Beverage Emulsions and the Utilization of Gum Acacia as Emulsifier/Stabilizer

By Roberto Buffo, and Gary Reineccius, Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN

Beverage emulsions are O/W emulsions comprised of two categories: flavor emulsions and cloud emulsions. The former provides the beverage with flavor and cloudiness in certain formulae, whereas the latter provide only cloudiness with minimal or no flavor.[30]

A typical composition includes flavor oils (often essential oils) and weighing agents in the oil phase, and water, hydrocolloid, citric acid, preservatives, colorings and sweetener in the water phase. In a cloud emulsion, the flavorless oil can be orange terpenes or vegetable oils. Hydrocolloids, traditionally gum arabic (gum acacia) and more recently modified starches, serve as emulsifier and stabilizer. Citric acid is used for pH control, bringing the pH below 4.5, and benzoate as preservative. Both are for the purpose of preventing microbial spoilage, the reason being that most bacteria grow best at pH ranges 6.0 to 8.5, and pathogens do not grow below pH 4.5. Also, pH lowering is essential to activate the preservative since it is the undissociated form of benzoic acid that exhibits antimicrobial action. Colorings are for aesthetic purposes (e.g., FDC colors, marigold extract and b-carotenes for either yellow or orange shades). When the emulsion is just a cloudifier, no colors are added; the natural color of cloudifiers is milky white. Weighing agents can also be called density adjusting agents, that is, oil-soluble materials with no flavor of their own and density higher than the flavor oil. As the name implies, they are added to the flavor oil to increase the oil phase density. The importance of good quality water in the emulsion is no less than that in soft drinks and similar standard treatments should be applied. Treatment should remove colloidal and suspended matter, undesirable taste, odor and microorganisms. The carbonated water hardness or alkalinity should be reduced since it neutralizes the acid and causes the beverage to "go flat" and taste insipid. In addition, it can affect the stability of the emulsion due to neutralization of electrostatic charges. That is why water hardness level recommended for beverage emulsions is ≤ 50 mg of $CaCO_3$ per liter.[28,29]

Beverage emulsions are a unique class of emulsions, different from other food emulsions in that they are to be consumed in a highly diluted form rather than in their original concentrated form. They are first prepared as an emulsion concentrate which is later diluted in sugar solution in order to produce the finished beverage, either carbonated or non-carbonated. The emulsion must have a significant degree of stability in both the concentrated and diluted forms; typically, at least six months are required by the beverage industry.[29]

Preparation

The preparation of a beverage emulsion can be divided into the following steps:

- Preparation of water and oil phases: For the water phase, one dissolves preservative, citric acid, coloring, gum and sweetener in water and makes a complete solution, following this order of addition. For the oil phase, one dissolves the weighing agent completely in the oil. Usually the maximum permissible amount of weighing agent is used to take full advantage of it, which is not a simple task because dissolution of the weighing agent into the oil is a lengthy and tedious process.
- Prehomogenization: The oil and water phases are mixed to make a crude emulsion or premix. As a general rule, the premix should contain droplets which are all under about 20mm. This is achievable by the use of a high-speed mixer.
- Homogenization: This is the most important step. The crude emulsion is passed through a homogenizer at high pressure. This creates turbulence and cavitation forces that shatter the oil droplets into fine particles. For beverage emulsions, two-stage homogenizers are preferred. In a two-stage homogenizer, the second stage provides controlled back-pressure ensuring the maximum efficiency of the first stage, and at the same

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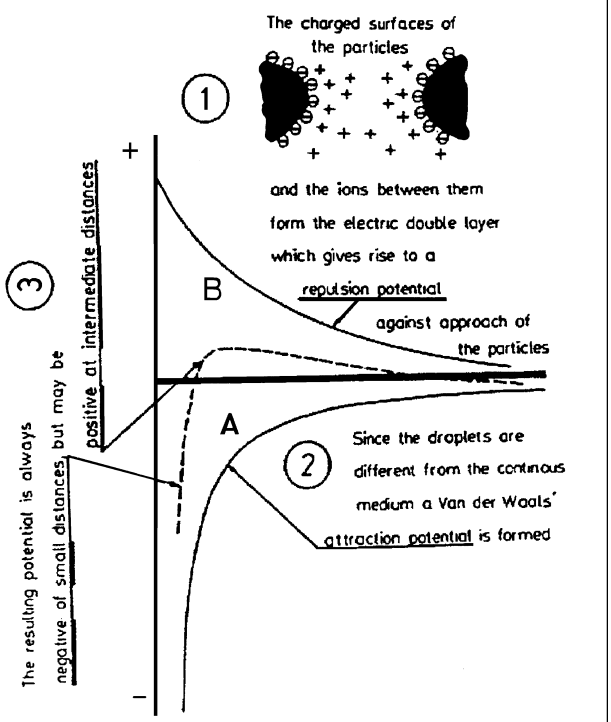

Figure 1. The DLVO theory: Colloidal stability on the relative distance between independent potentials

time it minimizes the possibility of clumping and coalescence of the oil droplets in the emulsion. Generally, two passes of the emulsion through the homogenizer are applied in order to obtain a more uniform particle size.[29]

Colloidal Interactions

Electric repulsion and the DLVO theory: The stability of a colloidal suspension is essentially dependent on the distance (relation) of two independent interactions between colloidal particles. These interactions are (a) Van der Waals attraction, and (b) electrostatic repulsion between electrical double layers of identical sign (Figure 1). The theory predicts that if the repulsion potential B exceeds the absolute value of the attraction potential A by a value [*B*-*A*]>>kT, kT being the thermal energy, k the Boltzmann constant and T the absolute temperature, at any distance between the particles, the suspension will be stable. For low values of B, that is [B-A]<<kT, the suspension will collapse as soon as the particles approach each other by the diffusion process. The DLVO theory does not cover all phenomena which affect the stability of an emulsion. It does, however, give a satisfactory estimation of the resistance to the approach of two droplets in aqueous solution when the emulsifiers are charged.[37]

Steric Repulsion: Some adsorbed molecules, mostly polymers, have flexible molecular chains ("hairs") that protrude into the continuous phase. These may cause steric repulsion by two mechanisms: (a) Hairs are restricted in the conformations they can assume, which implies loss of entropy, that is, an increase in free energy, and repulsion occurs; (b) Hairs overlap causing an increased concentration of protruding chains, increased osmotic pressure, movement of water to the overlap region and repulsion. However, this is true only if the continuous phase is a good solvent for the hairs; if it is not, attraction may result. Solvent quality usually is of overriding importance for strong repulsion. Only for large particles (strong Van der Waals attraction) and a fairly thin adsorbed layer (short hairs) will aggregation occur in a good solvent (Walstra, 1996).

Mechanisms of Destabilization

Instability of beverage emulsions, observed both in concentrates and in finished soft drinks, may lead to the following occurrences:

Creaming: Creaming is defined as the movement of oil droplets under gravity to form a concentrated layer at the top of O/W emulsions with no accompanying change in the droplet size distribution.[15] In the industry, the common term for creaming in bottled soft drinks is "ringing" because the flavor emulsion separates from the soda, floats to the top, and shows a white creamy ring at the neck of the bottle. Ringing can be considered as a separation of one emulsion into two emulsions, that is, one richer in the oil phase than the original emulsion, and the other, just the opposite, richer in the water phase. When creaming occurs, the emulsion richer in the oil phase rises to the top and forms a creamy layer that is not only undesirable but also indicates the breakdown of oil distribution in the soft drink.[28] The rate of creaming obeys Stokes' law:[23]

$$v = \frac{2r^2g(d_1-d_2)}{9\eta}$$

where:
v: velocity of separation (cm/s)
g: acceleration of gravity (980 cm/s^2)
r: droplet radius (cm)
d_1: density of the dispersed phase (g/cm^3)
d_2: density of the continuous phase (g/cm^3)
η: viscosity of the continuous phase (centipoise)

According to this equation, creaming in dilute beverage emulsions may be minimized by:

