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Storage Stability of Microencapsulated Cloudberry (*Rubus chamaemorus*) Phenolics

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Cloudberries (*Rubus chamaemorus*) contain phenolics (mainly ellagitannins), which have recently been related to many valuable bioactivity properties. In general, phenolics are known to react readily with various components, which may create an obstacle in producing stable functional components for food and pharmaceutical purposes. In this study, the aim was to improve the storage stability of cloudberry phenolic extract by microencapsulation. The phenolic-rich cloudberry extract was encapsulated in maltodextrins DE5−8 and DE18.5 by freeze-drying. Water sorption properties and glass transition temperatures (T_g) of microcapsules and maltodextrins were determined. Microcapsules together with unencapsulated cloudberry extract were stored at different relative vapor pressures (0, 33, and 66% RVP) at 25 °C for 64 days, and storage stability was evaluated by analyzing phenolic content and antioxidant activity. Compared to maltodextrin DE18.5, maltodextrin DE5−8 had not only higher encapsulation yield and efficiency but also offered better protection for phenolics during storage. Without encapsulation the storage stability of cloudberry phenolics was weaker with higher storage RVP. Microencapsulation improved the storage stability of cloudberry phenolics. The physical state of microcapsules did not have a significant role in the stability of cloudberry phenolics because phenolic losses were observed also in amorphous glassy materials. The antioxidant activity of the microencapsulated cloudberry extract remained the same or even improved slightly during storage, which may be related to the changes in phenolic profiles.

KEYWORDS: Cloudberry phenolics; ellagitannins; microencapsulation; storage stability; glass transition; water sorption; antioxidant activity

INTRODUCTION

Cloudberries (*Rubus chamaemorus*) are reddish orange berries that are widespread especially in the northern part of Finland. Besides having a delicious taste, cloudberries contain many valuable compounds such as vitamins C and E, essential oils, and phenolic compounds (*1*). Berry phenolics were shown to have beneficial properties such as antioxidant and antimicrobial activities (*2, 3*). They may also be associated with lower risk for heart diseases and cancer (*4, 5*). Even though cloudberries consist of many phenolic compounds such as ellagic acids and its glycoside derivatives, *p*-coumaric acid, gallic acid, flavan-3-ols, and quercetin (*3*), the dominating phenolic class is ellagitannins (*6*) (**Figure 1**). The main ellagitannins are sanguiin H6 dimer and lambertianin C trimer (*6*).

From a food technological perspective, it would be most applicable to benefit from cloudberry phenolics in powdered form. Phenolic-rich powders would be easy to handle and to use in food and pharmaceutical purposes. However, the susceptibility of most phenolics toward various chemical reactions may cause problems in the production of phenolic-rich cloudberry powders. Thus, storage may result in changes in phenolic content (*7−9*) and antioxidant activities (*10*) of berries and berry products, even though antioxidant activity can also remain constant during storage (*7*). For commercial interest, the phenolic-rich berry powder should maintain its quality (including phenolic content, bioactivity, and safety for human consumption) during prolonged storage under various storage conditions.

One promising way to stabilize phenolics is microencapsulation, which can be used to extend the shelf life of sensitive food components. Microcapsules are defined as microsize particles that consist of capsule material(s) and encapsulated component(s) (*11*), and they can be prepared by methods such as freeze- and spray-drying (*12, 13*). Depending on the case, microencapsulation can provide many advantages for phenolics such as improving either bioavailability or processing and storage stability (*14−17*), controlling release (*18*), enhancing solubility (*17*), and masking unpleasant taste. Phenolics have been encapsulated with various capsule materials such as yeast cells (*14*), cyclodextrins (*17*), mixtures of alginate and chitosan (*18*), gum arabic, and maltodextrins (*15, 16*). The natural

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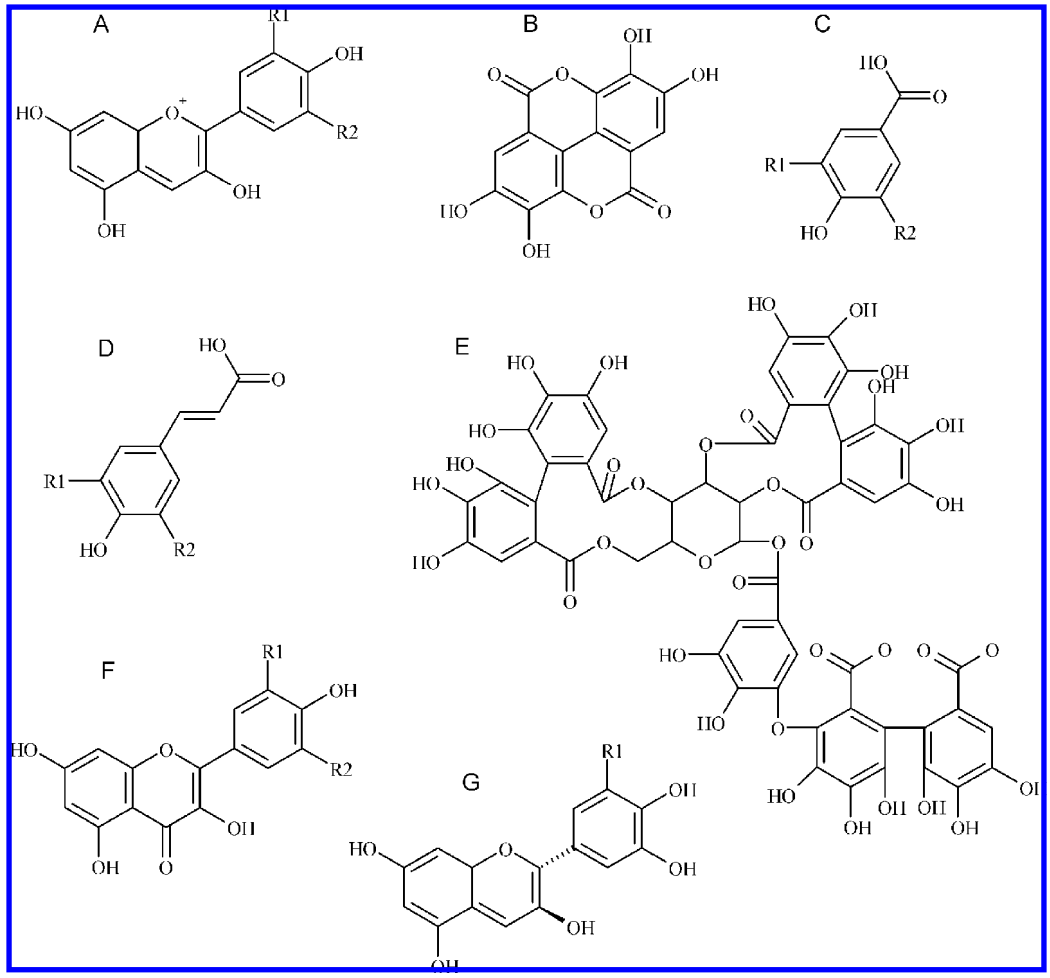

Figure 1. Chemical structures of anthocyanins (**A**), ellagic acid (**B**), hydroxybenzoic acids (**C**), hydroxycinnamic acids (**D**), ellagitannin dimer (**E**), flavonols (**F**), and flavan-3-ols (**G**).

phenolics that have been encapsulated in previous studies have mainly belonged to the phenolic classes of anthocyanins (*16*), flavonols (*17*), phenolic acids (*14*), or condensed tannins (*15*), which are the main phenolic classes of most berries and fruits. On the contrary, research related to the encapsulation of ellagitannins is scarce. One recent paper (*19*) introduced an ellagitannin-rich milk powder with oak flavor. The powder was prepared by boiling oak shavings with milk, after which the solution was filtered and spray-dried. In that powder, ellagitannins can be considered to be encapsulated into a milk powder matrix.

Maltodextrins with various average molecular weights are defined as hydrolyzed products of starch (*20*), and they belong to the most popular capsule materials used in the food field. Both hydrophobic and hydrophilic components such as natural pigments (*21, 22*), flavors (*13*), and oils rich in polyunsaturated fatty acids (*23*) have been encapsulated in maltodextrins. The encapsulation power of maltodextrins is based on their ability to form amorphous glassy matrices during the encapsulation process (*24*). Amorphous material can be produced at nonequilibrium conditions by removing the dispersing medium using evaporation or freezing or alternatively by rapid cooling of carbohydrate melts (*24*). The glassy matrix formed by carbohydrates, for example, maltodextrins, is a kind of solid network, which is thought to hold up by hydrogen bonds between carbohydrate chains (*25*). Encapsulated components are enclosed within the capsule matrix and, thus, protected from outward conditions.

