	15	84.47	NA	Ahmadi *et al.*, 2012.
		35	41.01	NA	Ahmadi *et al.*, 2012.
	Sage seed gum	40	32.26	15.07	Razavi *et al.*, 2015.

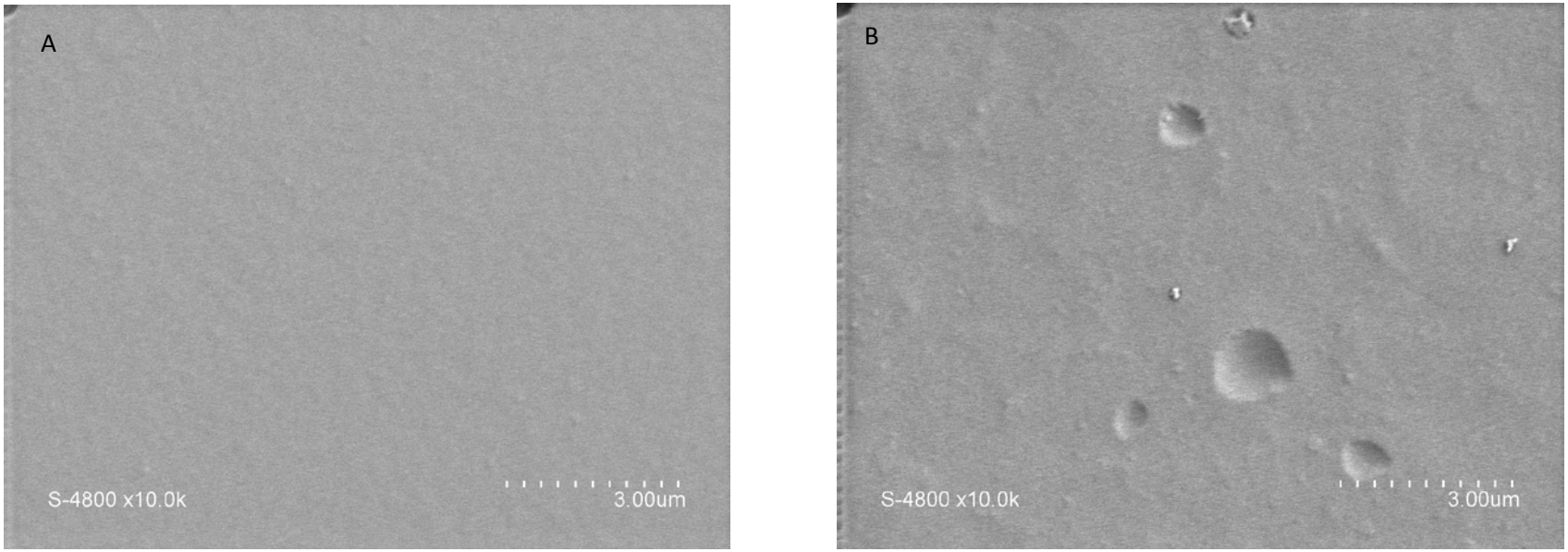
		Glycerol (%)	θ water (°)	γ (mN.m^{-1})	Refs.
		100	55.98	12.64	Razavi *et al.*, 2015.
	Cress seed gum	0	79.80	NA	Jouki *et al.*, 2013.
		50	43.76	NA	Jouki *et al.*, 2013.
	Lipidium perfoliatum seed gum (LPSG)	40	72.90	NA	Seyedi *et al.*, 2013.
		70	68.36	NA	Seyedi *et al.*, 2013.
	Gum ghatti	15	77.25	NA	Zhang *et al.*, 2016
		30	73.25	NA	Zhang *et al.*, 2016
		45	70.00	NA	Zhang *et al.*, 2016
	Apricot gum from Vayots Dzor	0	50.6	NA	Chichoyan., 2015
Proteins	Soy protein	10	67.6	33.NA9	Silva *et al.*, 2007.
	Whey protein	0	93	63.7	Basiak *et al.*, 2016.
	Gelatin	0	65.93	37.39	Farris *et al.*, 2011.

Supplementary table V.2: Water vapor permeability (WVP) of different polysaccharide and protein films. NA stands for non-available value.

