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Comparison of Microencapsulation Properties of Spruce Galactoglucomannans and Arabic Gum Using a Model Hydrophobic Core Compound

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In the present study, microencapsulation and the physical properties of spruce (*Picea abies*) *O*-acetyl-galactoglucomannans (GGM) were investigated and compared to those of arabic gum (AG). Microcapsules were obtained by freeze-drying oil-in-water emulsions containing 10 wt % capsule materials (AG, GGM, or a 1:1 mixture of GGM−AG) and 2 wt % α-tocopherol (a model hydrophobic core compound that oxidizes easily). Microcapsules were stored at relative humidity (RH) of 0, 33, and 66% at 25 °C for different time periods, and their α-tocopherol content was determined by HPLC. X-ray microtomography analyses showed that the freeze-dried emulsions of GGM had the highest and those of AG the lowest degree of porosity. According to X-ray diffraction patterns, both freeze-dried AG and GGM showed an amorphous nature. The storage test showed that anhydrous AG microcapsules had higher α-tocopherol content than GGM-containing capsules, whereas under 33 and 66% RH conditions GGM was superior in relation to the retention of α-tocopherol. The good protection ability of GGM was related to its ability to form thicker walls to microcapsules and better physical stability compared to AG. The glass transition temperature of AG was close to the storage temperature (25 °C) at RH of 66%, which explains the remarkable losses of α-tocopherol in the microcapsules under those conditions.

KEYWORDS: Spruce *O*-acetyl-galactoglucomannans; arabic gum; microencapsulation; α-tocopherol; storage stability

INTRODUCTION

Wood compounds have been used in the food industry for several years. Phytosterols, xylitol, and carboxymethylcellulose are examples of valuable wood-based compounds, which are widely used in different types of foods all around the world. One potential new wood-based compound for food use is *O*-acetyl-galactoglucomannans (GGM) that is the predominant hemicellulose in spruce (*Picea abies*) (*1*). GGM consists of a linear 1→4-linked chain of β-mannopyranosyl and β-glucopyranosyl units to which branches of single α-galactopyranosyl units are attached (*2*,*3*). The hydroxyl groups in mannopyranosyl units of GGM are partly substituted by acetyl groups (degree of acetylation ∼ 16%). Compared to common commercial galactomannans, for example, locust bean gum, guar gum, or konjac gum, GGM has lower molecular weight and less viscous character in concentrated solutions (*3*−*5*). GGM can be recovered from the process water of the pulping industry, and methods for effective extraction and recovery have been developed (*2*,*5*). GGM has not yet been approved for food use and is not commercially available, but several studies have been done or are ongoing regarding the usage of GGM in different applications including use in edible films (*6*) and as emulsion stabilizers in beverages (*7*).

At its simplest, microcapsules can be produced by preparing emulsions from the capsule material (e.g., polysaccharide and/or protein) and the desired sensitive component which needs protection (e.g., vitamin, flavor or unsaturated lipids) and by drying the achieved emulsions to the amorphous glassy powders (*8*,*9*). There are numerous ways for microencapsulation of which, for example, freeze-drying, spray-drying, and extrusion, produce glassy powders (*10*). The ability of the glassy matrix to protect the encapsulated component is based on its very high viscosity, and thus, the molecular mobility and oxygen permeation are extremely slow (*11*). The state of the capsule matrix may change from glassy to rubbery (= glass transition) if the capsules are stored under such conditions that the glass transition temperature (T_g) of the matrix is lower than the storage temperature. The physical properties of the capsule materials are important to know in order to find the proper storage conditions for microcapsule powders. If the microcapsules are stored under unfavorable conditions,

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the powder quality may be weakened (because of phenomena related to exceeding of T_g such as caking and stickiness) (*9*) and further the ability of the capsule to protect the encapsulated components may decrease (*8*).

Properties of ideal capsule materials for edible products have been listed by many authors (e.g., ref *12*). First, the capsule material has to be approved for food use, but other important matters are good protection properties (e.g., during storage), water-solubility, low viscosity in solutions, low costs, and emulsification ability. When considering a component for food use, the sensory properties should not be underestimated either. In the end, the selection of capsule material is dependent on the component to be encapsulated and on the aim of the microencapsulation. The same capsule material may not be suitable for every purpose. At the moment, quite a limited selection of capsule materials exists: modified and unmodified starches, milk compounds (proteins/lactose), and arabic gum (AG) dominate the field. The latter, probably the most common of the ones in the food field, is an exudate from *Acacia* trees. AG has a complex structure with a backbone of 1→3-linked galactopyranose units and side-chains of 1→6-linked galactopyranose units terminating in glucuronic acid or 4-*O*-methylglucuronic acid residues (*13*). The structure contains also some protein. AG has been used to encapsulate a wide variety of hydrophobic compounds, such as flavors and unsaturated oils, through the oil-in-water emulsification process (*14*−*21*). Undoubtedly, the most common goal in encapsulating sensitive components by AG is to protect them against oxidation, or at least to diminish this phenomenon. AG fulfills almost all the requirements of a perfect capsule material, which explains its wide usage. Surprisingly few new capsule materials have been proposed for the food field during the last years. In one recent review with an industrial viewpoint, food scientists and technologists were encouraged to search for inspiration for microencapsulation also from other (than food) fields such as agricultural, pharmaceutical, and chemical fields (*22*).

GGM could be an interesting choice for the microencapsulation of food compounds, since GGM has some properties, for example, water-solubility (*4*), emulsion stabilizing capacity (*7*), and low solution viscosity in concentrated solutions (*4*, *5*) that may be useful when considering it as a microcapsule material. Recently, a Swedish research group prepared hydrogel microspheres from GGM by cross-linking in water-in-oil emulsions (*23*). The capacity of the microsphere system for diffusional release of the encapsulated model compounds, water-soluble caffeine (small molecule) and bovine serum albumin (macromolecular protein), was then studied (*23*). The aim of the present study was to find out whether GGM could be used as a capsule material alone or in a mixture with AG for α-tocopherol (hydrophobic model core compound) and compare its encapsulation properties to those of AG. In addition, the physical properties of the capsule materials were characterized to better understand the ability of the capsule materials to protect the encapsulated components under different storage conditions.