The glass transition temperature (T_g) is considered to be a landmark for the stability of amorphous material because below T_g molecular mobility is extremely slow due to the high viscosity of the matrix and, as a consequence, the occurrence of many diffusion-controlled reactions is hindered (*24*). As long as the amorphous capsule matrix is stored at temperatures below the T_g, the capsule matrix remains glassy-like and it may protect the encapsulated component from various deteriorative changes (such as oxidation). If the capsule matrix is instead stored at temperatures above the T_g, the matrix changes from a glassy- to a rubbery-like state, and often its ability to protect the encapsulated component suffers. Water has a plasticizing effect on amorphous materials because it can disturb hydrogen bonds between carbohydrate chains and, as a consequence, decrease the T_g very efficiently (*25, 26*).

Although there have been studies concerning the encapsulation of natural phenolics originating from various sources, no studies exist using encapsulation to improve the storage stability of cloudberry phenolics. In this study, the aim was to investigate whether the microcapsules prepared with maltodextrins DE5−8 and DE18.5 could improve the storage stability including phenolic content and antioxidant activity of cloudberry phenolics at 25 °C under different relative vapor pressure (RVP) conditions. The second goal was to clarify whether the physical state of microcapsules could explain the storage stability of encapsulated components.

MATERIALS AND METHODS

Capsule Materials. The following maltodextrins were used as capsule materials: C*Dry A 01318 with DE18.5 (AP1891, Cerestar Scandinavia A/S, Holte, Denmark) and C*Dry MD 01955 with DE5−8 (AR2337, Cerestar Scandinavia A/S, Holte, Denmark), which were kindly donated by Cerestar Finland/A Cargill Co. (Helsinki, Finland). These maltodextrins were chosen because they differ from each other in their molecular weight and, as a consequence, in their physical properties. Therefore, their abilities to protect cloudberry phenolics were expected to be different. Water contents for maltodextrin DE5−8 and DE18.5 determined gravimetrically after oven-drying at 130 °C for 1 h were 4.7 and 4.2% (w/w of powder), respectively. Water content was taken into consideration when solutions were prepared for microencapsulation purpose.

Preparation of Cloudberry Phenolic Extract. The isolation of phenolic compounds from the cloudberries was carried out in triplicate as follows: 2.0−3.0 g of freeze-dried berry material was weighed into a centrifuge tube as six replicates, 20 mL of 70% aqueous acetone was added, and the sample was homogenized with an Ultra-Turrax for 1 min. Samples were centrifuged (1570*g*, 15 min), and the supernatants were collected. The procedure was repeated with another 20 mL of 70% aqueous acetone. Supernatants were combined, evaporated to dryness with a rotary evaporator, and dissolved in 15 mL of water. Samples were then applied to the glass column (300 mm × 40 mm) filled with XAD to remove sugars and organic acids with 6% aqueous acetonitrile. Phenolic compounds were eluted from the column with 100% acetonitrile, and the fraction was dried with a rotary evaporator, dissolved in water, and freeze-dried. Settings for the freeze-drying procedure for both berry material and extract were as follows: prefreezing, −20 °C for 10 min; primary drying, −20 °C/0.2 mbar for 2 h, −10 °C/0.2 mbar for 5 h, 0 °C/0.5 mbar for 10 h, 5 °C/0.5 mbar for 10 h, 10 °C/0.5 mbar for 4 h, 15 °C/0.5 mbar for 4 h, 20 °C/0.5 mbar for 1 h; secondary drying, 25 °C for 1 h; total time, 37 h (Heto FD8, Jouan Nordic A/S, Allerød, Denmark).

Preparation of Microencapsulated Cloudberry Phenolics. Maltodextrin DE5−8 or DE18.5 (9% w/w) and cloudberry phenolic extract (1% w/w) were dissolved into distilled water. The mixture was slightly heated (∼50 °C) and stirred for 30 min. The pH of solutions was measured and, in all cases, it was 2.9. Solutions were pipetted into the 20 mL brown glass vials as a portion of 4.5 mL. After that, the solutions were frozen at −20 and −80 °C for 2 and 19 h, respectively, and then placed into the freeze-dryer (Lyovac GT2 freeze-dryer, Amsco Finn-Aqua GmbH, Hürth, Germany) and dried for 48 h (pressure <0.1 mbar). As a reference, phenolic extract−water solution without maltodextrin was freeze-dried similarly. To prepare amorphous matrices without phenolics, maltodextrins (9% w/w) were dissolved in distilled water and solutions were freeze-dried as described above. The dried samples were powdered using a glass rod and placed into vacuum desiccators over P_2O_5 (Merck, Darmstadt, Germany) for at least 1 week before further usage. The abbreviations, which are used later in this text for the above introduced maltodextrins and microcapsules, are as follows: MD5−8, freeze-dried maltodextrin with DE5−8; MD18.5, freeze-dried maltodextrin with DE18.5; MC5−8, microencapsulated cloudberry phenolics prepared with maltodextrin DE5−8; MC18.5, microencapsulated cloudberry phenolics prepared with maltodextrin DE18.5.

Microscopical Observations. The surface morphology of dry microcapsules and unencapsulated phenolics was characterized by scanning electronic microscope Zeiss DSM962 (Carl Zeiss, Oberkochen, Germany). The samples were coated with platinum and placed on double-sided adhesive tape. Electron micrographs were obtained at 200× magnification at an acceleration voltage of 5−8 kV.

Analysis of Water Sorption. Approximately 30 mg of freeze-dried maltodextrins and microcapsules was weighed into the 4 mL brown glass vials. Samples were stored at 25 °C in vacuum desiccators over saturated salt solutions of LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $NaNO_2$, and NaCl (Merck) under RVP conditions of 11, 24, 33, 44, 54, 66, and 76%, respectively, providing water activity (a_w) of RVP/100 at equilibrium state (*27*). Water sorption of triplicate samples was determined gravimetrically as a weight gain along with time up to 7 days. Sample vials were weighed every day using a Mettler AT20 balance (Mettler Toledo AG, Greifensee, Switzerland). To avoid uncontrolled moisture sorption from the surrounding air, the sample vials were equipped with caps before each weighing. The Guggenheim−Anderson−de Boer (GAB) model was fitted to the data as described by Roos (*24*). The GAB equation has the form

$$m/m_m = (KCa_w)/[(1 - Ca_w)[1 + (K - 1)Ca_w]]$$

where m is experimental steady-state water content obtained after 24 h of storage, m_m is a monolayer water content, a_w is water activity, and C and K are parameters. For the GAB model, the a_w range of 0.11−0.76 was used to fit experimental sorption data.

Analysis of T_g. Freeze-dried maltodextrins and microcapsules (10−15 mg) were placed into the preweighed 40 μL aluminum crucibles (ME-51119870, Mettler Toledo AG) and rehumidified in vacuum desiccators at 25 °C for 24 h under the same RVP conditions as in the water sorption study. After rehumidification, sample crucibles were sealed hermetically with lids (ME-51119871, Mettler Toledo AG) before reweighing. The onset temperature of glass transition of triplicate samples was determined using a differential scanning calorimeter (DSC823, Mettler Toledo AG) equipped with software STARe DP V9.00 20070621. The equipment was calibrated with melting temperatures and enthalpies of *n*-pentane (−129.7 °C, 116.7 J g^{-1}), *n*-hexane (−95 °C, 151.8 J g^{-1}), mercury (−38.8 °C, 11.4 J g^{-1}), distilled water (0.0 °C, 334.5 J g^{-1}), gallium (29.8 °C, 80.0 J g^{-1}), and indium (156.6 °C, 28.5 J g^{-1}). To purge the measuring cell and prevent moisture condensation, a flow of dry nitrogen (50 mL min^{-1}) was used. The samples were scanned three times over the glass transition region (first heated, then cooled, and again heated) at a rate of 5 °C min^{-1} using an empty crucible as a reference. The data (together with corresponding water contents from water sorption study) were modeled by the Gordon−Taylor equation

$$T_g = (w_1T_{g1} + kw_2T_{g2})/(w_1 + kw_2)$$

where T_g is the T_g of the mixture containing solids and water, T_{g1} is the T_g of the solids, T_{g2} is the T_g of amorphous water [−135 °C according to Johari et al. (*28*)], w_1 is the weight fraction of solids, w_2 is the weight fraction of water, and k is a parameter (*26*).

