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Gum Arabic: More Than an Edible Emulsifier

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1. Introduction

Gum Arabic (GA) or *Acacia* gum is an edible biopolymer obtained as exudates of mature trees of *Acacia senegal* and *Acacia seyal* which grow principally in the African region of Sahe in Sudan. The exudate is a non-viscous liquid, rich in soluble fibers, and its emanation from the stems and branches usually occurs under stress conditions such as drought, poor soil fertility, and injury (Williams & Phillips, 2000).

The use of GA dates back to the second millennium BC when the Egyptians used it as an adhesive and ink. Throughout the time, GA found its way to Europe and it started to be called "gum arabic" because was exported from Arabian ports.

Chemically, GA is a complex mixture of macromolecules of different size and composition (mainly carbohydrates and proteins). Today, the properties and features of GA have been widely explored and developed and it is being used in a wide range of industrial sectors such as textiles, ceramics, lithography, cosmetics and pharmaceuticals, encapsulation, food, etc. Regarding food industry, it is used as a stabilizer, a thickener and/or an emulsifier agent (e.g., soft drink syrup, gummy candies and creams) (Verbeken et al., 2003).

In the pharmaceutical industry, GA is used in pharmaceutical preparations and as a carrier of drugs since it is considered a physiologically harmless substance. Additionally, recent studies have highlighted GA antioxidant properties (Trommer & Neubert, 2005; Ali & Al Moundhri, 2006; Hinson et al., 2004), its role in the metabolism of lipids (Tiss et al., 2001, Evans et al., 1992), its positive results when being used in treatments for several degenerative diseases such as kidney failure (Matsumoto et al., 2006; Bliss et al., 1996; Ali et al., 2008), cardiovascular (Glover et al., 2009) and gastrointestinal (Wapnir et al., 2008; Rehman et al., 2003).

Therefore, there is substantial evidence that GA can play a positive health-related role in addition to its well-known properties as an emulsifier. Therefore, the aim of this chapter is to describe general aspects of the source, composition, and already known uses of GA as well as some new aspects of its antioxidant capacity against some reactive oxygen substances (ROS), and its antimicrobial activity (AMA).

2. GA sources and processing

Acacia senegal and *Acacia seyal* trees are the main sources of GA. These species grow naturally in the semi-arid sub-Saharan regions of Africa. There are over 1000 species of acacia and a summary of their botanical classification was reported by Phillips and Williams, 1993. Sudan has traditionally been the main GA producer and its derivatives until the early 60s with a production of 60 kTn/year. Nevertheless, such a production decreased from 60 kTn/year to 20 kTn/year in the '70s and '80s due to extensive drought and unstable governments. These facts prompted new GA-producing countries such as Chad and Nigeria which produce mainly *Acacia seyal* (Abdel Nour, 1999). Europe and U.S. are the most important GA markets importing 40 kTn/year, on average, while Japan, the largest Asian consumer, imports about 2 kTn/year.

The crude exudate of GA is processed differently according to the quality finally required for it to be marketed. Air drying is the easiest method to be applied which, together with mechanical milling (kibbling), are used in order to produce a granular material that is much more soluble than the raw product. Other processing methods are spray drying and roller drying. These methods involve dissolving exudate in water under controlled heating conditions and constant stirring. Heating must be mild to avoid distortion of the gum which could have a detrimental effect on its functional properties. After removing the insoluble material by decantation or filtration, the solution is pasteurized and subjected to spray or roller drying. Spray drying involves spraying the solution into a stream of hot air. The water completely evaporates and the dry powder is separated from air by a cyclone, resulting in 50 to 100 mμ particles. During the roller-drying, the solution is passed to the hot rollers and the water is evaporated by the air flow. The thickness of the resulting GA film is controlled by adjusting the distance between the rollers. The film is separated from the roll by scraping blades giving way to particle scales of several hundred μm in size. GA samples produced by spray drying and drying rollers have an advantage over raw gum as they are virtually free of microbial contamination and dissolve much faster.

3. Chemical composition and structure

In recent years, several investigations have been conducted in order to reveal the molecular structure of GA and relate it to its exceptional emulsifying and rheological properties. The chemical composition of GA is complex and consists of a group of macromolecules characterized by a high proportion of carbohydrates (~97%), which are predominantly composed of D-galactose and L-arabinose units and a low proportion of proteins (<3%) (Islam et al., 1997). The chemical composition of GA may vary slightly depending on its origin, climate, harvest season, tree age and processing conditions, such as spray dying (Al-Assaf, et al., 2005 (a,b); Flindt et al., 2005; Hassan et al., 2005; Siddig et al., 2005). Therefore, there are some differences between the chemical composition of the GA taken from *Acacia senegal* and *Acacia seyal*. In fact, both gums have the same sugar residues but *Acacia seyal* gum has a lower content of rhamnose and glucuronic acid and a higher content of arabinose and 4-O-methyl glucuronic acid than *Acacia senegal* gum. Instead, *Acacia seyal* gum contains a lower proportion of nitrogen, and specific rotations are also completely different. The determination of the latter parameters may clearly spot the difference between the two species (Osman et al., 1993).

Table 1 presents the chemical composition and some properties of both gums reported by Osman et al., 1993 and Williams & Phillips, et al., 2000. Despite having different protein content, amino acid composition is similar in both gums. Recently, Mahendran et al., 2008, reported the GA amino acid composition in *Acacia Senegal*, being rich in hydroxyproline, serine, threonine, leucine, glycine, histidine, **Table 2.**

Parameter	*Acacia senegal*	*Acacia seyal*
% Rhamnose	14	3
% Arabinose	29	41
% Galactose	36	32
% Glucuronic acid	14.5	6.5
% Nitrogen	0.365	0.147
% Protein	2.41	0.97
Specific rotation (degrees)	-30	+ 51
Average molecular mass (kDa)	380	850

Table 1. Comparative chemical composition and some properties of Gum arabic taken from *Acacia senegal* and *Acacia seyal* trees (Osman et al., 1993 and Williams& Phillips, et al., 2000).

Aminoacid	(nmol/ mg) GA	% Aminoacid
Hydroxyproline	54.200	0.711
Serine	28.700	0.302
Threonine	15.900	0.208
Proline	15.600	0.180
Leucine	15.100	0.198
Histidine	10.700	0.166
Aspartic acid	10.600	0.141
Glutamic acid	8.290	0.122
Valine	7.290	0.085
Phenylalanine	6.330	0.105
Lysine	5.130	0.075
Alanine	5.070	0.045
Isoleucine	2.380	0.031
Tyrosine	2.300	0.042
Arginine	2.120	0.037
Methionine	0.110	0.002
Cysteine	0.000	0.000
Tryptophan	0.000	0.000

Table 2. Aminoacid content in Gum Arabic taken from *Acacia senegal* (Mahendran et al., 2008).