- Reducing particle size and narrowing particle size distribution: To produce small emulsion droplets, the coarse mixture of oil, water and surfactant must be emulsified under vigorous mechanical conditions. As aforementioned, this means using a valve homogenizer at high operating pressure with a generous ratio of emulsifier to oil.[15]
- Minimize density difference of the two phases: One way to produce a closer density match between food oils and water is by artificially increasing the oil phase density through the use of "weighing agents," such as brominated vegetable oil (BVO), SAIB (sucrose acetate isobutyrate) or glyceryl abietate (ester gum). However, there is little room for maneuvering in adjusting the density difference when accounting for the chemical, legal and toxicological constraints related to weighing agents.[15]

Coalescence: Coalescence is the process whereby two or more liquid droplets merge together to form a single larger droplet. It involves the following events: coming together of two oil droplets, rupture of the interfacial film, joining of the oil droplets, reduction in interfacial area, and finally separation of the dispersed phase from the continuous phase. The process is irreversible and causes breakdown of the emulsion.[28,34] In relation to emulsion shelf life, coalescence is a much more severe form of instability than creaming: when perceived by the consumer in a food product, it is almost invariably considered unacceptable. In O/W emulsions, coalescence eventually leads to the formation of a layer of oil on top of the material, which is refer to as "oiling off".[15] An efficient hydrocolloid that has the ability to form a film around the droplets will avoid reaching this extreme stage.[28]

Flocculation: Instability by flocculation essentially means that the oil globules move as aggregates rather than individually. Since flocculation does not involve rupture of the interfacial film surrounding each oil droplet, there is no change in basic droplet size or droplet size distribution. Flocculation is always closely linked to creaming, especially in highly polydispersed emulsion systems. Differential creaming rates of small and large droplets lead to an enhanced droplet encounter rate and therefore a greater degree of flocculation. This enhancement of flocculation simultaneously causes faster creaming. Flocculation can usually be distinguished from coalescence by its reversibility with respect to dilution, change of pH, or stirring.[15] Effectively, in a soft drink system droplet concentration is so low that flocculation is often reversible. The aggregates can readily be redispersed when the interactions between droplets are weak.[28]

Ostwald Ripening: Ostwald ripening is the growth of larger droplets at the expense of smaller ones due to mass transport of soluble dispersed phase through the dispersion medium. The thermodynamic driving force is the changing chemical potential of the dispersed phase component with increasing radius of curvature of the surface bounding the material; in other words, there is a greater tendency for the dispersed phase to dissolve into the continuous phase from a small droplet rather than for a larger one.[15] This phenomenon becomes significant when there is an appreciable solubility of the oil phase in water. This is precisely the case for essential oils used in soft drinks. Dickinson et al.[13] considered Ostwald ripening as the main destabilization mechanism for D-limonene-in-water emulsions. Buffo and Reineccius[7] found Ostwald ripening to be highly significant in dilute beverage emulsions.

Principles of Stabilization

For a beverage emulsion, the most critical criterion of stability is its stability in the finished soft drink where the emulsion concentrate is further dispersed in sugar solution. Emulsion stability in the concentrate is much easier to achieve than in the soft drink because concentrate viscosity is high due to the high concentration of hydrocolloid. In a

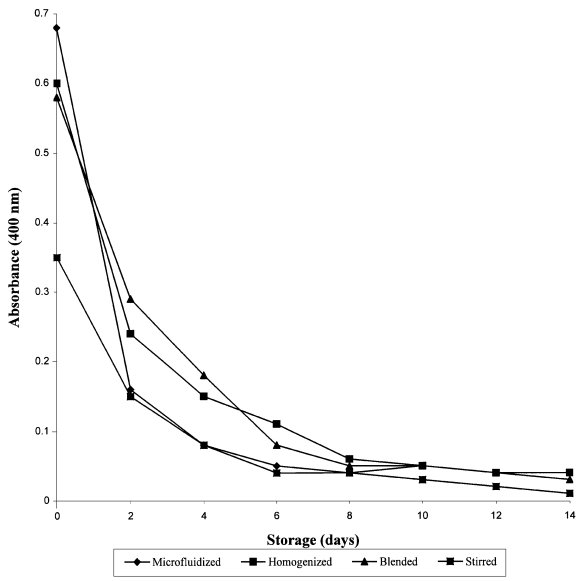

Figure 2. Stability of 0.3% emulsions (without ester gum) made with gum arabic during storage at 25°C.

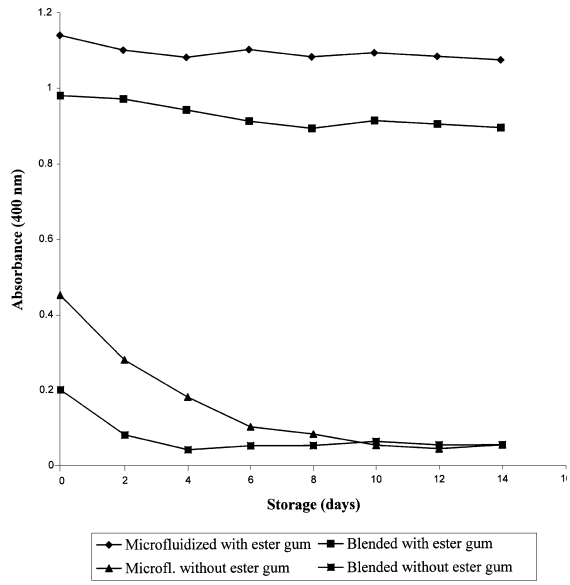

Figure 3. Comparison of stability of blended and microfluidized (MF) emulsions (gum arabic) made with and without ester gum during storage at 25°C

soft drink, the emulsion concentrate is redispersed in sugar solution at a very high dispersion ratio. It can almost be described as the emulsion concentrate being dispersed in a second water phase, that is, from a gum-solution water phase to a sugar-solution water phase.[30]

Principles involved in stabilizing beverage emulsions in sugar solutions such as soft drinks are discussed as follows:

Stokes' law: The ringing test is the most popular method to evaluate the stability of beverage emulsions in soft drinks. It is a simple test in which bottles of soft drink containing the beverage emulsion are held in an upright or horizontal position for observation of ringing. The rate of ringing of an oil droplet in sugar solution is ruled by Stokes' law as previously discussed. As an example of the use of the law, consider the case of orange flavor emulsions. Typically, orange oils have a density of 0.85 g/cm^3, and the sugar solution in a soft drink has a density of 1.040 g/cm^3 for a 10% sugar concentration or 1.048 g/cm^3 for a 12% sugar concentration. Applying these density data to Stokes' law, the resulting velocity carries a negative sign, which indicates that the emulsion will ring. Since orange oil is lighter than the sugar solution, weighing agents must be added to the orange oil to increase the density. However, as aforementioned, it is impossible to adjust the density of the oil to that of the sugar solution due to regulations on the usage level of weighing agents.[30]

Before 1970, BVO was allowed to be used with no limitation. It was banned in the United Kingdom in 1970 because of concerns about the accumulation of bromine in body fat from ingestion. In the United States, it was regulated by the Food and Drug Administration (FDA) in 1970 to allow a maximum of 15 ppm in soft drinks, well below the level required for a stable emulsion. BVO is unique in the sense that it has a high density of 1.33 g/cm^3 and can be easily added to citrus oils to increase the density. Since the regulation of BVO, it was imperative for the industry to find other materials as weighing agents. In the United States, ester gum was approved by FDA to be used to a maximum of 100 ppm in soft drinks. In Canada, the permitted agent is sucrose acetate isobutyrate (SAIB), and for the countries in the European Community, dammar gum. The shortcoming of all these agents is that they all have a density much lower than BVO: ester gum 1.08 g/cm^3, SAIB 1.15 g/cm^3, and dammar gum 1.06 g/cm^3. For example, an equal weight of ester gum added to orange oil can only bring the density from 0.85 to 0.97 g/cm^3, which still is significantly different from 1.05 g/cm^3. Because of the consumer's preference for the strength of the orange flavor and the 10-12% sugar sweetness level in the beverages, there is little a soft drink processor can do to further narrow the density difference between phases. The exception is making diet drinks, where the use of artificial sweeteners results in a water phase of density equal to 1 g/cm^3.[28]