Critical a_w and Water Content (m). Critical values are those values of a_w and m that decrease the T_g to the storage temperature, meaning 25 °C in this study (*29*). The critical w_2 was calculated using the Gordon−Taylor equation, and w_2 was further used in calculation of the critical m. After solving for critical m, critical a_w was taken from the GAB water sorption isotherm.

Analysis of Phenolics. Phenolic contents of the cloudberry extract and microcapsules were determined by high-performance liquid chromatography (HPLC) coupled with diode array detection (DAD).

Preparation of Cloudberry Extract and Microcapsules for the HPLC Analysis. One milligram of freeze-dried cloudberry extract was dissolved in 1 mL of water and filtered through a 0.45 μm PTFE filter (GHP Acrodisc, hydrophilic polypropylene, Pall Lice Sciences) prior to analysis by HPLC. Microcapsules (1 mg) were weighed (Sartorius ME5, Hamburg, Germany), and 1 mL of methanol was added. The solution was vortexed (10 s) and centrifuged (Eppendorf Minispin) for 60 s at 9300*g*, and the methanol fraction was collected. A pellet was then dissolved in 1 mL of water by vortexing until dilution to obtain water fraction. Both methanol and water fractions were analyzed using HPLC. Phenolics in the methanol fraction were considered to be surface phenolics, and phenolics in the water fraction were considered to be encapsulated phenolics.

HPLC Analysis. The HPLC system consisted of a Waters 2690 separations module (Waters, Bedford, MA), a Waters PDA 996 diode array detector, and Millennium 32 software. The column was a Nova-Pak C18 (150 × 3.9 mm, 4 μm, Waters) equipped with a C18 guard column. For detection, 280 nm (for hydroxybenzoic acids, ellagitannins, and proanthocyanidins), 320 nm (for hydroxycinnamic acids), 365 nm (for ellagic acids and flavonols), and 520 nm (for anthocyanins) were the wavelengths recorded. The HPLC analysis was performed according to the method outlined by Kähkönen et al. (*3*).

Encapsulation Yield (EY) and Encapsulation Efficiency (EE). EY defines the percentage of the initially added ellagitannins that were inside microcapsules after the freeze-drying process. EY was calculated as follows:

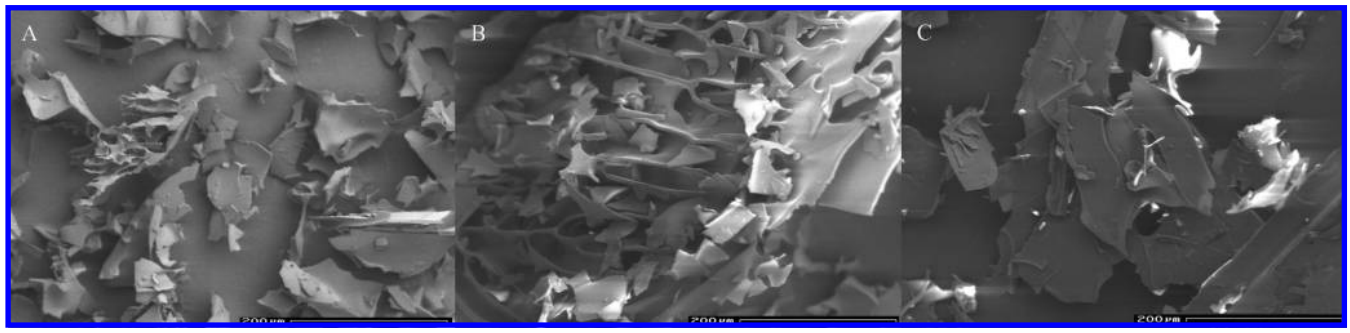

Figure 2. Scanning electron micrographs of dry microcapsules MC5−8 (**A**) and MC18.5 (**B**) and unencapsulated cloudberry extract (**C**).

$$\text{EY}\ (\%) = \text{encapsulated ellagitannins (mg/g)} / \text{calculated value of added ellagitannins (mg/g)} \times 100$$

EE describes the percentage of each phenolic compound that really was encapsulated into microcapsules. EE was calculated as follows:

$$\text{EE}\ (\%) = \text{encapsulated phenolics (mg/g)} / [\text{encapsulated phenolics (mg/g)} + \text{surface phenolics (mg/g)}] \times 100$$

Analysis of Sugar and Organic Acid Content. The sugar content of the cloudberry extract was analyzed by using gas chromatography (GC). The GC instrument used was a Hewlett-Packard 5890 series II gas chromatograph equipped with a flame ionization detector (FID) using splitless injection, injector, and autosampler (Hewlett-Packard, Palo Alto, CA). The column used for all separations was an HP 5 capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA). The monosaccharide standards used were arabinose, xylose, glucose, and galactose. The samples were first reduced with $NaBH_4$ and then acetylated with acetic acid anhydride according to the method of Blakeney et al. (*30*).

Organic acids were analyzed according to a modified method originally described by Tatár et al. (*31*). The chromatographic instrument used was an Agilent 1200 series HPLC equipped with a PDA, an autosampler, an injector, a binary pump, a column oven, and an Agilent Zorbax SB-C18 Stable Bond column (4.6 × 150 mm, 5 μm) with a C18 guard column (Agilent Technologies). For detection, a wavelength of 210 nm was recorded. Organic acid standards used were malic acid, L-ascorbic acid, citric acid, benzoic acid, and sorbic acid.

Antioxidant Activity Assay. Antioxidant activities of microcapsules and unencapsulated cloudberry extract were tested by using liposome lipid oxidation test. The liposome oxidation model system was performed as described earlier (*32*). The liposomes were prepared from soybean lecithin (concentration of 0.8 mg/mL in samples) and water. Samples in triplicate were dissolved in methanol and put into the Erlenmeyer flasks at concentrations of 4.2 and 8.4 μg/mL and dried under nitrogen. Oxidation was started by adding 30 μL of 3 μM cupric acetate. The inhibition against liposome oxidation was calculated at day 3 by measuring the formation of conjugated diene hydroperoxides spectrometrically at a wavelength of 234 nm (Perkin-Elmer λ 25 UV−VIS spectrometer, Norwalk, CT) and hexanal by headspace gas chromatography (Perkin-Elmer Autosystem XL gas chromatography equipped with Perkin-Elmer HS40XL headspace autosampler, Shelton, CT).

Storage Stability Test. Unencapsulated cloudberry extract and microcapsules were stored in desiccators at 0, 33, and 66% RVP at 25 °C for 0, 16, 32, and 64 days. These storage RVP conditions were chosen because they represent typical RVP values of room air during the winter (~30%) and summer (~60%) in Finland. Storage stability was monitored by analyzing the phenolic content and antioxidant activity on each sampling day.

Statistical Analysis. Statistical analyses were done by one-way ANOVA using SPSS 15.0.1 (SPSS Inc., Chicago, IL). The level of significance, p, was <0.05.

RESULTS AND DISCUSSION

Composition of Unencapsulated Cloudberry Extract. The phenolic profile of cloudberry extract consisted of ellagitannins (61.7%), proanthocyanidins (11.8%), hydroxybenzoic acids (10.0%), hydroxycinnamic acids (10.0%), ellagic acid (3.2%), flavonols (2.8%), and anthocyanins (0.4%). Similar results were also obtained earlier (*3*). Cloudberry extract also contained 6% (w/w) fructose and glucose and a trace amount of organic acids (data not shown).

Visual Images of Microcapsules. Dry microcapsules were obtained as salmon-pink powders. SEM images showed that microcapsules had smooth surfaces and flake-like structure (**Figure 2**), which is typical of microcapsules prepared by freeze-drying (*13*). Powder particles differed in their sizes and shapes because they were powdered after the encapsulation process (*12*). The SEM images of MC5−8 and MC18.5 did not differ from each other, whereas unencapsulated cloudberry extract seemed to have a slightly thinner flake structure compared to the encapsulated versions.

Encapsulation Yield. The EY was remarkably better in MC5−8 (EY = 79%) than in MC18.5 (EY = 48%). These EY values are fairly high and comparable to values obtained by Che Man et al. (*13*). In their study, a water-soluble durian flavor was encapsulated in maltodextrins with various DE by freeze-drying and spray-drying. It was observed that even though the highest EY (58.7−83.9%) was obtained with the higher molecular weight (DE12−15) maltodextrin, also the lower molecular weight maltodextrins (DE14−16 and DE18) had fairly high EY values (50.1−73.2 and 48.3−71.6%, respectively).