Table 3 shows some physicochemical properties used as international GA quality parameters, for example: moisture, total ash content, volatile matter and internal energy, with reference to gums taken from *Acacia senegal* species in Sudan (FAO, 1990, Larson & Bromley, 1991). The physicochemical properties of GA may vary depending on the origin and age of trees, the exudation time, the storage type, and climate. The moisture content facilitates the solubility of GA carbohydrate hydrophilic and hydrophobic proteins. The total ash content is used to determine the critical levels of foreign matter, insoluble matter in

acid, calcium, potassium and magnesium (Mocak et al., 1998). The compositions of cations in the ash residue are used to determine the specific levels of heavy metals in the gum arabic quality (FAO, 1990, 1996). The volatile matter determines the nature and degree of polymerization of the compositions contained in sugar (arabinose, galactose and rhamnose) which exhibits strong binding properties to act as emulsifiers and stabilizers in the manufacture of cough syrups in the pharmaceutical industry (Phillips & Williams, 2001). The GA internal energy is the required energy to produce an amount of carbon by heating at 500 °C to release carbon dioxide. Optical rotation is used to determine the nature of GA sugars as well as to identify the source of production.

Property	Value
Moisture (%)	13 - 15
Ash content (%)	2 - 4
Internal energy (%)	30 - 39
Volatile Matter (%)	51 - 65
Optical rotation (degrees)	(-26) - (-34)
Nitrogen content (%)	0.26 - 0.39
Cationic composition of total ash at 550 °C	
Copper (ppm)	52 - 66
Iron (ppm)	730 - 2490
Manganese (ppm)	69 - 117
Zinc (ppm)	45 - 111

Table 3. International specifications of Gum Arabic quality (FAO, 1990).

Gel permeation chromatography studies using both refractive index and UV(260 nm) absorption detections have confirmed that both A*cacia senegal* and *Acacia seyal* gums consist of three main components (Islam et al., 1997, Idris et al., 1998, Williams & Phillips, 2000, Al Assaf 2006):

i.   A main fraction (88-90%) of a polysaccharide of β-(1→3) galactose, highly branched with units of rhamnose, arabinose and glucuronic acid (which is found in nature like salts of magnesium, potassium and calcium). This fraction is called Arabinogalactan (AG) and contains a low protein content (~0.35%) and MW≈ 300 kDa (Renard et al., 2006, Sanchez et al., 2008);

ii.   A secondary fraction constituting ~10% of the total, with a protein content of 11% and a molecular weight of 1400 kDa, corresponding to a complex Arabinogalactan-Protein (AGP) (Goodrum et al., 2000), and finally,

iii. A smaller fraction (1% of total) composed by a glycoprotein (GP) consisting of the highest protein content (50 wt%) with an amino acid composition different from the complex AGP (Williams et al., 1990).

Although the total content of carbohydrate fractions of the three components is similar, as reported by Williams et al., 1990, it was found that protein-rich fractions have a significantly lower glucuronic acid content. Circular dichroism studies conducted on different GA fractions showed that only the AGP and GP components have a secondary structure (Renard et al., 2006). The AGP fraction was isolated by gel filtration chromatography and subjected to deglycosylation with hydrofluoric acid (HF) to separate the protein (Qi et al., 1991). About 400 amino acids were contained by the AGP protein fraction (~33% are

hydroxyproline residues). In addition, it was shown that the AGP fraction is composed of blocks of carbohydrates attached to the polypeptide chain by covalent bonds through serine and hydroproline residues (Mahendran et al., 2008). Further SDS-PAGE studies conducted after deglycosylation with HF indicated the presence of two proteins moieties, one with a mass of about 30 kDa corresponding to a polypeptide chain of approximately 250 amino acids, and the second one with about 45 amino acids (~5 kDa). This minor protein fraction is thought to be associated with the main AG fraction. It was proposed that in the structure of AGP, the polypeptide chain of 400 amino acids acts as "cable connector" of the blocks of carbohydrates (≤ 40 kDa) which are covalently linked to the protein ("wattle blossom" model) (Fincher et al., 1983; Mahendran et al., 2008).

4. Physicochemical properties

The GA is a heterogeneous material having both hydrophilic and hydrophobic affinities. GA physicochemical responses can be handled depending on the balance of hydrophilic and hydrophobic interactions. GA functional properties are closely related to its structure, which determines, for example, solubility, viscosity, degree of interaction with water and oil in an emulsion, microencapsulation ability, among others.

4.1 Solubility and viscosity

GA has high water solubility and a relatively low viscosity compared with other gums. Most gums cannot dissolve in water in concentrations above 5% due to their high viscosity. Instead, GA can get dissolved in water in a concentration of 50% w/v, forming a fluid solution with acidic properties (pH ~4.5). The highly branched structure of the GA molecules leads to compact relatively small hydrodynamic volume and, consequently GA will only become a viscous solution at high concentrations. Solutions containing less than 10% of GA have a low viscosity and respond to Newtonian behavior (Williams et al., 1990). However, steric interactions of the hydrated molecules increase viscosity in those solutions containing more than 30% of GA resulting in an increasingly pseudoplastic behavior. Its high stability in acidic solutions is exploited to emulsify citrus oils. The viscosity of GA solutions can be modified by the addition of acids or bases as these ones change the electrostatic charge on the macromolecule. In very acidic solutions, acid groups neutralize so inducing a more compact conformation of the polymer which leads to a decreased viscosity; while a higher pH (less compact molecule) results in maximum viscosity around pH 5.0-5.5. In very basic solutions, the ionic strength increment reduces the electrostatic repulsion between GA molecules producing a more compact conformation of the biopolymer and thus reducing the viscosity of the solution (Anderson et al., 1990; Williams et al., 1990).

4.2 Emulsifying properties

GA is well recognized as emulsifier used in essential oil and flavor industries. Randall et al., 1998, reported that the AGP complex is the main component responsible for GA ability to stabilize emulsions, by the association of the AGP amphiphilic protein component with the surface of oil droplets, while the hydrophilic carbohydrate fraction is oriented toward the aqueous phase, preventing aggregation of the droplets by electrostatic repulsion. However, only 1-2% of the gum is absorbed into the oil-water interface and participates in the emulsification; thus, over 12% of GA content is required to stabilize emulsions with 20%

orange oil (Williams et al., 1990). If there is not enough GA amount to cover all the gum drops, unstable emulsion is formed and flocculation and coalescence occurs.

