Enzymatic mediated modification of gum Arabic by curcumin oxidation products: physicochemical and self-assembly study

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Highlights:

- The laccase assisted modification of gum Arabic by curcumin oxidation allowed the grafting of phenolic compounds.
- Contrary to the native one, modified gum Arabic presented a phase transition temperature.
- Due to a higher hydophobicity, modified gum Arabic formed spherical particles in water.

ABSTRACT: Gum Arabic (GA) is a polysaccharide widely used in industry as an emulsifier and encapsulating agent. Nevertheless, its physicochemical properties can be largely improved. The objective of this study was then to modify its structure to improve its functionalities or to provide new ones. To do so, a heterogeneous catalysis process using a laccase and a phenolic compound, curcumin, in aqueous solution was performed. The laccase allowed the oxidation of curcumin, which was then able to be grafted onto the polymer. Therefore, this environmentally friendly process allowed the modification of GA with

curcumin oxidation products (OXP) by creating ester bonds between GA and OXP. The molecular mass of GA was largely increased after functionalization. This product contained two fractions of approximately ~1.165 x 10^8 g.mol^{-1} and ~5.057 x 10^6 g.mol^{-1}. All these modifications impacted greatly the polymer properties such as its thermal behaviour, with a significant decrease of its glass transition, its hydrophilicity and its hygroscopicity. They also brought antioxidant properties to the modified GA. Moreover, the modification led interestingly to the spontaneous formation of spherical monodisperse micrometric particles in water.

KEYWORDS: gum Arabic, curcumin, self-assembly, thermal behaviour, phenol grafting, laccase

1. Introduction

Gum Arabic (GA), also known as Acacia gum, is a complex polysaccharide exuded by acacia trees such as *Acacia senegal* and *Acacia seyal* to protect themselves against insects and heal their wounds (Lopez-Torrez, Nigen, Williams, Doco, & Sanchez, 2015; Osman, Williams, Menzies, & Philips, 1993; Verbeken, Dierckx, & Dewettinck, 2003). It was already used 70 000 years ago in Africa and by the Egyptians more than 4000 years ago (Renard, Garnier, Lapp, Schmitt, & Sanchez, 2012; Sanchez et al., 2018; Sanchez, Renard, Robert, Schmitt, & Lefebvre, 2002). Nowadays, GA, thanks to its remarkable properties (high solubility in water), is used as a stabilizer or an emulsifier under the European code E414 according to the European food safety authority (EFSA) (Masuelli 2013; Mortensen, Aguilar, Crebelli, Domenico, & Frutos, 2017; Sanchez et al. 2018). It is a common ingredient in confectionery to delay the crystallization of sugar or in wine to prevent pigment precipitation. It is also used in non-food industries, for example as an ingredient in the micro-encapsulation

process for the production of carbonless paper (Dickinson, Murray, Stainsby, & Anderson, 1988; Osman et al., 1993; Sanchez et al., 2018).

GA is a highly branched polysaccharide and is composed of main chains of 1,3 linked β-D-galactopyranosyl. The side chains are composed of β-D-galactopyranosyl linked to the main chain in 1,6. Both chains contain α-L-arabinofuranosyl and α-L-rhamnopyranosyl while most of the β-D-glucuronopyranosyl and 4-O-methyl-β-D-glucuropyranosyl are located at the end of the side chains (Aphibanthammakit, Nigen, Gaucel, Sanchez, & Charlier, 2018; Lopez-Torrez et al., 2015; Masuelli 2013; Verbeken, et al., 2003). The exact chemical composition of GA can vary depending on different conditions: origin, age of trees or climatic conditions (Cozic, Picton, Garda, Marlhoux, & Le Cerf, 2009; Lopez-Torrez et al., 2015; Masuelli 2013). Its structure can be separated in three different fractions: arabinogalactan-peptide (AG), arabinogalactan protein (AGP) and glycoprotein (GP), which represent 85-90%, 10% and about 2% of the whole gum, respectively. As suggested by the names of the different fractions, GA also contains a protein part linked to the polysaccharide part by serine and hydroxyproline rich domains. The GP is then the minor fraction but it is the one containing the most proteins, contrary to the AG, which contains the least amount of protein but is the major fraction of the gum. The AGP fraction contains less protein than GP but more than AG. (Lopez-Torrez et al., 2015; Osman et al., 1993; Renard, Lavenant-Gourgeon, Ralet, & Sanchez, 2006; Sanchez et al., 2018).

Despite its many functions, GA has certain limitations. Only one fraction (AGP) is currently valued because it is the only one responsible for the ability of GA to stabilize emulsions (Dickinson et al., 1988; Randall, Phillips, & Williams, 1988; Nakauma et al., 2008).

In addition, compared to other polysaccharides, GA is not the most effective for emulsions stabilization compared to lemon or beet pectin for example (Wu, Kong, Zang, Hua, & Chen, 2015). Nakauma et al (2008) were able to determine that for a 15% wt water : triglyceride emulsion made by high-pressure homogenisation, a higher concentration of GA was required to form a stable emulsion than with beet pectin or soluble polysaccharides from soybean (Nakauma et al., 2008). Another study showed that emulsions formed by agitation in a rotor stator system by GA, would be less stable than those formed by the gum extracted from *Prosopis alba*; for a similar initial droplet size initially. In addition, the oil/water interface tension decreased less by GA than by this other gum (Vasile, Martinez, Pizones, Judis, & Mazzobre, 2016). However, from its reactive group, it is possible to modify its properties by modification with exogenous molecules. This has already been recorded in the literature on other polysaccharides (Karaki, Aljawish, Humeau, Muniglia, & Jasniewski, 2016; Li et al. 2016).

GA modifications were then reported. Gum Arabic was functionalized *via* a Maillard reaction with proteins like casein or canola protein to enhance its emulsifying properties (Hou, Wu, Xia, Phillips, & Cui, 2017; Pirestani, Nasirpour, Keramat, Desobry, & Jasniewski, 2017). It has also been modified by chemical pathway to form particles as shown by Sarika, James, Kumar, Raj, & Kumary (2015) where the binding of curcumin has shown to change the physicochemical properties of GA. The formation of spherical particles closed to 200 nm of diameter was demonstrated as well as an increase in anticancer properties compared to free curcumin.

The latter paper, such as most papers dealing with polysaccharide modification used a chemical pathway to obtain the modified polymer. Softer processes were rarer but still exist. For example, chitosan and pectin were functionalized with phenolic compounds produced by laccase catalyzed oxidationof of ferulic acid and ethyl ferulate. The grafting was found to be

covalent between carboxyl groups of pectin or the amine groups of chitosan and the oxidation products of the ferulic acid. The polymer hygroscopicity was decreased and hydrophilicity increased because the oxidation products inserted into the pectin backbone increased the space between the polymer chains. A significant improvement in antioxidant properties was also noted in both polysaccharides (Aljawish et al., 2014; Karaki Aljawish, Muniglia, Humeau, & Jasniewski, 2016).

GA was also modified through an enzymatic process. A laccase was used to oxidize ferulic acid to form reactive derivatives (oxidation products), which could then react with the functional groups of GA such as amines or carboxylic acids. The resulting modified polymer exhibited a greater ability to reduce the surface tension of water compared to GA. This modification also induced a decrease in the interfacial tension between water and oil, which could lead to improved emulsifying capabilities. Another interesting aspect of this study was the increase of the antioxidant properties of the modified polysaccharide. These modifications also led to structural changes, modification of its water solubility, its thermal behaviour and its colour (Vuillemin et al., 2020).