MATERIALS AND METHODS

Materials. Spray-dried GGM, with an average molecular weight of 39 kDa, was obtained from an industrial-scale isolation trial from the process water of a Finnish pulp mill (*2*, *5*). AG (*Acacia seyal*, Valspray F 25500) used in the study was a purified and spray-dried powder (Valmar, Aubagne, France). The water content of GGM and AG determined after oven-drying (130 °C, 1 h) was 9.1 and 11.8% (w/w), respectively. This was taken into account when emulsions were prepared for microencapsulation. An α-tocopherol (purity >99.5%, Merck, Darmstadt, Germany) was chosen for a model hydrophobic core compound because it is prone to degradation when the water activity, storage temperature, or molar ratio of oxygen increases (*24*).

Preparation of Freeze-Dried Capsule Materials. The capsule materials used were GGM, AG, and a 1:1 (w/w) mixture of GGM−AG. The capsule material(s) (10% w/w or 5% + 5% w/w in the case of a mixture) were dissolved in distilled water at 60 °C under constant stirring. In a mixture containing both AG and GGM, the capsule materials were mixed with each other before dissolving them into water. The solutions were cooled and stored in a refrigerator overnight. After one day of storage in the refrigerator, the AG solution appeared as translucent and had yellowish/brownish color whereas the GGM-containing solutions were opaque and had beige/cafe-latte-like color. The flow curves of solutions of GGM, GGM−AG, and AG were determined by using a ThermoHaake Rheostress600 instrument (Thermo Electron GmbH, Dreieich, Germany) at a shear rate range of 0.03−300−0.03 s^{-1} (at 25 °C), and the apparent viscosities recorded at 50 s^{-1} were 56, 23, and 10 mPa s, respectively.

The solutions were pipetted into 20 mL brown glass vials (5 mL per vial) and frozen at −20 and −80 °C and then freeze-dried (Lyovac GT 2 freeze-dryer, Amsco Finn-Aqua GmbH, Hürth, Germany) for 72 h at a pressure of < 0.1 mbar and finally powdered using a pestle and mortar. The residual moisture content was removed by storing the powders for 2 weeks in vacuum desiccators over P_2O_5 in the dark.

Amorphous/Crystalline Character of Capsule Materials. The amorphous/crystalline character of the dry capsule materials (freeze-dried AG and freeze-dried GGM) was analyzed by X-ray powder diffraction (XRPD). To further characterize the presence of possible crystalline impurities in the GGM powders, the original GGM (without any extra purifying or drying procedure) and GGM that was purified were analyzed. Purification of GGM was done by dialyzing (dialysis tubing with 12−14 kDa, Medicell International Ltd., London, U.K.) GGM−water solution against distilled water for 24 h, and the solution obtained was freeze-dried. XRPD data were obtained by using a PANalytical X'Pert Pro diffractometer (PANalytical B.V., EA Almelo, The Netherlands) utilizing copper radiation ($\lambda K\alpha_1$ = 1.5406 Å; 45 kV/40 mA). Automatic divergence and receiving slits with the irradiated length of 2.00 mm and a PIXcel detector were used. The comparative data were collected at scattering angles 2θ from 10−15° to 40° (step size 0.04°; scan time 100 s for AG and 600 s for the others). To improve the identification of the crystalline part of the freeze-dried GGM, more detailed data were collected by measuring the 2θ range of 5−60° three times using a scan time of 1000 s and step size of 0.025°. The patterns were compared to the reference patterns in the PDF-2 database vol. 44 (ICDD-International Centre for Diffraction Data, PA).

Water Sorption Properties and Glass Transition Temperatures of Capsule Materials. The freeze-dried and powdered capsule materials were weighed (~100 mg) into 4 mL brown glass vials and then stored at 25 °C in vacuum desiccators under RH conditions of 11−85%. Saturated salt solutions of LiCl (11%), CH_3COOK (24%), $MgCl_2$ (33%), K_2CO_3 (44%), $Mg(NO_3)_2$ (54%), $NaNO_2$ (66%), NaCl (76%), and KCl (86%) (Merck, Darmstadt, Germany) were used to obtain the desired RH. The water sorption of the capsule materials was determined gravimetrically by daily weighing during 7 days. The water sorption was also modeled by the Guggenheim−Anderson−de Boer (GAB) model as described previously (*25*).

The capsule materials were weighed (~10−15 mg) into the DSC pans and kept in the vacuum desiccators over various salt solutions at 25 °C for 2 days. The glass transition temperature (T_g) of triplicate samples was determined by using a differential scanning calorimeter (DSC, DSC823, Mettler Toledo AG, Greifensee, Switzerland) equipped with software STARe DP V9.00 20070621 as reported previously (*25*).

Preparation of Microcapsules. The capsule materials were dissolved in distilled water as described before (in the section preparation of freeze-dried capsule materials) and stored in a refrigerator overnight. On the next day, 2% (w/w) α-tocopherol was added into the capsule-material−water solutions. Emulsions were homogenized, first with a Heidolph homogenizer (Diax900, Heidolph, Germany) for 2 × 2 min at 26 000 rpm and then with a microfluidizer (Microfluidics model 110y, Newton, MA) for 10 min at 50 MPa using recirculation. During preparation, the temperature of the emulsions was kept below 10 °C with a cooling ice bath. The obtained emulsions were poured into the capped brown glass bottles (and stored ~2 h in a refrigerator) before portioning (5 mL per vial) them into 20 mL brown glass vials in which they were freeze-dried as described previously.

The droplet size distribution of the emulsions (measured size range 0.04−2000 μm) was determined using a laser diffraction particle size analyzer (LS230, Coulter, Fullerton, CA) equipped with polarization intensity differential scattering. A refraction index of 1.6 (a rough estimation) was used, and two measurements were taken from each emulsion. The droplet size distribution of emulsions is reported as volume distribution, and mean droplet size of emulsions as volume-length diameter, d_{43} ($=\sum_{i=1}\phi_i d_i$), which is the sum of the volume ratio of droplets in each size-class (ϕ_i) multiplied by the midpoint diameter of the size-class (d_i) (*26*).

X-ray Microtomography Analyses of Freeze-Dried Emulsions. The computed X-ray microtomography (μCT) measurements were carried out using a custom-made μCT device nanotom supplied by Phoenix|Xray Systems + Services GmbH (Wunstorf, Germany). The main hardware components of the device are a high-power nanofocus transmission-type X-ray tube with a tungsten anode, a high resolution computer-controlled translation/rotation stage for the sample, and a CMOS flat panel detector with size of 2304 × 2284 pixels of 50 μm (Hamamatsu Photonics, Japan).