4.3 Molecular association

It is well known the tendency of polysaccharides to associate in aqueous solution. These molecular associations can deeply affect their function in a particular application due to their influence on molecular weight, shape and size, which determines how molecules interact with other molecules and water. There are several factors such as hydrogen bonding, hydrophobic association, an association mediated by ions, electrostatic interactions, which depend on the concentration and the presence of protein components that affect the ability to form supramolecular complexes.

Al-Assaf et al., 2007, showed that molecular associations in GA can lead to an increase in molecular weight in the solid state by maturation under controlled heat and humidity. The process does not involves change in the basic structural components and, while the maturation takes place, the level of association increases giving way to AGP with higher molecular weight and protein content. This process mimics the biological process which produces more AGP throughout the tree growth, and gets maturation to continue during the storage of GA after harvest. Subsequently, Al-Assaf et al., 2009, analyzed the role of protein components in GA to promote molecular association when the gum is subjected to different processing treatments such as maturation, spray drying and irradiation. Results demonstrate the ability of protein components to promote hydrophobic associations that influence the size and proportion of the high molecular weight component AGP. When GA undergoes maturation (solid state heat treatment) there is an increase in the hydrophobic nature of the gum and therefore an increase of its emulsifying properties. Spray drying involves not only the aggregation through hydrophobic associations but also changes in the surface properties of peptide residues increasing GA hydrophilicity compared with the association promoted by the treatment of maturity in the solid state. Ionizing radiation in both aqueous solutions and solid state induces cross-linking between polysaccharide blocks by the formation of –C-C- bonds.

It was also reported that, by using mild UV-radiation, it is possible to induce GA cross-linking (Kuan et al., 2009). The process reduced the solution viscosity and improved emulsification properties. This GA modification can be used in food products requiring better reduced viscosity emulsifying properties such as dressings, spreads, and beverages, as well as in other nonfood products such as lithographic formulations, textiles, and paper manufacturing.

5. Pharmacological action

Although GA is being widely used as an experimental vehicle for drugs in physiological and pharmacological experiments, and it is supposed to be an inert substance, recent reports have confirmed that GA has some biological properties as an antioxidant (Trommer & Neubert, 2005; Ali & Al Moundhri, 2006, Hinson et al., 2004) on the metabolism of lipids (Tiss et al., 2001, Evans et al., 1992), positive contribution in treating kidney, (Matsumoto et al., 2006; Bliss et al., 1996, Ali et al., 2008), cardiovascular (Glover et al., 2009) and gastrointestinal diseases (Wapnir et al., 2008, Rehman et al., 2003).

GA has been extensively tested for its properties as non-digestible polysaccharide which can reach the large intestine without digestion; in the small intestine, it can be classified as dietary fiber. Due to its physical properties, it reduces glucose absorption, increases fecal mass, bile acids and has the potential to beneficially modify the physiological state of humans (Adiotomre et al., 1990). GA is slowly fermented by the bacterial flora of the large intestine producing short chain fatty acids (Annison et al., 1995). Therefore, its tolerance is excellent and can be consumed in high daily doses without intestinal complications. In addition, GA is able to selectively increase the proportion of lactic acid bacteria and biphidus bacteria in healthy subjects.

A daily intake of 25 and 30 g of GA for 21 to 30 days reduced total cholesterol by 6 and 10.4%, respectively (Ross et al., 1983, Sharma 1985). The decrease was limited only to LDL and VLDL, with no effect on HDL and triglycerides. However, Topping et al. (1985) reported that plasma cholesterol concentrations were not affected by the supply of GA, but triglyceride concentration in plasma was significantly lower than in controls.

Various mechanisms have been proposed to explain the hypocholesterolemic effect of GA (Annison et al., 1995; Tiss et al., 2001). Some studies have suggested that the viscosity of fermentable dietary fiber contributes substantially to the reduction of lipids in animals and humans (Gallaher et al., 1993; Moundras et al., 1994). However, other studies suggested that this property is not related to plasma lipids (Evans et al., 1992). The mechanism involved is clearly linked to increased bile acid excretion and fecal neutral sterol or a modification of digestion and absorption of lipids (Moundras et al., 1994).

6. Applications

GA is being widely used for industrial purposes such as a stabilizer, a thickener, an emulsifier and an encapsulating in the food industry, and to a lesser extent in textiles, ceramics, lithography, cosmetic, and pharmaceutical industry (Verbeken et al., 2003). In the food industry, GA is primarily used in confectionery, bakery, dairy, beverage, and as a microencapsulating agent.

6.1 Confectionery and baking

GA is employed in a variety of products including gum, lozenges, chocolates, and sweets. In these products, GA performs two important functions: to delay or to prevent sugar crystallization, and to emulsify fat to keep it evenly distributed throughout the product. In baking, GA is extensively used for its low moisture absorption properties. GA solubility in cold water allows greater formation of clear solutions than in sugar solutions. It has also favorable adhesive properties to be used in glace and meringues, and it provides softness when used as an emulsion stabilizer. Baking properties of wheat and rye flours can be improved by adding a small amount of GA since its capacity for retaining moisture reduces the hardening of bread.

6.2 Dairy products

GA is used as a stabilizer in frozen products like ice-cream due to its water absorption properties. The role of GA in these products is to cause a fine texture and growth by inhibiting the formation of ice crystals which is achieved by combining a large amount of

water and holding it as water of hydration, being its higher melting point the main attraction of ice-cream.

6.3 Beverages

GA is used as an emulsifier in beverages such as citrus juices, beer, and cola drinks. GA ability to stabilize foams is used in the manufacture of beer and soft drinks. Besides, it can be used for clarifying wines.

6.4 Microencapsulation

In the food industry, microencapsulation is an important process to improve the chemical stability of sensitive compounds, to provide the controlled release of microencapsulated compounds and to give a free flowing powder with improved handling properties (Anandaraman and Reineccius, 1986; Sheu and Rosenberg, 1993). The encapsulating material must preserve and protect the encapsulated compounds during manufacture, storage, and handling to release them into the final product during manufacture or consumption.

Solubility and low viscosity emulsion properties have facilitated the use of GA as an encapsulating agent for retention and protection of chemically reactive and volatile commercial food flavoring. Reineccius (1988) has reported on the encapsulation of orange oil using GA as wall material. Its main drawback is its cost for the oversubscription. However, due to its efficacy with regard to other wall materials such as maltodextrin (Krishnan et al., 2005) and modified starch, reported by various studies (Reineccius, 1989), the cost may not be relevant as long as extra protection or stability are achieved for microencapsulated high-value products, and in food or pharmaceutical fields.