Examination of Stokes' law reveals that velocity of an oil droplet going upward is directly proportional to r^2 (droplet radius) keeping all other parameters constant. Thus, when oil density is maximized through weighing agents, reducing

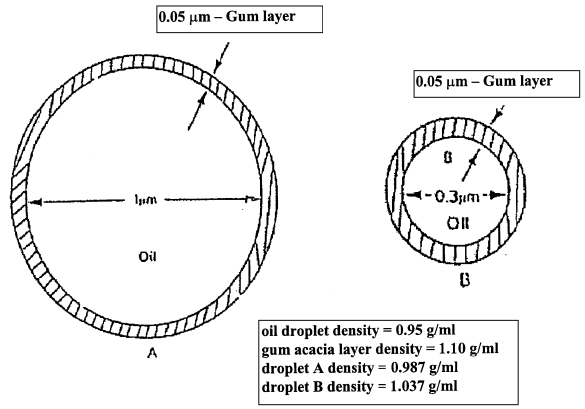

Figure 4. Weight contribution to orange oil droplets of different sizes from the gum acacia interfacial membrane

the droplet size is the most effective way to control creaming velocity. For instance, a particle of 0.1 mm in diameter will travel upward at a velocity 100 times slower than a particle of 1.0 mm in diameter. However, a creaming velocity of the order of 1 mm in 24 hr is usually sufficiently counteracted by normal thermal convection.[30]

Tse[34] studied the stability of orange oil-based beverage emulsions with respect to the means of homogenization (low-speed blending, high-speed blending, valve homogenization and high-pressure Microfluidization), formulation (amount of orange oil, 6.5% and 12.5%, and utilization or not of ester gum as a weighing agent) and emulsifier (gum acacia vs. modified starch). The rigor of the homogenization technique expectedly influenced the initial emulsion quality: the smaller the initial particle size, the better the initial emulsion quality (high-pressure Microfluidization > valve homogenization > high-speed blending > low-speed blending). No degree of homogenization or emulsification can compensate for the absence of a weighing agent. Ultimately, the most advantageous combination of the three factors should be searched in each particular system.

Adsorption at Interfaces

It has been known for many years that hydrocolloids (such as gum acacia) in a water phase are able to produce a film at the oil interface. This interfacial film is viscoelastic, approximately 0.1mm thick, and takes three to four days to mature to a "solid," possible multilayer film. Since a gum acacia solution is heavier than water, a layer of hydrated gum will provide an added weight to the oil droplet and actually change the density of the total droplet, that is, the oil droplet plus the gum acacia film, to a higher value. When an oil droplet is smaller, the percentage of weight contributed by the gum layer to the total droplet weight is larger than that for a larger droplet. This further indicates that the oil droplets should be made small in order to benefit more from the weight contributed by the gum layer.[30] The formation of the interfacial film by gum acacia or other hydrocolloid polymers on the oil droplets also helps to stabilize the emulsion in another way. The adsorbed hydrocolloid material prevents oil droplet coalescence that would lead to larger droplets and ultimately emulsion breakdown. The adsorbed layer keeps the droplets far enough apart so that the Van der Waals attraction force is minimized. The mechanism is evidently that of steric stabilization.[30] Figure 4 depicts this situation for two droplets of different diameter assuming a constant layer thickness over the range of oil droplet particle size. If densities of the oil droplet and gum acacia layer are taken as constants (0.95 g/ml and 1.10 g/ml, respectively), the resulting densities as a consequence of the additional weight from the emulsifier increase to 0.987 g/ml for droplet A (diameter = 1 mm) and to 1.037 g/ml for droplet B (diameter = 0.3 mm). As stated above, the smaller the droplet diameter, the greater the contribution from the gum layer.[31]

Electrostatic Interaction

Dispersed oil droplets may acquire an electric charge through the ionization of surface groups so that oppositely charged ions (counter-ions) are preferentially attracted towards the surface, whereas ions of the same charge (co-ions) are repelled from the surface. The region of unequal counter- and co-ion concentrations near the charged surface is called the electrical double-layer. The double-layer may be regarded as consisting of two regions: an inner region of strongly adsorbed ions and an outer region where ions are diffusely distributed according to a balance between electrical forces and random thermal motion. The electrical potential at any given distance from the particle surface is called the zeta potential. The determination of zeta potential is quite important in the study of emulsion stability. An emulsion should have a zeta potential more negative than -40 mV to be stable. Zeta potential values more positive than -15 mV usually result in flocculation. The threshold region of either flocculation or dispersion corresponds to the range -15 mV to -30 mV. Values that are more negative than -30 mV generally represent sufficient mutual repulsion to result in stability. Table 1 is a general stability guide for anionically dispersed systems as beverage emulsions, based on zeta potential measurements.[31]

Beverage emulsions are typical anionic systems because of two facts:[31]

- Oil droplets are always negatively charged since their dielectric constant is lower than that of water. An empirical rule states that a substance having a high dielectric constant is positively charged when in contact with another substance of lower dielectric constant.

Table 1. Stability characteristics of anionically-dispersed systemsaccording to zeta-potential ranges

Stability Characteristics	Zeta Potential (mV)
Maximum agglomeration and precipitation	0 to +3
Range of strong agglomeration and precipitation	+5 to -5
Threshold of agglomeration	-10 to -15
Threshold of delicate dispersion	-16 to -30
Moderate stability	-31 to -40
Fairly good stability	-41 to -60
Very good stability	-61 to -80
Extremely good stability	-81 to -100

- Gum acacia, as an acidic polysaccharide, presents numerous carboxyl groups (COO^-) at the periphery of the molecule which are very active in creating an anionic environment.

It should be stressed that zeta potential reflects both the electrolytes present in the system and the dissociated ions accompanying the original colloidal particles. When a cationic electrolyte is added to an emulsion containing a dispersed phase carrying negative charges, the electrolyte will be adsorbed and neutralize the zeta potential. This in turn will cause aggregation due to Van der Waals attraction forces. This electrolyte effect is much more evident in soft drinks than in emulsion concentrates because of the much higher degree of dilution and the lower effective amount of gum arabic.[30]

In the adsorption and desorption of electrolyte ions causing a change in zeta potential, the higher the valence of the ions, the greater the effect of compressing the electrical double layer around the droplets and the corresponding change of zeta potential. In other words, the higher the cation valence, the higher (less negative) the zeta potential, and the higher the ion concentration, the more significant the change in zeta potential. Thus, trivalent ions such as Al^{+++} have 10 to 100 times the effect of equivalent concentrations of divalent ions, like Ca^{++}, which in turn have 10 to 100 times the effect of equivalent concentrations of univalent ions, like Na^+. The effects of electrolytes on emulsion stability are shown in Table 2. For this reason, the soft drink industry uses only treated water to make both emulsion concentrates and finished beverages.[30] Tan and Wu Holmes[28] and Tse and Reineccius[35] have emphasized the importance of developing rapid quality control procedures based on zeta potential measurements.

Table 2. Reagent dosages required to bring a diluted silica suspension to zero zeta potential

Type of Reagent	Reagent	Dosage (ppm)	Dosage (mmol)
3:1	$AlCl_3$	4	–
2:1	$CaCl_2$	12,000	100
1:1	KCl & NaCl	22,000	300
1:2	K_2SO4	55,000	300
1:4	$Na_2P_2O_7$	110,000	450

However, other researchers[22,19] have challenged the validity of any "absolute index of emulsion stability" status associated with zeta potential based on the fact that the parameter considers exclusively the colloidal interactions involved in the DLVO theory (i.e., electrostatic repulsion and Van der Waals attraction) and not others as important, specially polymeric steric and hydrophobic. Thus, zeta potential should only be viewed as one of many criteria that define stability, mutually complementing each other (e.g., particle size, layer thickness of emulsifier around dispersed droplets, packing density of emulsifier at the O/W interface, protein load at the O/W interface, etc.).