For the μCT measurements, small pieces of approximately $3 \times 3 \times 3$ mm^3 were cut from the freeze-dried emulsions using a razor blade. The small samples were mounted on top of steel pins using beeswax. The samples were measured using 50 kV and 240−350 μA power. The measurements consisted of 1800 X-ray transmission images, taken at intervals of 0.2° around a full circle of rotation. The exposure time was 6−10 s for each image. The pixel size in the images was 1.1−1.5 μm.

Three dimensional (3D) reconstructions were made using datos|x rec software supplied with the μCT device. The voxel size of the reconstruction was the same as the pixel size of the measured images. Visualization of the reconstructed volumes was carried out using the program VGStudio MAX 1.2.1 (Volume Graphics GmbH, Germany). From the samples, a volume of 0.9−1 mm^3 was selected for analysis and visualization. For visualization, random noise in the 3D reconstructions was suppressed using a Gaussian convolution filter with a 5 × 5 × 5 voxel kernel.

The porosity of the samples was determined from unfiltered μCT reconstructions using the programs Blob3D (University of Texas (*27*)) and Matlab (The Mathworks Inc.). For the porosity analysis, air and the solids were segmented into separate components using the seeded grayscale threshold algorithm of Blob3D. As a result of this process, all voxels belonging to the solid content were given a single nonzero grayscale value and the voxels representing air were given the value 0. The porosity ϕ was calculated from the air-occupied volume V_{air} and the total volume V_{tot} in the sample using the formula:

$$\phi = \frac{V_{air}}{V_{tor}} \times 100\% \quad (1)$$

The distribution of volume weighted wall thickness (denoted d_W) in the dried emulsions was also determined from the segmented reconstructions. This was done by using the package Local Thickness of the program ImageJ (National Institutes of Health). The analysis was based on fitting spheres with maximal radius inside the segmented material, and d_W was obtained as the diameter of these spheres (*28*, *29*). The d_W values were transformed into a unit-normalized probability density distribution, from which the expectation value $\langle d_W \rangle$ and the standard deviation $\delta(d_W)$ were calculated using Matlab. The lower limit and the step of the d_W distribution were both twice the voxel size, 2.2−3.0 μm.

Storage Test for Microcapsules. Unencapsulated α-tocopherol (~50 mg) and microcapsules (~50 mg) were weighed into 4 mL brown glass vials and stored in desiccators at 25 °C at 0% RH for 39 days, and at 33% and 66% RH for 60 and 120 days in the dark. The content of α-tocopherol was analyzed in each sampling day. The analyses were carried out at least in two replicates.

α-Tocopherol Analysis. The content of α-tocopherol in the unencapsulated and encapsulated samples was determined by high performance liquid chromatography (HPLC). All samples were protected from the light-induced instability by preparing them under subdued light. The methods used were based on the ones presented and validated by Schwartz et al. (*30*).

Preparation of Unencapsulated α-Tocopherol for the HPLC Analysis. Replicate α-tocopherol samples were dissolved in heptane (HPLC-grade, Rathburn Chemicals Ltd., Walkenburn, Scotland) before injection into the HPLC system.

Preparation of Microcapsules for the HPLC Analysis. To release the α-tocopherol from the capsule matrices, microcapsules were broken down by adding 2 × 2 mL of distilled water and then transferred into 30 mL test tubes containing ascorbic acid (L-ascorbic acid, BDH, AnaloR) as an antioxidant. The test tubes were vortexed for 1 min, after which 2 mL of ethanol was added and the tubes were left slightly shaking (220 rpm) for 30 min at room temperature.

Saponification (in order to break the capsule matrix thoroughly) was started by adding 0.25 mL of saturated KOH into the mixture. Before and after adding KOH, the samples were flushed with nitrogen gas for 20 s. The samples were hot-saponified in a water bath at 85 °C for 15 min, after which they were let to cool at room temperature. The saponification caused deacetylation of the GGM samples, and as a consequence GGM lost its solubility and the capsules precipitated into the bottom of the test tube. It is possible that the precipitation re-encapsulated (similar to coacervation) some amount of the α-tocopherol and further decreased the α-tocopherol yields. We also tried the extraction procedure without saponification, but we decided to use saponification since it gave higher α-tocopherol yields with narrower standard deviation.

After the saponification, 4 mL of distilled water and a small amount of NaCl (Merck, Darmstadt, Germany) were added into the test tubes. Then the samples were extracted two times with 6 mL of 50:50 v/v heptane/diethyl ether (Baker analyzed, anhydrous 8254, J.T. Baker). The upper phases were collected, combined, washed first with 10% (w/v) aqueous NaCl solution and then with 2 × 5 mL of distilled water, and finally evaporated to dryness with a rotary evaporator. The residue (= α-tocopherol) was dissolved in 10 mL of heptane (and further diluted with heptane if needed) and filtered through Millex-LCR filters (0.45 μm; 13 mm) before injection into the HPLC system.

HPLC Analysis. The content of α-tocopherol was analyzed by normal phase HPLC with an Inertsil silica column (5 μm particle size, 4.6 mm × 250 mm; Varian Chromapack, Middelburg, The Netherlands). The mobile phase (flow rate 2 mL/min) contained 3% 1,4-dioxane in heptane, and α-tocopherol was detected with fluorescence detection (FLD) using an excitation wavelength of 292 nm and an emission wavelength of 325 nm and quantified with an external standard method. The column temperature was 30 °C. The amount of α-tocopherol was expressed as grams of its original amount in the emulsions (calculated value).

Statistical Analysis. One-way analysis of variance (SPSS 15.0.1, SPSS Inc., Chicago, IL) was used to test the differences in storage stability of the different microcapsules. Tests of normality and the equality of variances were carried out with Shapiro-Wilk test and Levene's test, respectively. Tamhane's post hoc test was used (instead of Tukey's test because of uncertainty in normalities and variances) for pairwise comparison of the means. Differences were considered significant when P was < 0.05.