		RH gradient	Glycerol (%)	WVP ($\times 10^{-11}$ mol m^{-1} Pa^{-1} s^{-1})	Refs.
Polysaccharides	Corn hull arabinoxylan	54%RH at 22°C	0	0.26	Zhang *et al.*, 2004.
			0.5	0.17	Zhang *et al.*, 2004.
	Potato starch	33%RH at 20°C	0	2.03	Garcia *et al.*, 1999.
			2	1.34	Garcia *et al.*, 1999.
	Corn starch	33%RH at 20°C	0	2.04	Garcia *et al.*, 1999.
			2	1.43	Garcia *et al.*, 1999.
	Amylomaize	33%RH at 20°C	0	1.46	Garcia *et al.*, 1999.
			2	1.19	Garcia *et al.*, 1999.
	Gellan	54%RH at 21±2°C	50	1.08	Yang *et al.*, 2000.
			75	3.01	Yang *et al.*, 2000.
			50	1.35	Jouki *et al.*, 2013.
	Kefiran	75%RH	0	0.28	Ghasemlou *et al.*, 2011.
			25	0.31	Ghasemlou *et al.*, 2011.

		RH gradient	Glycerol (%)	WVP ($\times 10^{-11}$ mol m^{-1} Pa^{-1} s^{-1})	Refs.
			35	0.33	Ghasemlou *et al.*, 2011.
	Sage seed gum	97%RH at 22°C	40	0.24	Razavi *et al.*, 2015.
	Gum ghatti	75%RH at 25°C	15	0.32	Zhang *et al.*, 2016
			30	0.63	Zhang *et al.*, 2016
Proteins	Sesame protein	70%RH at 30°C	10	0.07	Sharma *et al.*, 2016.
	Casein	NA	25	6.02	Wagh *et al.*, 2013.
			50	10.80	Wagh *et al.*, 2013.
	Whey protein	NA	25	10.49	Wagh *et al.*, 2013.
			50	14.04	Wagh *et al.*, 2013.
		100%RH at 25°C	37.5	7.70	McHugh *et al.*, 1994.
			50	9.94	McHugh *et al.*, 1994.
	Gelatin	75%RH at 20°C	0	1.5	Rivero *et al.*, 2010.
			20	0.78	Rivero *et al.*, 2010.
	Soy protein isolate	75%RH at 25°C	40	1.06	Kokoszka *et al.*, 2010.

	RH gradient	Glycerol (%)	WVP ($\times 10^{-11}$ mol m^{-1} Pa^{-1} s^{-1})	Refs.
	50%RH at 25°C	20	0.94	Nassar *et al.*, 2017.



Supplementary figure V.1: SEM images of upper surface of A. *senegal* (A) and A. *seyal* (B) cast films.

II. Major outcome

In this chapter, we reported, for the first time, the in-depth investigation of Acacia gum film surface. The effect of film formation process and the addition of glycerol as plasticizer on film surface structure and hydrophobicity were prior evaluated. Then, the spin-coated films were used as a model to determine the surface properties, i.e. surface free energy and affinity with different nature compounds. The obtained results have been related to the functionalities of film as water vapor permeability and ability to retain aroma compounds. The relationship between film properties and the gums nature and biochemical composition can be therefore established. The main results and some remarks are shown in the following table:

Film forming process
• Spin coating allowed to produce Acacia gum thin film with and without glycerol addition • The addition of glycerol was indispensable to produce a peeled off self-support films with a higher thickness compared to spin-coating technique.
Spin coated film surface structure
• A *senegal* films without and with glycerol addition: Smooth homogeneous surface with some aggregated particles was observed due to the high accessible protein to lower interfacial tension at air interface. • A. *seyal* without glycerol addition: Irregular surface with a repetitive organization as numerous large particle uniformly distributed on surface. This could be due to the low degree of branching, rich in arabinose favoring intra and inter molecular hydrogen bonding, high hydration ability causing aggregation of polysaccharide chain. • A. *seyal* with glycerol addition: Decrease number of aggregation making more smooth and homogeneous surface may be due to the hydrogen bonding between glycerol and hydroxyl group of A. *seyal* sugar.
Water contact angle (Surface hydrophobicity)

- A. *senegal* films was more hydrophobic than A. *seyal* films. This could be related to the higher protein-rich AGPs content and accessibility of the former resulting in the different structuration of surface.
- Addition of glycerol allowed to increase the contact angle for both gum films but the increase level was higher for A. *seyal* than for A. *senegal*. This could be due to relatively decrease of the available hydroxyl groups at the film surface and consequently the hydrophilic contribution, and induce conformational changes of Acacia gum molecules favoring the richness at the surface of hydrophobic residues such as acetyl group of 4-O-Me-Glucuronic acid and of hydrophobic amino acids.

Film surface properties

- The surface energy of both gum films was composed of polar and dispersive components with the lower value of the former than the one of the latter. This was consistent with the amphiphilic characteristic of Acacia gum.
- The dispersive component of A. *senegal* gum films was higher than the one of A. *seyal* gum films. This was in agreement with the higher protein content of the former.
- Independently of gum specie, strong affinity between organic volatile apolar compounds and Acacia gum films was observed. The better affinity was found between Acacia gum films and alkane than alcohol compound.