Emulsion Liquid Membrane Encapsulation

Emulsion liquid membranes are double emulsions formed by mixing two immiscible liquids and then dispersing the resulting emulsion in another continuous phase under agitation. The beverage flavor emulsion is definitely fit to be classified as an emulsion liquid membrane. In actual practice of the flavor and beverage industry, the flavor oil is microencapsulated by an emulsion liquid membrane (hydrated gum layer). Once the emulsion concentrate is made, it is dispersed in another continuous phase, the sugar solution, to become the beverage. Therefore, the beverage flavor emulsion is a type of liquid membrane microencapsulation.[31]

Gum Acacia: General Characterization

Acacia gums, commonly called gum arabic, are natural vegetable colloids obtained as exudations from the trunk and branches of leguminous plants of the Acacia family. There are several hundred species of Acacia, only a few of which are able to produce gum and these are mainly concentrated in the sub-desert region of Africa: the Sahel. Main producing countries are Sudan (about 75-85% of the world's supply), Senegal, Mali, Mauritania and Nigeria. Lesser quality gums are produced in Morocco, Tanzania, Ethiopia and Somalia. Acacia senegal is the main species, and has been used occasionally as a reference for pharmacopoeias and different regulatory standards, although the related species of Acacia are being increasingly sold in the market. Acacia trees reach a height of 4.5-6 m, and have a life span of 25-30 years (Thevenet, 1988).

Gum acacia is unique in that it is produced by trees only when they are in an unhealthy condition. Healthy trees are apparently unable to yield the gum. The production of gum seems to be a response to some type of aggression upon the tree: injury of tissues or infection by microorganisms.[32]

The gum is collected during the dry season, which runs from November to May. Trees are tapped by the villagers and then the exudation begins. Gum collects in the wound within 3-8 weeks, depending on environmental conditions. After exudation and drying, the gum forms nodules resembling tear drops that are then collected by hand and transported to a central market. The gum is then sorted by hand to standardize the product, bagged and exported. Despite two harvests per year (December and April), the quantity of gum obtained from each tree generally does not exceed 300g. Some production comes from "wild" trees but the majority is harvested in cultivated tree plantations. In addition, there are research efforts to get a third generation of Acacia plants by in-vitro cultivation. The application of biotechnology has immediate advantages: perfect selection of species, consistent gum yields, increased gum output and improved standardization of exudates.[32]

There are four major grades of gum acacia:[16]

- Selected: These are the cleanest and largest pieces, having the lightest color and commanding the highest price.
- Cleaned and sifted: The color varies from pale to dark amber and the gum contains a minimum of material smaller than 6.35 mm (four-mesh size).
- Clear amber: The color varies from light to dark amber and the gum contains various amounts of fine pieces (smaller than four-mesh size).
- Siftings: This is the cheapest grade of gum and is the residue found by sorting the above choice grades; siftings are fine chips containing considerable sand, dirt and bark.

In crude form (as exported from Africa) gum acacia contains a percentage of impurities comprising mineral and vegetable matter. To eliminate them, the old method treated the product by a dry process using different stages: pulverization, selection and sifting. Increasingly, gum acacia is sold in spray dried form. Before spray drying, the gum is dissolved in water and is then filtered and centrifuged, which ensures levels of impurities less than 0.04%. In addition, the solution undergoes pasteurization to improve bacteriological quality and inactivate enzymes, and the production batches are standardized to be uniform. All these gums are sold with guarantees of color, viscosity and levels of impurities. Further treatment such as sterilization by β- or χ-radiation are applicable without altering the product's functional properties.[32,16]

Chemical Composition and Structure

Acacia gums belong to the group of complex acid polysaccharides of primarily uronic acid type, and occur as a mixed neutral or slightly acidic salt (sodium, calcium, magnesium and potassium).[32] They have been described as heteropolymolecular substances, consisting of molecules that differ in their sugar composition, mode of linkage and molecular mass.[25]

Basically, gum arabic (acacia) is composed of six carbohydrate moieties: galactose, arabinopyranose, arabinofuranose, rhamnose, glucuronic acid, and 4-O-methylglucuronic acid.[16] Some authors also report glucose[32] although it is generally accepted that the presence of any other carbohydrate material beyond the six moieties aforementioned indicates that the gum is not pure or that has been adulterated.[16] The main structural feature is a central chain of β-galactopyranose units with 1,3 bonds and side chains of 1,6-linked galactopyranose units terminating in glucuronic acid or 4-O-methylglucuronic acid residues. Rhamnose and arabinose groups are connected via 1,4 and 1,3 linkages, respectively.[32]

The gum contains 0.5% to 4.5% of proteinaceous matter, the protein content showing a significant range of variation depending on the species.[1] Vandevelde and Fenyo[36] have proposed that the gum is composed of two different fractions: one that constitutes around 70% of the total and has a low molecular mass and contains very little protein, and another that has a high molecular mass and is relatively rich

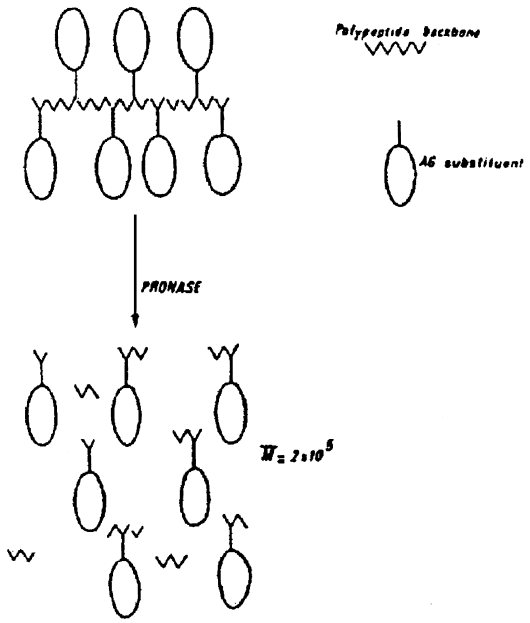

Figure 5. The "Wattle Blossom Model" of gum acacia before and after treatment with pronase

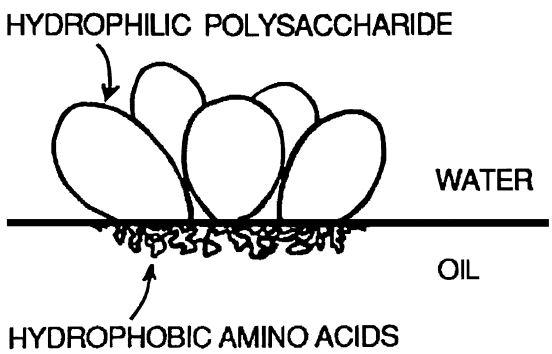

Figure 6. Schematic illustration of the AGP complex structure at the O/W interface

in protein (probably an arabinogalactan-protein complex). Randall et al.,[25] using hydrophobic affinity chromatography, have shown that the gum consists of three distinct fractions: a high molecular mass arabinogalactan-protein complex (AGP), containing most of the total protein, a glycoprotein (G1), containing the rest of the protein, and a lower molecular mass fraction, an arabinogalactan polysaccharide, which is protein-deficient (AG). They represent 10.4%, 1.2% and 88.4% of the total weight of the gum, respectively. The AGP fraction can be degraded by proteolytic enzymes with the molecular mass decreasing to that of the AG fraction.[25]

Connolly et al.[9] have described the structure of the AGP fraction in terms of the "Wattle Blossom" model, where carbohydrate blocks of molecular mass near $2x10^5$ are covalently linked to a main polypeptide chain of around 1,600 amino acid residues. It is likely that the polypeptide chain is located at the periphery of the molecule, thus facilitating its adsorption onto hydrophobic substances (Figure 5).