GA is mainly used for fat microencapsulation because it produces stable emulsions in the case of most oils in a wide pH range, and it has the ability to form films (Kenyon, 1995). Barbosa et al., 2005 studied the photostability of the microencapsulated carotenoid bixin in different edible polysaccharide. They found out that microencapsulated bixin in GA was three to four times more stable than the one microencapsulated with maltodextrin, and about ten-fold than in homogeneous solvents.

7. Antioxidant action

Several reports suggest that GA has antioxidant capacity. However, there are controversial results of it, mainly *in vivo* studies. For example, GA has been reported to exert a protective effect against gentamicin and cisplatin nephrotoxicity (Al-Majed et al., 2002, 2003), and doxorubicin cardiotoxicity (Abd-Allah et al., 2002) used as biological models in rats. However, Ali et al., (2003) reported that treatment of rats with GA causes only a slight palliative effect of gentamicin nephrotoxicity. Later, Trommer & Neubert (2005) studied lipid peroxidation antioxidant and reducing effects in vitro of various polysaccharides (including GA). They found that GA reduces lipid peroxidation of skin in a dose-dependent. In contrast, Ali (2004) reported that administration of GA at concentrations of 2.5%, 5.0% and 10.0% in drinking water for eight consecutive days to rats did not significantly alter the concentrations of free radical scavenger's glutathione (GSH) and acid ascorbic acid (AA), and superoxide dismutase (SOD), or lipid peroxidation.

Consequently, the antioxidant activity of GA in biological systems is still an unresolved issue, and therefore it requires a more direct knowledge of the antioxidant capacity of GA that can be obtained by *in vitro* experiments against different types of oxidant species. The total antioxidant activity of a compound or substance is associated with several processes that include the scavenging of free radical species (eg. HO•, ROO•), ability to quench reactive excited states (triplet excited states and/or oxygen singlet molecular $^1O_2$), and/or sequester of metal ions (Fe^{2+}, Cu^{2+}) to avoid the formation of HO• by Fenton type reactions. In the following sections, we will discuss the *in vitro* antioxidant capacity of GA for some of these processes.

7.1 Quenching of electronically excited states

From the viewpoint of food and biological systems, Vitamin B_2 (riboflavin, RF) is a widely distributed molecule. **Table 4** shows the most relevant spectroscopic and photophysical properties in aqueous solution (Valle et al., 2011). As many isoalloxasine derivatives, RF is a well known photosensitizer by absorbing near UV radiation and visible light (blue-edge), generating both reactive excited states and reactive oxygen species (ROS) in the presence of ground state molecular oxygen ($^3O_2$), **Scheme 1**.
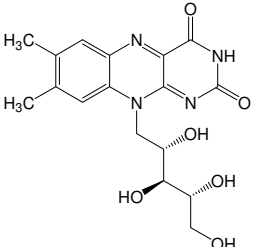

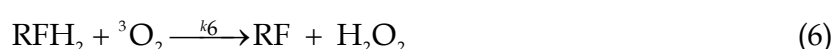|	
λ_{ab}^{max} **(nm) [$\varepsilon_\lambda^{max} \times 10^{-3}$ ($M^{-1}cm^{-1}$)]:**	267 [2.94]; 375 [0.96]; 445 [1.12]
λ_{fl}^{max} **(nm); τ_{fl} (ns); Φ_{fl}; E_S (kJ/mol):**	520; 4.7; 0.2; 239
λ_T^{max} **(nm); τ_T (µs); Φ_T; E_T (kJ/mol):**	720; 25; 0.58; 209

Table 4. Chemical structure and spectroscopical and photophysical properties of riboflavin (RF) in aqueous phosphate buffer pH 7.4 (Valle et al, 2011).
λ_{ab}^{max}, λ_{fl}^{max}, λ_T^{max} are the spectral maximum of UV-Vis absorption, emission, and triplet state absorption, respectively. ε_λ is the molar extinction coefficient. τ_{fl} and τ_T, Φ_{fl} and Φ_T, E_S and E_T; are the lifetime, quantum yield, and energy content of the singlet and triplet excited states of RF, respectively.
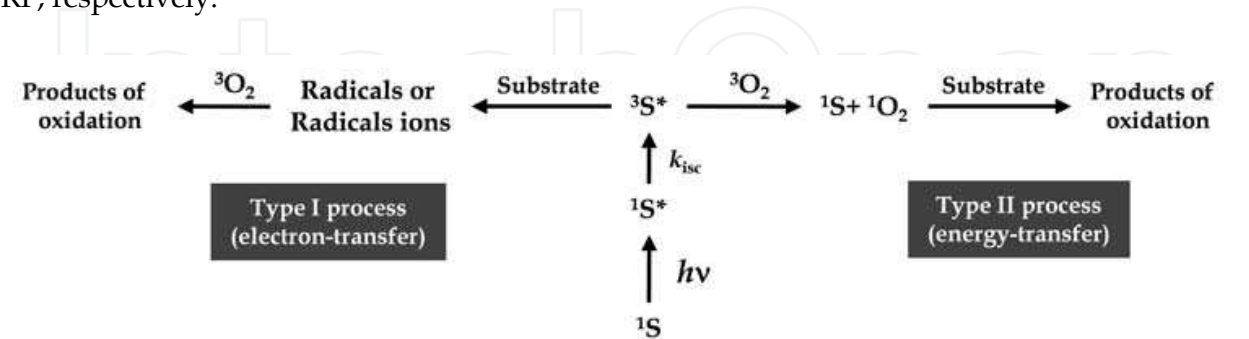
Scheme 1. Photosensitized Type I and Type II oxidative processes involving a sensitizer molecule S.

Depending on the relative concentration of reactive substrate and dissolved molecular oxygen ($^3O_2$), RF is able to induce photosensitized oxidation of molecular targets by either Type I (electron-transfer) or Type II (energy-transfer) processes (Foote, 1991). In Type I

process, the excited triplet state of RF, i.e. $^3RF^*$, can react with aminoacids residues (A) of peptides and proteins to produce radicals ions pair $RF^{\bullet-}$ and $A^{\bullet+}$ (eqns 1 and 2). The semi reduced radical anion $RF^{\bullet-}$ undergoes secondary reactions that can subsequently generate ROS, such as superoxide anion $O_2^{\bullet-}$, hydrogen peroxide H_2O_2, eqns 3-6. In the presence of heavy metal cations, e.g. Fe^{2+}, H_2O_2 can produce the very reactive hydroxyl radical $HO^\bullet$ by Fenton reaction, eqn. 7.