RESULTS AND DISCUSSION

Physical State and Water Sorption of Capsule Materials. X-ray diffraction analysis showed that anhydrous AG had an amorphous character (**Figure 1**). The glass transition temperatures (T_g) of AG after storing at different RH values at 25 °C are presented in **Figure 2**. T_g could not be determined for anhydrous AG. Water, as an effective plasticizer of amorphous materials, decreased T_g close to room temperature at 66% RH (T_g = 23 °C). The AG powder showed features of stickiness and caking at 66% RH, and at 86% RH the structure of the powder was totally collapsed, being just a translucent button on the bottom of the vial.

Visual caking of the GGM powder was observed at 76% RH, but the extent of observed caking was smaller than that in the AG powder stored under the same conditions. Moreover, GGM did not collapse at 86% RH as dramatically as the AG powder. The X-ray diffractogram of GGM showed that the anhydrous material was mostly amorphous but also exhibited some crystallinity (**Figure 1**). The diffraction pattern clearly exhibited nine peaks corresponding to sodium sulfate Na_2SO_4 (ref code 024-1132), which may exist as an impurity left after the isolation procedure. The obtained peaks were quite wide, indicating small crystals.

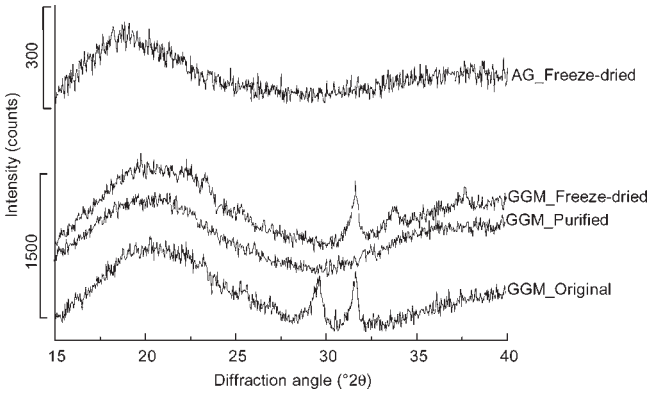

Figure 1. X-ray powder diffractograms for freeze-dried arabic gum (AG), freeze-dried spruce galactoglucomannans (GGM), purified and freeze-dried GGM, and original GGM.

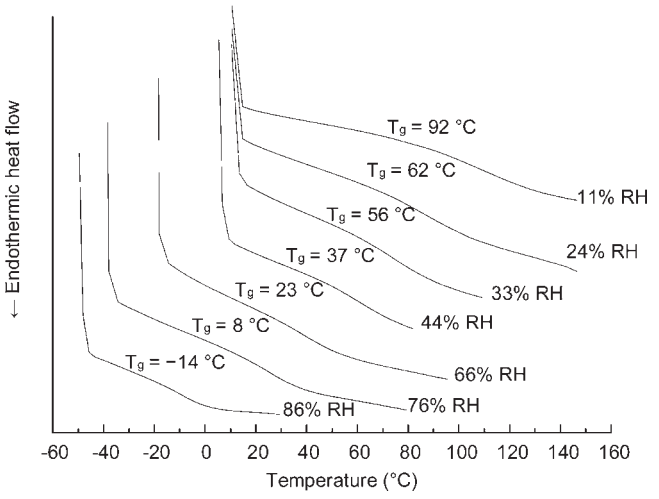

Figure 2. DSC thermograms (second heating) for freeze-dried arabic gum (AG) after storing at different RH (11−86%) at 25 °C.

After purification (by dialyzing) and freeze-drying of the original GGM, the peaks mentioned above disappeared (**Figure 1**), further suggesting that the crystallinity was not due to GGM itself. However, contrary to expectations, the analysis of the diffraction pattern of the original GGM powder (without any extra purifying or drying procedures) did not show the peaks representing sodium sulfate but instead revealed the presence of probably several forms of calcium−sodium sulfates, the closest fits being to $Na_2Ca_5(SO_4)_6 \cdot 3H_2O$ (040-0718) and $CaSO_4 \cdot 0.6H_2O$ (043-0605) (**Figure 1**). The reason for the differences in the peak positions remains unknown. Relatively little is known about the physical state of galactomannans or other hemicelluloses as compared to other polysaccharides such as starches and cellulose. The recent study of Vendruscolo et al. (*31*) provided data on the physical state of spray- and oven-dried galactomannans of *Mimosa scabrella* Bentham. They found that both materials were mostly amorphous, but also a low degree of crystallinity was observed especially in oven-dried powders.

DSC thermograms revealed that GGM showed an endothermic transition only during the first heating (**Figure 3**). However, this transition was not characterized as a glass transition because glass transition is a reversible change (*9*) and, thus, it should have been observable also during the second heating. In addition, the observed endothermic transition occurred in the same temperature range irrespective of RH conditions, an onset temperature varying from 40 to 55 °C. The glass transition, instead, typically

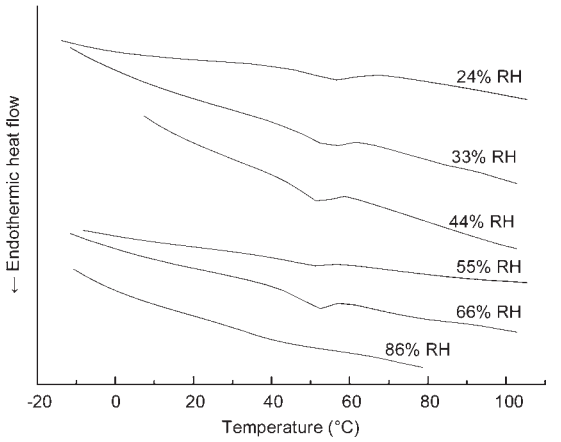

Figure 3. DSC thermograms for freeze-dried spruce galactoglucomannans (GGM) after storing at different RH (24−86%) at 25 °C. Thermograms were obtained from the first heating because no transitions were found during the second heating.