Functionalities

- The higher water vapor permeability was found for A. *seyal* films than for A. *senegal* ones. This result was in agreement with the outcome of contact angle measurement.
- The higher retention of limonene compared to the one of linalool was consistent with the greater affinity of limonene with Acacia gum films observed from contact angle measurement.

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General conclusions and perspectives

Chapter 6 –General conclusions and perspectives

La gomme d'Acacia est une gomme naturelle unique par ses propriétés techno-fonctionnelles. Dans l'industrie alimentaire, elle est principalement utilisée dans le domaine des boissons pour stabiliser les arômes sous forme d'émulsions ou pour encapsuler les composés d'arômes ou les colorants, et dans le domaine de la confiserie comme agent d'enrobage. La gomme d'Acacia, également appelée gomme arabique, est un continuum d'arabinogalactanes-protéines (AGPs) qui différent entre eux par leurs compositions biochimiques, propriétés physicochimiques et structurales à l'origine de leurs diverses fonctionnalités. La connaissance précise de la composition, de la structure et des propriétés physicochimiques, et techno-fonctionnelles, des AGPs constitutifs de la gomme d'Acacia devrait permettre de développer des produits innovants et à haute valeur ajoutée. Seulement deux espèces de gommes sont utilisées et autorisées comme additifs, l'A. *senegal* et l'A. *seyal.* Des fonctionnalités de ces deux gommes sont différentes qui s'expliquent par de faibles variations dans leur composition en particulier leur teneur en protéines mais aussi par des propriétés structurales (l'A. *senegal* étant plus ramifiée mais moins compacte que l'A. *seyal)* et physicochimiques (A. *senegal* est plus visqueuse que l'autre) qui accentue leur disparité. De plus, s'il existe beaucoup d'études sur l'A. *senegal* qui est considérée comme la plus « noble » c'est à dire ayant de meilleures propriétés émulsifiantes grâce à la présence d'AGPs de haute masse molaire et riche en protéines, il y a encore peu d'études concernant la gomme A. *seyal* qui est pourtant la plus produite.

Dans ce contexte, le but général de mes travaux de thèse était d'aboutir à une meilleure compréhension des propriétés interfaciales et émulsifiantes des AGPs de gomme d'A. *senegal*, d'A. *seyal* et de leurs fractions. Pour atteindre ce but, les objectifs scientifiques suivants ont été fixés :

(i) Etablir les relations entre la composition biochimique et les propriétés structurales des gommes d'A. *senegal* et A. *seyal* et leurs propriétés interfaciales.

(ii) Evaluer l'impact de la nature de la phase dispersée sur les propriétés interfaciales des gommes d'A. *senegal* et A. *seyal.*

(iii) Caractériser les émulsions produites par les gommes d'A. *senegal* et A. s*eyal*, et une gomme reconstituée en utilisant des fractions bien caractérisées issues de gomme d'A. *senegal.*

(iv) Caractériser la microstructure et les propriétés interfaciales de films séchés à base de gommes et établir les relations entre ces propriétés et la perméabilité à l'eau mais aussi la capacité de rétention de composés d'arômes.

Les conclusions clés issues de mes travaux de thèse sont expliquées ci-dessous.

- La comparaison des propriétés interfaciales entre les gommes d'A. *senegal* et d'A. *seyal* a permis de montrer qu'à concentration égale en gomme, celles d'A. *senegal* étaient plus efficaces pour abaisser rapidement la tension interfaciale. Cela est lié de façon évidente à (i) une concentration plus élevée en protéines, (ii) à une plus grande teneur en AGPs de haute masse molaire riches en protéines qui peuvent, de plus, être sous forme d'agrégats, (iii) à une plus grande accessibilité de la partie protéique et (iv) à une plus grande flexibilité des AGPs et donc une adsorption facilitée. L'importance de la teneur en protéines des gommes d'Acacia a été clairement confirmée en faisant varier sa concentration au sein d'une même gomme alors que l'importance des AGPs de haute masse molaire et riches en protéines et de leur flexibilité est mise évidence lorsque l'on compare les gommes en fonction de leurs teneurs en protéines. Par contre il est important de souligner que pour une concentration en protéines équivalente, la gomme A. *seyal* de par sa plus grande compacité atteint plus vite l'interface que l'A. *senegal.*

Une autre conclusion de l'étude sur la tension interfaciale est qu'il est important de faire varier la concentration en gomme pour mieux différencier les gommes entre elles. De plus, il ne faut pas se limiter à la comparaison de leur tension à l'équilibre mais aussi caractériser plus finement les cinétiques de début d'adsorption.

