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Effects of Cross-Linking, Capsule Wall Thickness, and Compound Hydrophobicity on Aroma Release from Complex Coacervate Microcapsules

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Microcapsules were produced by complex coacervation with a gelatin−gum acacia wall and medium-chain-triglyceride core. Dry capsules were partially rehydrated and then loaded with model aroma compounds covering a range of volatility, hydrophobicity, and molecular structure. An experimental design was prepared to evaluate the effects of cross-linking, wall/core ratio, and volatile load level on aroma release from capsules in a hot, aqueous environment. The real-time release on rehydration was measured by monitoring the headspace of a vessel containing the capsules to proton transfer reaction mass spectrometry (PTR−MS). Data collected showed no effects of cross-linking or wall/core ratio on volatile release in hot water for any of the volatiles studied. When comparing real-time release of the prepared coacervates to a spray-dried equivalent, there was no difference in the release from hot water but the release was slower when coacervates were added to ambient-temperature water. We found volatile release to be primarily determined by compound partition coefficients (oil/water and water/air) and temperature.

KEYWORDS: Complex coacervation; encapsulation; release; cross-linking; PTR−MS; hydrophobicity

INTRODUCTION

Encapsulation refers to techniques by which a material is coated or entrapped within another material forming a protective shell or wall (*1*, *2*). The main purposes of producing dry flavorings are to convert liquid compounds into a powder easy to handle and to provide a protection against oxidation and evaporation (*3*, *4*). The materials composing the wall or coating vary from technique to technique as well as with the ultimate application. The most common wall materials are carbohydrates (e.g., maltodextrins, modified starches, and gum acacia), proteins (e.g., gelatin or whey protein), cellulose, or combinations of these materials. The flavoring material can be either entrapped as such or diluted in a matrix, such as oil (*3*). Several literature reviews detail the various encapsulation methods along with their strengths and weaknesses (*3*−*5*).

Several techniques exist to manufacture dry flavorings through a variety of processes each providing unique characteristics. This study focuses on encapsulation via complex coacervation. Complex coacervation is a "true" encapsulation (shell−single-core structure) of oil droplets into a colloidal material in solution. Coacervation is based on electrostatic interactions between one or more polymers formed around an emulsified phase. Complex coacervate formulations and process parameters have been extensively studied. Schmitt et al. (*6*) and Burgess (*5*) have provided in depth reviews regarding the optimization of several manufacturing parameters in forming complex protein−polysaccharide coacervates.

Cross-linking in capsule formation is an optional process that can modify the structure and properties of the coacervate microcapsules. The role of cross-linking is described as to harden the wall material after the formation of the capsules (*7*). The goal of hardening the capsules is to make them more stable during drying and also to confer some unique properties to the wall material, such as modifying the physical state (change of the glass transition temperature). The chemical cross-linking agents used link hydroxyl residues on polysaccharides and/or amine residues on the protein polymer. Typically, formaldehyde and glutaraldehyde have been used as cross-linking agent in the fertilizer and pharmaceutical industries (*8*, *9*). There are some toxicology issues on using formaldehyde and glutaraldehyde in food applications. No published data could be found on the effects of cross-linking on the release of encapsulated material, in particular, for encapsulated volatiles.

Complex coacervation has been investigated intensively for pharmaceutical applications and as drug carriers for targeted delivery (*10*). For these applications, capsule formation parameters have been optimized to obtain a desired drug release profile. The parameters focused on include particle size, water-transport dynamics (*8*), wall composition and ratio (*11*), effect of the degree of cross-linking (*7*), and drug solubility (*12*). While

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there is extensive literature available on the release of drugs, genes, or proteins from complex coacervates, limited published data are available to date on the release of volatiles (i.e., aroma compounds) from such microcapsules.

It is generally accepted that aroma is a key factor determining food acceptance. However, it is well recognized that it is not the absolute presence of volatiles in a food that determines their perception but their release (rate and quantity) (*13, 14*). For this reason, to fully characterize a flavor encapsulation system, it is desirable to characterize the release of aroma compounds from it in a given application. In the past decade, technological developments in analytical instruments allow online, real-time measurements via mass spectrometry (MS) (*15, 16*), such as with proton transfer reaction mass spectrometry (PTR−MS). The principles behind this method have been described in detail elsewhere (*16*−*18*). PTR−MS has been extensively used in such applications including breath analysis (medical applications) (*16*), aroma release during eating (*13*), and nose and sensory perceptions (*19*−*21*).