In the present study, a similar procedure was used, based on the enzymatic oxidation of curcumin, which then reacted with the functional groups of GA. Curcumin is already in itself a food-grade compound widely used for its colour but especially for its various properties (antioxidant and anticancer activities). Moreover, it is largely hydrophobic, which could modify the hydrophilic/hydrophobic balance of the GA by increasing the hydrophobicity and thus induce a self-assembly in water. This modification was made in order to modify the physicochemical properties of the GA. This one has indeed a great solubility in water (up to 37% at 25 °C) (Sanchez et al. 2018) and grafting an hydrophobic compound such as oxidation products of curcumin would therefore modify its behaviour in aqueous media. Furthermore,

phenolic compounds could also bring a certain antioxidant activity to the gum which could be an advantage for the new polymer.

The grafting of the oxidation products (OXP) was studied by Fourier transform infrared spectroscopy and SEC-MALS (size exclusion chromatography multi angle light scattering) analysis. The modification of physicochemical properties was analysed by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and measurement of surface tension in water and antioxidant properties. The behaviour in water was then studied.

2. Materials and methods

2.1 Materials

Gum Arabic (100% w/w) purified Instantgum AA from *Acacia senegal* was a gift from Nexira (France). Curcumin ≥ 65% w/w (Cur) and salt for buffer solution (Na_2HPO_4, KH_2PO_4) were purchased from Sigma Aldrich (France). Ethanol (analytical grade) was purchased from Carlo Erba (France).

The laccase used was Novozym 51003 from Novozymes (BGAsvaerdt, Denmark) which is a fungal phenol oxidase from *Myceliophtora thermophila* produced in *Aspergillus oryzae.* The activity of the laccase stock is expressed in Laccase *Myceliophthora* Units (LAMU). One LAMU is defined as the amount of enzyme that oxidizes 1 micromole of syringaldazine per minute. The laccase activity was 22 315 ± 2 732 $LAMU.g^{-1}$. This activity was calculated by oxidation kinetics of syringaldazine and a Bradford titration of proteins.

2.2 Methods

2.2.1 Functionalization of Acacia gum by curcumin oxidation products

The method used was adapted from literature (Vuillemin et al., 2020; 2021). 10 g of GA was added in 450 mL of 50 mM phosphate buffer; pH 7.4 and stirred overnight at 4 °C.

GA solubility in phosphate buffer is very low but it has been shown that functionalization can be achieved under heterogeneous conditions (Vuillemin et al., 2021; 2020; Aljawish et al.,2012; Karaki, et al., 2016). The resulting dispersion was then heated under stirring to 30 °C using a magnetic heating plate. 50 ml of 50 mM curcumin solution in ethanol was then added to the GA dispersion. Then 10.25 $LAMU.mL^{-1}$ of laccase was added. The reaction was stopped after 1 h by adding 3 volumes (1.5 L) of frozen ethanol and was then kept at -21 °C for 24 h. This procedure also allowed the separation of functionalized and non-functionalized polymers contained in the mixture at the end of the reaction (Vuillemin et al., 2020). Indeed, gum Arabic-oxidized curcumin (GAC) was soluble in a water/ethanol 1:3 mixture, unlike native GA, which precipitated in this solvent. The native GA was then eliminated by centrifugation at 12 100 *g* for 10 min. The salts from the buffer were distributed between the two phases. Ethanol contained in the supernatant was evaporated by a BUCHI R144 rotary evaporator at a boiling point of 40 °C at 175 mbar (BUCHI SARL, Rungis, France). The sample (water-suspended GAC) was then frozen and freeze-dried for 24 h. The yield was 20 g of functionalized gum for 100 g of native gum. Dialysis (MWCO 10 000 Da from Membrane Filtration Products Inc.) of the freeze-dried sample against pure water was then performed to remove buffer salts and recover pure modified GA referred to GACD to differentiate it from GAC, which was not dialyzed.

2.2.2 Structure of functionalized Acacia gum

Fourier-transform infrared spectroscopy (FTIR) was used to investigate the impact of functionalization on GA chemical structure. The different compounds were analysed in the solid state so that they would not be affected by water absorption in the region of the amide I band (1720-1580 cm^{-1}). Measurements were performed using a Tensor 27 spectrometer equipped with a DTGS detector (Bruker, Germany). The GACD powder was placed on a diamond ATR top-plate (ATR Platinum, Bruker, Germany). Recording was performed with

64 scans, 10 kHz scanning, spectra from 400 to 4000 cm^{-1} and 6 cm^{-1} of resolution. The background was measured on air to be subtracted from the raw spectra. The raw data were collected by OPUS software version 7.2 (Bruker, Germany) and then underwent different treatments which were in sequence: extended ATR correction; transmittance to absorbance spectrum conversion; H_2O/CO_2 compensation: straight line generation on the blind area of the diamond detector; 9 points smoothing; baseline correction; offset normalization. All the measurements were performed in triplicate. The degree of esterification was calculated using equation 1 (Karaki et al. 2017; Chatjigakis et al. 1998).

esterification degree = peak area at 1730 cm^{-1} / (peak area at 1730 cm^{-1} + peak area at 1650 cm^{-1}) * 100 Eq (1)

2.2.3 Polymer molecular weight

SEC experiments were performed with a HPLC pump (LC10AD, Shimadzu) coupled to an autosampler (Autosampler VE 2001, Malvern Panalytical) and a multi-detectors system recording UV, light scattering (RALS: right angle light scattering and LALS: left angle light scattering), intrinsic viscosity and refractive index signals (Viscotek TDA305, Malvern Panalytical). Two SEC columns (A4000-A6000, 10 or 13 µm, 8 mm ID x 300 mm, void volume ~6 mL, total volume ~ 12.5 mL, Malvern Panalytical) were mounted in series and equipped with a post-column nylon filter (0.22 µm). The columns were equilibrated with DMAC/H_2O 50:50. The flow rate was 0.1 $mL.min^{-1}$ and the temperature was 30 °C. Data were processed with the Omnisec software (v5.12, Malvern Panalytical). The calibration procedure was performed with Bovin Serum Albumin (Sigma) and cross-validations were performed with Dextran 70 kDa standard (Viscotek PolyCal standards, Malvern Panalytical).

The refractometer was used as the concentration detector and the refractive index increment value (dn/dc) used to determine the molecular weight was 0.136 $mL.g^{-1}$ (Grein, Da Silva, Wendel, Tischer, & Sierakowski, 2013). GACD samples were solubilized in the aforementioned buffer at 10 $g.L^{-1}$ and filtered through a 0.22 µm RC-filter just before injection. All the measurements were performed in triplicate and expressed with mean ± standard deviation.

2.2.4 Antioxidant activity

The antioxidant activity of GAC and curcumin were determined by carrying out tests of $ABTS^{\bullet+}$ radical scavenging. The method was adapted from Karaki et al., (2016b) and Vuillemin et al., (2020). Solutions of $ABTS^{\bullet+}$ (2.45 mM) were diluted to obtain an absorbance of 0.680 ± 0.02 AU (arbitrary units) at 734 nm. 1 ml of this solution was then added to 10 µL of sample with a concentration ranging from 0.5 to 0.0005% (wt). After incubation at 30 °C for 20 min and colour stabilization, the absorbance was measured at 734 nm in a quartz cell. All the measurements were performed in triplicate and expressed with mean ± standard deviation.

The percentage of inhibition of the studied molecules was calculated using the equation 2 where: final A was the absorbance of the $ABTS^{\bullet+}$ solution which has reacted with the studied molecule and where initial A was the absorbance of the $ABTS^{\bullet+}$ solution which had not yet reacted. The values were then expressed in EC50 ± standard deviation. This was the concentration for which the molecule of interest inhibits 50% of the $ABTS^{\bullet+}$ radicals.

$$\text{inhibition (\%) } ABTS^{\bullet+} = 1 - (\text{final A} / \text{initial A}) * 100 \qquad \text{Eq (2)}$$

2.2.5 Thermal behaviour analysis

Thermogravimetric Analyses (TGA) were performed on GA, GACD and Curcumin to investigate the impact of functionalization on GA thermal stability. The experiments were performed using a thermogravimetric analysis apparatus (TGA 5500, TA Instruments, Waters, France) under N_2 gas. The heating rate was 20 °C.min^{-1} from 30 °C to 600 °C and the mass of the sample was approximately 6 mg ± 2 mg. Raw data were collected and analysed using TRIOS software (TA Instruments, Waters, France) to recover thermograms and their derivatives. Each step of compound degradation was determined using the obtained derivatives. All the measurements were performed in triplicate.