- En ce qui concerne l'étude rhéologique à l'interface, les films formés par les 2 gommes ont un caractère élastique. Les résultats indiquent que deux phénomènes se succèdent et qu'ils sont dépendants de la teneur en protéines et en AGPs de haute masse molaire riches en protéines qui viennent s'adsorber à l'interface. D'abord, on observe la formation du film élastique qui se traduit par une augmentation du module et se termine par la saturation de l'interface sans doute par la formation de nouvelles liaisons inter- et intra-chaines. Ce phénomène dépend directement de la concentration en gomme et de sa nature. En effet, plus on augmente la concentration en gomme et plus la vitesse de formation des films est élevée et la saturation atteinte rapidement. Si la saturation est atteinte pour l'A. s*enegal* avec les 3 concentrations testées (0,5, 1 et 5 wt%), ce n'est pas le cas pour l'A. *seyal* à 0.5 et 1wt%. Ceci est sans doute parce que la teneur en AGPs d'intérêt reste en dessous de celle de l'A. *senegal* à 0,5%. Le 2ème phénomène s'accompagne d'une diminution du module et correspond à la réorganisation des molécules adsorbées à l'interface. Ce phénomène dépend évidemment de la concentration en gomme et en protéines mais aussi de l'espèce des gommes. Le réarrangement n'a été observé pour l'A. *seyal* qu'à la plus forte concentration. On peut supposer que son apparition est décalée dans le temps par rapport à la gomme A. *senegal*. Cette réorganisation peut s'expliquer par la mise en place de nouvelles liaisons entre les blocs polysaccharidiques ou encore une compétition des différentes molécules riches en AGP pour l'interface due à la modification de concentration dans le milieu.

La démarche utilisée dans cette étude, c'est à dire le choix des mêmes concentrations pour les 2 gommes n'a pas permis d'apporter toutes les

informations nécessaires à une bonne compréhension et comparaison des 2 gommes. Il aurait été plus judicieux de comparer des gommes à teneur équivalente en protéines ce qui aurait fait mieux ressortir le rôle des AGPs riches en protéines. De plus cette comparaison est rendue difficile par le fait que les molécules d'AGPs de l'A. *seyal* sont différentes de celles de l'A. *senegal*.

- Toutefois, dans le but de confirmer le rôle prépondérant des AGPs de masse molaire élevée riche en protéines sur les propriétés interfaciales, des expériences ont été réalisées avec une fraction obtenue à partir d'A. *senegal* à teneur élevée en ces molécules (IEC-F1). Il a été clairement montré que les cinétiques des tensions interfaciales (vitesse de diminution) et la rhéologie des films à l'interface (valeur du module de viscoélasticité, et réorganisation des molécules à l'interface) étaient similaires pour la fraction IEC-F1 issue de la gomme d'A. *senegal* à une concentration de 0,415 wt% et pour la gomme d'A. *senegal* à 5 wt%. Pourtant, il s'avère que la teneur en AGPs de masse molaire élevée riche en protéines contenues dans la gomme d'A. *senegal* à 5wt% est plus élevée que celle présente dans la fraction. De plus, l'augmentation de la concentration en fraction IEC-F1 a permis encore d'accroitre les propriétés interfaciales avec une diminution de tension interfaciale plus rapide et plus importante. En ce qui concerne le comportement rhéologique, l'utilisation de IEC-F1 à une concentration de 4,15wt%, c'est à dire avec une concentration en AGPs de haute masse molaire riche en protéines abondantes ne conduit pas à une diminution du module et à un réarrangement comme décrit pour la gomme entière. Cette fraction n'est pas constituée seulement par les AGPs de haute masse molaire, ce qui peut expliquer les différences trouvées avec la gomme initiale. Il faut aussi souligner une présence de sels non négligeables dans la fraction IEC-F1 qui a pu modifier la force ionique du tampon et impacter les propriétés interfaciales.

Ceci souligne d'une part l'importance d'étudier les propriétés des gommes en fonction de la force ionique et du solvant et d'autre part de considérer non pas les fractions purifiées seules mais de maitriser leurs concentrations en reconstituant les gommes pour mieux comprendre leur comportement, comme il a été fait dans notre cas pour les émulsions.

- Concernant l'effet de la phase dispersée sur les propriétés interfaciales des gommes, les résultats ont montré avec les trois composés sélectionnés que plus la tension interfaciale initiale des composés est élevée et leur solubilité dans l'eau est faible, plus les gommes d'Acacia diffusent rapidement à l'interface et plus la valeur du module élastique est élevée. La différence entre les deux gommes au niveau de la capacité à diminuer la tension interfaciale et le module élastique est plus accentuée à l'interface de l'octanol, le composé le plus polaire.