The two main mechanisms for aroma release from coacervate microcapsules are the mechanical destruction of the capsule wall leading to rapid leakage of the encapsulated material into the surrounding system and the slower diffusion of the active component from the core through the intact wall. Both approaches are used in pharmaceutical applications. For food applications, if capsules are above a certain size, they maybe degraded by chewing, liberating the encapsulated material in the mouth. For all other situations, release principally occurs via diffusion, i.e., hydration of the wall such that it becomes permeable to the core material (*4*). The diffusion rate will be affected primarily by the shape and speed of the water front entering the particle shell and the characteristics of the shell polymer, i.e., glass transition, strength, and cohesiveness of the network. As a result, the dynamic swelling or rate of water (solvent) uptake of dried particles is a critical parameter for release. One would also expect particle size and thickness of the capsule wall to affect the particle swelling rate.

The diffusional release rate also depends upon the ease and rate at which the encapsulated material can migrate through the porous wall material. For this reason, works published in the pharmaceutical area do not necessarily apply in flavor applications because aroma compounds are relatively small molecules and differ greatly in chemical properties (e.g., water solubility and volatility) versus typical pharmaceuticals. The release profiles of aroma compounds from coacervate capsules are, therefore, expected to be very different from published data on drug molecules.

In the work presented herein, we report on the dynamic release of aroma compounds from capsules produced by complex coacervation. This study focused on determining the influence of capsule manufacturing parameters, such as wall thickness and cross-linking of wall materials, on the release profiles of various aroma compounds differing in chemical properties.

MATERIALS AND METHODS

Materials and Chemicals. Gelatin 250 Bloom strength, 20 mesh, type A provided by PB−Leiner (Davenport, IA) and gum Arabic (Acacia seyal, FT powder, TIC Gums, Belcamp, MD) were used as wall materials in the formation of microcapsules. Medium-chain triglyceride oil (MCT, Lumulse CC-33K, Lambent Technologies, Gurnee, IL) was used as core material.

Capsul, a octanyl-succinate anhydrous substituted starch (National Starch Corp., Bridgewater, NJ), was used as an encapsulation matrix in spray drying.

The aroma compounds used were purchased from Aldrich Chemicals (St Louis, MO) at the highest purity available, except for β-damascenone, which was provided by Robertet Flavors, Inc. (Piscataway, NJ). 2-Butanone, β-damascenone, and methyl-pyrrole were used at 100 ppm (i.e., each 12.5% of the pure compounds mixture), and methylpropanal was used at 500 ppm (w/w of oil present, 62.5% of the pure compounds mixture).

Preparation of Microcapsules. Microcapsules were prepared by complex coacervation using the following process. A total of 8 g of gum acacia and 12 g of gelatin were dispersed in 450 mL of deionized (DI) water heated at 45 °C in a stainless-steel beaker using an overhead stirrer (RW20 digital, IKA works, Wilmington, NC) rotating at 350 rpm. pH was adjusted to 4.5 with hydrochloric acid (10% aqueous solution). The unflavored liquid core material (MCT, 40, 80, or 120 g for the 3 wall-thickness levels) was emulsified into the hydrocolloid dispersion (600 rpm on stirrer) for 25 min, maintaining the temperature at 45 °C. After emulsification, 400 mL of DI water (35 °C) was added, the stirring rate was reduced to 300 rpm, and the system was slowly cooled to 13 °C: first to room temperature (about 25 °C in about 2 h) and second using a water bath filled with ice water (cooling from 25 to 13 °C in about 1.5 h).

In the case of cross-linked capsules, the following step was added: while maintaining the system at about 13 °C and stirring at 300 rpm, the pH was adjusted to 9 with sodium hydroxide (5% aqueous solution) and 2 g of cross-linking agent was added (glutaraldehyde, 50% solution in water, Aldrich Chemicals). The cross-linking stage was continued for about 2 h at 13 °C and then allowed to reach room temperature for the next 12 h.

Capsules were collected: scooped from the surface of the vessel and rinsed with DI water. Collected capsules were deposited on stainless-steel trays and cooled to −30 °C in a blast freezer. After 24 h, the frozen capsule slurry was freeze-dried (FTS Systems, Stone Ridge, NY) for 48 h, under the following parameters: chamber temperature of −30 °C and vacuum of 100 mTorr.