Because freeze-drying as a very mild encapsulation method is recommended for sensitive compounds such as hydrolyzable tannins (*33*), it was expected that freeze-drying would not cause remarkable losses of cloudberry ellagitannins. However, it is possible that before the drying process, during the preparation of water dispersions, some ellagitannins were hydrolyzed or oxidized because dispersions were prepared in the presence of ambient oxygen and, besides, the dispersions were slightly heated (~50 °C) and the pH of dispersions was very acidic (pH 2.9). Lei (*34*) found that major ellagitannins of oaks (castalagin and vescalagin) underwent chemical transformations in acidic water solution even at 23 °C, even though the biggest changes were observed at 60 °C. The degradation of ellagitannin was dependent on time and the type of ellagitannin, and the degradation rate increased if oxygen was present instead of nitrogen. Degradation yielded ellagic acids and affected discoloration of aqueous solutions. However, in the present study, the water dispersions were prepared in a 0.5 h; thus, no dramatic changes in ellagitannin content were expected to occur. No visible changes in the color of water dispersions were observed.

Encapsulation Efficiency. MD5−8 appeared to be a clearly better capsule material alternative than MD18.5 for ellagitannins because the EE values were 99 and 56%, respectively. Also, all other phenolic compounds were encapsulated more efficiently into MD5−8 (EE = 54−94%) than into MD18.5 (EE =

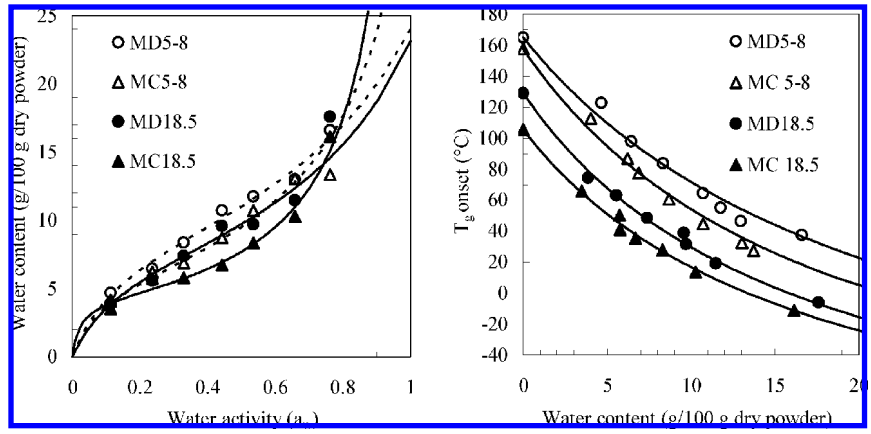

Figure 3. (**A**) GAB water sorption isotherms of microcapsules (MC5−8 and MC18.5, solid lines) and maltodextrins (MD5−8 and MD18.5, dashed lines) at 25 °C. (**B**) Glass transition temperature as a function of water content for microcapsules (MC18.5 and MC5−8) and maltodextrins (MD18.5 and MD5−8). The glass transition temperatures were predicted using the Gordon−Taylor equation (solid lines).

41−69%). Interestingly, it seemed that MD5−8 encapsulated best those phenolics with higher molecular weight (ellagitannins, proanthocyanidins, and flavonols, EE = 99, 94, and 90%, respectively), whereas the best EE values of MC18.5 were for low molecular weight acids (hydroxycinnamic acids and hydroxybenzoic acids, EE = 69 and 68%, respectively).

These results suggest that MD5−8 (with higher molecular weight) possibly formed tighter complexes with phenolics than MD18.5 (with lower molecular weight), and as a consequence higher EE values were obtained. It is a known phenomenon that phenolics such as tannins and flavonols may form complexes with polysaccharides (e.g., starch), and the affinity of phenolics to polysaccharides depends on the water solubility, molecular size, conformational mobility, and shape of the polyphenol (*35*).

Water Sorption Properties. Water contents increased with increasing storage RVP (**Figure 3A**). In contrast to previously reported results (*29*), MD5−8 adsorbed slightly more water than MD18.5 up to $a_w = 0.66$, having higher water sorption only at a_w 0.76. The difference between the results may be explained by the somewhat different compositions of maltodextrins used in these studies. Maltodextrins are mixtures of oligo- and polysaccharides with different chain lengths, and this fact makes variability between the compositions of maltodextrin products (*20*). The other explanation for differences between the results may be attributed to the differences in sample sizes. In this study, very small sample sizes were used for both freeze-dried maltodextrins and microcapsules due to limited supply of cloudberry extract. Identical conditions in the water sorption study were desired for both microcapsules and maltodextrins and, therefore, a small sample size was also used for maltodextrins. Maltodextrins as such adsorbed water more readily than microcapsules. This suggests that maltodextrins and cloudberry phenolics interacted during the microencapsulation process by forming complexes and, thus, the hydrophobic nature increased compared to maltodextrins alone.

The GAB model proved to be suitable for modeling experimental data over the $a_w$range up to 0.54, whereas at higher a_w water sorption isotherms did not settle themselves on the experimental data points (**Figure 3A**). Water sorption isotherms of the maltodextrins and microcapsules showed sigmoidal shape, which is typical of amorphous food material (*24*). The shapes of isotherms for MD5−8 and MC5−8 appeared to be quite similar over the whole a_w range. On the contrary, the shapes of isotherms for MD18.5 and MC18.5 resembled each other only at the a_w range up to 0.66. The monolayer water content (m_m) could be calculated using the GAB model. m_m is defined as the amount of water that is sufficient to form a layer of water molecules of the thickness of one molecule on the adsorbing surface (*24*). m_m values for maltodextrins MD5−8 and MD18.5 (**Table 1**) were in line with those of previous studies, where m_m for maltodextrin DE4−12 and for maltodextrins DE20−23 (at 25 °C) varied between 6.36−9.47 and 4.79−6.46, respectively (*25, 29*), all of these showing that m_m values of maltodextrin increased with decreasing DE values. m_m values for MC5−8 and MC18.5 were slightly lower compared to those for maltodextrins, which means that under the same storage conditions microcapsules may lose their stability more easily than maltodextrins as such. m_m values of microcapsules differed slightly from those values reported by Serris and Biliaderis (*22*) for maltodextrin DE5 and DE20 encapsulated beetroot pigment at 30 °C (6.26 and 5.30 g/100 g of dm, respectively).

Table 1. GAB Monolayer Water Content (m_m), Parameter k (Gordon−Taylor Equation), Critical Water Content (Critical m), and Critical Water Activity (Critical a_w) for Microcapsules and Freeze-Dried Maltodextrins

material		m_m (g/100 g of dm)	k	critical m (g/100 g of dm)	critical a_w
microcapsules	MC5−8	7.5	5.47	15.2	0.79
	MC18.5	4.1	5.84	8.6	0.55
maltodextrins	MD5−8	8.8	4.50	19.4	0.88
	MD18.5	6.2	6.04	10.8	0.57

Glass Transition Temperatures. The anhydrous T_g values were estimated to be 165 and 129 °C for maltodextrins MD5−8 and MD18.5, respectively, and 158 and 105 °C for microcapsules MC5−8 and MC18.5, respectively. These results confirm the previous findings according to which increasing M_w increased also the T_g of maltodextrins (*26*). However, determination of the anhydrous T_g of each freeze-dried material was found to be quite challenging because clear stepwise change in the baseline of the DSC thermograms could not be observed. The unencapsulated cloudberry extract was also analyzed by DSC using a temperature range of −50 to 180 °C. However, glass transitions could not be found, which was apparently due to the heterogeneous character of the extract. Only an exothermic peak at ∼150 °C and an endothermic peak at ∼160−170 °C were observed, with the latter most likely related to the degradation of the phenolics.