Differential Scanning Calorimetry (DSC) was performed to investigate the impact of functionalization on GA thermal behaviour (DSC 250, TA Instruments, Waters, France). 6 ± 2 mg of GACD, GA or curcumin were filled in a Tzero hermetic aluminium pan to be analysed (TA Instruments). A Heat-Cold-Heat method was chosen. First, the samples were equilibrated at -50 °C, then heated from -50 °C to 100 °C at 10 °C.min^{-1} to eliminate the thermal history of the samples. Next, they were cooled to -50 °C at 10 °C.min^{-1}, and finally, heated again to 100 °C at 10 °C.min^{-1}. All steps were separated by an isothermal step of 2 min. Data were collected using TRIOS software (TA Instruments), which allowed automatic detection of the Tg (glass transition temperature). All the measurements were performed in triplicate and expressed with mean ± standard deviation.

2.2.6 Hygroscopy

The water sorption isotherm of GA and GACD was performed to determine whether the functionalization had an impact on the hygroscopy of the polysaccharide. The results were obtained by depositing 90 ± 10 mg for GA and 50 ± 10 mg for GACD in the sealed chamber of the dynamic vapour sorption analyser (DVS, Surface Measurement Systems Ltd., London,

UK). This weight difference was due to the greater volume taken up by the GACD powder (lower powder density) in the capsule of the machine. The temperature was set at 25 °C. The relative humidity (RH) was first brought as low as possible (approx. 0.1 %) and then increased by 10% steps to 85%. The balancing times for the individual steps were set manually such as: (1) for GACD: 500 min for 0.1%; 200 min for 10 to 60%; 300 min for 70%; 500 min for 80% and 250 for 85% and (2) for GA: 800 min for 0.1%; 400 min for 20 to 40%; 200 min for 50%; 450 min for 60%; 700 min for 70%; 800 min for 80% and 900 min for 85%. The weights were measured continuously but only the weights at equilibrium were recorded. All the measurements were performed in triplicate and expressed with mean ± standard deviation.

2.2.7 Preparation of aqueous samples: particle dispersion

GAC was dispersed in ultra-pure water at 0.5 wt.% and stored overnight at 4 °C under stirring. This solution was then diluted in ultra-pure water at a chosen concentration for specific investigations.

2.2.8 Surface tension measurement

The surface tension of GAC dispersions in ultrapure water was measured at 25 °C using a Kruss K100 tensiometer (Krüss, Paris, France) equipped with a Wilhelmy plate. In this method a platinum plate was placed at the water/air interface and allowed the capillary force of the solution to be measured. The data were collected continuously over a maximum duration of 21000 s in order to reach the equilibrium. Before each measurement, the cleanliness of the equipment used was checked by carrying out a measurement on ultra-pure water in order to avoid any interference; a value of 72.5 ± 0.3 $mN.m^{-1}$ had to be reached.

The evolution of surface tension as a function of time was modelled using equation 3 (Mahfoudhi et al., 2014). This equation provides the equilibrium surface tension value (γf) for

infinite time. The parameters τ1, τ2, γ1 and γ2 were the one to characterize the decay of the surface tension values. τ1 corresponded to the migration time of the emulsifier to the interface and τ2 to the time needed for the reorganization of the molecules at the interphase. γ1 and γ2, in turn, corresponded to the surface tensions corresponding to these different times. γ represented the surface tension at infinity. t corresponded to the time of the measurement. The measurements were performed in triplicate and expressed with mean ± standard deviation.

$$\gamma = \gamma_f + \left(\gamma_1 - \gamma_f\right).e^{-t/\tau 1} + \left(\gamma_2 - \gamma_f\right).e^{-t/\tau 2} \quad \text{Eq (3)}$$

2.2.9 Size and electrophoresis mobility measurements

Size of GA and self-assembled GAC particles was determined with Zetasizer nano-ZS apparatus (Malvern Panalytical, United Kingdom) equipped with a He/Ne ion laser (λ = 532 nm) in a backscattering configuration (measurement angle was of 173 °). The refractive index used for the GAC is 1.47. The refractive index of water is also taken into account (1.33). The analysis model used is general purpose for the size and Smoluchowski for the charge. Experiments were performed at 25 °C with an equilibrating time of 120 s in a disposable folded capillary cell (DTS1070, Malvern Panalytical). Measurements were made in triplicate and expressed with mean ± standard deviation.

2.2.10 Morphological study

Particles morphology of GAC was visualised by a Hitachi S-4800 Field-Effect scanning electron microscopy (SEM) operating at 1.0 kV with a field emission gun (cold cathode). A droplet of a sample at 0.05 wt.% in ultra-pure water was dropped off onto an aluminium plot and let air-dried at room temperature during 48 h and then coated with carbon.

Epifluorescence microscope observations with a LEITZ DMR microscope (Germany) and with an H3 Micfilter were also performed in order to visualize the presence of phenols in the

particles. Image capturing was carried out by a monochrome CCD COHU camera and was captured at magnification of × 40 with an ocular magnification of ×10. The excitation wavelength was of 420 nm. Observations were made by smearing a 0.05% aqueous solution of GAC with a magnification × 400 and × 1000 by immersion.

Surface structure of freeze-dried particles was recorded using atomic force microscopy (AFM) (Flex-Axiom, Nanosurf, Liestal, Switzerland) with the Nanosurf C3000 controller software. Powders were gently dispersed on a thin layer of epoxy glue spread onto a circular glass slide. Particle surface properties (topography and elasticity) were recorded at 22 ± 2 °C using an AFM cantilever (Stat0.2LauD cantilevers from Nanosurf, Liestal, Switzerland) having a spring constant of 0.2 $N.m^{-1}$. Topography acquisition was performed in air, in contact mode on a 5 µm × 5 µm surface area. The average surface roughness (Sa) was calculated for single particles on 1 µm ×1 µm according to Equation 4.

$$Sa = \frac{1}{n} \sum_{i=1}^{n} |y_i| \qquad \text{Eq (4)}$$

where y is the height (z) measured by the piezoelectric scanner at a given pixel (i) in the image. At least 3 particles were analyzed to be able to average the surface roughness value.

Mechanical properties of GAC were probed at the nanoscale by AFM nanoindentation technique. The force-distance curves recorded by AFM were analysed according to the theoretical Hertz model to extract the mechanical properties (e.g. elasticity). Defined areas of 5 µm × 5 µm were probed to generate elasticity maps. The cantilever spring constant was calculated thanks to the thermal calibration method and deflection sensitivity calibrated onto a silicon surface. Elasticity maps of 1024 force curves were processed with the ANA Software (Nanosurf, Liestal, Switzerland). At least three topography images and 3 elasticity maps were recorded and analysed.

3. Results and discussion

3.1 Structure of modified Acacia gum

FTIR analyses were performed to evidence changes in the chemical structure of GA induced by the functionalization process. The polymer modification can lead to diverse changes in GA structure observable on FTIR spectra. Polysaccharide modification with the OXP of phenolics compounds can lead to the formation of ester and/or amide bonds. Indeed, the oxidation of phenol by phenol oxidases may lead to the formation of semi-quinones which then react with carboxylic acid groups of glucuronic acid of GA (Lopez-Torrez et al., 2015) to form ester bonds. The protein fraction of GA may also react to form amide bonds with oxidised phenol (Vuillemin et al., 2020). Formation of ester bonds have already been reported between pectin and oxidation products obtained from ferulic acid (Karaki, Aljawish, Muniglia, Bouguet-Bonnet, & Laclerc, 2017). The oxidation of phenols such as tannic acid, quercetin, caffeic acid or gallic acid could lead to the formation of ester or amide bond on polysaccharides such as chitosan (Božič, Gorgieva, & Kokol 2012a; 2012b). Indeed the modification *via* phenol oxidases mediated-system has led to the formation of quinones, which can react with nucleophilic groups such as amines to produce Schiff bases (Aljawish et al., 2014; Kumar, Smith, & Payne 1999).