Flavor Loading Method. On the basis of preliminary experiments, loading aroma compounds into the microcapsules during their formation led to significant losses. This was due to volatilization (the process is carried out at 45 °C for at least 30 min) and partitioning into the water phase (which is discarded). An alternative loading method based on the procedure detailed in U.S. patent 6,106,875 (*22*) was used. The procedure consisted of spreading 10 g of freeze-dried capsules on a sieve (#140, mesh 106 μm) over a steam flow (2 m s^{-1}) until all capsules were moist. Capsules were then transferred into a 50 mL glass jar with a Teflon lid. A total of 20 μL of a mixture of pure compounds (listed above and in the proportion desired) was added to the jar, which was then closed, shaken vigorously for 5 min, and allowed to equilibrate for 24 h. A total of 1 g of finely ground silica (Syloid 244, Grace Davison, Columbia, MD) was then added to the jar and mixed well to absorb moisture from the capsule walls, thereby sealing them from volatile loss. Capsules remained in the closed jar until analysis.

Capsule Characterization. *Microscopy.* The structure, shape, and formation of microcapsules during manufacture were observed by microscopy using a bright field microscope (Carl Zeiss, Inc., Thornwood, NY), mounted with a digital camera (Olympus Evolt E330, Japan). Images were analyzed with ImageJ software (National Institute of Health, Bethesda, MD). The images obtained were used to determine the structure of capsules (mononuclear versus aggregates or polynuclear) and wall shape and to estimate the wall thickness. Pictures of similar capsules have been published in previous work (*23*).

Wettability. Wettability of dry capsules is being defined as the capacity to swell in the presence of a solvent or water. Dry particles were fixed onto double-faced tape on a microscopy slide. A drop of DI water (room temperature) was added to the slide, and a slip cover was added, moving the water onto the capsules. To determine the time to complete hydration of the dry particles, the microscope was mounted with a digital camera in "video" mode. Time to complete hydration is reported as the time difference between the addition of the water to the slide and the moment the capsules cease swelling, judged visually.

Size Distribution. Particle size distribution of capsules was determined using light scattering (Malvern Series 2600 particle size analyzer, Malvern Instruments, Inc., Malvern, Worcestershire, U.K.) using

methanol as the solvent (spectrophotometric grade, 99% purity, Sigma-Aldrich). The size distribution was characterized by its mean diameter, standard deviation, and type of distribution (i.e., uni- or bimodal). Data gathered are the De Broucker means, measured by the laser scattering instrument. Results reported are the average of triplicate samples. Particle size measurements were confirmed by data collected by microscopy.

Flavor Load. Surface "oil" of capsules was determined by first weighing 0.5 g of capsules into a 20 mL headspace vial. A total of 5 mL of dodecane (Aldrich Chemicals) containing 1000 ppm of internal standard (heptane, Aldrich Chemicals) was added to the vial, which was then capped with a Teflon septa and shaken at 2000 rpm for 2 min (Table Shaker Laboratory Line Orbit No. 3590, Lab-line Instruments, Inc., Melrose, IL). A total of 3 mL of solvent was removed with a 3 mL glass syringe mounted with a syringe filter (0.45 μm pores, nylon, Fisher Scientific, Pittsburgh, PE) to remove any floating capsules. A total of 1 μL of the solvent was then injected into gas chromatograph (GC, 5890, Hewlett-Packard, Wilmington, DE). The GC−FID was equipped with a DB-5 column (J&W Scientific, Folsom, CA), 30 m × 0.25 mm × 0.25 μm. The GC operating parameters were injection port, 225 °C; detector, 250 °C; column head pressure, 12 psi; split ratio, 1:50; oven temperature program, 43 °C/6 min/15 °C min^{-1}/110 °C/20 °C min^{-1}/200 °C/2 min. Quantification was performed by dividing the peak area of the aroma compound by that of the internal standard and comparing this ratio to a pre-established calibration curve created under the same analytical conditions. Data reported represent the average of triplicate extractions (one injection per solvent extraction).