Maltodextrins as such seemed to have higher T_g as compared to microcapsules. This indicates that the cloudberry extract

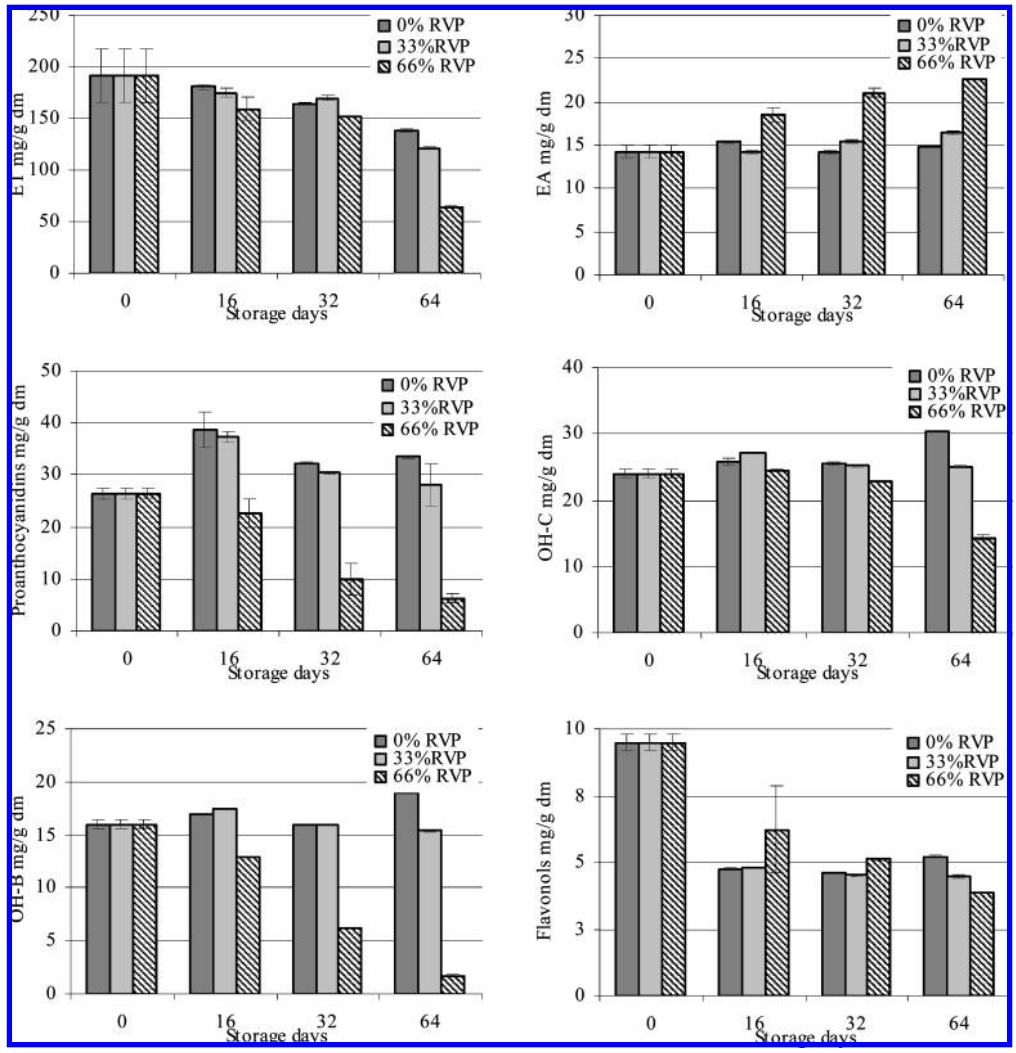

Figure 4. Effect of storage at 25 °C at different RVPs (0, 33, and 66%) on content of unencapsulated cloudberry extract. Phenolic contents are expressed as milligrams per gram of dry matter. Abbreviations: ET, ellagitannins; EA, ellagic acids; OH-C, hydroxycinnamic acids; OH-B, hydroxybenzoic acids.

contained low M_w components, which decreased the T_g of microcapsules. In cloudberry extract, phenolic acids and flavonols (representing 26.4% of phenolics) had fairly low molecular masses (approximately 300−350 and 600 Da, respectively), whereas ellagitannins (61.7% of phenolics) and proanthocyanidins (11.8% of phenolics) were of higher molecular mass (<2000 and <3000 Da, respectively). Organic acids decrease the T_g of amorphous materials because their T_g values are very low; for example, Adhikari et al. (*36*) reported that the T_g of anhydrous citric acid (the common acid in fruits) can be as low as 12 °C. Besides phenolics, cloudberry extract contained also 6% (w/w) of fructose and glucose, for which anhydrous T_g values were reported to be 5 °C (*37*) and 38 °C (*36*), respectively.

Water clearly had a plasticizing effect on maltodextrins and microcapsules, which was seen as a decrease in T_g with increasing water content of amorphous materials (**Figure 3B**). This result agreed well with previous studies (*25, 26*). The Gordon−Taylor equation, by which experimental data were modeled, was seen to be applicable to predict the plasticizing effect of water on maltodextrins and microcapsules (**Figure 3B**).

Critical Values. Critical m and a_w, meaning the values that decrease the T_g of the material to 25 °C, were higher for MD5−8 than MD18.5 (**Table 1**). These results are parallel to those of a previous study (*29*), even though the reported critical values for maltodextrins DE4−7 and DE20−23 were lower ($a_w = 0.70$ and 0.55 and $m = 11.2$ and 9.4, respectively) than our values. It appeared that critical m differed notably from the m_m values, being about 2 times higher (**Table 1**) as also observed by Roos (*29*). Indeed, m_m as a measure of food stability has been criticized because it totally ignores the plasticizing effect of water and, therefore, the critical values were considered to be more usable tools for predicting dry food stability (*29*). According to critical values, both microcapsules should be in a glassy state when stored at ≤33% RVP, whereas only MC5−8 is in glassy state when stored at ≤66% RVP.

Effect of Storage on the Phenolic Content. The storage stability of the unencapsulated cloudberry extract and microcapsules when stored at different RVPs (25 °C) was followed for 64 days.

Ellagitannins (ET) and Ellagic Acids (EA). Unencapsulated ellagitannins were sensitive to storage under all conditions tested (**Figure 4**). At 0 and 33% RVP, a statistically significant decrease in ET content was observed after 64 days of storage, but at 66% RVP, the degradation of ET was significant already after 32 days. At the end of the storage period, only 73% (at 0% RVP), 63% (at 33% RVP), and 34% (at 66% RVP) of the original amount of ET remained in the powder. In contrast, the content of EA was higher with greater storage RVP. After 64

days, EA contents were 104% (at 0% RVP), 116% (at 33% RVP), and 159% (at 66% RVP) of the original EA content.

The losses of ET in cloudberry extract can probably be attributed to hydrolysis of ET, which liberates EA. Hydrolysis of ET has been suggested to occur during various processes and storage in previous studies. Aaby et al. (*8*) observed that strawberry puree made from flesh retained all ET during 16 weeks of storage at 22 °C. On the contrary, purees made from strawberry homogenate and achene-enriched fraction lost 20−28% of their initial ET and, at the same time, the concentration of EA almost doubled, which was explained by the hydrolysis of ET. In another study, the free EA content of raspberries increased 2.5-fold during jam processing (*9*). The authors speculated that cooking released EA from the ET structure or, alternatively, EA could be more easily extracted from processed product than from berries as such. Lei (*34*) found that the main ET of oak wood degraded in aqueous solutions even at room temperature in the absence of oxygen. Hydrolysis was thought to be one of the changes oak ET underwent because the results from the HPLC analyses of the degradation reactions indicated the formation of EA.

All above-mentioned ET losses occurred under moist conditions (purees, jams, aqueous solutions). In our study, a decrease in ET content occurred also under low RVP conditions. Thus, the losses of ET could not be explained only by hydrolysis reaction but also oxidation. Oxidation may probably explain the results obtained by Salminen (*33*). He observed that in freeze-dried and powdered birch leaves the concentration of the main ET (pedunculagin derivate) decreased during 1 year of dry storage in sealed plastic bags at 22 °C in the dark. In the presence of oxygen radical or other catalysts (such as iron, which might be present as trace amounts in maltodextrins), ET may polymerize into oligomeric and polymeric structures via a radical-mediated oxidation pathway (*35, 38*). The reaction is analogous to autoxidation of polyunsaturated lipids (*35*). The oxidation of phenolics may result in the formation of quinones and other yet unknown products. In our study, the disappearance of ET may partly be explained by the formation of ET oligomers and polymers even though ET only up to trimeric structures could be analyzed with the HPLC method used.

The color of phenolic powders changed from reddish to brown only when stored at 66% RVP. A reason for this discoloration may be the hydrolysis or oxidation of ET. Previously, Lei (*34*) reported that hydrolysis and nonspecific reactions of oak ET led to brown discoloration of water solutions. In another study (*39*), it was observed that autoxidative reactions yielded visual browning in the ET-rich wines and juices. However, it should be mentioned that, besides ET, also other phenolics might form brown discoloration (*35*).