The complete spectra of GACD, curcumin and GA are visible on Figure 1A. The fingerprint of these compounds was found between 1800 and 400 cm^{-1}. A focus on this specific area is presented in Figure 1B.

From 3600 to 3000 cm^{-1} the broad band corresponded to the characteristic stretching of O-H and N-H bonds (Božič et al., 2012b; Liu, You, Tarafder, Zou, & Fang 2019). It was possible to see two peaks on the curcumin spectrum, one at 3510 cm^{-1} and the other one at 1279 cm^{-1} corresponding to the stretching and the bending of the phenolic -OH groups,

respectively (Božič et al., 2012b; Liu et al., Ying, Cai, & Le, 2017; Nascimento da Silva, de Matos Fonseca, Feldhaus, Soares, & Valencia, 2019). Peaks located at 1600 and 1508 cm^{-1} corresponded to the stretching of the C=C bonds of the cyclic alkenes (Božič et al., 2012b; Hasan et al., 2016; Poljanšek, Šebenik, & Krajnc 2006). These peaks were correlated to aromatic rings in curcumin. C-O-C bonds sign at 1026 cm^{-1} corresponding to the $COCH_3$ groups in its structure (Liu et al., 2019; Poljanšek et al., 2006). The peak at 1279 cm^{-1} corresponded to the phenolic C-OH (Liu et al., 2019; Poljanšek et al., 2006)

On the spectrum of GA, peaks corresponding to the amide bands I and II at 1649 cm^{-1} and 1597 cm^{-1} respectively were visible. The amide III band was located at 1225 cm^{-1}. (Liu et al., 2019; Liu, Cai, Jiang, Wu, & Le, 2016; Lopez-Torrez et al., 2015)

On the GACD spectrum the same two peaks as visible on curcumin spectra were reported at 1600 and 1508 cm^{-1} which corresponded to the stretching of the C=C bonds of the cyclic alkenes (Božič et al., 2012b; Hasan et al., 2016; Poljanšek et al., 2006). These peaks suggested that aromatic rings were also present in GACD structure. However, the peak at 1600 cm^{-1} was, on GACD spectrum, broadened by the presence of two other peaks and in particular a peak at 1589 cm^{-1} corresponding to the signals of the amide II band (Liu et al., 2019; Liu et al., 2016) indicating the presence of proteins brought by the GA. The amide III band was not clearly visible because it is assigned to a region where too many bonds vibrated. The amide I band was present at 1627 cm^{-1} but this peak also corresponded to the signals of the C=O from curcumin and from COO- groups notably brought by the polysaccharidic part of the GA (Abdul Rohman, Sudjadi, Devi, Dwiky Ramadhani, & Ardi Nugroho, 2015; Lopez-Torrez et al., 2015; Mundlia, Ahuja, Kumar, & Pillay, 2019).

The peak corresponding to the phenolic C-OH at 1279 cm^{-1} on the curcumin spectrum shifted to 1265 cm^{-1} on the GACD spectrum. This change could be induced by the formation

356 of ester bonds since this type of bond brings C-O bonds that stretched asymmetrically at this
357 wave number (Liu et al., 2019; Poljanšek et al., 2006).

358 This hypothesis was correlated by the appearance of a new peak on the GACD spectrum
359 at 1730 cm^{-1}. This peak was characteristic of the stretching vibration of the C=O carbonyl
360 bonds of esterified carboxyl functions (Božič et al., 2012a; 2012a; Karaki et al., 2017; 2017;
361 Vuillemin et al., 2020). There was therefore a combination of clues that an ester bond was
362 formed between the hydroxyl groups of curcumin and the carboxyl groups of GA.

363 The degree of esterification of GACD, calculated from the intensity of the free and
364 esterified carbonyl peaks, was 11.4% whereas it was equal to 2.6% for native GA. The
365 functionalization allowed to esterify 8.8% more of COO^- group. This result should be taken
366 with caution because the presence of the amide I band at the same wavenumber as the
367 carbonyl groups did not allow to evaluate precisely the esterification degree. However, the
368 presence of the amide band suggested that some of the protein fraction of the GA was present,
369 or it may also be possible that bonds were formed between the curcumin OXPs and the
370 proteins, but this cannot be verified here.

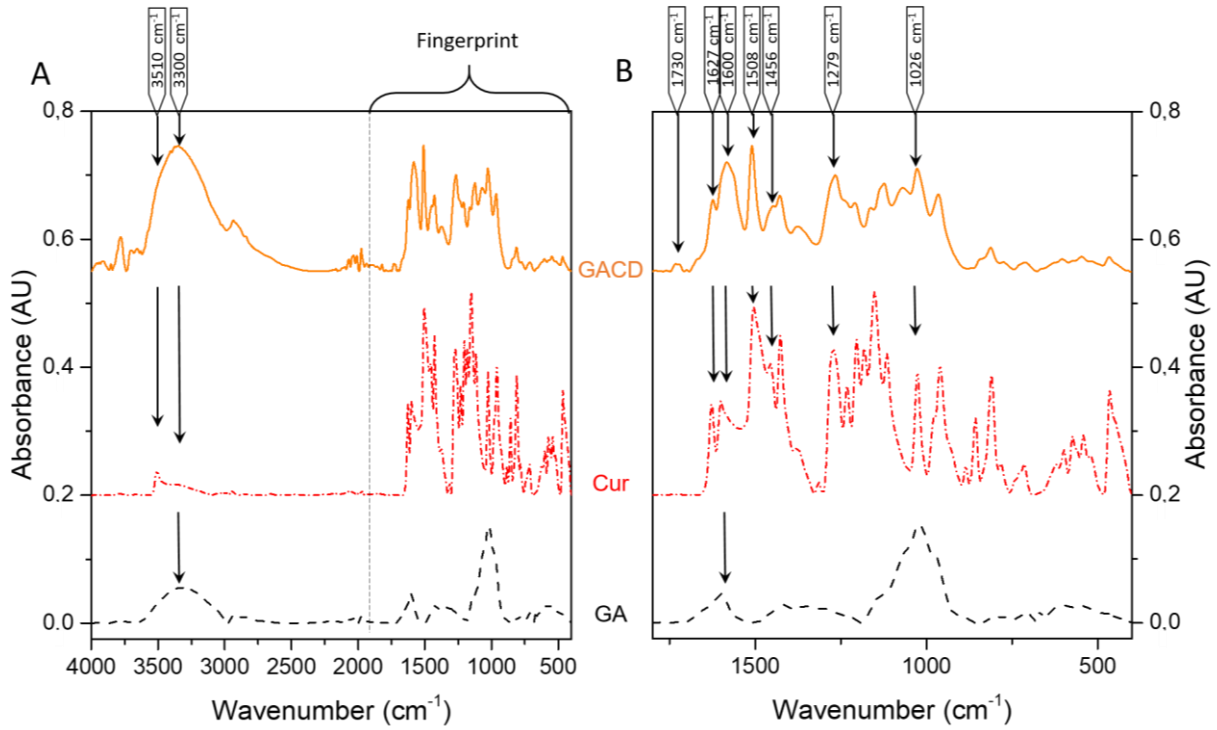


Figure 1: (A) FTIR spectra of gum Arabic (GA) (black dash line), of curcumin (Cur) shifted by 0.2 AU (red dash dot line) and GACD shifted by 0.55 AU (solid orange line); (B) focused FTIR spectra of GA, Cur and GACD between 600 and 1800 cm^{-1}