Total flavor load of the capsules was determined by first weighing 2 g of capsules into a 20 mL headspace vial and then adding 7 mL of DI water containing 0.025 g of protease (Validase BNP L, Valley Research, South Bend, IN). The vial was sealed with a Teflon septum, heated at 60 °C for 5 min, and then placed on the shaker Table (1500 rpm) at room temperature for 18 h. The sample was allowed to rest for 1 h after shaking, and then 3 mL was transferred into a new 20 mL headspace vial. A total of 3 mL of propylene glycol (Aldrich Chemicals) containing 1000 ppm of internal standard (heptane, Aldrich Chemicals) was added to the vial, which was then sealed and vortexed for 1 min. A total of 1 μL of this extract was then injected into the GC−FID setup as detailed above. Quantification was performed by dividing the peak area of the aroma compound by that of the internal standard and comparing this ratio to a pre-established calibration curve created under the same analytical conditions. Data reported represent the average of triplicate extractions (one injection per solvent extraction).

Dynamic Release: PTR−MS Setup. The objective of this part of the study was to evaluate the release of encapsulated volatiles from the prepared capsules in the presence of water. For this determination, the headspace purging system published by Lindinger et al. (*24*) was used. The system is described below.

A total of 50 mg of capsules were weighed into a water jacketed glass cell (total volume of 250 mL), thermostatted at 70 °C. The glass cell was closed at the top by a stainless-steel lid, which also supported a heated, double-jacketed burette (100 mL total volume) setup to empty its contents into the sample cell. The burette−cell system was placed in an oven (85 °C) to maintain temperatures while manipulating the samples and avoiding any condensation of released volatiles. Sample purge gas (150 sccm) entered the burette and then flowed through the sample vessel (when opened to allow water to enter the sample cell). The purge gas coming from the cell (loaded with any volatiles released from the sample) was diluted by air (2000 sccm) to avoid overloading of the PTR−MS. This diluted sample effluent was directly sampled by the PTR−MS (Ionicon Analityk, Innsbruck, Austria). Only about 20 sccm of the diluted sample gas (2150 sccm) was introduced into the PTR−MS.

The PTR−MS parameters were set as follows: drift tube voltage, 600 V; drift tube temperature, 60 °C; drift tube pressure, 2.1 mbar; quadrupole (SEM) voltage, 2800 V; quadrupole pressure, 3.5 × 10^{-5} mbar.

The best ion (on the basis of abundance and uniqueness) for each volatile compound monitored was selected in preliminary experiments. The instrument was setup using multiple-ion detection, using 0.1 s dwell time on each mass. The following *m*/*z* were monitored in the study: 21, 37 (water cluster), 73 (methyl-propanal), 82 (*N*-methyl-pyrrole), 87 (diacetyl), and 191 (β-damascenone).

Table 1. Aroma Compounds Used in This Study and Their Physical−Chemical Properties

compound	molecular mass (ion used in PTR−MS measurement)	vapor pressure (mm Hg at 75 °C)	log *P* value
diacetyl	86 (87)	23	−1.34
methyl-propanal	72 (73)	33	0.74
N-methyl-pyrrole	81 (82)	1.1	1.43
β-damascenone	191 (191)	<1	4.21

Data Analysis (from PTR−MS). The PTR−MS instrument software provides data in counts per second for each mass recorded. The counts are then transformed into concentration as given by the following equation (*18*, *25*). The equation takes into account the operating parameters of the reaction chamber

$$(RH^+)_{ppb} = \frac{(RH^+)_{counts} 10^9 U_{drift} 2.8 \cdot 22400 P_{atm}{}^2 T_{drift}{}^2}{k 9.2 (H_3O^+)_{corr.counts} P_{drift}{}^2 N 273.15^2 transm_{(RH^+)}}$$

where $(RH^+)_{ppb}$ is the concentration of the compound in the gas phase, $(RH^+)_{counts}$ is the counts per second of the ion representing the compound, $(H_3O^+)_{corr.counts}$ is the counts per second of ion 21 corrected with m/z_{21} transmission factor and multiplied with the isotopic factor (500), U_{drift} is the voltage in the drift tube (V), P_{atm} is 1013 mbar, T_{drift} is the temperature in the drift tube (333.15 K), k is the reaction rate constant ($\approx 2 \times 10-9$ cm^3 s^{-1}), P_{drift} is the pressure in the drift tube (2.1 mbar), N is Avogadro's number (6.022×10^{23} mol $^{-1}$), and $transm_{(RH^+)}$ is the transmission factor in quadrupole of *m*/*z* value of RH^+.

The transformed data were then plotted in terms of ppb versus time.