Randall et al.[25] proposed another structural model for the AGP complex (Figure 6), in which five carbohydrate blocks of molecular mass around $2.8x10^5$ are linked together by amino acid residues to form a very compact structure. As in the previous model, it is most likely that the polypeptide chain is located at the periphery of the molecule with exposed hydrophobic amino acids, thus facilitating adsorption onto hydrophobic substrates. This fully explains the capability of the gum to act as an emulsifier/stabilizer with the hydrophobic groups from the protein moiety of the AGP complex adsorbing on the O/W interface and the hydrophilic groups from the polysaccharide moiety facing the water, thus preventing droplet flocculation and coalescence through steric repulsion forces.[18]

Proximate composition of the gum obtained from acacia senegal and its fractions are presented in Table 3,[25] whereas Table 4 shows the amino acid profile of Acacia senegal from two different sources.[25,2] Hydroxyproline is the most abundant amino acid followed by serine, proline, aspartic acid and threonine. Hydroxyproline is not incorporated directly into peptides/proteins; rather, it arises through the post-translational hydroxylation of proline. In a polypeptide or protein containing ca. 300 hydroxyproline residues per 1,000 amino acid residues, every third or fourth residue must be hydroxyproline or, alternatively, blocks of contiguous hydroxyproline residues must occur. The latter is a well established feature of arabino-galactan proteins, and indeed, a characteristic property of the proteinaceous fraction of gum acacia.[2] More recently, it has been reported that the polysaccharide structures are covalently attached to the protein component by linkage to hydroxyproline and, perhaps, also serine units.[5]

Regulatory Status

Regulatory specifications are designed to identify the actual chemical entity which is introduced into a particular product. This has been a problem regarding gum acacia because of vague definitions of the substance in the Food Chemicals Codex, U.S. Pharmacopoeia, European Pharmacopoeia, and FDA regulations. Although all definitions recognize that commercial gum acacia (gum arabic) originates from more than one species of Acacia, none of them is thorough enough to avoid any possible misinterpreta-

Table 3. Proximate composition of gum from *acacia senegal* and isolated fractions

Component	Whole Gum	AG	AGP	G_1
% of total recovered[a]		88.4	10.4	1.0
% galactose	36.2 ± 2.3	34.5 ± 2.2	29.3 ± 0.7	12.3 ± 0.5
% arabinose	30.5 ± 3.5	27.6 ± 1.9	31.4 ± 1.0	15.0 ± 1.3
% galact. / % arab.	1.19	1.25	0.93	0.82
% rhamnose	13.0 ± 1.1	11.8 ± 2.2	12.9 ± 1.0	6.7 ± 1.1
% glucuronic acid	19.5 ± 0.2	23.1 ± 0.4	17.6 ± 0.1	11.2 ± 0.3
% protein[b]	2.24 ± 0.15	0.35 ± 0.10	11.8 ± 0.5	47.3 ± 3.0

All percentages expressed on a dry weight basis.
[a] Yield > 99%
[b] Calculated using a nitrogen conversion factor of 6.60 (Anderson, 1986a).

tion. Nevertheless, in 1996, the FAO Joint Committee for Food Additives and the European Union achieved a uniform and ultimate definition that reflects current practices:[20]

> *"Acacia gum/gum acacia/gum arabic is the dried exudation obtained from the stems and branches of natural strains of Acacia senegal (L) or closely related species of Acacia (family Leguminosae). It consists mainly of high molecular mass polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid."*

To prevent adulteration by non-acacia gums such as Combretum and Abizia, it is required that mannose, xylose and galacturonic acid are absent in the hydrolysis products. In the European Union specification, the additional requirement has been introduced that the molecular mass shall be ≈350,000, which seems to have little scientific justification according to the above discussion on the gum structure. Possible specifications for specific rotation and nitrogen content have been considered but not presently included. In practice, the "related" or "closely related" species which form more than 90% of the gum arabic of commerce are Acacia senegal and Acacia seyal.[20]

Gum acacia has been classified as a GRAS (Generally Recognized As Safe) food additive by the FDA after an overall review of toxicological, teratological and mutagenic tests. Furthermore, toxicological testing of selected exudate gums in Europe by the European Community showed no toxicological effects on laboratory animals. Minimum standards for food quality have been defined in the U.S. Pharmacopoeia XX and food grade standards published in the Food Chemicals Codex III.[16]

Physicochemical Properties: Solubility

Gum acacia is a unique hydrocolloid because of its high water solubility. It is possible to prepare solutions containing up to 50-55% of the gum with the solubility increasing as the temperature increases. Most other gums cannot be dissolved in solutions containing a concentration greater than 5%. Both freeze dried and spray dried gum acacia are more soluble than the crude powdered material. Drying does not affect viscosity or pH of solutions. High quality gum grades are required for tasteless and colorless solutions.[16]

Viscosity

At relatively low concentrations, gum acacia yields solutions that are essentially Newtonian in behavior and have very low viscosities compared to other polysaccharides of similar molecular mass. Viscous solutions are only obtained at much higher concentrations, i.e. above 25-30% w/w. Behavior is similar to that of globular proteins such as β-lactoglobulin up to 20% w/w (Figure 7), lending further support to the concept of the gum having a highly branched compact structure.[38] A typical Newtonian behavior is observed at concentrations of less than 40%; however, solutions become pseudoplastic at concentrations above 40%, as viscosity decreases with increasing shearing stress. There is no data available regarding the viscosity of the fractions but it can be assumed that AG will have a higher viscosity than AGP.[18]

The viscosity of gum acacia is affected by tree age, environmental factors, storage conditions, and physical product form (e.g., chipped vs. powdered gums). Variations in viscosity as a result of these factors can be affected by as much as 50%. Evidently, viscosity is proportional to temperature although variation is relatively small. Both storage and preparation temperatures influence viscosity of gum acacia solutions upon aging. Solutions are acidic with a pH of 4.5 to 5.5, which is in the region of maximum viscosity. Typically, viscosity rises sharply with increasing pH to a maximum at about 6 and then falls gradually to a value of about 12, where it stabilizes.[16]

The addition of electrolytes results in decreased viscosity which is proportional to the increase in the volume of the cation or the increase in the electrolyte concentration. The addition of more than one electrolyte gives an additive effect.[16] The behavior of gum arabic regarding viscosity in the presence of electrolytes and at high and low pH is characteristic of that of most complex hydrocolloids. However, it is believed that the decrease is not through compaction of the polymer coils as result of the screening of the intramolecular charge repulsions, but rather a reduction in its effective volume due to compaction of the electrical double layer surrounding the molecules.[20]

Gum arabic solutions also show an irreversible decrease in viscosity by cleavage of glycosidic bonds due to hydrolysis, oxidation or mechanical energy due to the disruption of

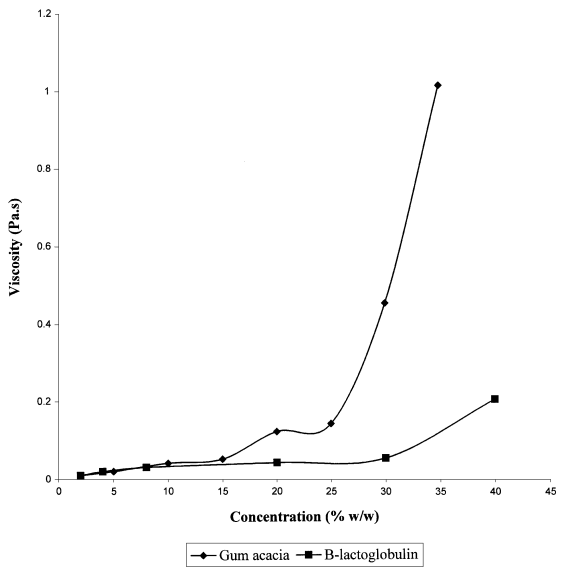

Figure 7. A comparison of the viscosities of gum arabic (acacia) and b-lactoglobulin as a function of polymer concentration