3.2 Polymer molecular weight

The impact of curcumin OXP grafting on the molecular weight and hydrodynamic radius of GA has been determined using size exclusion chromatography coupled to a multi-detectors system (UV, LS, IV and RI signals were recorded). GAC was analyzed (Figure 2) and two peaks were observed, probably corresponding to the modified AGP and AG fractions of 1.249 x 106 $g.mol^{-1}$ (Vuillemin et al., 2021). Indeed, due to the low UV signal at 280 nm, it was concluded that GP fraction was eliminated during ethanol precipitation realized after the enzymatic reaction leaving only a purified and functionalized AGP and AG fractions. The proportion of each fraction was determined using RI detector (2.5% for AGP and 97.5% for AG). The determination of *Mw* using light scattering gave *Mw* of ~1.165 x 10^8 $g.mol^{-1}$ and ~5.057 x 10^6 $g.mol^{-1}$ for these respective fractions. Finally, the determination of the hydrodynamic radius (*Rh*) was performed thanks to IV detector, giving values of ~222 and 59

nm (see Table S1 in supplementary materials for a summary of the derived parameters). Compared to the native gum Arabic (Vuillemin et al., 2021), the calculated molecular weights of the two fractions of the GAC were very high, suggesting an important grafting of curcumin OXP onto the polysaccharide and probably a crosslinking between the fractions.

A very low UV signal at 280 nm was observed, the determination or estimation of the protein content of each fraction was not possible. However, a significative absorbance at 300 nm was observed, certainly due to curcumin OXP present on GAC. Surprisingly, no UV signal at 420 nm was observed suggesting the absence of accessible curcumin onto GAC.

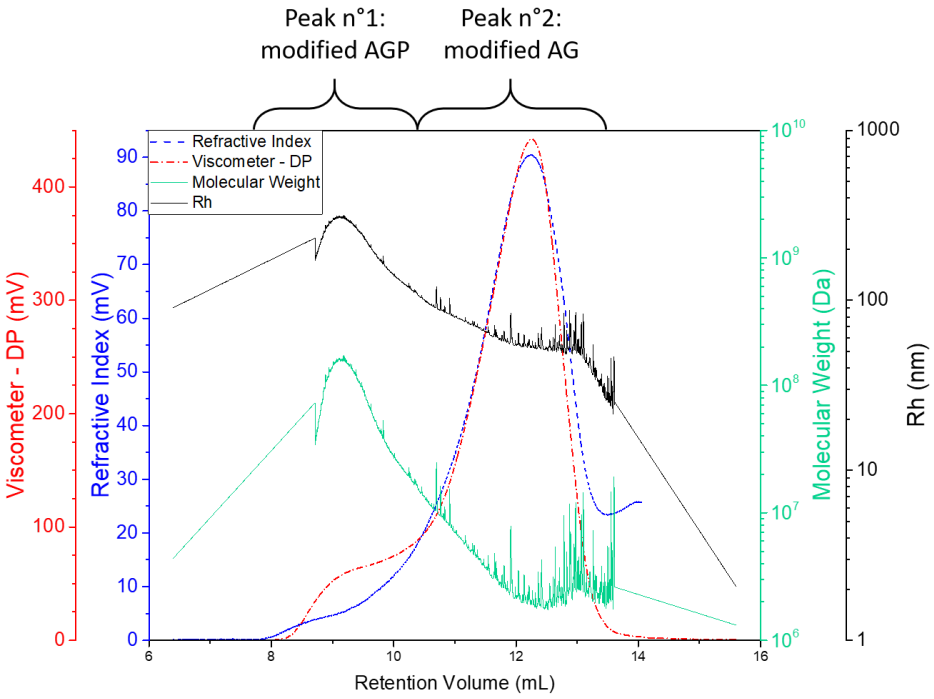


Figure 2: Size exclusion chromatography characterization (refractive index blue dash line, viscometer red dash dot line, M_w green solid line and Rh black solid line) of GAC. AG = arabinogalactan-peptide, AGP = arabinogalactan protein.

3.3 Antioxidant activity

GA is a polymer that contains a small proportion of phenolic compounds such as catechin and gallic acid. These phenols give to the GA a very low antioxidant activity (Hamdani, Wani, Bhat, & Masoodi, 2018; Tahir et al., 2018). Their quantity is so small in relation to the total amount of GA that it shows only a very low antioxidant activity. Its EC50 is around 560 $g.L^{-1}$ according to the literature (Vuillemin et al., 2020). The addition of phenol derivatives improved its antioxidant activity. The EC50 of the GAC was 20.9 ± 0.8 $mg.L^{-1}$ (about 0.002% wt). The antioxidant activity of the gum was stepped up by 25 000, due to the intake of OXP. In comparison, pure native curcumin, which is well known for its antioxidant activity, presents an EC50 of 3.7 ± 0.5 $mg.L^{-1}$. The modification of GA from the grafting of antioxidant molecules such as oxidation products of ferulic acid, a structural analogue of curcumin with only one phenolic ring, using a similar pathway as the one use in this study, has already allowed to increase the antioxidant activity of GA to 1.33 $g.L^{-1}$ (Vuillemin et al., 2020). Using OXP coming from curcumin allowed an even better antioxidant activity.

3.4 Thermal analysis

Modification of GA with curcumin derivatives modified many physicochemical properties of GA. With this mind, thermal analyses were carried out by TGA and DSC.

Thermal stability has been determined to observe the change in GA degradation upon temperature after functionalization. Figure 3 shows the thermograms of GA, GACD and curcumin.

The first weight loss recorded before 100 °C corresponded to weakly bound water whereas more strongly bound water was detected up to 150 °C. In this temperature range, different behaviours between the native and the functionalized gum were reported. This first stage of degradation before 150 °C represented about 6 and 3% of their total weight for GA

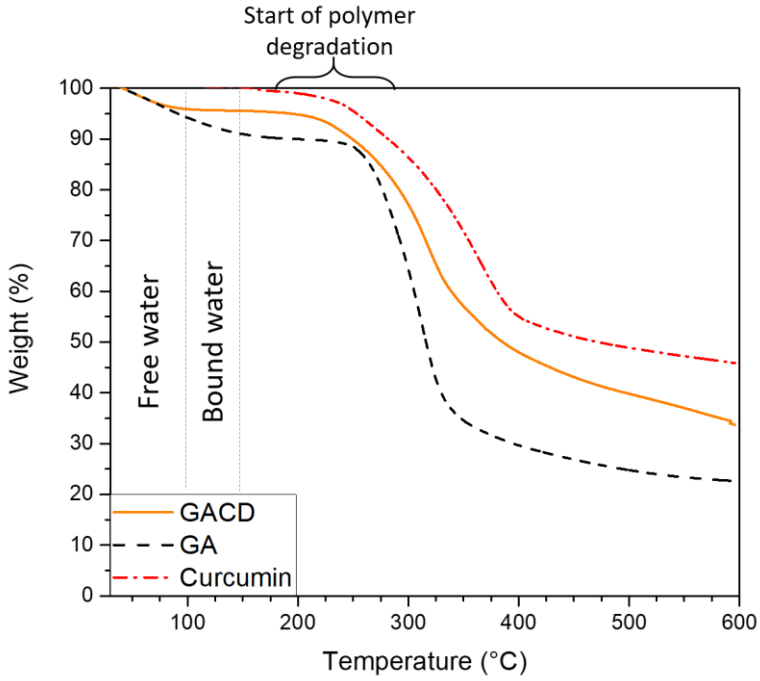
and GACD, respectively. Moreover, GACD lost all of its water before 100 °C, whereas GA weight losses were about 6% before 100 °C and 4% between 100 and 150 °C. It seemed then that GACD was relatively less hygroscopic than GA since it contained a lower amount of strongly bound water. GACD was also more hydrophobic than GA because it contained less water, and the greater the degradation step in this temperature range is, the more hydrophilic the polymers are (Zohuriaan & Shokrolahi 2004). These differences could be due to the presence of grafted OXP that would have modified the hydrophilic properties of gum Arabic by increasing its hydrophobicity. This was already noticed by Vuillemin et al., (2020) for gum Arabic modified by oxidation products of ferulic acid. It may also modify the inter-chain spacing of the polymer, giving more space to water adsorption which then modified the hygroscopy. Curcumin contained almost no water without any weight loss below 150 °C. This seemed to make sense since it was a very hydrophobic molecule.