Data Analysis. The release is considered only in the context of a hot beverage application; thus, time zero on all figures is when hot water (75 °C) was added to hydrate the microcapsules. The release was monitored for a total of 5 min after water addition. The release is typically characterized by the maximum intensity, time to maximum intensity, and persistence (or burst). A minimum of three replicates per sample type were analyzed and used for statistical analysis. The curves presented are the average replicated for a given sample.

Statistical Analysis. Analysis of variance (ANOVA) was conducted to determine the effects of, respectively, capsule cross-linking (2 levels), wall thickness (3 levels), and aroma properties (4 hydrophobicity levels) on both the time to maximum intensity and relative intensity at 0.5 min (i.e., persistence). Statistical significance was determined at $\alpha = 0.05$ for each factor. Analyses were performed with the R package software (R-2.7.1, http://www.r-project.org/). Modeling of decay curves was performed using the R package software as well.

RESULTS AND DISCUSSION

The four compounds studied in this paper were chosen because of their differences in physical and chemical properties, namely, molecular structure and size, volatility, and hydrophobicity. A summary of these properties is presented in **Table 1**.

Effect of Aroma Compound Properties on Release. A typical release profile from cross-linked coacervated capsules is presented in **Figure 1a**. First of all, one should note that the maximum intensity (I_{max}) is reached almost instantaneously for the four compounds after the addition of hot water. Looking more closely, there are differences in I_{max} between the four compounds, even though *N*-methyl-pyrrole, diacetyl, and β-damascenone were present in similar initial concentrations (surface and total load). However, the time to I_{max} is not significantly different for any of the compounds, even if slightly longer in

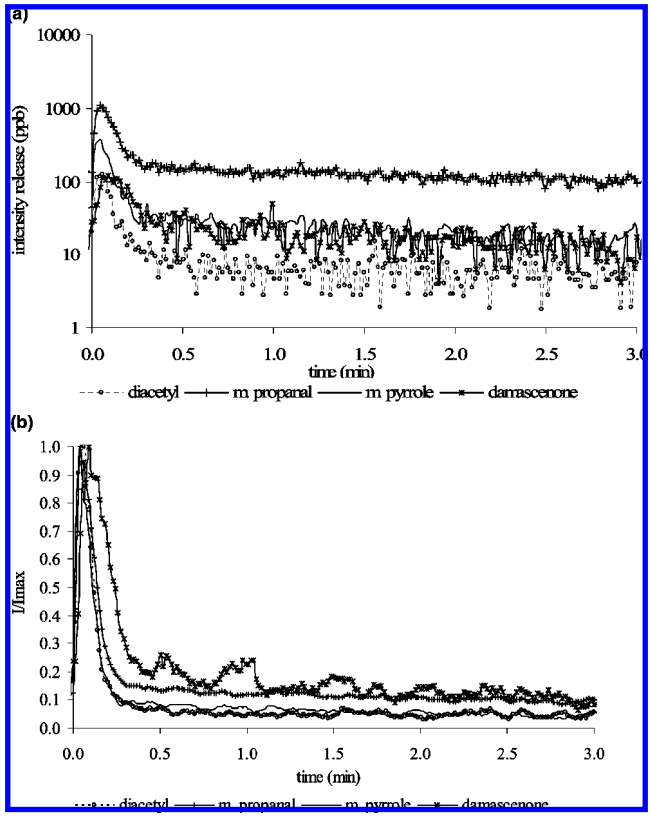

Figure 1. (**a**) Absolute release and (**b**) relative release (I/I_{max}) of volatiles from coacervate microcapsules, cross-linked and intermediate wall thickness (made with 80 g of oil). Time 0 is the moment when hot water was added. Each curve is an average of triplicate runs.

the case of β-damascenone (3−4 versus 6−8 s). The difference in volatility and hydrophobicity of the compounds does not affect the initial release profile.

The differences in amounts of the compounds released makes comparisons of release persistence difficult. For this reason, data have been converted to an I/I_{max} format and then plotted against time (**Figure 1b** and the following). This presentation format allows us to determine more confidently that the release occurs as a burst for the four compounds. Most of the release occurs within 0.5 min of the addition of hot water. There is a substantial difference between the compounds regarding the length of the burst. At 0.5 min, the relative intensity of diacetyl and *N*-methyl-pyrrole is about 10% of I_{max}, whereas it is about 15 and 20% for methyl-propanal and β-damascenone, respectively. However, the difference between the two groups cannot be explained by either the difference in volatility (methyl-propanal and β-damascenone are the most and least volatile compounds, respectively), molecular mass, or structure and only partially by their hydrophobicity (β-damascenone has the highest hydrophobicity and highest persistence at 0.5 min). It is important to note as well that the complete purging of the headspace volume occurs within 1 min of water addition (150 mL of headspace purged at 150 sccm). This indicates that the release is mostly immediate.