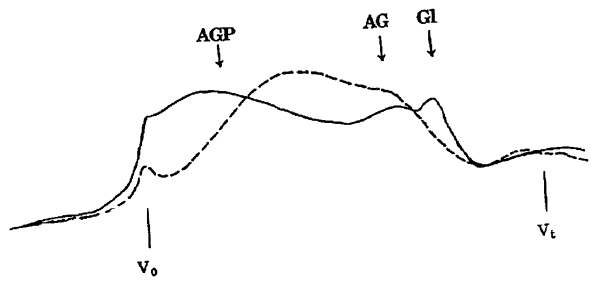

Figure 8. Gel permeation chromatograms of 1.5% w/w spray dried gum arabic solutions before (—) and after (——) heat treatment (102ºC for 4 hr). chromatographic conditions were: column, Sephacryl 500; solvent, 0.5M NaCl; detector, UV at 218 nm

the intramolecular entanglement, reduction of the effective molecular volume and less overall friction.[38]

Heat Sensitivity

Gum acacia has been shown to be heat sensitive and some precipitation will occur on prolonged heating. The precipitation consists essentially of proteinaceous material. Gel permeation chromatograms obtained before and after heating a gum solution (Figure 8) indicate that it is both the AGP and G_1 fractions that are removed from solution.[38] The optical rotation of the dissolved gum remains unaltered following heat treatment indicating that the AGP fraction (main polysaccharide macrostructure) is not affected.[26] According to Anderson and McDougall,[4] the protein undergoes autohydrolysis upon heating. Quantitatively, the precipitate is 72% protein. As a consequence of the loss of the high molecular mass AGP fraction, the solution viscosity decreases considerably.[38] There is a selective precipitation of amino acids: alanine, aspartic acid, glutamic acid, glycine, leucine, isoleucine, phenylalanine and valine, whereas proline, hydroxyproline, aspartic acid, glutamic acid, glycine and serine remain in solution. It is evident from these lists that a differential precipitation of hydrophobic amino acids occurs which is of paramount importance regarding emulsifiying capabilities of the gum).[4]

Emulsifying Properties

As aforementioned, the traditional view is that proteins make good emulsifying agents because of their substantial hydrophobicity and molecular flexibility that allow rapid adsorption and rearrangement at the interface to give a coherent macromolecular protective layer. Polysaccharides make good stabilizing agents because of their hydrophilicity, high molecular weight and gelation behavior leading to the formation of a macromolecular barrier of appreciable thickness between dispersed droplets by being able to protrude significantly in the aqueous medium. It would seem then that the conflicting requirements of substantial hydrophobicity for strong adsorption at the interface and substantial hydrophilicity for thick steric layers can be reconciled by covalent linkage of protein and polysaccharide moieties to form a single macromolecule with amphiphilic features. The main advantage of a covalent linkage over other types of non-covalent bonding is the retention of solubility and molecular integrity over a wide range of solution conditions (temperature, pH and ionic strength). One of the best examples of a natural protein-polysaccharide hybrid in this regard is gum acacia).[14]

The film-forming ability of gum acacia enables adequate formation and stabilization of beverage emulsions both as an emulsion concentrate and as the diluted final form. Its mode of action is unique among food hydrocolloids which generally confer stability by modifying the dispersion medium rheology at concentrations above the overlap polymer concentrations, and which therefore cannot sustain stabilization on extensive dilution of the aqueous continuous phase. Gum acacia is a genuine emulsifier in its own right although its surface activity is rather low compared with most food proteins. To compensate for this,

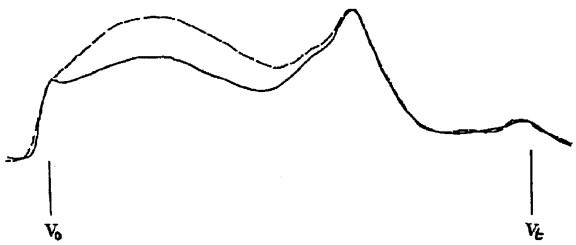

Figure 9. Gel permeation chromatograms of gum arabic in solution (19% w/w gum solution in distilled water, 20% w/w orange oil) before (—) and after (——) emulsification. Conditions similar to those in Figure 7

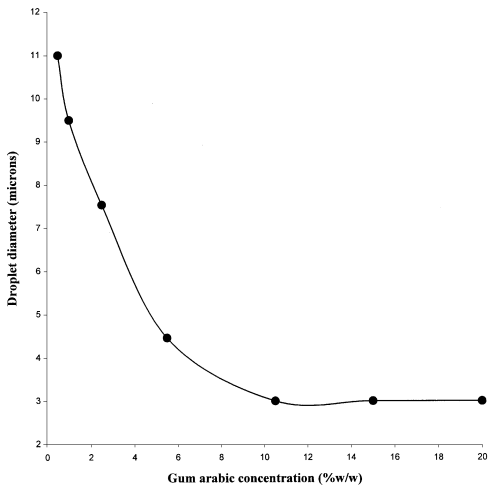

Figure 10. The average droplet diameter of emulsions (20% w/w orange oil) as a function of gum arabic composition. Measurements performed on dilute dispersions (1/10000) with distilled water using a ZM model Coulter Counter equipped with a 50 mm orifice electrode

a high concentration of the emulsifier must be used in practice. A gum-to-oil ratio of roughly 1:1 is required as compared with a 1:10 protein-to-oil ratio for an equivalent protein-stabilized emulsion.[14] Randall et al.[24] showed that stable 20% orange oil emulsions can only be obtained with gum acacia concentrations at ≥ 12%.

The ability of gum acacia to form a coherent adsorbed film at the oil-water interface is readily demonstrated by the visible "wrinkling" of the surface of a macroscopic oil droplet upon reducing its volume after prolonged exposure to an aqueous solution of the gum. The surface viscosity of an adsorbed gum acacia film is little affected by subsequent extensive dilution of the aqueous sub-phase. This means that once a macromolecular film has formed at the O/W interface by adsorption from a gum acacia solution, its viscoelastic properties are maintained even when the major part of the gum is removed from the aqueous phase in contact with the adsorbed layer. Thus, only a small proportion of the gum used to make an emulsion is actually involved in the stabilization mechanism.[14]

Randall et al.[25] demonstrated that the high molecular mass AGP fraction is responsible for the gum's emulsifying ability due to its unique structural features as previously described. This is evident from Figure 9, which shows the gel permeation chromatogram of a solution of gum acacia monitored before preparing an orange oil emulsion and also the chromatogram of a solution of the continuous phase recovered following emulsification. The difference between the two profiles represents the portion of gum that is adsorbed onto the oil droplets. Only a small amount of the gum is actually adsorbed, as mentioned above, and it can be seen that the adsorbing species is the AGP fraction based on the position of the depletion in the chromatogram.

The AG fraction has very little, if any, affinity for the oil surface due to its lack of protein to anchor at the interface and hence contributes little to the emulsification process apart from enhancing the viscosity of the continuous phase. Consequently, since AG is the most abundant fraction in the gum, relatively high concentrations are required to produce stable emulsions of relatively small droplet size as aforementioned. This is illustrated in Figure 10, which shows the average droplet size of orange oil emulsions produced using various concentrations of gum acacia. In this particular example, using 20% w/w orange oil and shearing with a bench-top mixer, over 10% w/w gum acacia solution is required to produce emulsions of minimum droplet size. This indicates that at lower gum concentrations, there is insufficient surface-active material present to fully coat all the droplets produced on shearing the system so that droplet flocculation and coalescence occur yielding emulsions of higher average droplet size. Obviously, the optimum concentration will vary with the nature of the oil, the type of mixer and conditions used since increased shearing would generate even smaller droplets thus requiring increased amounts of emulsifier.[38]

Dickinson et al.[13] noted the importance of nitrogen content on the time-dependent droplet size distribution of n-hexadecane-in-water emulsions in several acacia gums within a range of 0.1-5.27% N. It was found that the higher