Encapsulation was advantageous for the storage stability of ET (**Table 2**). However, the stability of encapsulated ET was strongly dependent on storage RVP and the capsule material used. MD5−8 proved to be a better capsule material than MC18.5. Most of the ET was completely encapsulated into MC5−8, and only traces of ET occurred on the surfaces of microcapsules. During the storage period, the ET content in MC5−8 remained constant at 0 and 33% RVP, whereas at 66% RVP it decreased significantly already at an early stage of storage. MD18.5 was found to be a poor capsule material for ET because almost half of the total amount of ET was located on the surfaces of microcapsules. ET content in MC18.5 declined at every RVP (least at 0% and most at 66% RVP), even though a statistically significant drop could be observed only at 66% RVP. The content of surface ET in MC18.5 decreased during storage, which was expected because the unencapsulated surface phenolics were readily available to interact with the surrounding moisture and oxygen.

Encapsulation with either type of maltodextrin resulted in decreased formation of EA during 32 days of storage. However, after 32 days of storage, the content of EA in microcapsules increased rapidly, being >2-fold at 33% RVP and >3-fold at 66% RVP. The content of EA was lower in MC5−8 than in MC18.5. The proportion of EA on the surface of MC5−8 remained unchanged during the whole storage period, whereas in the MC18.5 the contents of surface EA increased after 32 days.

Proanthocyanidins. Proanthocyanidins in unencapsulated cloudberry extract remained unaltered at 0 and 33% RVP (**Figure 4**). Indeed, their content even increased slightly during the first 16 days but started to decline thereafter. At 66% RVP, the content of proanthocyanidins decreased significantly during the whole storage period, and only 24% of the initial proanthocyanidins were left after 64 days of storage. The instability of proanthocyanidins was also found in the other studies: for example, concentration of proanthocyanidins in strawberry purees decreased when the purees were stored at 22 °C for 8−16 weeks (*8*), and the content of proanthocyanidin monomers, catechins, in liquid blueberry extract decreased remarkably during storage at 23 °C for 60 days (*10*). Microencapsulation improved the stability of proanthocyanidins at 66% RVP even though losses were observed (**Table 2**).

Hydroxycinnamic Acids (OH-C). The content of OH-C in unencapsulated extract remained constant or even increased slightly at low RVP (**Figure 4**). On the contrary, OH-C was not stable at 66% RVP, and after 64 days of storage, only 60% of the original OH-C was left. In the study of Srivastava et al. (*10*), the content of OH-C decreased even more drastically when liquid blueberry extracts were stored in the capped glass bottles at 23 °C for 60 days. García-Alonso et al. (*40*) observed that the total OH-C content in the jelly-type berry dessert decreased slowly (from 72 to 57 mg/kg) during storage at 21 °C for 12 months. In our study, the encapsulation improved the storage stability of OH-C. MC5−8 protected OH-C against loss under any storage conditions tested—even at 66% RVP the content of OH-C remained unchangeable (**Table 2**). As opposed to the excellent protecting ability of MC5−8, MC18.5 could not prevent the loss of OH-C at ≥33% RVP.

Hydroxybenzoic Acids (OH-B). OH-B were stable under dry storage conditions (**Figure 4**). When stored for 64 days at 33% RVP, the content of OH-B decreased only slightly (6%), whereas during storage at 66% RVP, the content decreased to the level of 11% of the original content. Compared with all other cloudberry phenolics, OH-B seemed to be more susceptible to moisture. OH-B are probably the most hydrophilic phenolic compounds among the cloudberry phenolics, which can undoubtedly explain their higher instability compared to other phenolics during storage at elevated RVP.

MC5−8 maintained all OH-B when stored at 0 and 33% RVP (**Table 2**). At 66% RVP, the microcapsules could not provide perfect protection for OH-B, but after 64 days of storage, 72% of the original OH-B remained inside microcapsules, which is a good result when compared to the content of OH-B of unencapsulated version. Not only MC5−8 but also MC18.5 offered some protection for OH-B at 66% RVP—63% of original OH-B was left after the storage period.

Flavonols. At all RVP, the flavonol contents in unencapsulated extract declined significantly already during 16 days of storage to the level (<55% of the initial amount) maintained until the end of storage (**Figure 4**). At 66% RVP, the flavonol losses were highest (59%). These results are consistent with the study of Zafrilla et al.

Table 2. Phenolic Contents on the Surface of Microcapsules (Surface) and Inside Microcapsules (Encapsulated) during Storage at Different RVPs at 25 °C (Mean ± Standard Deviation)

phenolic compound	storage time (days)	MC5−8[b]						MC18.5[b]					
		0% RVP		33% RVP		66% RVP		0% RVP		33% RVP		66% RVP	
		surface	encapsulated	surface	encapsulated	surface	encapsulated	surface	encapsulated	surface	encapsulated	surface	encapsulated
ellagitannins (mg/g)[a]	0	0.07 ± 0.04 a	6.74 ± 1.91 a	0.07 ± 0.04 a	6.74 ± 1.91 a	0.07 ± 0.04	6.74 ± 1.91 a	3.26 ± 0.27 a	4.14 ± 1.10 ab	3.26 ± 0.27 a	4.14 ± 1.10 a	3.26 ± 0.27 a	4.14 ± 1.10 a
	16	nd	9.55 ± 2.76 a	0.01 ± 0.02 a	6.93 ± 1.33 a	nd	3.99 ± 1.56 ab	1.40 ± 1.02 b	2.67 ± 0.35 b	0.40 ± 0.05 b	2.52 ± 0.67 a	0.17 ± 0.04 b	3.23 ± 0.97 ab
	32	nd	10.48 ± 2.08 a	0.02 ± 0.02 a	6.88 ± 2.23 a	nd	1.77 ± 0.71 b	0.90 ± 0.86 b	6.32 ± 2.26 a	1.93 ± 1.35 ab	3.73 ± 1.55 a	0.12 ± 0.03 b	1.90 ± 0.33 b
	64	0.01 ± 0.01 b	8.44 ± 0.86 a	0.01 ± 0.01 a	8.08 ± 3.80 a	nd	0.69 ± 0.16 b	0.54 ± 0.20 b	2.03 ± 0.54 b	0.55 ± 0.21 b	1.76 ± 0.57 a	0.19 ± 0.03 b	1.68 ± 0.43 b
proanthocyanidins (μg/g)[a]	0	134 ± 39 a	2162 ± 296 c	134 ± 39 a	2162 ± 296 b	134 ± 39 a	2162 ± 296 a	473 ± 46 b	1611 ± 408 b	473 ± 46 c	1611 ± 408 ab	473 ± 46 a	1611 ± 408 a
	16	374 ± 475 a	3518 ± 455 a	73 ± 34 a	3168 ± 186 a	73 ± 63 a	1957 ± 159 a	1435 ± 140 a	1775 ± 279 b	1491 ± 95 a	1410 ± 165 b	695 ± 267 a	1554 ± 277 ab
	32	nd	2593 ± 183 bc	44 ± 76 a	2427 ± 581 ab	nd	1038 ± 108 b	1262 ± 325 a	1675 ± 172 b	1156 ± 362 ab	1479 ± 116 b	384 ± 23 a	942 ± 66 b
	64	83 ± 2 a	3284 ± 326 ab	115 ± 75 a	2816 ± 222 ab	nd	1214 ± 172 b	732 ± 62 b	2873 ± 386 a	724 ± 56 bc	2254 ± 331 a	nd	1029 ± 51 ab
hydroxycinnamic acids (μg/g)[a]	0	390 ± 12 a	1772 ± 171 b	390 ± 12 a	1772 ± 171 a	390 ± 12 a	1772 ± 171 a	663 ± 15 a	1489 ± 28 a	663 ± 15 b	1489 ± 28 a	663 ± 15 a	1489 ± 28 a
	16	359 ± 247 a	2101 ± 17 a	254 ± 38 a	1967 ± 57 a	158 ± 121 b	1726 ± 152 a	863 ± 779 a	1103 ± 3b	1209 ± 131 a	1049 ± 73 b	747 ± 226 a	1147 ± 89 b
	32	154 ± 6 a	2110 ± 10 a	276 ± 141 a	1873 ± 161 a	63 ± 5 b	1866 ± 62 a	935 ± 42 a	1295 ± 91 ab	1047 ± 55 a	1090 ± 31 b	693 ± 9 a	1156 ± 28 b
	64	213 ± 5 a	2204 ± 9 a	265 ± 170 a	2002 ± 146 a	82 ± 50 b	1875 ± 36 a	1093 ± 23 a	1291 ± 128 ab	1064 ± 78 a	1172 ± 62 b	676 ± 53 a	1247 ± 36 b
hydroxybenzoic acids (μg/g)[a]	0	229 ± 7 a	1275 ± 40 b	229 ± 7 a	1275 ± 40 a	229 ± 7 a	1275 ± 40 a	467 ± 19 b	1024 ± 32 a	467 ± 19 b	1024 ± 32 a	467 ± 19 a	1024 ± 32 a
	16	148 ± 90 ab	1519 ± 58 a	95 ± 34 b	1403 ± 16 a	59 ± 64 b	1236 ± 94 ab	746 ± 55 a	817 ± 20 b	806 ± 64 a	762 ± 61 b	483 ± 78 a	845 ± 68 b
	32	69 ± 2 b	1511 ± 73 a	89 ± 20 b	1313 ± 89 a	13 ± 3 b	1129 ± 6 b	682 ± 46 a	883 ± 29 b	767 ± 47 a	761 ± 78 b	339 ± 31 b	749 ± 3b
	64	95 ± 6 b	1504 ± 14 a	76 ± 70 b	1376 ± 105 a	11 ± 15 b	921 ± 15 c	658 ± 7 a	891 ± 57 b	650 ± 108 a	793 ± 45 b	268 ± 28 b	643 ± 18 c
flavonols (μg/g)[a]	0	74 ± 9 a	636 ± 32 b	74 ± 9 a	636 ± 32 a	74 ± 9 a	636 ± 32 a	262 ± 10 a	487 ± 18 a	262 ± 10 ab	487 ± 18 a	262 ± 10 a	487 ± 18 a
	16	7 ± 12 b	796 ± 35 a	5 ± 9 b	610 ± 148 a	6 ± 11 b	462 ± 75 c	110 ± 10 b	361 ± 24 c	127 ± 10 c	350 ± 28 b	93 ± 11 c	406 ± 59 ab
	32	5 ± 9 b	738 ± 29 a	34 ± 23 ab	625 ± 77 a	nd	538 ± 17 ab	223 ± 76 a	415 ± 13 b	296 ± 72 ab	354 ± 22 b	128 ± 8 b	358 ± 14 bc
	64	20 ± 4 b	441 ± 6 c	28 ± 19 b	421 ± 31 b	5 ± 5 b	393 ± 14 c	189 ± 1 ab	273 ± 16 d	195 ± 4 bc	253 ± 12 c	130 ± 5 b	283 ± 2 c
ellagic acids (μg/g)[a]	0	523 ± 133 a	598 ± 10 b	523 ± 133 a	598 ± 10 b	523 ± 133 a	598 ± 10 b	754 ± 50 b	517 ± 37 b	754 ± 50 b	517 ± 37 b	754 ± 50 b	517 ± 37 b
	16	462 ± 146 a	675 ± 37 b	518 ± 205 a	577 ± 29 b	442 ± 119 a	564 ± 34 b	817 ± 208 b	484 ± 98 b	701 ± 36 b	409 ± 24 bc	780 ± 29 b	454 ± 22 b
	32	590 ± 201 a	673 ± 69 b	636 ± 182 a	540 ± 100 b	508 ± 101 a	512 ± 23 b	1013 ± 106 ab	469 ± 128 b	879 ± 186 b	355 ± 51 c	751 ± 68 b	400 ± 44 b
	64	1106 ± 636 a	948 ± 91 a	696 ± 149 a	776 ± 57 a	610 ± 38 a	1851 ± 121 a	1340 ± 218 a	1283 ± 213 a	1229 ± 162 a	1081 ± 97 a	1256 ± 181 a	1813 ± 395 a