Parmi les composés étudiés, un fort réarrangement du film après saturation de l'interface est observé pour le limonène. Ceci pourrait être provoqué par la structure cyclique de ce composé qui est moins favorable à la formation de liaisons intra et intermoléculaires par rapport à la structure linéaire de l'hexadécane et de l'octanol. Mais aussi à sa plus faible viscosité par rapport aux autres composés qui pourrait induire des phénomènes de déstabilisation plus importants.

La différence de comportement à l'interface des 2 gommes mise en évidence pour le limonène que ce soit au niveau de la tension interfaciale ou du module élastique se retrouve lors de la formation d'émulsions en utilisant ce composé.

En effet, l'utilisation de gomme d'A. *senegal* a permis d'obtenir des émulsions avec une taille des gouttelettes plus faible que celles produites par l'A. *seyal* pour toutes les concentrations étudiées. De plus, les émulsions produites par l'A. *senegal* avaient une meilleure stabilité (delay time élevé et faible CI) que celles produites par l'A. *seyal*.

Ceci était en accord avec l'augmentation plus rapide du module élastique, et la saturation du film interfacial de l'A. *senegal* par rapport à l'A. *seyal* (Tableau VI.1).

Tableau VI.1 : Propriétés interfaciales et émulsifiantes des gommes d'A. *senegal* et d'A. *seyal* à 5 wt% avec le limonène 5 wt%.

	A. *senegal*	A. *seyal*
Teneur en protéines	0.122 wt%	0.045 wt%
Paramètres caractérisant les propriétés interfaciales		
Onset time (s)	12	12
Tension interfaciale à 7 200 s ($mN.m^{-1}$)	11.8	15.5
Vitesse de diminution de tension interfaciale au Régime II ($mN.m^{-1}.s^{-1}$)	6.5	4.1
Vitesse de formation du film à l'interface ($mN.m^{-1}.s^{-1}$)	32.1	14.8
Temps mis pour atteindre la valeur maximale du module élastique (s)	1 020	9 000
Valeur maximale du module élastique ($mN.m^{-1}$)	35.3	36.6
Paramètres caractérisant les émulsions		
Taille des gouttelettes (µm)	0.73	3.46
Delay time (min)	75.8	13.5
CI	10.4	23.42

De la même façon que pour les propriétés interfaciales, les propriétés émulsifiantes ne sont pas seulement dépendantes de la teneur en protéines. La taille des gouttelettes produites par l'A. *seyal* à une concentration de 20 wt% était en effet plus élevée que celles produites par l'A. *senegal* à 5 wt% contenant moins de protéines. De plus la stabilité de cette dernière était aussi plus importante. Ces résultats suggèrent que c'est à la fois la composition biochimique (teneur en protéines mais aussi en sucres chargés), les propriétés structurales et plus précisément la compacité, la flexibilité des AGPs, le taux d'agrégats ainsi que l'accessibilité de la fraction protéique et donc la polarité relative des deux gommes qui affectent leurs propriétés émulsifiantes. En effet, si l'A. *senegal* est caractérisée par de meilleures propriétés émulsifiantes que l'A. *seyal*, c'est parce qu'elle a un caractère plus hydrophobe, une teneur plus élevée en protéines et en acide uronique, une plus grande flexibilité moléculaire entraînant une plus grande accessibilité de sa fraction protéique. En revanche, l'A. *seyal* possède une plus grande teneur en arabinose qui est susceptible de favoriser la formation des liaisons hydrogènes

intra et intermoléculaires conduisant à des phénomènes de floculation/coalescence des gouttelettes pendant l'émulsification et le stockage. La viscosité des gommes contribue aussi à la stabilité des émulsions : la plus grande viscosité des dispersions de gommes d'A. *senegal* permet d'obtenir des émulsions plus stables que celles avec la gomme d'A. *seyal*. L'importance de ce paramètre sur la stabilité a d'ailleurs était confirmée par l'ajout de glycérol qui permet d'augmenter la viscosité des suspensions de gommes.

La purification et la caractérisation des fractions de la gomme d'A. *senegal* par HIC et IEC qui ont été en réalisées en parallèle de cette étude, nous ont permis de reconstituer des gommes en utilisant des mélanges de la fraction HIC-F1 essentiellement constituée d'AGPs de faible masse molaire et pauvre en protéines et de la fraction IEC-F1 constituée d'AGPs de haute masse molaire et riches en protéines. Par cette approche innovante, nous avons pu étudier des dispersions à différentes teneurs en protéines et en AGPs contrôlées. Les résultats ont montré une synergie entre la fraction de haute masse molaire riche en protéines de l'A. *senegal* (IEC-F1) et la concentration totale en gomme sur la formation de gouttelettes de petite taille et sur la stabilité à court terme de la gomme d'A. *senegal*. En revanche, seule la concentration totale avait un rôle déterminant dans le maintien de la stabilité à long terme des émulsions. A des concentrations en protéines égales, la gomme d'A. *senegal* reconstituée permet une meilleure stabilité que l'A. *senegal* ce qui peut s'expliquer par une teneur en agrégats plus importante dans la fraction IEC-F1, se traduisant par une plus grande flexibilité moléculaire et une plus grande facilité à aller s'adsorber à l'interface et à stabiliser le film mais aussi par une plus grande viscosité. Ce comportement est en accord avec les propriétés rhéologiques à l'interface de la fraction IEC-F1 précédemment mises en évidence.