$$RF \xrightarrow{h\nu} {}^1RF^* \xrightarrow{k_{isc}} {}^3RF^* \tag{1}$$

$$^3RF^* + A \xrightarrow{k_2} A^{\bullet+} + RF^{\bullet-} \tag{2}$$

$$RF^{\bullet-} + H^+ \xrightarrow{k_3} RFH^\bullet \tag{3}$$

$$2RFH^\bullet \xrightarrow{k_4} RFH_2 + RF \tag{4}$$

$$RF^{\bullet-} + {}^3O_2 \xrightarrow{k_5} RF + O_2^{\bullet-} \tag{5}$$

$$RFH_2 + {}^3O_2 \xrightarrow{k_6} RF + H_2O_2 \tag{6}$$

$$H_2O_2 \xrightarrow{M^{n+}/Fenton} 2HO^\bullet \tag{7}$$

On the other hand, Type II process competes efficiently with the electron-transfer pathway in aerobic environments where the concentration of ground triplet state molecular oxygen is relatively high (~0.27 mM), and singlet molecular oxygen ($^1O_2$) is the most abundant ROS generated under these conditions, with a quantum yield $\Phi_\Delta \approx 0.48$ (Valle et al., 2011), eqn. 8. It is also possible an electron-transfer reaction from $^3RF^*$ to $^3O_2$ to form anion superoxide, but this reaction occurs with very low efficiency <0.1% (Lu et al., 2000).

$$^3RF^* + {}^3O_2 \xrightarrow{k_8} RF + {}^1O_2 \tag{8}$$

In turn, $^1O_2$ is a very electrophilic excited state species of molecular oxygen that interacts efficiently with electron-rich molecules, such as aminoacid residues of proteins like histidine, metionine, tryptophan, tyrosine, etc., by both physical and chemical quenching processes, eqns. 9 and 10 (Davies, 2003; Bisby et al., 1999).

$$^1O_2 + A \xrightarrow{k_q} {}^3O_2 + A \tag{9}$$

$$^1O_2 + A \xrightarrow{k_r} AO_2 \text{ (oxidation product)} \tag{10}$$
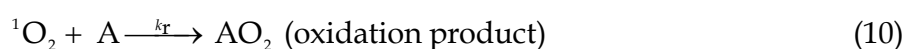
A larger k_q/k_r ratio for the molecule A, better is its ability as catalytic quencher, since physical quenching of $^1O_2$ (eqn. 9) does not consume the antioxidant molecule (Montenegro et al, 2002; Morán Vieyra et al., 2009).

Therefore, it is a very relevant issue the evaluation of molecules and macromolecules that can efficiently act as quenchers of electronically excited states, such as $^3RF^*$ and $^1O_2$ as examples, to avoid the formation of ROS and/or eliminate them (Wondrak et al., 2006).

F**igure 1a** shows that the addition of GA (Powdered food grade MW = 3.5 x 10^5 g/mol of Colloids Naturels Brazil, Saõ Paulo, Brazil) in RF aqueous solutions produces additive absorption changes, since GA showed a slight scattering effect (dashed line spectrum) that distorted the absorption spectrum of RF at the UV edge. This effect did not modify the fluorescence emission spectra of RF obtained by excitation at 445 nm. These results indicate that no strong interactions are occurring with GA either in the ground or singlet excited state of RF.
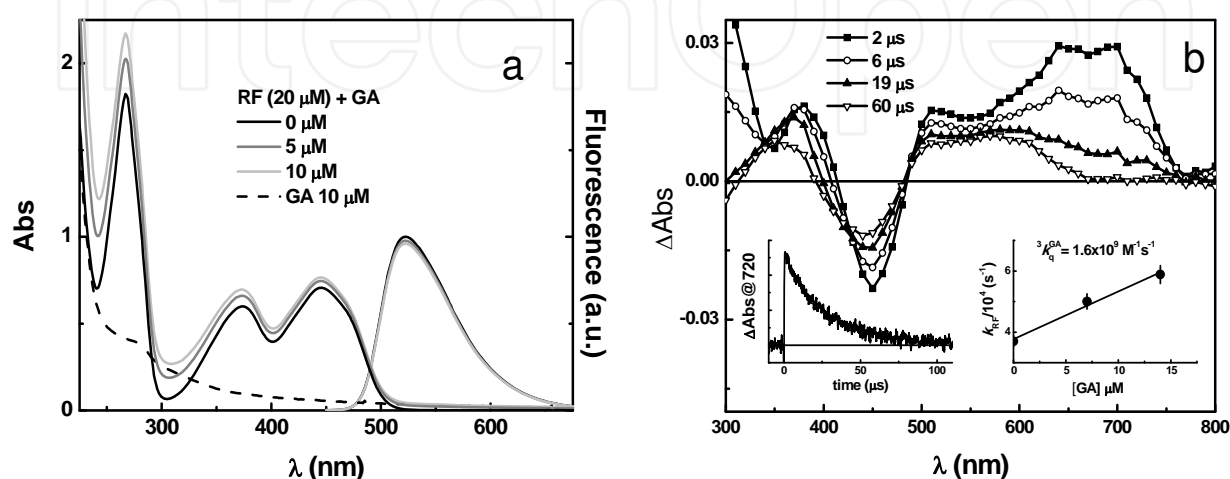
Fig. 1. **a)** UV-Vis absorption and fluorescence emission spectra of riboflavin (RF, 20 μM) and Gum Arabic aqueous solutions at pH 7 (phosphate buffer 100 mM). **b)** Transient absorption spectra of RF (35 μM) in N_2-saturated MeOH-Water (1:1) solution. The insets show the transient decay at 720 nm for the ^{3}RF* species and the Stern-Volmer plot for the quenching of ^{3}RF* by GA, eqn 11.

Figure 1b shows the transient absorption spectra of RF (i.e. the difference between the ground singlet and excited triplet states) obtained by laser-flash photolysis using a Nd:Yag pulsed laser operating at 355 nm (10 ns pulse width) as excitation source. At short times after the laser pulse, the transient spectrum shows the characteristic absorption of the lowest vibrational triplet state transitions (0 ← 0) and (1 ← 0) at approximately 715 and 660 nm, respectively. In the absence of GA, the initial triplet state decays with a lifetime around 27 μs in deoxygenated solutions by dismutation reaction to form semi oxidized and semi reduced forms with characteristic absorption bands at 360 nm and 500-600 nm and (Melø et al., 1999). However, in the presence of GA, the ^{3}RF* is efficiently quenched by the gum with a bimolecular rate constant $^3k_q^{GA} = 1.6\times10^9$ M^{-1}s^{-1} calculated according to eqn 11, where k_{RF} and k_{RF}^0 represent the observed rate constant for the triplet decay in presence and absence of GA, respectively.