[a] Values obtained at different storage times for each phenolic compound followed by different letters are significantly different ($p < 0.05$). nd, not detected. [b] Surface, microcapsules were extracted with methanol; encapsulated, microcapsules were extracted with water.

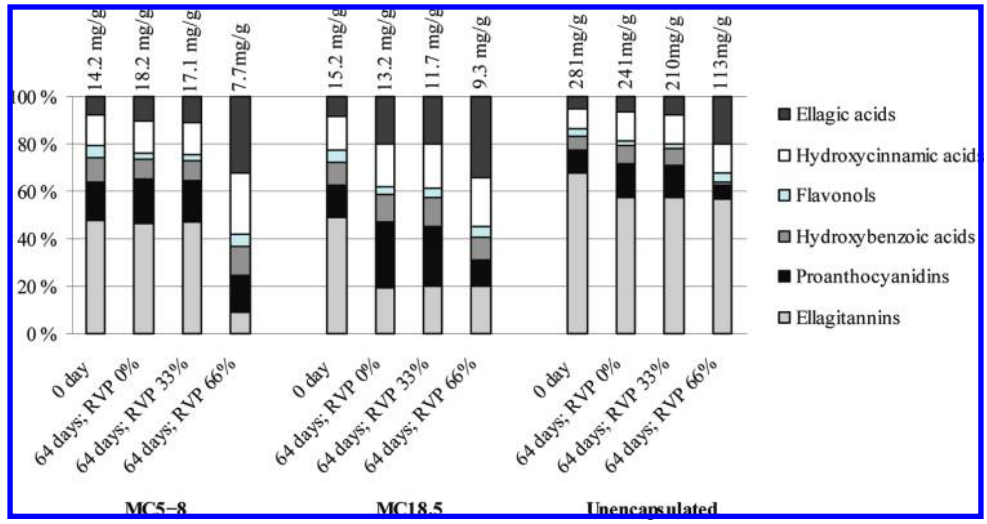

Figure 5. Phenolic profile of microcapsules (MC5−8 and MC18.5) and unencapsulated cloudberry extract after storage at 0, 33, and 66% RVP (25 °C) for 0 and 64 days.

(*9*), in which flavonols of raspberry jam were observed to be sensitive to storage. Already after 1 month of storage of jam at 20 °C, notable losses of flavonols (kaempferol 3-glucoside and quercetin 3-glucoside) were observable. Six months of storage of raspberry jam at 20 °C resulted in 40−50% flavonol losses. García-Alonso et al. (*40*) stored berry desserts at 8, 21, and 30 °C for 12 months and found that the flavonol losses were higher with higher storage temperature.

MD5−8 improved the stability of flavonols at ≤33% RVP for up to 32 days, whereas the stability at 66% RVP could not be enhanced (**Table 2**). MD18.5 was not able to protect flavonols at any RVP. It seemed that flavonols were better protected when the capsule material was more hydrophobic. Previously, flavonols (myricetin and quercetin) have been encapsulated with cyclodextrins, which are cyclic oligosaccharides derived from starch (*17*). Flavonol oxidation was inhibited in the presence of cyclodextrins due to the complexation of the flavonol in the hydrophobic cavity of cyclodextrin.

Total Phenolic Content (Calculated as a Sum of Contents of Individual Phenolic Compounds Analyzed). In unencapsulated extract and MC18.5, phenolic losses were observed at all RVP, and the losses increased (from 13 to 39% and from 14 to 60%, respectively) with increasing RVP from 0 to 66% (**Figure 5**). In MC5−8, total phenolic content remained constant or surprisingly even increased during storage at 0 and 33% RVP, whereas at 66% RVP only 54% of the phenolics were left at the end of the storage. Increases in berry phenolics during storage have also been reported earlier: for example, the levels of total phenolics of fresh raspberries increased 1.5-fold during 8 days of storage at 20 °C (*41*). In another study (*7*), the content of total phenolics in raspberries increased from 100 to 111% when storage conditions after the harvest of fresh berries to the supermarket and onto the consumer's table were mimicked (4 °C for 3 days and then 18 °C for 24 h). Also, Nohynek et al. (*38*) showed that the content of total phenolics in frozen cloudberries increased during 12 months of storage. They stated that this unexpected increase was due to structural changes in ET molecules or in other phenolic compounds.