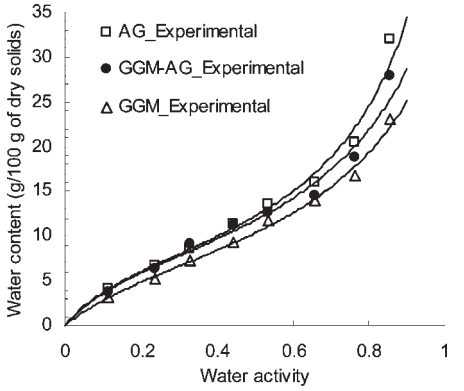

Figure 4. Water sorption of freeze-dried arabic gum (AG), a 1:1 mixture of spruce galactoglucomannans and arabic gum (GGM−AG), and spruce galactoglucomannans (GGM) at 25 °C. Isotherms were predicted with the GAB model (shown as solid lines).

notably decreases with increasing water content (*11*). The obtained endothermic transition might be due to crystal melting or enthalpy relaxation (related to glass formation kinetics and packing of molecules) which is often accompanied with glass transition (*11*). Xu et al. (*5*) reported that purified GGM at 20% (w/w) water content showed an endothermic peak at 43 °C (onset) and this transition was suggested to be the glass transition. The expected T_g region was scanned only once, and thus, it can be speculated whether the transition would also have been observed during a second heating.

The water sorption isotherms of each capsule material appeared as a sigmoidal shape, as can be seen in **Figure 4**. The water sorption properties of all materials were quite similar at low RH but differed from each other at higher RH. AG adsorbed the most and GGM the least water at all RH. The water sorption of the GGM−AG (a 1:1 mixture) settled between the values of AG and GGM. AG and GGM have different chemical structures (average molecular weight, composition, amount of branches in the structure) which can result in differences in water sorption. The equilibrium moisture content of AG at each RH in this study parallels quite well the results from the study of Pérez-Alonso et al. (*32*) despite the different AG (AG originated from *Acacia seyal* vs *Acacia senegal*, respectively) used in the studies. The GAB parameters were also close to each other (m_o = 8.24 g/100 g and 8.11 g/100 g; C = 7.51 and 15.9; K = 0.855 and 0.841 for the

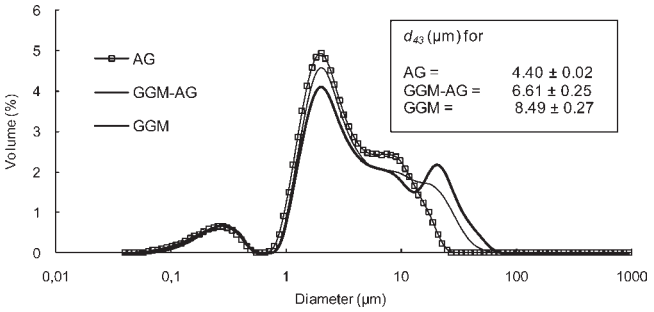

Figure 5. Droplet size distribution and mean droplet size (as volume-length diameter, d_{43}) of arabic gum (AG), a 1:1 mixture of spruce galactoglucomannans and arabic gum (GGM−AG), and spruce galactoglucomannans (GGM) emulsions containing α-tocopherol.

present study and the study of Pérez-Alonso et al. (*32*), respectively). The water sorption isotherm for GGM has not been reported previously. The water sorption of its structural relative guar gum at 25 °C has been characterized (*33*). The equilibrium moisture content of GGM at each RH obtained in the present study was clearly lower compared to those values obtained for guar gum (*33*), which may be explained by the differences in molecular weights (guar gum ∼ 220 kDa vs GGM = 39 kDa), physical states, and particle sizes.

Emulsion Droplet Sizes. An emulsion that has a unimodal droplet size distribution is often more stable than an emulsion with a multimodal droplet size distribution (*26*). **Figure 5** illustrates the influence of the capsule material on the mean droplet size (d_{43}) and droplet size distribution. The mean droplet size of GGM emulsion was almost 2 times bigger (8.49 μm) and that of GGM−AG emulsion (6.61 μm) about 1.5 times bigger compared to that of the AG emulsion (4.40 μm). All emulsions had multimodal droplet size distributions; a plot for the AG emulsion was trimodal, whereas the plots for the GGM and GGM−AG emulsions were tetramodal. The results parallel the study of Kim et al. (*15*), according to which the emulsions prepared with 10% (w/v) arabic gum and orange oil gave multimodal droplet size distribution, but differ from the study of McNamee et al. (*17*) in which emulsions containing 10% (w/v) arabic gum and soya oil (at oil/AG ratios 0.25−5.0) showed unimodal droplet size distribution. The multimodal character of the droplet size distributions of the emulsions in the present study can mainly be interpreted as a consequence of an insufficient emulsifying ability of the capsule materials used. Also the homogenization conditions (homogenizer type, pressure, and time) could have influenced the emulsion droplet size and the droplet size distribution. Increasing the homogenization pressure or duration of homogenization may create narrower droplet size distribution (*34*). The homogenization pressure cannot be increased at will, since too high pressure may cause the degradation of polysaccharide chains and, thus, the emulsion stabilizing ability of polysaccharides may be weakened (*35*). However, the homogenization conditions play only a small role in the formation of a stable emulsion if the amount of emulsifier is limited (*26*).

Obviously, the emulsion recipe used was not optimal for neither AG or GGM. Although having multimodal character in the droplet size distribution, the emulsions did not show observable phase separation. Given that AG showed narrower droplet size distribution and a smaller d_{43} value than GGM-containing emulsions, it can be said that AG showed a slightly better ability to form emulsions than either GGM or GGM−AG. The optimal recipe for AG and GGM emulsions may, however, differ from each other. The ability of AG to form emulsions is often related to the protein part of the AG structure (*13*). GGM contains also a small amount of protein, but contrary to AG the protein part has not been found to correlate with the emulsion stability (*7*).

Structural Properties and Storage Stability of Microcapsules. *Visual Images*. Freeze-dried (e.g., refs *18* and *36*) and spray-dried (e.g., refs *14*, *17*, *19*, *36*, and *37*) microcapsules containing AG have most commonly been visualized by scanning electron microscopy. In the present study, the microstructure of the freeze-dried emulsions (before grinding them into powders) was analyzed using X-ray microtomography.

Sample preparation was very straightforward; nothing else other than cutting samples of suitable volume was required prior to the measurements, and the measurements were carried out at room temperature and pressure. Thus, the possibility of producing structural artifacts to the samples during preparation was negated. A further significant benefit of X-ray microtomography was that a large volume, also in terms of depth, of each sample could be analyzed based on a single reconstruction. **Figure 6** shows the visualization of the reconstructed volumes of the dried emulsions (**Figure 6A**, **C**, **E**), along with 2D slices from within the volumes (**Figure 6B**, **D**, **F**, in which the solids are shown in gray whereas air is shown in black). The large difference in the X-ray absorption between the solid content and the air within the dried emulsions produced good contrast, enabling the segmentation of the two components.