- En ce qui concerne les films de gommes, la différence de structuration de la surface observée par AFM entre les films d'A. *seyal* et d'A. *senegal* s'est traduite par une variation de mouillabilité par l'eau et d'énergie libre de surface. Les films de gomme

d'A. *seyal* qui présentent une surface irrégulière mais organisée se sont montrés plus hydrophiles et avaient une énergie libre de surface et une composante polaire plus élevées que les films d'A. *senegal* qui eux avait une surface très homogène. Alors que les parties protéiques de nature apolaire de la gomme A. *senegal* peuvent facilement se positionner à la surface (de par leur nombre élevé, leur accessibilité et la flexibilité des AGPs), la gomme A. *seyal* est obligée d'adopter un comportement différent à l'interface solide/air en raison de son faible taux de ramification, sa compacité et son manque de flexibilité qui rendent encore plus difficile l'accessibilité des quelques AGPs riches en protéines qui la composent. Par contre, grâce à sa richesse en arabinose, elle est capable de former des liaisons hydrogènes intra- et inter-chaines favorisant ainsi l'agrégation de la partie polysaccharidique qui vient se positionner à la surface. Malgré la forte teneur en sucres des deux gommes, les deux types de films présentent à la fois une affinité pour l'eau et pour des composés plus apolaires. Cette dualité qui est retrouvée pour les deux gommes indépendamment de la structuration spécifique des films se traduit par une énergie libre de surface du même ordre de grandeur et la prédominance des composantes dispersives. Celle-ci est toutefois plus prononcée pour le film à base de gomme A. *senegal* que pour l'A *seyal* (Tableau VI.2). La légère différence en ce qui concerne le caractère hydrophile des films se traduit bien par des variations dans la valeur de perméabilité à l'eau.

Tableau VI.2 : Comparaison de l'énergie de surface et ses composantes polaire et dispersive à l'interface air/solide (film) et air/liquide des gommes d'A. *senegal* et d'A. *seyal* à 20 wt%.

	Interface air/solide		Interface air/liquide	
Gommes	A. *senegal*	A. *seyal*	A. *senegal*	A. *seyal*
Energie de surface ($mN.m^{-1}$)	40.7	46.5	67.8	67.8
Composante polaire ($mN.m^{-1}$)	1.5	9.5	58.5	61.3
Composante dispersive ($mN.m^{-1}$)	39.2	37.0	9.3	6.5

Nous avons aussi pu montrer qu'au sein d'une même famille chimique, plus les composés étaient apolaires, plus l'affinité pour les films mesurés par angle de contact

était faible. **Il serait cependant intéressant de le vérifier pour plus de familles chimiques et de composés.**

Pour un film de gomme donné, la plus faible affinité du linalool par rapport au limonène mise en évidence par angle de contact se retrouve lors de la formation des émulsions par des suspensions de gommes. Des émulsions du composé ayant moins d'affinité avec les films de gomme avaient des gouttelettes de taille plus élevée et une rétention dans l'émulsion séchée sous forme de films plus faible. Cette similitude de comportement entre les films et les suspensions de gommes vis à vis des composés d'arômes devra être confirmée avec plus de composés. En effet, si elle s'avère vérifiée pour nombre de composés, une mesure d'angle de contact permettra de prévoir de façon rapide le comportement d'une phase dispersée lors de la réalisation d'une émulsion. Il apparaît aussi nécessaire de vérifier si la réalisation de films avec des solutions de gommes moins concentrées (5 ou 10 wt%) conduit à la même structuration des films et aux mêmes types de réponses vis à vis des arômes et si on peut toujours relier l'affinité avec les propriétés émulsifiantes.

Celles-ci sont habituellement décrites comme reliées aux propriétés interfaciales liquides/liquides et c'est ce que nous avons montré pour le limonène. Qu'en est-il maintenant des valeurs d'angles de contact des composés d'arômes par rapport aux pressions de surface mesurées pour ces mêmes composés ? Les valeurs vont dans le même sens mais les différences entre les composés sont moins prononcées si on regarde les valeurs d'angles de contact et la différence entre les gommes est plus difficile à anticiper. C'est d'ailleurs ce que nous avons observé pour le limonène qui bien qu'ayant des valeurs d'angles de contact qui sont très proches d'une gomme à l'autre (Tableau VI.3) conduisent à des émulsions avec des tailles de gouttelettes différentes (Tableau VI.1).