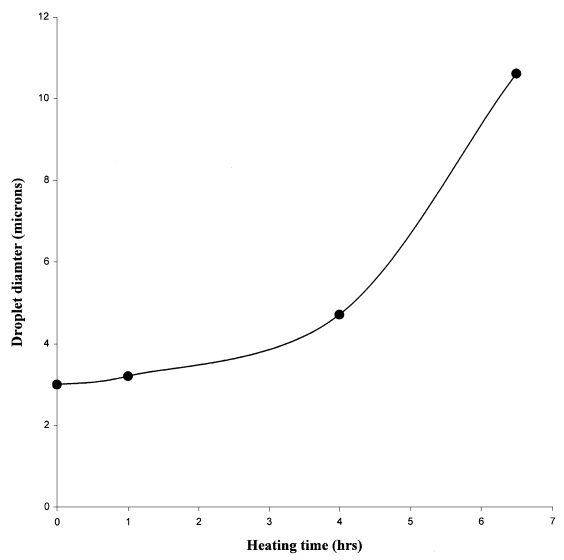

Figure 11. The average droplet diameter in orange oil (20% w/w) emulsions using gum acacia (19% w/w) heated for various times at 100°C. Conditions as in Figure 9

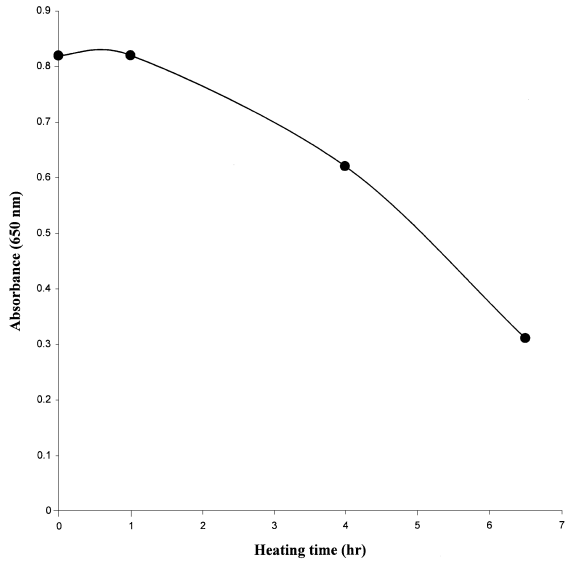

Figure 12. Emulsification efficiency of heat-treated gum arabic as monitored by the absorbance at 650 nm. Temperature: 100°C

the nitrogen content, the better the emulsion capacity and emulsion stability (regarding both coalescence and flocculation), which evidently correlates with the effective amount of AGP available for emulsification. Those emulsions also exhibited high surface viscosity that did not change upon dilution.[12] However Buffo et al.[6] found no correlation between the protein content of gum acacia and emulsion formation/stability. They hypothesized that other factors, mainly a favorable unfolding of the protein moiety at the O/W interface to expose hydrophobic amino acids towards the oil phase, were more important than the amount of protein in the gum or at the interface.

According to Dickinson et al.,[11] for a given nitrogen content, two main molecular factors would be expected to influence the rate of lowering of the interfacial tension: the molecular weight of the protein-polysaccharide complex and the accessibility of the protein component within the macromolecular complex. Low molecular weight fractions will diffuse quickly to the interface leading to a rapid lowering of the tension, whereas high molecular weight fractions will diffuse slowly and lower the tension slowly. Once the Acacia gum molecule has diffused to the interface, it will become adsorbed if the protein moiety is accessible to the interface and is not buried in the center of the molecule. That is why it is difficult to reconcile good surface and emulsifying properties with an Acacia gum model that places all the protein (or polypeptide chains) at the center of a bulky, tightly packed macromolecule. Experimental evidence definitely suggests a model with hydrophobic amino acids loaded at the periphery of the AGP complex, readily available for adsorption. Dickinson et al.[11] also reported some Acacia gums with low nitrogen content but nevertheless a rapid lowering of the interfacial tension. It appears that in these samples the low nitrogen content is compensated by the fast-adsorbing action of small proteinaceous fragments.

The gum loses its emulsifying ability upon sufficient heating to cause denaturation and precipitation of the AGP complex. This is demonstrated in Figure 11, which shows that the average droplet size of an orange oil emulsion (20% w/w) prepared using a 19% (w/w) gum acacia solution increases as the heat treatment time increases, thus reflecting a decrease in emulsification efficiency.[38]

Randall et al.[26] studied the effect of heat on the emulsifying properties of gum acacia in orange oil emulsions using gel permeation chromatography (GPC). They observed a decrease in the intensity of the peak corresponding to the AGP complex and an increase in the intensities of the lower molecular mass peaks during heating at 100°C for up to 3 hr. Heat led to protein precipitation in the form of a brown solid and an overall decrease in area under the GPC curve after refluxing for 6.5 hr at 100°C. The emulsification efficiency of the heated gum solution was assessed from turbidity measurements at 650 nm (Figure 12). Turbidity decreased with refluxing time, indicating an increase in the average droplet size and hence a reduction in

Table 4. Amino acid composition of *Acacia senegal* gum samples reported in the literature

Amino Acid	Randall et al. (1989a)	Anderson (1986b)
Hydroxyproline	250	274
Aspartic Acid	72	60
Threonine	72	77
Serine	136	137
Glutamic Acid	38	36
Proline	79	77
Glycine	57	49
Alanine	30	31
Cystine	–	1
Valine	36	45
Methionine	2	0
Isoleucine	12	14
Leucine	72	75
Tyrosine	17	11
Phenylalanine	37	29
Histidine	54	51
Lysine	26	26
Arginine	9	7

Values quoted in residues per 1000 residues.

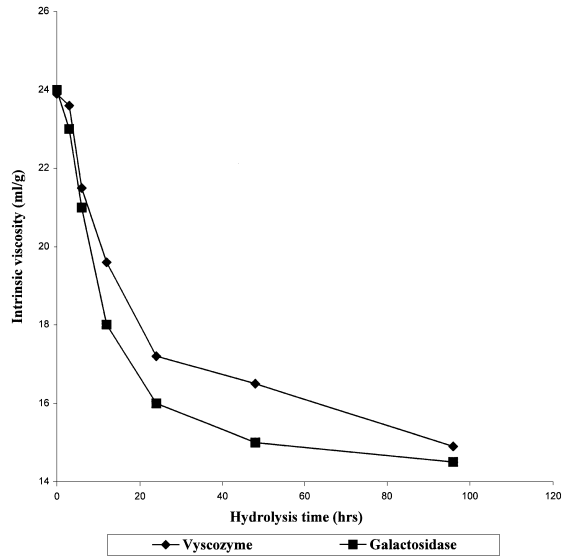

Figure 13. Changes in intrinsic viscosity after treatment with Viscozyme and β-D-galactosidase

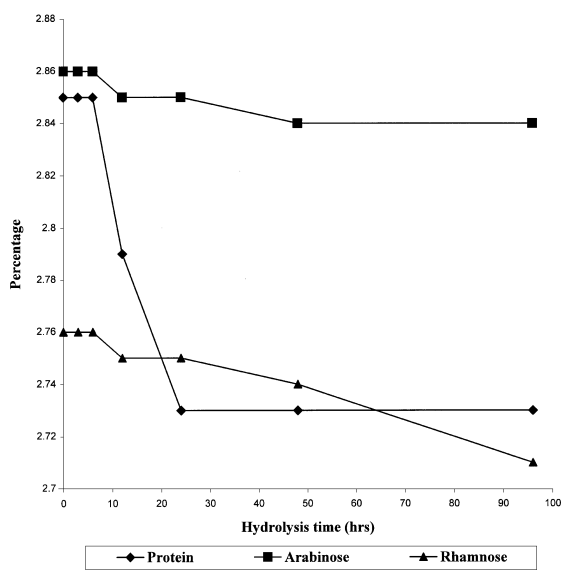

Figure 14. Changes in protein, arabinose and rhamnose after treatment with Viscozyme

emulsification efficiency. Dickinson, cited by Garti and Reichman,[18] indicated that although pre-heating the gum can be detrimental in terms of reduction of emulsifying capacity due to denaturation (and eventually precipitation) of the glycoprotein complex, it could be also beneficial in terms of unfolding the protein moiety and exposing hydrophobic groups readily available to adsorb at the O/W interface. This would improve both emulsifying capacity and long-term stability. Demetriades et al.[10] suggested that temperatures around 65 to 80ºC cause a partial unfolding of the protein at the interface which in fact can be beneficial as aforementioned or detrimental. Proteins do not necessarily and effectively rearrange so that all hydrophobic amino acids are directed towards the oil phase: those that remain oriented towards the water phase promote droplet aggregation because of an unfavorable thermodynamical interaction.