A second and more important stage of degradation was observed at higher temperatures. This weight decrease was the greatest for GA (~ 60% of the total weight between 225 °C and 400 °C and ~ 40% of the total weight for GACD in the given temperature range) and could correspond to a decomposition step of the polymer. This degradation step showed that GACD was less temperature stable than native GA since it started to degrade before.

At 600 °C, at the end of the heating process, more residue remained for GACD (~ 40%) than for GA (~20%). These obtained data with GA have been correlated with those reported in the literature (Cozic et al., 2009; Mothé & Rao 2000; Zohuriaan et al., 2004).

Therefore, functionalization had an impact by changing the thermal stability of the polymer. It had already been noted in the literature that the functionalization of gum Arabic modified its temperature stability (Ahmad, Mazumdar, & Kumar 2013; Zohuriaan- Mehr,

447 Motazedi, Kabiri, & Ershad- Langroudi, 2005) and especially its functionalization by
448 phenols (Vuillemin et al., 2020).



449

450 Figure 3: Thermograms of GA (black dash line), GACD (orange solid line) and Curcumin
451 (red dash dot line) obtained by TGA

452 Thermal behaviour was studied by DSC. GA and curcumin thermogram did not show any
453 significant event in the studied temperature range. It is quite frequent that in the studied
454 temperature range no Tg was detected on the GA thermograms as it has been reported by
455 various authors such as Mothé et al., (2000), Zohuriaan et al., (2004) or Sabet, Rashidinejad,
456 Melton, Zujovic, & Akbarinejad et al., (2021). The difference that can be observed on DSC
457 thermograms closed to 60 °C between the first heating vs. cooling and second heating
458 corresponded to an enthalpic relaxation due to the thermal history of the sample.

459 After functionalization with OXP of curcumin, gum Arabic exhibited a glass transition (a
460 significative endothermic thermal event) at 51 °C ± 7 °C. The presence of this new event
461 suggested that oxidized compounds fixed onto the polysaccharide modified the inter-chain
462 spaces of the polymer by filling steric hindrance. This steric modification allowed gum Arabic

to obtain a rubber amorphous state over 51 °C. These type of results were already reported onto pectin or inulin modified by ferulic acid or catechin (Karaki et al., 2016; Spizzirri et al., 2010) and on GA modified by ferulic acid (Vuillemin et al. 2020).

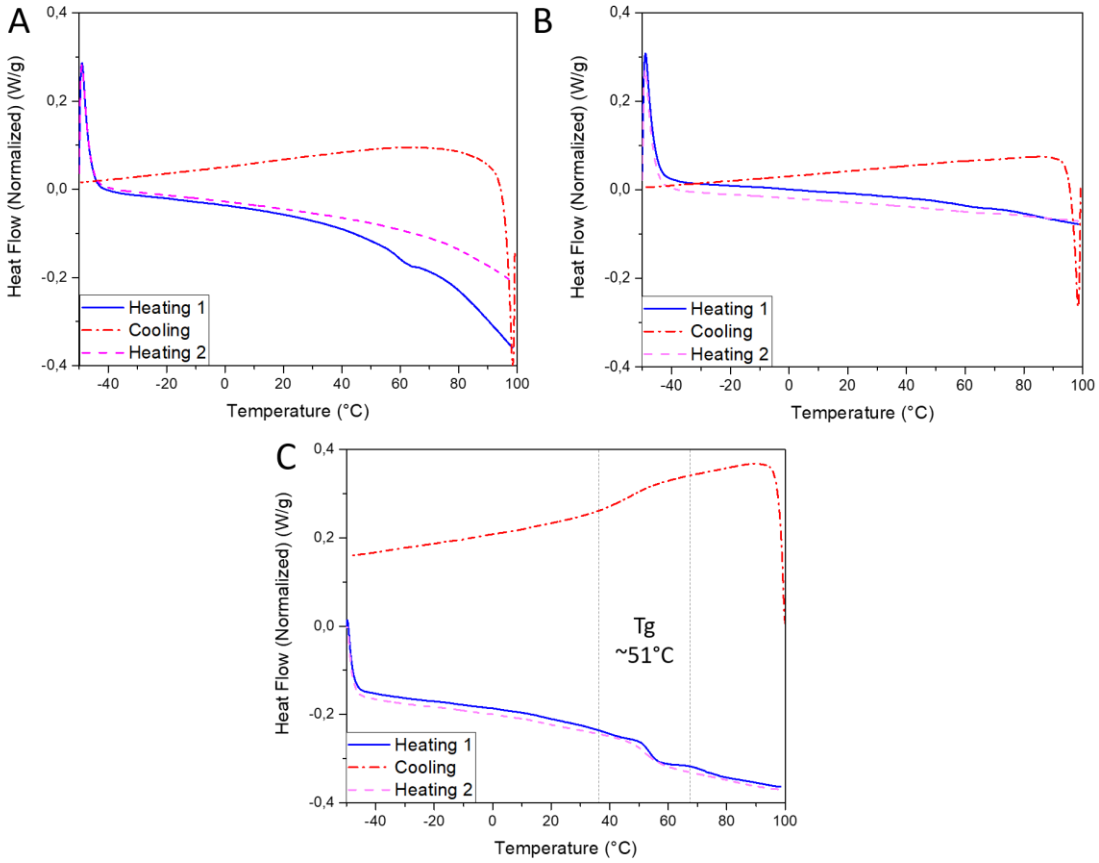


Figure 4: Thermograms obtained by DSC of (A) GA,(B) Curcumin and (C) GACD with in solid blue line the first heating, in red dash dot line the cooling and in pink dash line, the second heating. Tg : glass transition

3.5 Hygroscopy

Gum Arabic is a hydrophilic polymer soluble up to 42.5 ± 2.6% w/v (Vuillemin et al., 2020) and curcumin is an hydrophobic molecule (Peng et al., 2018). By grafting OXP of curcumin, GA seemed to become less hydrophilic and less hygroscopic as suggested by the TGA results. Indeed, modification by hydrophobic compounds such as phenols could modify the affinity of the polysaccharide with water (Karaki et al., 2016). The sorption curves of the

GA and GACD were then carried out to determine whether the functionalization changed interaction of modified GA with water (Figure 5).

The water sorption isotherm of GA was of type II according to international union of pure and applied chemistry (IUPAC). The part at low relative humidity (RH), corresponded to the strongly bound water related to the hygroscopy of the material. The second part in the middle RH range corresponding to the quasi linear section which matched to the weakly bound multilayer water absorbed by capillarity. The third region (high RH) corresponded to free water (Lykiema, Sing, Haber, Kerker, & Wolfram, 1984). For GA the first part could be between 0.1 and 20% of RH, which would correspond to the water necessary to form a monolayer around the polymer. Multilayer adsorption then starts up till 60%. Above this humidity, only free water is added around the polymer. This behaviour has already been reported by some authors (Frascareli, Silva, Tonon, & Hubinger, 2012; Pérez-Alonso, Beristain, Lobato-Calleros, Rodríguez-Huezo, & Vernon-Carter, 2006).