All samples had a porous structure, which is typical of freeze-dried emulsions (*36*, *38*) and freeze-dried material in general (**Figure 6**). Quantitative analysis of the structures revealed some differences in the porosity values and in the wall thickness distributions (**Table 1**, **Figure 7**). The dried GGM−AG emulsion showed slightly lower porosity than the sample containing only GGM, but the difference to the sample containing only AG was within the accuracy of the porosity value. The slightly higher expectation value $\langle d_W \rangle$ in the GGM sample was due to the larger contribution of d_W values in the range 9−24 μm (**Figure 7**). The maximum value of d_W was 26 μm in the AG-containing samples and 28 μm in the sample containing only GGM.

Effect of Storage on the α-Tocopherol Content. The content of unencapsulated α-tocopherol decreased during storage at 25 °C at and below 33% RH and especially at 66% RH (**Figure 8**). Already 30 days of storage at both RH values decreased the α-tocopherol content statistically significantly. After 120 days of storage, 81 and 67% of the original α-tocopherol was left at 33 and 66% RH, respectively. The results are in line with the storage stability study of Widicus et al. (*24*) which showed that the degradation rate of α-tocopherol in a model nonfat food system increased with increasing water activity (from a_w 0.10 to 0.65). The results of unencapsulated α-tocopherol could not be compared with the results of encapsulated α-tocopherol, since the preparation procedure was different. However, these results evidenced the instability of α-tocopherol at elevated RH in the presence of oxygen.

The α-tocopherol content of dry microcapsules (0% RH) was determined after 39 days of storage (**Figure 9**). AG retained the highest amount of α-tocopherol (59% of the original content), GGM followed (45%), and the mixture GGM−AG showed the lowest α-tocopherol content (40%). The values obtained for GGM-containing samples may be somewhat too low because of the observed difficulties in the extraction of α-tocopherol from the dry GGM capsules. However, this was just as expected, since of those three capsule types AG had the best ability to form emulsions (as was shown in the droplet size analyses), and hence, during freeze-drying the oil droplets were more evenly distributed and better protected by the capsule matrix compared to the GGM or GGM−AG capsules. A proper formation of emulsion

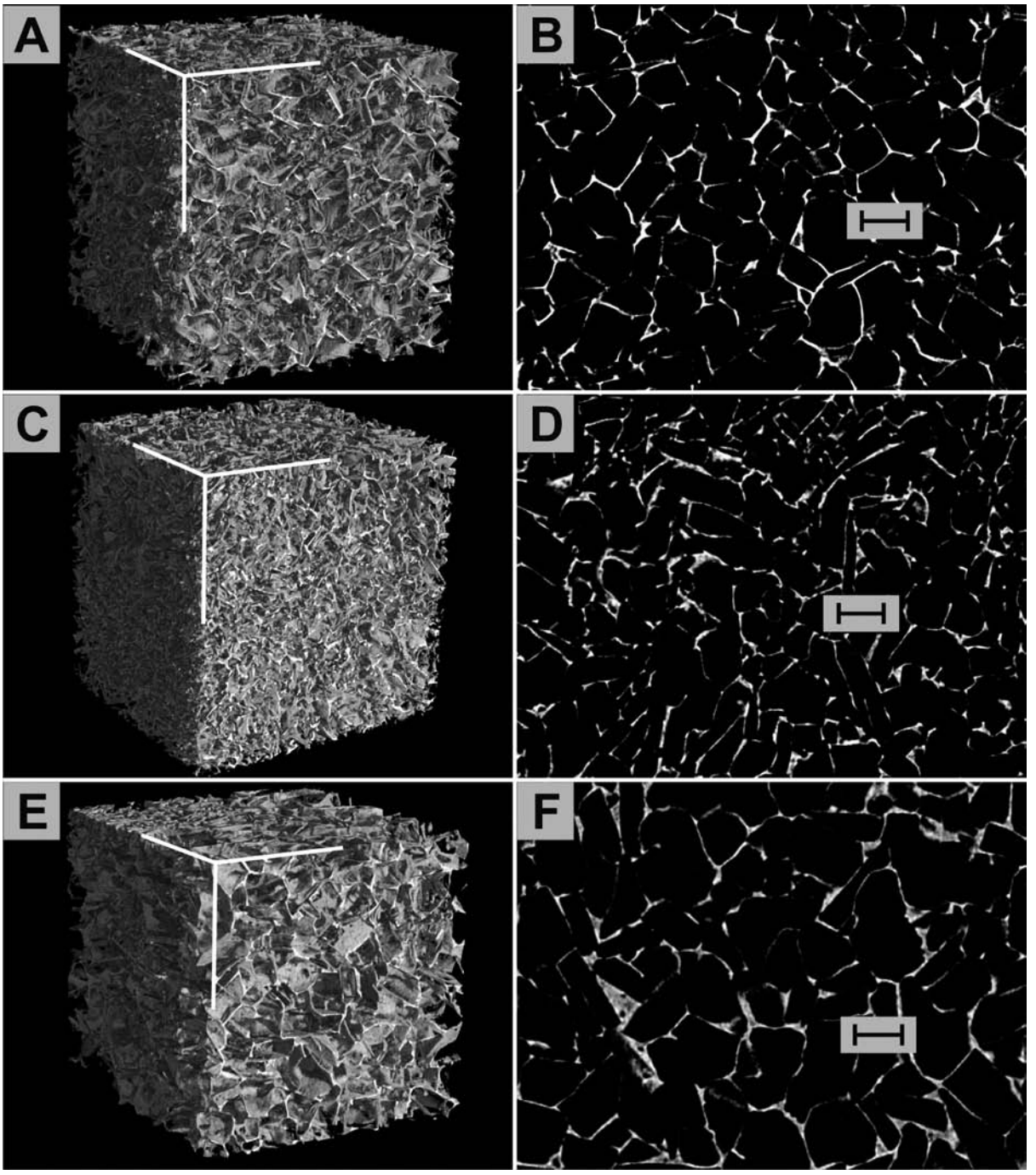

Figure 6. X-ray microtomography images of freeze-dried emulsions of arabic gum (**A** and **B**), a 1:1 mixture of spruce galactoglucomannans and arabic gum (**C** and **D**), and spruce galactoglucomannans (**E** and **F**). Panels **A**, **C**, and **E** show pseudo-3D renderings of the samples (the scale bar denotes 500 μm), while panels **B**, **D**, and **F** show 2D cross sections inside the samples (the scale bar denotes 100 μm).