Ray et al.[27] undertook a detailed study of the emulsification properties of gum arabic in concentrate and dilute beverage emulsions using fractions obtained by GPC and hydrophobic affinity chromatography (HAC). The results are summarized in Table 5. In general, emulsion stability increased with increasing molecular mass and protein content. For emulsions prepared using the GPC fractions, the amount of gum used was adjusted so that the protein content remained constant. It was noted that fractions 2-1 and 2-2 (according to Table 5) produced emulsions as good as the whole gum at much lower gum concentrations. Fraction 3 required three times the amount of gum as the whole gum and was too viscous to homogenize properly. For the HAC fractions, the results corresponded to emulsions prepared using equal weights of gum. Fraction 2, of a high protein content, produced much better emulsions than fraction 1 and the whole gum.

Chikamai et al.[8] investigated the effects of two processing approaches on gum acacia with respect to its emulsify-

Table 5. Characterization of gum arabic fractions and evaluation of their emulsification efficiency

Fraction	% Recovery	Molecular Mass	% Protein	Droplet Size (μm)[a]	Emulsion Characteristics[a]	Beverage Characteristics[b]
GPC Fractions						
Whole gum control				0.63	no creaming; some free oil	OK
1	0.9	1.0E7	15.4	1.25	no creaming; trace of free oil	light ring
2		6.5E6		0.66	no creaming; trace of free oil	
2-1	10.7		6.0	0.65	no creaming; trace of free oil	OK
2-2	9.8		2.5	0.61	no creaming; trace of free oil	OK
3	67.1	1.4E5	1.0	0.84	no creaming; some free oil	
4	5.2	3.4E3	2.1	5.03	emulsion separated; much free oil	heavy ring; complete clearing
HAC Fractions						
Whole Gum Control				0.95	no creaming; no separation at bottom	very slight ring
1	80.0		0.7	1.45	very slight free oil; no creaming; no separation at bottom	moderate ring
2	8.0		8.7	0.64	no creaming; no separation at bottom	OK
3A	0.6		18.7			
3B	0.4		36.8			

[a] GPC fractions after 4 days; HAC fractions after 3 days
[b] For GPC fractions after 2 days; for HAC fractions after 1 day

ing properties: heating and enzyme biotechnology. Studies on heat processing were carried out at 100°C and 65°C. Results showed that heating the gum solution at 100°C for more than 6 hours caused significant degradation of the protein component and subsequent loss of emulsion capacity though a reduction in viscosity was achieved. Heating at 65°C allowed a reduction in viscosity to desirable industrial levels within 24 hours without a drastic loss in emulsification functionality. Process time and temperature were confirmed as important parameters during heat processing. The three enzymes studied (pronase, Viscozyme and β-D-galactosidase) caused losses in viscosity but with different implications for the other gum properties. Pronase was very effective in reducing intrinsic viscosity to industrial levels (i.e., below 16 ml/gr) within 12 hours, but there was also a significant loss in protein and emulsion capacity. It is therefore not suitable for gum processing when emulsification and stabilization are important properties. Both Viscozyme and β-D-galactosidase attained reductions in viscosity to levels desired for industrial uses within 24 hours (Figure 13). The former, however, did cause some degradation of the protein component giving rise to unstable emulsions. The amino acids selectively released were arginine, histidine, hydroxyproline, leucine, serine and threonine and there was also release of neutral sugars, particularly rhamnose (Figure 14). The effect of β-D-galactosidase was to increase the overall protein content and subsequent emulsification properties recalling high specificity for the carbohydrate moiety. The enzyme

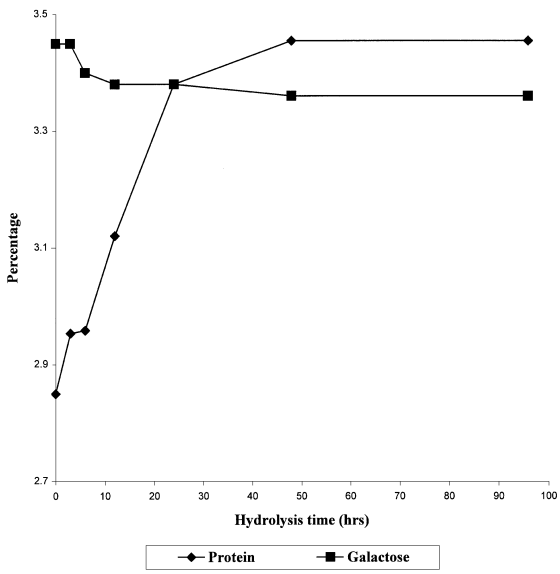

Figure 15. Changes in protein and galactose after treatment with β-D-galactosidase

has the advantage that it requires pH values within the range of the natural conditions of pH for gum arabic and room temperature for processing (Figure 15). However, Thevenet[33] questioned the validity of this enzymatic treatment. The compact molecular structure of gum arabic

based on the Wattle Blossom model and the fact that the galactopyranose side chains have terminal units of glucuronic acid indicate a difficult access for the enzyme to the attacking sites in the carbohydrate moiety which does compromise its effectiveness in terms of quantity of enzyme needed and treatment time. Besides an increase in protein content from 2.85 to 3.45% as shown in Figure 15, is not significant enough to reflect a substantial improvement in emulsion capacity.

Buffo et al.[6] studied a number of factors potentially related to the performance of gum acacia as emulsifier/stabilizer in beverage emulsions, including proximal composition of the gum (protein and mineral content), gum processing prior to emulsion preparation (pasteurization and demineralization), and pH of the dilute emulsion. Protein content was not linked to emulsion stability as aforementioned, whereas minerals decreased stability presumably due to an electrostatic screening effect (i.e., compression of the double electrical layer around the dispersed droplets and decrease of the electrostatic repulsion potential). Pasteurization and demineralization of the gum were considered of paramount importance to promote emulsion stability, most feasibly in relation to an effective unfolding of the protein moiety at the O/W interface (exposure of hydrophobic amino acids towards the oil phase causing a tenacious adsorption of the emulsifier) and elimination of the electrostatic screening effect, respectively. Dilute emulsions were significantly less stable at pH = 2.5 than at higher pH levels (4.5 & 5.5) because of an increased electrostatic screening effect from the electrolytes (H^+) added for pH adjustment. Viscosity of emulsion concentrates was increased by gum demineralization due to elimination of the screening effect from indigenous cations and decreased by pasteurization due to breakage of the molecular entanglement and reduction of the effective molecular volume. The study was run for a pool of gum acacia samples corresponding to the two most commercially important species: Acacia senegal and Acacia seyal. The natural variability among Acacia species and even within the same species among batches from different growing regions was clearly put in evidence through significantly different emulsifying properties. Thus, similar processing factors aiming to enhance their performance may have quite different effectiveness according to this natural variability. Van der Waals attractive forces, electrostatic repulsive forces and polymeric steric repulsive forces were identified as the main colloidal interactions playing a role in the determination of stability of dilute beverage emulsions.

It seems then that the protein-hybrid in gum acacia meets all the necessary requirements in a capacity comparable to that of emulsifying proteins via its numerous adsorption sites, flexibility, conformational change at the interface and the entropy gain. Therefore, although the gum is basically a hydrocolloid, its interfacial and emulsifying properties are derived from its small proteinaceous fraction.[18]

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Address correspondence to Gary Reineccius, Department of Food Science aned Nutrition, university of minnesota, 147 Food Science and Nutrition Building, 1334 Eckles Avenue, St. Paul, MN.

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