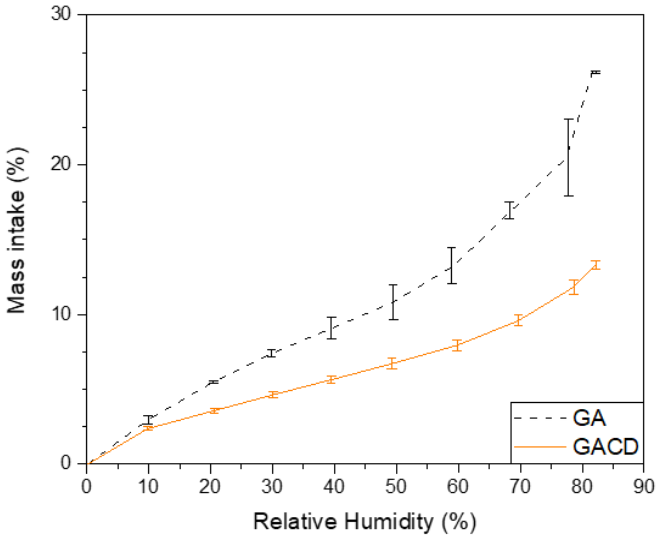
The sorption isotherm obtained for GACD was also of type II but was considerably different from GA. The monolayer adsorption of water was terminated at the very latest at 10% relative humidity. The formation of multilayer water around the polymer was terminated at 60% and then only free water was added. In addition, less water was adsorbed onto the polymer.

The effect of the grafting of phenolic groups could therefore be seen at lower RH since the adsorbed water content was lower, this meant that GACD was less hygroscopic than GA. At high RH the weight intake of GACD was also lower than for GA suggesting that GACD was less hydrophilic than native gum.

As previously supposed from TGA experiments, the functionalization due to the addition of OXP, decreased hygroscopy and hydrophilicity. Karaki et al., (2017) were able to show

that oxidation product of ferulic acid bound to pectin induced a higher hydrophilicity than native pectin. Authors argued that this result was due to the opening of the structure by the oxidation products creating larger spaces between the chains and allowing more water to be absorbed. In the present case, for GACD, hydrophilicity is lower than that of the native polymer. It is therefore at this stage complicated to affirm something about inter-chain space with absolute confidence. However, it was not the same polysaccharide nor the same phenol that in this study. GA was slightly more hydrophilic and less hygroscopic than pectin. Moreover, thanks to the DSC thermograms, it was possible to show a Tg on the GACD and not on the GA whereas the GA was more hydrophilic and water has a plasticizing effect on polymers. It was then possible to consider that the oxidation products from curcumin were much more hydrophobic and repelled more water and due to this hydrophobicity, the spaces created between the chains were insufficient to accept as much water as the GA. It is also possible that the spaces between the chains were filled with OXP and thus water cannot get into them and these OXP may had a plasticizing effect on GACD, allowing it to exhibit a phase transition.

It has also already been shown that the functionalization of gum Arabic could lead to a reduction of its hydrophilicity, in particular through the addition of hydrophobic phenol derivatives (Vuillemin et al., 2020). It has also already been shown on other polysaccharide like chitosan (Aljawish et al., 2016).



519

520 Figure 5 : Water sorption isotherms of GA (black dash line) and GACD (orange solid line)
521

522 3.6 Behavior in water

523 Gum Arabic is a naturally amphiphilic polymer. The grafting of hydrophobic phenolic
524 compounds has made the GAC more hydrophobic and thus modifying its behavior in water.
525 Indeed, GA has a natural ability to lower the surface tension of water. Various studies have
526 been conducted on this subject (Wu et al., 2015; Ebrahimi, Homayouni Rad, Ghanbarzadeh,
527 Mohammadali, & Pasquale, 2020; Cao, Zhang, Zhang, & Du, 2013; Wang, Williams, &
528 Senan, 2014; Vuillemin et al., 2020). It is able to lower the water surface tension about 65
529 $mN.m^{-1}$ at a concentration of 0.5% (wt). Once modified by phenols, the GAC lowered even
530 more the water surface tension. It was then able to lower the water surface tension to 51.6 ±
531 0.3 $mN.m^{-1}$ at 0.5% (wt). A decrease of the surface tension is shown in Figure S1 in
532 supplementary materials.

533 It has already been shown that modification of GA with hydrophobic compounds
534 improves the ability of the gum to decrease the surface tension of water (Vuillemin et al.,
535 2020; Wang et al., 2014). It should also be noted that the ability of GA to decrease surface
536 tension is primarily due to the AGP fraction (Dickinson et al., 1988; Randall, Phillips, &

Williams, 1988; Nakauma et al., 2008). In GAC, the AG fraction is also functionalized and thus could participates in the lowering of surface tension. Functionalization thus allowed the valorisation of an important part of the GA which was not exploited until now.

The modification of the amphiphilicity of the GA may also induced a self-assembly phenomenon since it depended on the balance between hydrophobic and hydrophilic interactions to minimize energetically unfavourable interactions as reported by Sarika et al. (2015). By increasing the hydrophobicity, the interactions with water were less favourable and therefore the polymer promoted particle formation (Akiyoshi & Sunamoto 1996; Huie 2003; Six & Ferji 2019), whereas, for the same concentration, the native polymer was solubilized in water. GAC preparation in water thus led to very turbid sample even at very low concentrations. The presence of this cloudiness indicated the spontaneous formation of particles in water. This behavior had not, to this day, been shown on enzymatically modified GA with phenols such as ferulic acid (Vuillemin et al., 2020). However it has been reported in literature that the polysaccharides chemical modification using phenolic compounds could lead to the formation of particles as demonstrated with pectin and curcumin (Mundlia et al., 2019). It has already also been shown that a chemical grafting of curcumin onto gum Arabic lead to the formation of particles in water composed of an hydrophobic curcumin core and a hydrophilic polysaccharide shell (Sarika et al., 2015). A recent study also shows that dextran enzymatically modified with curcumin could form self-assemblies in water (Curcio et al., 2019) as it is shown today with GA.

Measurements of particles hydrodynamic diameter by dynamic light scattering have shown that the size of these particles was in mean 1621 ± 94 nm in diameter. The polydispersity index of the particle size populations was 0.130 ± 0.068. Size distributions expressed in intensity, volume and number are presented on Figure 6. Peaks were centred at 1616, 1496, and 1401 nm for intensity, volume, and number, respectively. Such close values

illustrated that the three curves were overlapping suggesting monodisperse size distribution. The polydispersity appeared then to be very low and did not correspond to particles formed by a simple aggregation but rather a self-assembly phenomenon.

Furthermore, the surface charge of the particles has been determined. The particles surface was negatively charged and the corresponding electrophoretic mobility was equal to -3.062 ± 0.039 µm.cm/V.s (approx. Zeta potential : -39.1 ± 0.5 mV) (pH of the sample : 6.23 ± 0.12). The GA electrophoretic mobility was measured at -2.492 ± 0.091 µm.cm/V.s (approx. Zeta potential : -31.8 ± 1.1 mV). This negative charge could prevent aggregation although the large particle size lead to rapid sedimentation. A simple agitation then allowed the particles to be resuspended.