Table 1. Porosity (%) of the Freeze-Dried Emulsions of Arabic Gum (AG), a 1:1 Mixture of Spruce Galactoglucomannans and Arabic Gum (GGM−AG), and Spruce Galactoglucomannans (GGM) and the Expectation Value $\langle d_W \rangle$ and Standard Deviation of the Wall Thickness $\delta(d_W)$ Distribution

sample	AG	GGM−AG	GGM
porosity [%][a]	82	80	86
$\langle d_W \rangle$ [μm]	6.5	6.5	8.2
$\delta(d_W)$ [μm]	3.0	2.6	4.3

[a] The accuracy of porosity was estimated to be 3%.

has been found to create a basis for the elevated encapsulation yields for microcapsules prepared by spray-drying (*16*, *20*), but undoubtedly, a stable emulsion is also necessary when capsules are formed by freeze-drying. Soottitantawat et al. (*21*) reported that AG capsules prepared by spray-drying from emulsions with large droplet size (~3 μm) had a greater amount of surface oil compared to capsules prepared from emulsions with small droplet sizes (~1 μm). Gejl-Hansen and Flink (*38*) showed that the freezing (before freeze-drying) rate was critical when considering the amount of surface oil/encapsulated oil of microcapsules and the further oxidative stability of the oil. The capsules prepared from slowly frozen emulsions contained clearly more surface oil and less encapsulated oil than those from rapidly frozen emulsions, with the surface oil accounting for 79% versus 65% and the encapsulated oil 21% versus 35%, respectively.

The researchers explained that slow freezing enabled the oil droplets to agglomerate and coalesce to give large droplet sizes. Further, the large droplets could not be incorporated into the concentrated solute phase, and as a consequence a large amount of oil remained on the surface. The surface oil was observed to be responsible for oxidative instability, whereas the encapsulated oil remained unoxidized. Another study (*39*) showed that, despite the unchanged structure of microcapsule matrices, fish oil oxidation occurred in microcapsules when stored at 20 °C for 21 days

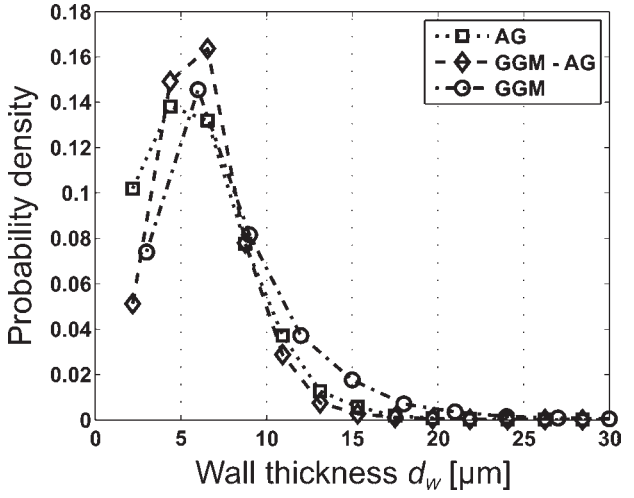

Figure 7. Unit-normalized, volume-weighted wall thickness distributions of the freeze-dried emulsions of arabic gum (AG), a 1:1 mixture of spruce galactoglucomannans and arabic gum (GGM−AG), and spruce galactoglucomannans (GGM).

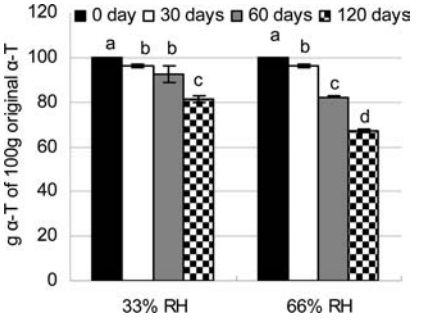

Figure 8. Stability of unencapsulated α-tocopherol (α-T) after storage at 25 °C at 33 and 66% RH for 0−120 days. The error bars show the standard deviation and different letters means statistically significant differences ($P < 0.05$).

over silica (dry conditions). The researchers explained that the strong increase in fish oil oxidation within the first week of storage was due to the oxidation of unencapsulated oil. Possibly, also in the present study, the large amounts of unencapsulated α-tocopherol, which was easily attainable to oxygen, could explain the considerably small amounts of α-tocopherol observed in the microcapsules under dry conditions. Another explanation for the small amount of encapsulated α-tocopherol may be a too high α-tocopherol/capsule material ratio used. It might be possible to increase the amount of encapsulated compound by increasing the amount of capsule material. Kaushik and Roos (*40*) showed that the amount of encapsulated limonene retained better during freeze-drying when an AG/limonene ratio of 9:1 (retention of 75.3%) was used instead of ratio 8.5:1.5 (retention of 60.2%).

Even though AG was superior in relation to the protection of α-tocopherol at the beginning in the dry state, the situation was opposite at higher RH (**Figure 8**). During 60 days of storage at 33% RH, the GGM and GGM−AG capsules showed their effectiveness and retained α-tocopherol significantly better compared to the AG capsules. However, the differences got smaller during storage, meaning that only small differences (not even statistically significantly different) benefiting GGM and GGM−AG were observed after 120 days of storage. A downward trend of α-tocopherol content in all capsules was evident.

The results on the capsules stored for 60 days at 66% RH seemed to parallel those of 33% RH (**Figure 8**). Again, the microcapsules containing GGM offered greater protection for α-tocopherol. In fact, the difference in the protection ability between the GGM-containing capsules and the AG capsules increased as compared to the values obtained after storage at 33% RH. At the end of the storage period (after 120 days), the amount of α-tocopherol in AG capsules decreased into a low level: only 7% of the original α-tocopherol remained. The losses of α-tocopherol in the GGM-containing capsules were high but not as remarkable as those in the AG capsules (losses of 78−81% vs 93%, respectively). It was interesting to note that the amount of α-tocopherol in the GGM-containing capsules after 120 days of storage was the same or even slightly higher than that in the AG capsules after 60 days of storage.