$$k_{RF} = k_{RF}^{\ 0} + {}^3k_q^{GA}[GA] \qquad (11)$$

The value of $^3k_q^{GA}$ was similar to those obtained for the quenching of ^{3}RF* by aminoacids such as histidine (His) and tyrosine (Tyr) with $^3k_q^{His} = 3.8\times10^8$ M^{-1}s^{-1} y $^3k_q^{Tyr} = 1.8\times10^9$ M^{-1}s^{-1}, respectively. The values of $^3k_q^{His}$ and $^3k_q^{Tyr}$ obtained were in very good agreement with 2.0×10^8 M^{-1}s^{-1} and 1.4×10^9 M^{-1}s^{-1} reported by Huvaere and Skibsted (2009) and Cardoso et al.,

(2004), respectively. Thus, the quenching of $^3RF^*$ can be associated with the protein moiety of GA, which is rich in His and Tyr, see **Table 2**. Further evidence of the reaction of $^3RF^*$ with aminoacid residues of GA, is the blue-shift of about 20 nm observed for the long-lived transient band at the UVA (~350 nm) with the increment of the concentration of GA, **Figure 2a**. The same behavior was previously reported by Lu & Liu (2002) for the reaction of the $^3RF^*$ with Tyr or Trp due to the formation of the neutral radicals $RFH^\bullet$ and $Tyr^\bullet/Trp^\bullet$ by one-electron transfer coupled with proton-transfer.
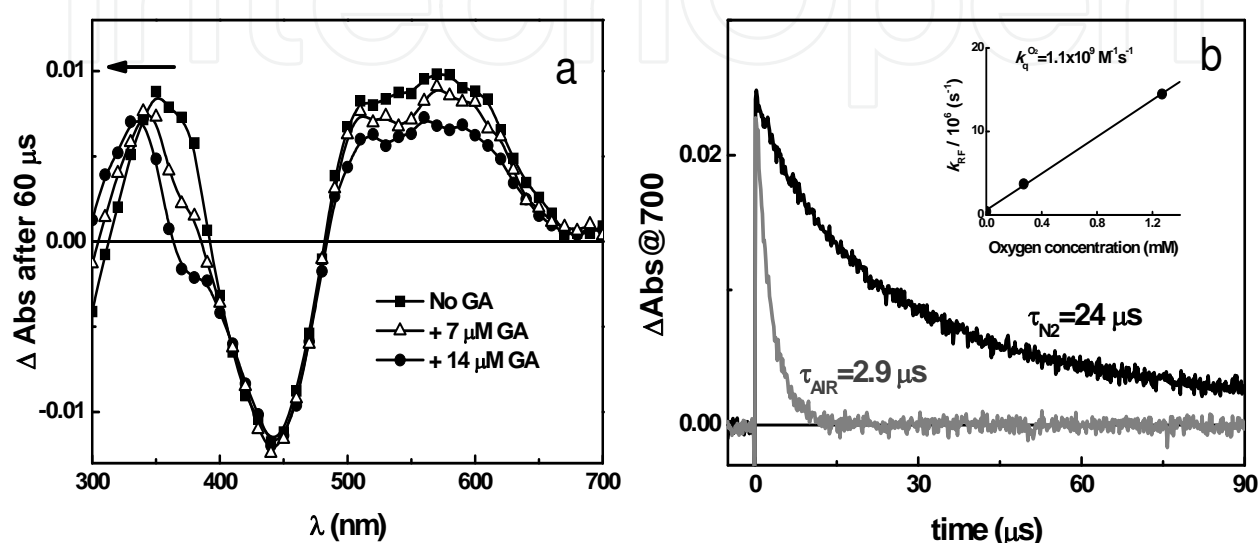
Fig. 2. **a)** Transient absorption spectra of RF (35 μM) in N_2-saturated MeOH-Water (1:1) solution observed after 60 μs of the laser pulse as a function of the concentration of GA. **b)** Effect of dissolved molecular oxygen ($^3O_2$) on the decay of the $^3RF^*$ at 700 nm. Inset: Stern-Volmer plot for the quenching of $^3RF^*$ by $^3O_2$.

Nevertheless, in aerobic media, molecular oxygen $^3O_2$ competes efficiently with GA for the interaction with $^3RF^*$, as showed by the large decreases of the decay time of $^3RF^*$ in air-saturated aqueous solutions, **Figure 2b.** The bimolecular quenching rate constant of $^3RF^*$ by $^3O_2$, i.e. $k_q^{O_2}$ = 1.1×10^9 M^{-1}s^{-1} (inset of **Figure 2b**). This value is almost the same that the obtained by the quenching of $^3RF^*$ by GA, see above. Thus, the predominant quenching process will modulated by the relative molar concentration between GA and $^3O_2$. Normally, the concentration of dissolved oxygen in air-saturated water at standard conditions is about 270 μM (Murov et al., 1997), and the concentration of GA it will depend on the type of food and pharmacological preparations. In any case, the combinatory effect will quench efficiently the harmful riboflavin excited triplet state. However, according to eqn 8, th quenching if $^3RF^*$ by $^3O_2$ produces mainly singlet molecular oxygen, $^1O_2$, and ground state RF. In a previous work, we reported that the total (physical + chemical) quenching rate constant of $^1O_2$ by GA, i.e. k_t^{GA} = 2.7×10^7 M^{-1}s^{-1}, as obtained by using time-resolved near-infrared phosphorescence detection of $^1O_2$ (Faria et al., 2010). In order to separate the contribution of physical and chemical quenching, we monitored the consumption of dissolved $^3O_2$ by GA using a FOXY-R oxygen-sensitive luminescent sensor coupled with to CCD detector from OceanOptics, **Figure 3a**. The dye methylene blue was used as sensitizer

($\Phi_\Delta \approx 0.52$) and histidine as actinometer, since this aminoacid reacts completely with $^1O_2$ with $k_r^{His} = 4 \times 10^7$ M^{-1}s^{-1}, (Bisby et al., 1999).