Tableau VI.3 : Comparaison des valeurs d'angles de contact, de pressions de surface et valeurs maximale de modules pour l'hexadécane, le limonène et l'octanol

	Angle de contact (°)		Pression de surface à 7200s ($mN.m^{-1}$)		Valeur maximales du module ($mN.m^{-1}$)	
Gommes	A. *senegal*	A. *seyal*	A. *senegal*	A. *seyal*	A. *senegal*	A. *seyal*
Hexadécane	13	11	25	21	52.1	54.3
Limonène	8.2	7.5	17	14	35.3	36.6
Octanol	15	17	4	3	11.5	9.5

On peut aussi se demander si les interfaces air/solide et air/liquide des 2 gommes présentent les mêmes caractéristiques et nous avons obtenu des éléments pour y répondre.

La comparaison des composantes polaires et dispersives associées aux énergies de surface liquide/air et solide/air (Tableau VI.2) met clairement en évidence la différence de structuration entre des solutions diluées et fortement concentrées (solide). Alors que les dispersions à 20 wt% se caractérisent par des composantes polaires élevées, les films ont une nature très apolaire. Ceci peut s'expliquer par la présence de l'eau et son rôle sur la conformation des molécules et leur organisation à la fois dans le milieu et à l'interface mais aussi par l'influence du support sur lequel est formé le film. De plus, la capacité d'hydratation entre les deux gommes diffère, elle est en effet plus importante pour l'A. *seyal* et impacte donc la valeur de la composante polaire de façon plus marquée.

Même si nous n'avons pas observé de réelles différences de structuration et de valeurs d'angles de contact entre les supports verre et LDPE qui sont pourtant l'un beaucoup plus polaire que l'autre, il est possible qu'ils influencent légèrement la structuration de l'interface. Ces hypothèses devraient pouvoir être vérifiées en réalisant des films avec des concentrations plus ou moins importantes ou en utilisant un support encore plus hydrophobe que le LDPE.

Parmi les perspectives issues de ces travaux de recherche, on peut souligner l'importance :

- De comparer les propriétés interfaciales des 2 gommes pour des teneurs en protéines similaires ce qui permettrait d'apporter des informations supplémentaires quant aux rôles des différents AGPs, l'idéal étant d'utiliser des gommes reconstituées à partir des fractions constitutives de A. *senegal* et A. *seyal*.
- D'étudier une plus grande gamme de composés d'arômes avec des polarités et des viscosités variables pour conclure sur l'importance de ces paramètres sur les propriétés interfaciales et ensuite les propriétés émulsifiantes des gommes d'Acacia.
- De mesurer l'impact de la force ionique sur les propriétés interfaciales et émulsifiantes en relation avec le taux d'agrégation des AGPs.

Les résultats obtenus à partir de ce travail permettent d'envisager diverses applications comme :

- L'utilisation de gommes reconstituées ciblées en fonction de l'application et de la stabilité recherchées.
- L'utilisation de la gomme A. *seyal* pour des applications comme la formation de films ou d'enrobage pour lesquelles la stabilité n'est pas cruciale.
- La production de films actifs ou enrobage réalisés à partir d'émulsions de gommes.

Valorisation

Scientific article

Aphibanthammakit, C., Nigen, M., Gaucel, S., Sanchez, C., & Chalier, P. (2018). Surface properties of Acacia senegal vs Acacia seyal films and impact on specific functionalities. *Food Hydrocolloids*, *82*, 519–533. https://doi.org/10.1016/j.foodhyd.2018.04.032

National and international communications

Journée de l'école doctorale GAIA 2016 (Poster session), Montpellier, France, "Parameters affecting the generation of emulsions from Gum Acacia".

30th EFFOST International Conference 2016 (Poster session), Vienna, Austria, "Characterization of interfacial properties of Gum Acacia thin film".