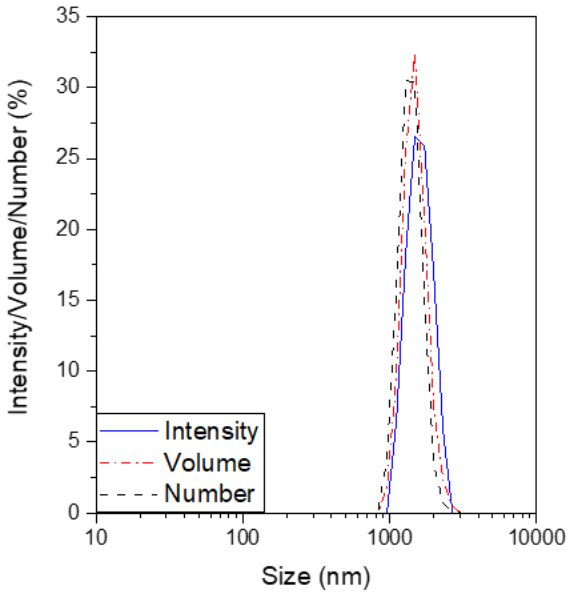
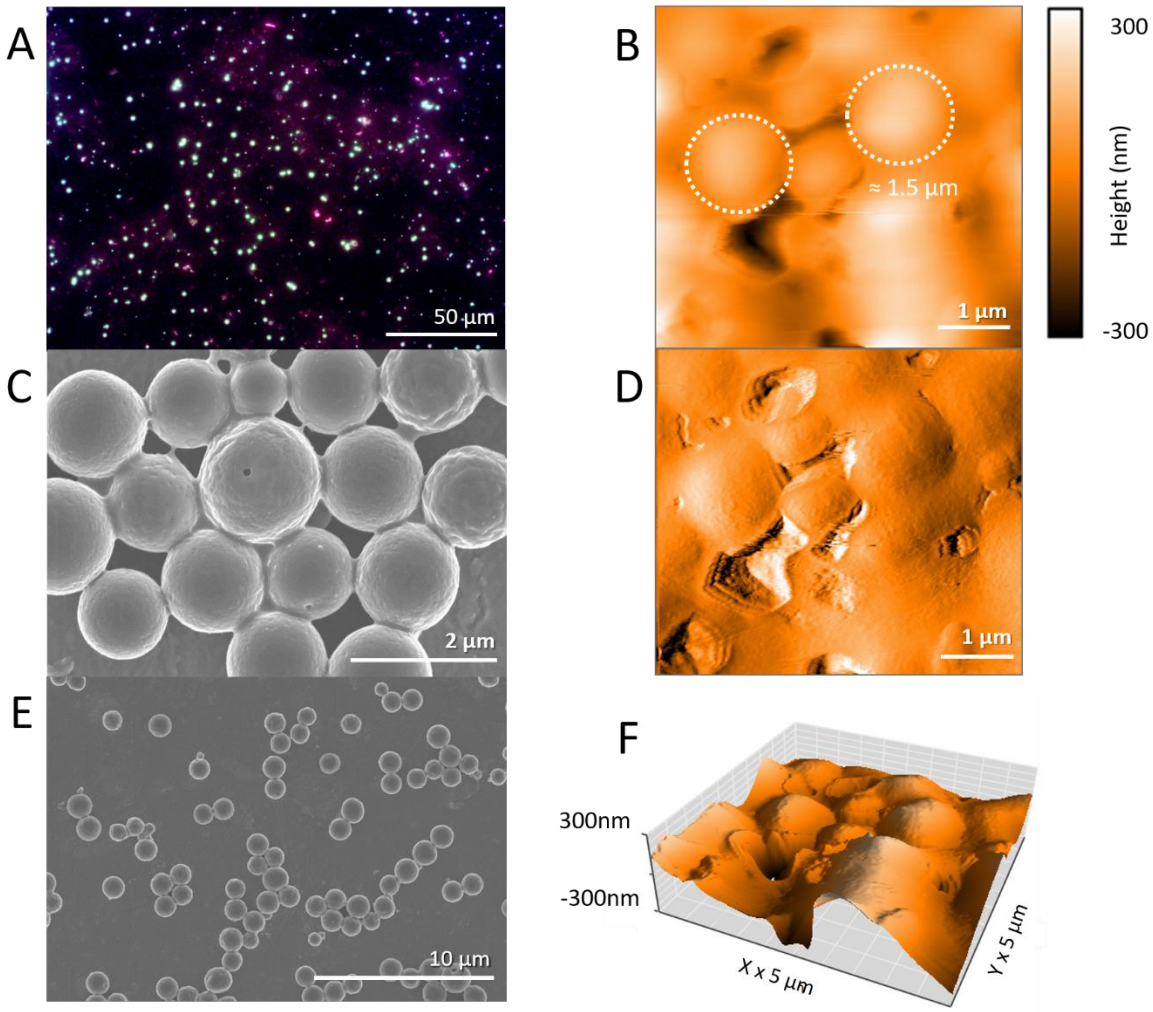


Figure 6 : Size distribution expressed in intensity (solid blue line), volume (red dash dot line) and number (blue dash dot line), of GAC dispersed in water at 0.005% w/v

3.7 Morphological study

In order to investigate further the self-assembly behaviour of the modified gum Arabic in water, the structure of the obtained particles had been studied by several microscopic technics. Obtained micrographs are shown in Figure 7. Epifluorescence microscopy images Figure 7A confirmed the presence of the OXP, that emit fluorescence from spherical particles. SEM (Figure 7C and E) confirmed the spherical shape of the particles. These particles presented a relatively smooth surface which was also observed by AFM topography measurements where particles presented a roughness of 24.50 ± 9.87 nm (Figure 7B, D and F). Size determined from the SEM micrograph analysis revealed the majority particles size was of 1.1 µm and AFM images showed a size below 1.5 µm, which was smaller than the one determined from DLS measurement. This was consistent since DLS measures the hydrodynamic radius of the particles, which includes a hydration layer. It is also possible that drying induces a slight contraction of the particles due to water loss.



589

590 Figure 7: Micrographs obtained by (A) fluorescence microscopy magnification × 400, AFM:
591 (B) height image (D) deflection view (F) 3D view and SEM magnification (C) × 20,000 and
592 (D) × 5,000

593

594 Additionally, the elastic modulus of the GAC particles was evaluated by AFM
595 nanoindentation (see Figure S2 in supplementary materials) and a value of 38 ± 9 MPa was
596 recorded. This value may seemed high if compared to alginate microbead (about 300 µm)
597 which has an elastic modulus slightly lower than 3 kPa (Chui, Bonilla-Brunner, Seifert,
598 Contera, & Ye, 2019). The assemblies were therefore rather strong, which may explain their
599 good resistance to drying imposed by the microscopy techniques used.

4. Conclusions

In this study, the modification of gum Arabic by an enzymatic process led to the addition of curcumin oxidation products to its structure which was confirmed by infrared spectroscopy. Ester bonds were created between GA and the products resulting from the oxidation of curcumin by the laccase. The polysaccharide molecular weight determined by SEC-MALS was greatly increased. The presence of these OXP modifies the properties of the polysaccharide. Indeed, the presence of these new compounds in the GAC structure have increased the antioxidant activity of the gum by 25,000 times. From DSC measurements, the grafting of the curcumin OXP entities induced a plasticizing effect and lead to a lowering of the polymer glass transition temperature to 51 °C. A lowering of the polysaccharide hygroscopy and hydrophily was also reported. The modification changed then the GA hydrophobicity/hydrophobicity balance. This change allows the GAC to obtain a greater capacity to decrease the surface tension of water than the GA until 51.6 $mN.m^{-1}$ compared to 65 $mN.m^{-1}$ for the native polymer at a polymer concentration in water of 0.5% (wt). As a result of these modifications, GAC became able to form spontaneously spherical particles of about 1.6 µm in aqueous solution with a very low polydispersity. It denotes the change of properties of the gum in water since it is naturally very polydisperse. It is therefore interesting to see that the modification by OXP of curcumin allowed to pass from a very soluble and polydispersed polymer (Atgié, Garrigues, Chennevière, Masbernat, & Roger, 2019; Renard et al., 2006) to an insoluble and quite monodispersed polymer at the same concentrations. It would be very interesting to study more in depth the characteristics of the assemblies such as defining the critical aggregation concentration or to study the impact of the physicochemical properties of the medium on the formed particles. It could also be very interesting to carry out encapsulation tests.

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6. Declaration of interest

Declarations of interest: none

7. Author contribution

Aurélie A. ADAM: Conceptualization; Formal analysis; Investigation; Data curation; Writing original draft
Jordane Jasniewski: Conceptualization; Investigation; Validation; Data curation; Supervision; Writing - reviewing & editing
Marie E. Vuillemin: Reviewing
Blandine Simard: Investigation; Data curation
Jennifer Burgain: Investigation; Data curation
Régis Badin: Investigation; Data curation
Lionel Muniglia: Validation & reviewing
Florentin Michaux: Conceptualization; Validation; Data curation; Supervision; Writing - reviewing & editing

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