Under photostationary conditions, the slopes of the linear plots of the consumption of dissolved oxygen are the observed pseudo-first order rate constant of the chemical quenchers, k_{obs} (Criado et al., 2008), and the rate constant for the reactive quenching of $^1O_2$ by GA is calculated with eqn. 12.
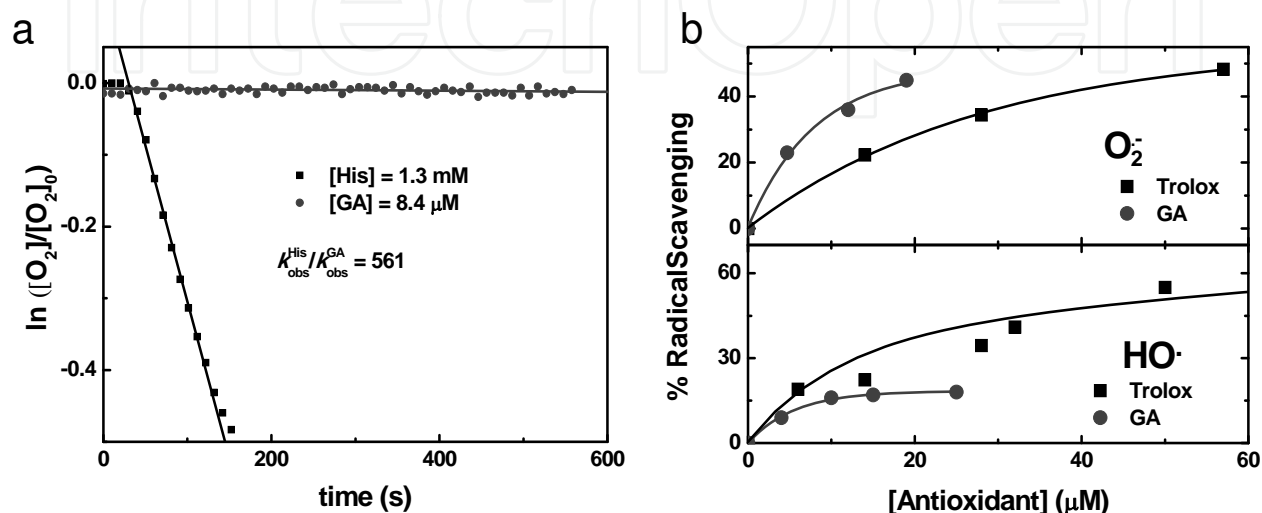

Fig. 3. a) First order plot of oxygen uptake in the Methylene-blue (MB)-sensitized photooxidation of GA 8.4 µM and 1.3 mM histidine (control) in phosphate buffer pH 7. b) Percentage radical scavenging activity for the control molecule Trolox and GA at pH 7.4 in phosphate buffer 10 mM (hydroxyl radical) and pH 10 in sodium carbonate buffer 50 mM (anion superoxide radical).

$$k_r^{GA} = k_r^{His} \times \frac{k_{obs}^{GA}[His]}{k_{obs}^{His}[GA]} \tag{12}$$

In the present case, $k_r^{GA} = 1.1 \times 10^7$ M^{-1}s^{-1}, a typical value for the reaction of aminoacid moieties with $^1O_2$ (Michaeli & Feitelson, 1994; Bisby et al., 1999). By comparison with the total quenching rate constant, $k_t^{GA} = 2.7 \times 10^7$ M^{-1}s^{-1}, it can be concluded that almost 60% of the interaction with $^1O_2$ is through physical quenching and about 40% of the reactive moieties of GA are oxidized by $^1O_2$.

In addition, Montenegro et al., (2007) determined that the photosensitized RF-mediated degradation of vitamins A, D$_3$, and RF itself in skimmed milk was strongly reduced by the addition of small amounts of lycopene-gum arabic-sucrose microcapsules, prepared by spray-drying. Under these conditions, the bulk properties of the skimmed milk were unmodified. The main photoprotection mechanism of the milk vitamins was the efficient quenching of the ^{3}Rf* by the protein moiety of GA. Small contributions (<5%) to the total photoprotection percentage was due to both inner filter effect and $^1O_2$ quenching by the microencapsulated lycopene.

These results show the functional ability of GA to act as quencher of electronically excited states in food systems either as non-processed gum or spray-drying microencapsulated preparations.

7.2 Scavenging of free radical species

The capacity of GA to scavenge *in vitro* the chemically generated free radicals hydroxyl ($HO^{\bullet}$) and superoxide anion ($O_2^{\bullet-}$) was determined by the Trolox Equivalent Antioxidant Capacity (TEAC) assay (Huang, 2005; Gliszczyńska-Świgł, 2006). The $HO^{\bullet}$ was generated by Fenton reaction at pH 7.4 (Aruoma, 1994), and the reaction was monitored spectrophotometrically at 532 nm, color created by the adduct formed between thiobarbituric acid with malonaldehyde, the product of oxidation of deoxyribose by $HO^{\bullet}$ (Gutteridge & Halliwell, 1988). The superoxide anion was detected by using the Nitro blue tetrazolium (NBT) method, as described by Sabu & Ramadasan, 2002. In this method the generation of $O_2^{\bullet-}$ is performed by auto-oxidation of hydroxylamine hydrochloride in presence of NBT, which gets reduced to nitrite, which in presence of EDTA gives a color measured at 560 nm. The radical scavenging (RS) activity of GA was reported as the percentage of inhibition of color formation, and calculated according to eqn. 13:

$$\%RS = \frac{A_0 - A_t}{A_0} \times 100 \qquad (13)$$

where A_0 in the absorbance value obtained for the control solution without GA or Trolox (TX), A_t is the absorbance value in the presence of these molecules. All the tests were performed by triplicate and, **Figure 3b** shows the increment of radical scavenging capacity with the TX or GA concentration. By comparing the initial linear slopes between the GA and TX curves (Re et al., 1999), obtained by fitting of experimental data with a second-order polynomial function, the TEAC value of 1.82 and 0.71 was calculated for the scavenging of $O_2^{\bullet-}$ and $HO^{\bullet}$, respectively. These results are consistent with the results reported by Liu et al., 1997, for the scavenging of $O_2^{\bullet-}$ and $HO^{\bullet}$ by polysaccharides extracts of mushrooms. These authors found that the radical scavenging efficiency by the polysaccharides was higher for $O_2^{\bullet-}$, and also increased by the protein content of the polysaccharide-protein complex.

8. Antimicrobial action

As for the antimicrobial activity of GA, few studies have been performed, mainly reporting growth inhibitory activity of certain periodontal pathogenic species (causal agent of tooth decay or agent involved in the plaque), such as *Prophyromonas gingivalis* and *Prevotella intermedia* (Clark et al., 1993). These results suggested that GA could inhibit the formation of plaque and improve dental remineralization, acting as a potential preventive agent in the formation of caries (Onishi et al., 2008). Such effects are attributed to the high salt content of Ca^{2+}, Mg^{2+} and K^+ of polysaccharides in GA, and the effect of the gum in the metabolism of Ca and possibly phosphate. It is also known that cyanogenic glucosides and GA contains many types of enzymes such as oxidases, peroxidases, and pectinases, some of which have antimicrobial properties (Tyler et al., 1997; Kirtikar & Basu, 1984).