Interfacial and emulsifying properties of Acacia gums and their fractions

Acacia gum (E414 EC) is mainly composed of arabinogalactan proteins (AGPs). It is widely used as a stabilizer, emulsifier and film-forming agent for food and non-food applications. However, the relationship between these properties and the biochemical composition and structural conformation of the gum is still poorly understood. The objective of this work was to establish a relationship between the interfacial (liquid-liquid and solid-liquid) and emulsifying properties of two Acacia gums (A. *senegal* and A. *seyal*) and their biochemical and structural properties. The main results on liquid-liquid interfacial properties confirmed the greater interfacial properties of A. *senegal* compared to those of A. *seyal*. These properties are partly correlated with a higher content of high molar masses protein-rich AGPs, greater accessibility of protein fractions and flexibility of its AGPs. Similarly, it has been shown that these three factors strongly involved in the better emulsifying properties of A. *senegal* compared to A. *seyal*. In addition, it was established that the biochemical composition, in particular the arabinose and uronic acid content, but also the apparent viscosity of the continuous phase, played a crucial role in the stability of the emulsions. An innovative approach using different fractions of A. *senegal* in order to control the content of high molar masses protein-rich AGPs and total gum concentration confirmed the predominant role of these AGPs and highlighted the existence of a synergism between the content of high molar masses protein-rich AGPs and total gum concentration on emulsion droplet size and stability. The study of solid-liquid interfacial properties showed that the two gums were differently structured at the air-film interface: A. *senegal* surface being more homogeneous and less hydrophilic than that of A. *seyal*. For A. *senegal*, this can be explained by a higher content of high molar masses protein-rich AGPs on the surface of the film. The more irregular films of A. *seyal* evidenced the formation of aggregates at the interface, which can be explained by the compactness and hydrophilic characteristic of its AGPs and its high arabinose content, which favors hydrogen bonds and the high hydration capacity of its AGPs. These two films have an amphiphilic characteristic with an affinity either for water but also for highly apolar compounds such as linear alcohols and alkanes. The measurement of contact angle also allowed to predict a better emulsification and retention of the more apolar compound, i.e. limonene, compared to linalool. The importance of the dispersed phase polarity on all the properties tested has been also demonstrated.
Keywords: Acacia gums, interfacial and emulsifying properties, arabinogalactan proteins, aroma compounds

Propriétés interfaciales et émulsifiantes de gommes d'Acacia *senegal*, Acacia *seyal* et de leurs fractions

La gomme d'Acacia (E414 EC) est principalement composée d'arabinogalactan-protéines (AGPs). Elle est largement utilisée comme stabilisant, émulsifiant et agent filmogène pour des applications alimentaires et non alimentaires. Cependant, le lien entre ces propriétés et la composition biochimique et la conformation structurale de la gomme reste encore mal connu. L'objectif de ce travail a été d'établir un lien entre les propriétés interfaciales (liquide-liquide et solide-liquide) et émulsifiantes de deux gommes d'Acacia (A. *senegal* et A. *seyal*) et leurs propriétés biochimiques et structurales. Les principaux résultats portant sur les propriétés interfaciales liquide-liquide ont confirmé les meilleures propriétés interfaciales d'A. *senegal* comparées à celles d'A. *seyal* en partie corrélées à une teneur plus élevée en AGPs de haute masse molaire riche en protéines, une plus grande accessibilité des fractions protéiques et flexibilité ses AGPs. De la même manière, il a été montré que ces trois facteurs sont fortement impliqués dans les meilleures propriétés émulsifiantes l'A. *senegal* par rapport à l'A. *seyal*. De plus, il a été établi que la composition biochimique en particulier la teneur en arabinose et en acide uronique mais aussi la viscosité apparente de la phase continue jouaient un rôle crucial dans la stabilité des émulsions. Une approche innovante mettant en jeu différentes fractions de gomme d'A. *senegal* permettant de contrôler la teneur en AGPs de haute masse molaire riches en protéines et la concentration totale en gomme a permis de confirmer le rôle prépondérant de ces AGPs et de mettre en évidence l'existence d'un synergisme entre la teneur en AGPs de haute masse molaire riches en protéines et la concentration totale en gomme à la fois sur la taille et la stabilité des gouttes. L'étude des propriétés interfaciales solide-liquide a prouvé que les deux gommes se structuraient différemment à l'interface air-film avec pour l'A. *senegal* une surface plus homogène et moins hydrophile que celle de l'A. *seyal*. Ceci peut s'expliquer pour l'A. *senegal* par une teneur plus élevée en AGPs de haute masse molaire riches en protéines à la surface du film. Les films plus irréguliers d'A. *seyal* montrent la formation d'agrégats à l'interface pouvant s'expliquer par la compacité et le caractères hydrophile plus important de ses AGPs et leur teneur élevée en arabinose favorisant les liaisons hydrogènes et la forte capacité d'hydratation de ses AGPs. Ces deux films présentent un caractère amphiphile avec une affinité à la fois pour l'eau mais aussi pour des composés fortement apolaires comme des alcools et alcanes linéaires. La méthodologie utilisée, c'est à dire la mesure de l'angle de contact, a aussi permis de prédire une meilleure émulsification et rétention du limonène composé plus apolaire comparé au linalool. L'importance de la polarité de la phase dispersée a d'ailleurs été démontrée pour toutes les propriétés testées.
Mots clés : gomme d'Acacia, propriétés interfaciales et émulsifiantes, arabinogalactane-protéines, arômes