The instability of the AG capsules at 66% RH (25 °C) was expected, since the glass transition temperature (onset value) of AG under those conditions was close to the storage temperature (**Figure 2**). The instability of the AG capsules at such RH has also been reported by other researchers. Rosenberg et al. (*19*) observed that the structure of spray-dried AG capsules containing water-insoluble aroma compounds remained unchanged when stored for about 30 days at 25 °C below 64% RH, and high retention of

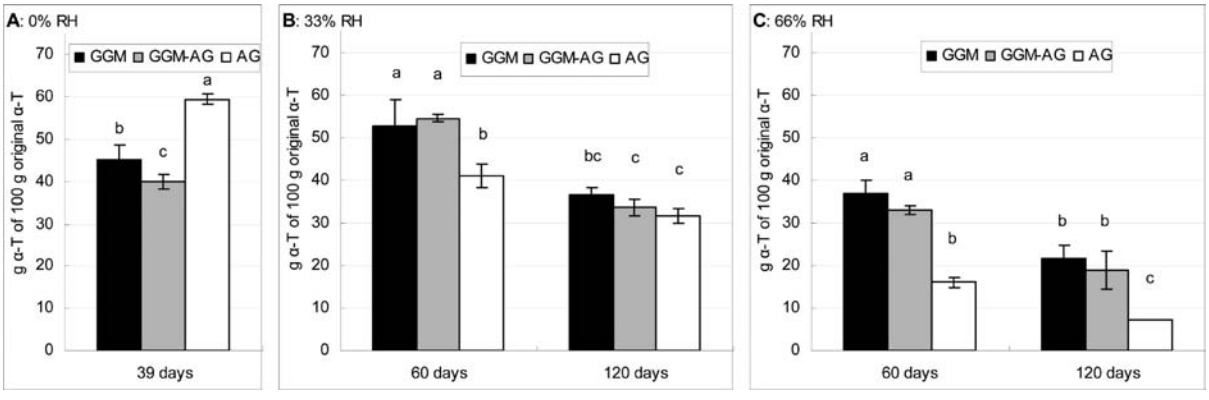

Figure 9. Effect of time on the α-tocopherol (α-T) content of microcapsules after storage at 25 °C at (**A**) 0% RH, (**B**) 33% RH, and (**C**) 66% RH. In each picture, the error bars show the standard deviation and different letters means statistically significant differences ($P < 0.05$).

the aroma compounds was observed. An increase in storage at RH to 75−97% was observed to be disadvantageous, since the capsules lost their structure and the aroma retention decreased notably. Minemoto et al. (*18*) encapsulated methyl linoleate with AG and stored capsules at different RH (at 37 °C) for 20 days. Oxidation of methyl linoleate was practically inexistent at 12 and 44% RH, whereas at 75 and 96% RH it was clearly observed. Recently, Jimenez et al. (*14*) reported that, after 60 days of storage at 35 °C, AG capsules showed structural damage at about 63−90% RH, which caused low retention of the encapsulated conjugated linoleic acid. The amount of oxidation products analyzed as *p*-anisidine value and hexanal content increased during 30 days of storage, with the highest being at 63% RH. The best retention and oxidative stability of conjugated linoleic acid in AG capsules was obtained at 44−52% RH. When stored at a higher temperature (at the same RH), the difference between storage temperature and glass transition temperature ($T - T_g$) has apparently been higher than that in the present study, meaning that the capsule matrices were more susceptible to change from the glassy to rubbery state.

Despite the bigger mean droplet size of the GGM emulsions compared to the AG emulsions, the GGM capsules were able to offer better protection for α-tocopherol at 33 and 66% RH. This result is in line with the study of Rusli et al. (*41*), in which emulsions of tuna oil/palm stearin and protein-glucose syrups were spray-dried to obtain microcapsules. They found that even though emulsions with the smallest droplet size (prepared with higher homogenization pressure) had the highest content of encapsulated oil, those emulsions oxidized more easily when stored at 23 °C for 4 weeks in the dark. The researchers suggested that the smaller droplets had a higher surface area compared to the bigger droplets and, thus, the encapsulating film around the oil droplets was thinner and further the protection ability of the capsule against oil oxidation was poorer than that of the capsule prepared from emulsion with the bigger droplets. The present study showed that GGM had the biggest droplet size in emulsion and the highest wall thickness in capsules, whereas in the case of AG the situation was the opposite. On the other hand, the mixture of GGM−AG had smaller droplets than GGM and similar wall thickness to AG, but it protected the encapsulated compound almost as well as GGM. However, the GGM and GGM−AG microcapsules differed from each other in their porous character, with GGM being more porous than GGM−AG. The air entrapped into the matrix during emulsification may take part in the oxidation of the encapsulated compounds, as suggested by Reineccius (*42*). However, the air intruded into the porous material after freeze-drying has a stronger impact on the oxidation of the encapsulated compound if oxygen is able to diffuse through the matrix. In the present study, several properties of the microcapsule matrices (e.g., physical state, glass transition temperature, wall thickness, and variation in the wall thickness) may have concurrently affected the ability of the microcapsule matrices to protect α-tocopherol. It was shown that GGM adsorbed less water and may have higher T_g under elevated RH conditions and, thus, have better physical stability, which means that α-tocopherol remains better protected against oxidation. The protection ability of GGM may also be the result of antioxidant compounds (phenolics) possibly present small in amounts in GGM (*43*).

To sum up, this study suggests that GGM, AG, and their mixture could be used as capsule material for hydrophobic compounds such as α-tocopherol. Among the capsule materials, AG showed the best ability to form emulsions, whereas the GGM microcapsules retained best the α-tocopherol during storage at elevated RH at 25 °C. Future research should focus on improving the encapsulation process with GGM. Moreover, the research examining the sensory properties of GGM or GGM microcapsules is needed when microcapsules are being considered for food purposes.

ABBREVIATIONS USED

AG, arabic gum; d_{43}, mean droplet size of emulsions as volume-length diameter; DSC, differential scanning calorimeter; FLD, fluorescence detection; GGM, *O*-acetyl-galactoglucomannans; HPLC, high performance liquid chromatography; RH, relative humidity; T_g, glass transition temperature; XRPD, X-ray powder diffraction.

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