In summary, volatile release from cross-linked, intermediate shell thickness coacervated capsules is similar for the four compounds studied regarding the I_{max}, time to I_{max}, and burst pattern. There are some differences in the length of burst, but they are small and, thus, of questionable significance in influencing flavor perception. It appears that differences in volatile release that one may expect across compounds because of their differing chemical and physical properties are minimized by the use of very hot water.

Effect of Cross-linking. Extensive literature is available on the effects of various cross-linking agents on coacervate capsule structure and water holding capacity (*6*, *8*, *9*, *24*−*27*). For our study, non-cross-linked (clk) capsules were prepared following the same manufacturing steps as the cross-linked capsules, but the cross-linking agent was omitted.

The release burst from non-clk capsules is similar to that from clk capsules (**Figure 1b**) and therefore not presented in the paper. The time to I_{max} for all four compounds in non-clk capsules does not statistically differ from the clk capsules. The absolute values for I_{max} were also similar in the two cases. In addition, the burst lasted about the same time (10−25% of I_{max} at 0.5 min for all four compounds) as for clk capsules. These observations indicate that, for the two types of capsules, the release was immediate and not significantly influenced by the cross-linking of the wall polymers.

In addition, the times to maximum swelling on water addition showed no statistical difference between clk and non-clk capsules (4 ± 1.2 s in both cases from the addition of water until no more visible increase in size). This indicates that the addition of cross-linking agent did not affect the water uptake kinetics and that, therefore, this mechanism does not limit volatile release. This observation is in agreement with some previous findings but in contradiction to others. For example, Nixon et al. (*30*) reported no slowing of drug release between cross-linked and non-cross-linked, polynuclear microcapsules. They indicated that release could be explained by a model assuming simple diffusion through a thin membrane. Factors such as particle size and surface area in contact with the aqueous environment were key. However, Robert and Buri (*7*) and Kumbar et al. (*31*) found that the degree of cross-linking significantly slowed drug release from capsules made by simple coacervation, i.e., using only one polymer (polyacrylamide-grafted chitosan). The difference in wall and capsule structure (simple coacervation versus complex coacervation) as well as potential for cross-linking might be responsible for the differences observed regarding the effect of cross-linking. In our study, our model compounds were very low in molecular weight and very volatile. We expect that the capsule wall offered no significant barrier to diffusion and, thus, the release irrespective of whether or not the wall was cross-linked.

Effect of the Wall Thickness and Volatile Load. In this study, we assume that the release from coacervate microcapsules occurred by diffusion of aroma from the core through the wall, into the aqueous environment. For this reason, we hypothesized that a thicker wall would slow the overall release. Capsules with three wall thicknesses were produced. The wall thicknesses were 50 μm (±12), 16 μm (±4), and 8.5 μm (±3) for capsules made with 40, 80, and 120 g of oil, respectively. Because the particle size distributions were similar for these three capsules, only the overall wall/core ratio was varied.

No statistical differences between the different samples can be detected for any of the four compounds, in terms of burst time and duration or in terms of persistence, compared to **Figure 1b**). Curves are therefore not presented here. The relative release from capsules with a thinner wall (made with 120 g of oil for 20 g of wall material, data not shown) also did not show any significant differences compared to the two other thicknesses. One can argue that, the greater the wall thickness, the greater the cross-linking effect would be and that they are therefore related factors. However, the addition of clk agent on various wall thicknesses did not influence volatile release. In summary, neither clk nor wall thickness was found to significantly alter aroma release from coacervate microcapsules. In addition, no

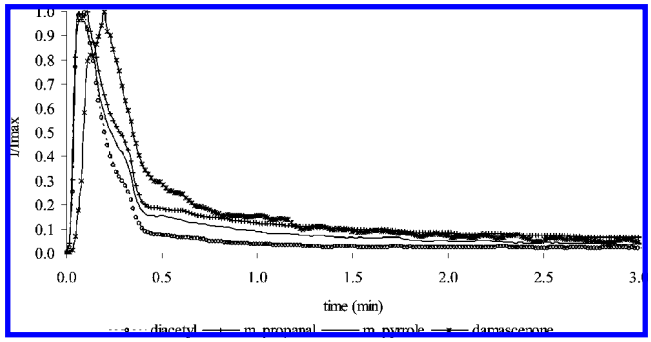

Figure 2. Relative release of aroma from spray-dried powder. The time 0 is the moment when hot water was added. Each curve is an average of triplicate runs.