Phenolic Profile. At the beginning of the storage (day 0) both microcapsules and unencapsulated extract had similar phenolic profiles having ET, proanthocyanidins, and OH-C as their main phenolics (**Figure 5**). During storage for 64 days at 0 and 33% RVP, the most significant changes in the phenolic profile of unencapsulated extract occurred in the proportion of ET that decreased from 68 to 57% and in the proportion of proanthocyanidins and OH-C, which increased from 9 to 12−14%. In contrast to the unencapsulated extract and MC18.5, phenolic profiles of MC5−8 remained practically unchanged at 0 and 33% RVP, further adding to the conclusion that microencapsulation with MD5−8 improved the storage stability of cloudberry phenolics. At 66% RVP neither unencapsulated extract nor microcapsules managed to avoid changes in phenolic profiles. The main phenolic compounds in unencapsulated extract after storage at 66% RVP for 64 days were ET (57%), EA (20%), and OH-C (13%), whereas EA (32%), OH-C (26%), and proanthocyanidins (16%) were the predominant phenolic compounds in MC5−8, and EA (34%), OH-C (21%), and ET (20%) dominated in MC18.5.

Effect of Storage on Antioxidant Activities. The antioxidant activities of encapsulated and unencapsulated cloudberry extract were at the same level and remained quite unaltered during storage (**Table 3**). The values of inhibition of conjugated diene and hexanal formation varied between 62 and 76 and between 71 and 84%, respectively, indicating moderate antioxidant activities. Interestingly, the highest antioxidant activity was obtained with MC5−8 after 64 days of storage at 66% RVP when the total amount of phenolic compounds decreased almost 50% and notable alterations in the phenolic profile were observed (**Figure 5**).

Phenolic content did not go hand in hand with antioxidant activity because a decrease or increase in phenolic content during storage did not seem to affect antioxidant activities. A similar phenomenon has been observed in other studies. Mullen et al. (*7*) found that during storage at 4 °C for 3 days and at 18 °C for 24 h the phenolic content of raspberries varied but that no changes were observed in antioxidant activities. In another study, despite the changes in phenolic content, the antioxidant activity of strawberry purees decreased only slightly when stored at 22 °C for 16 weeks (*8*). García-Alonso et al. (*40*) described that the phenolic content in berry dessert diminished during 1 year of storage at 8, 21, and 30 °C, whereas the total antioxidant activity of dessert remained practically unaltered (at 8 °C) or decreased slightly (at 21 and 30 °C).

The minor changes in antioxidant activities may be explained by the alteration in the phenolic profiles (**Figure 5**). The major changes in phenolic profiles were observed among the proportions of ET, EA, proanthocyanidins, and OH-C. All of these phenolics are known to possess antioxidant activities. For example, Zafrilla

Table 3. Antioxidant Activities (Expressed as Percent Inhibition of Hexanal or Conjugated Diene Formation) of Unencapsulated Cloudberry Extract and Microcapsules (MC5−8 and MC18.5) during Storage at Different RVPs at 25 °C for 64 Days[a]

storage		unencapsulated		MC5−8		MC18.5	
RVP (%)	time (days)	hexanal (%)	conjugated dienes (%)	hexanal (%)	conjugated dienes (%)	hexanal (%)	conjugated dienes (%)
0	0	80 ± 1 a	68 ± 1 a	77 ± 0 a	69 ± 0 a	81 ± 1 a	68 ± 1 a
	32	74 ± 4 a	67 ± 5 a	77 ± 5 a	66 ± 9 a	73 ± 5 b	63 ± 7 a
	64	79 ± 4 a	70 ± 5 a	79 ± 3 a	71 ± 3 a	78 ± 3 ab	70 ± 4 a
33	0	80 ± 1 a	68 ± 1 a	77 ± 0 ab	69 ± 0 ab	81 ± 1 a	68 ± 1 a
	32	72 ± 5 a	64 ± 8 a	71 ± 5 b	62 ± 5 b	72 ± 4 b	62 ± 5 a
	64	80 ± 4 a	72 ± 5 a	82 ± 1 a	75 ± 1 a	75 ± 2 ab	67 ± 5 a
66	0	80 ± 1 a	68 ± 1 a	77 ± 0 b	69 ± 0 ab	81 ± 1 a	68 ± 1 a
	32	77 ± 2 a	69 ± 3 a	76 ± 2 b	68 ± 3 b	73 ± 3 b	64 ± 6 a
	64	80 ± 0 a	73 ± 2 a	84 ± 2 a	76 ± 4 a	81 ± 3 a	72 ± 3 a

[a] Values obtained at different storage times at each RVP followed by different letters are significantly different ($p < 0.05$).

et al. (*9*) reported that in raspberry jams the antioxidant activity of EA was similar to that of gallic acid (belongs to OH-B), catechins (proanthocyanidin), and kaempferol (flavonol) but higher than that of caffeic acid and ferulic acid (belong to OH-C). Phenolic compounds are typically "team players", meaning that they work synergistically by supporting the antioxidant activities of one another (*42*). The loss of original phenolics might compensated by phenolics formed with equal or improved antioxidant activities (*42*). For example, oxidation of hydrolyzable tannins, such as ET, can lead to oligomerization through phenolic coupling, which consequently increases the number of reactive sites and enhances antioxidant activity (*43*). Moreover, hydrolysis of ET may improve the antioxidant activity by increasing the number of free hydroxyl groups (*8*).

Effect of Matrices and Their Physical State on Stability of Phenolics. In this study, microcapsules were stored below their critical values (except MC18.5 at 66% RVP) and, therefore, if the glassy state of the capsule matrix will be the determining factor for the stability of phenolics, the content of each phenolic compound should remain unaltered during the whole storage period. According to the same logic, MC18.5 at 66% RVP should not protect phenolics because those storage conditions exceeded critical values and the capsule matrix was expected to be in a rubbery state. However, the results showed that the physical state of matrices did not play the main role in the storage stability of cloudberry phenolics.

In the glassy state, MC5−8 maintained ET and all other phenolics at ≤33% RVP, whereas at 66% RVP, MC5−8 failed to protect cloudberry phenolics except OH-C. MC18.5 in the glassy state (at ≤33% RVP) offered some protection for ET and proanthocyanidins but no protection for other phenolics. However, it is noteworthy that the T_g values of MC5−8 and MC18.5 (at 66% RVP and 33% RVP, respectively) were quite close to storage temperature ($T - T_g = -8$ and -16 °C, respectively), which could explain the poor protection ability. The reason for the considerably better stability of encapsulated phenolics in MC5−8 than in MC18.5 can be due to the higher molecular weight of capsule material and as a consequence higher T_g values/critical values.

Maltodextrins protected cloudberry phenolics better at RVP 33% than at RVP 66% and, compared with MC18.5, MC5−8 had higher protection ability at both RVPs. Our results parallel the study of Serris and Biliaderis (*22*) in which the calculated half-life periods of maltodextrin DE5 and DE20 encapsulated beetroot pigment at 30 °C were the shortest at $a_w = 0.64$. The longest half-life periods were obtained at $a_w = 0.23$ with maltodextrin DE5 as capsule material, whereas under the same storage conditions maltodextrin DE20 provided a clearly shorter half-life for beetroot pigment. Cai and Corke (*21*) studied the retention of water-soluble betacyanin pigment in various maltodextrins (DE10−25) during 16 weeks of storage at different relative humidities (RH) (25 °C). They found that at 32% RH, the retention of pigment was lower in maltodextrins with higher DE values, whereas at 5% RH, the situation was opposite—pigment retention was higher, the lower was the DE value.

A glassy state does not necessarily guarantee the stability of encapsulated components. For example, Serris and Biliaderis (*22*) observed that the water-soluble beetroot pigment encapsulated in maltodextrin DE5 and DE20 degraded even when matrices were in the glassy state. In another study, Grattard et al. (*23*) found that T_g values of the maltodextrin (DE2, DE21, and DE40) matrices did not seem to control the rate of flaxseed oil oxidation in microcapsules. Moreover, Selim et al. (*44*) found that saffron carotenoids encapsulated in pullulan and PVP degraded also when stored at temperatures below T_g. The researchers proposed that the degree of protection offered by capsule matrices was dependent not only on the physical state but also on other factors such as the porosity and microstructure of polymeric matrices (*44*). It is possible that also in our study the porous structure of freeze-dried glassy matrices enabled oxygen to penetrate the matrix structure and, as a consequence, oxidization of phenolics even in glassy state was possible.

In conclusion, this study revealed that all cloudberry phenolics as such suffered from storage at elevated RVP (25 °C). Microencapsulation enhanced the storage stability of cloudberry phenolics. MD5−8 proved to be better than MD18.5 as a capsule material for cloudberry phenolics. The results demonstrate that the protective effect of microcapsules toward phenolics is associated not only with the physical state of microcapsule matrices but also with some other factors including the porous structure of the maltodextrin matrix. Neither microcapsules nor unencapsulated cloudberry extract lost their antioxidant activities during storage.

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