Saini et al., 2008 studied the antimicrobial effect of different acacia species, including *A. senegal*, using different plant parts (pods, bark, etc.), against three strains of Gram positive (*Bacillus cereus, Escherichia coli, Salmonella typhi*), two gram negative (*Pseudomonas aeruginosa, Staphylococcus aureus*) and three fungal strains (*Candida albicans, Aspergillus niger* and *Microsporum canis*). The study revealed that methanol extracts of the species *A. catechu* and *A. nilotica* showed the highest antimicrobial activity. This is due to the presence of hydrophilic components such as polyphenols, polysaccharides and tannins present in one or more parts of the plant. The hexane extracts of these species also showed significant activity. As for *A. senegal*, using the bark, determined that the hexane extract showed antimicrobial activity (AMA) against *S. aureus* and the fungus *C. albicans*, while the methanol extract showed AMA against *E. coli, B. cereus*, and fungi *C. albicans* and *A. niger*.

However, to date, no AMA studies have been conducted against spore-forming microorganisms. It is very important to search for a compound having action on the development of such organisms as *Bacillus subtilis* and *B. cereus*, since these microorganisms are able to withstand pasteurization conditions and contain hydrolytic enzymes, which generate off-flavor in the food.

For this reason, we evaluated the AMA of GA obtained from *Acacia* trees against "off-flavor" microbial producers in food such as *Pseudomonas aeruginosa, Bacillus subtilis* and *Micrococcus luteus*. **Figure 4a** shows the growth inhibition diameters of the three organisms analyzed by solutions with increasing concentration from 5 to 50 μM, as determined by the agar diffusion method (Ferreira et al., 2004). These results indicate that GA exerts only a moderated AMA against *P. aeruginosa*, while *Micrococcus luteus* and *Bacillus sutilis* were unaffected by the presence of GA. However, the in the presence of GA the inhibition zones measured were translucent, indicating that a bacteriostatic effect occurs, meaning that growth slows, but does not destroy the bacteria.
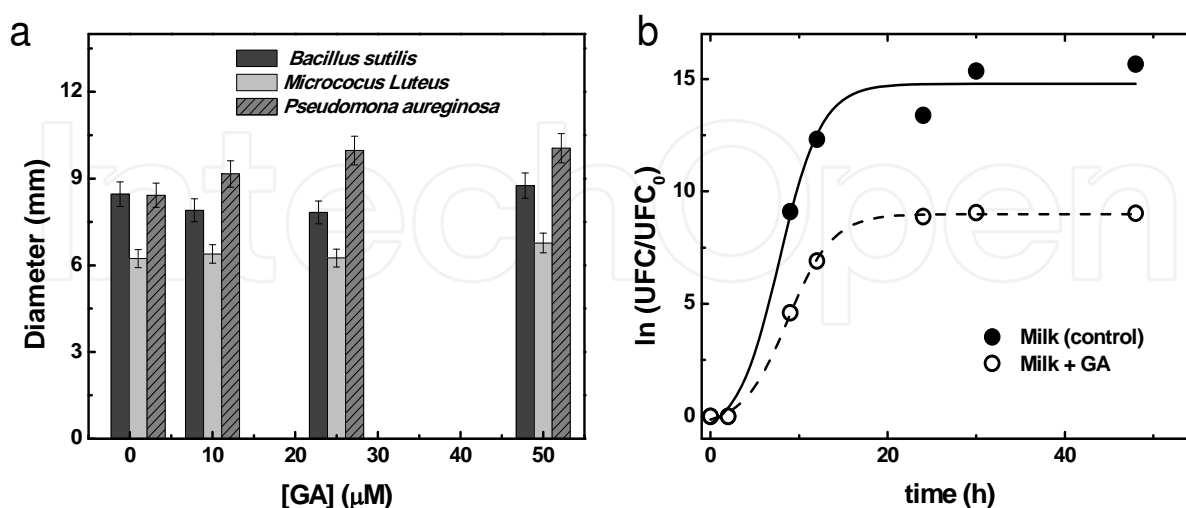

Fig. 4. a) Inhibition of microbial growth by GA, from *Bacillus subtilis*, *Micrococcus luteus* and *Pseudomonas aeruginosa*. b) Growth curve of *Bacillus subtilis* at 32 ° C (●) Milk, (O) milk with added GA 47 μM.

Additionally, the AMA of GA against *Bacillus subtilis* was evaluated in milk. The growth rates (h⁻¹) of the microbial culture in milk with and without added GA stored at 32 °C were determined from the slopes of the linear portion of the growth curves of the organism, which were built by standard plate counts determined according to the IDF standard No 100 B (IDF, 1991), **Figure 4b**. The growth rate determined for *Bacillus subtilis* in milk was 0.92 h⁻¹, whereas with the addition of GA in 47 μM the growth rate decreased almost 40% (0.56 h⁻¹), confirming a bacteriostatic effect on *Bacillus subtilis* by GA.

9. Conclusions

GA is a natural biopolymer with wide industrial use as a stabilizer, a thickener, an emulsifier and in additive encapsulation not only in food industry but also in textiles, ceramics, lithography, cosmetic and pharmaceutical industry (Verbeken et al., 2003).

Besides all the sensory and texturizing properties, GA has interesting antioxidant properties such as an efficient capacity for deactivation of excited electronic states and moderated radical scavenging capacity. There is increasing experimental evidence that associate the antioxidant function with its protein fraction, mainly by amino acid residues such as histidine, tyrosine and lysine, which are generally considered as antioxidants molecules (Marcuse, 1960, 1962; Park et al., 2005).

In summary, besides its use as texturing additive, the combined functionality of GA is an advantage over other edible biopolymers that do not show antioxidant activities

10. Acknowledgments

11. References

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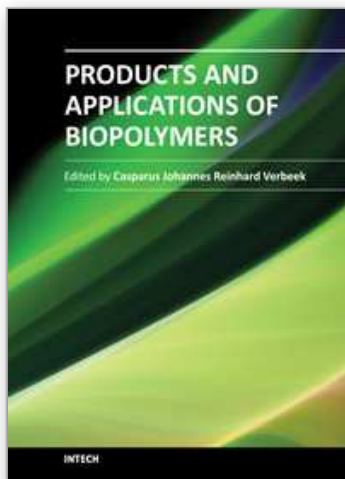
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It is interesting to consider that biopolymers are by no means new to this world. It is only because of our fascination with petrochemical products that these wonderful materials have been neglected for so long. Today we face a different challenge. Environmental pressure is pushing away from synthetic or petro-chemically derived products, while economic factors are pulling back from often more expensive "green" options. This book presents two aspects of biopolymers; potential products and some applications of biopolymers covering the current relevance of biopolymers.

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