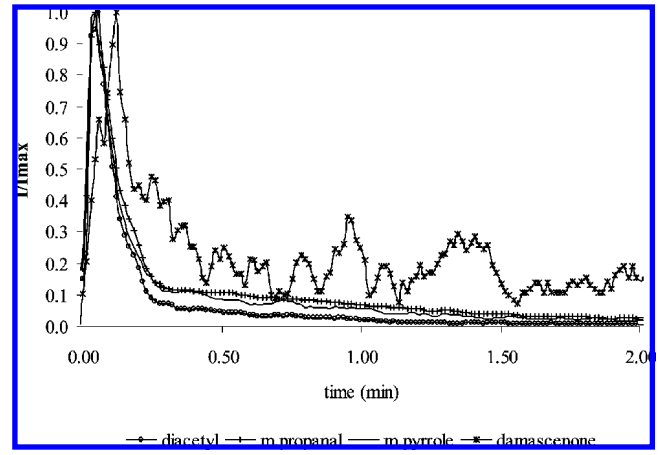

Figure 3. Relative release of aroma from flavored MCT. The time 0 is the moment when hot water was added. Each curve is an average of triplicate runs.

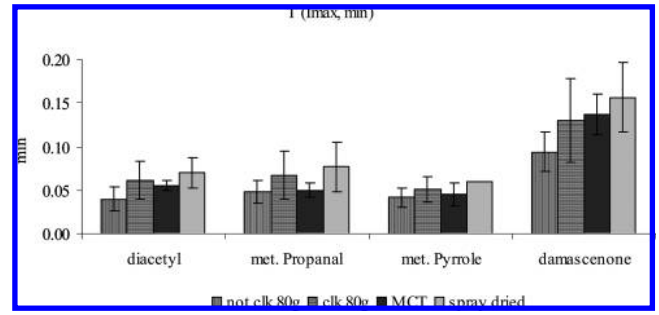

Figure 4. Time to I_{max} for various types of capsules tested: coacervates, intermediate wall thickness (80 g oil), not cross-linked (not clk) and cross-linked (clk), flavored oil only (no matrix, MCT), and spray-dried powder.

statistical interaction between these two factors could be detected on burst or persistence.

Wall thickness and wall/core ratio are also expected to have different influences irrespective of capsule structure (mono- or polynuclear). Our data are consistent with those of Nixon et al., who also found no statistical effect of wall thickness on drug release from mononuclear capsules.

Jégat and Taverdet (*32*) investigated the effect of stirring speed during manufacturing on drug release in water. Stirring speed would lead to differences in capsule structure (poly- versus mononuclear). They reported that the release was significantly faster from mononuclear compared to polynuclear capsules. However, their results were not reported in terms of the wall/core ratio; therefore, it is possible that the variation in wall/core ratio and variation in structure might be confounded in their conclusions. Several papers also indicate a slower, controlled release of hydrophobic drugs when encapsulated in polynuclear structures (*33*−*35*). The difference of release profiles might be due to the small molecular size of the aroma compounds (in our study) as opposed to large nonvolatile drugs (in the literature). A recent study by Hasan et al. (*36*) suggested the use of multilayer emulsions to slow the release of nanoparticles from coacervated microcapsules to reduce the burst effect and obtain a controlled, persistent release. More work in this area should be conducted to evaluate this technique with volatile molecules.

We also prepared capsules with a 10-fold higher load of volatiles. Relative release (I/I_{max}) from 10× load capsules was similar to that of its equivalent lower load, in terms of time to I_{max} and relative persistence. This observation confirms that the release was not influenced by volatile concentration but rather simple diffusion, and therefore, the structure of the capsules was not the limiting factor.

Comparison of the Release from Coacervates and Spray-Dried Powder. To evaluate if coacervate capsules have any effect on aroma release in a hot, aqueous environment, volatile release from a spray-dried powder (made with modified starch and pure compounds) was also determined. Because spray-dried particles are readily water-soluble, one might expect a more rapid release than observed for the coacervates particles because the coacervates are not soluble.

The relative release of our model volatiles from spray-dried powder is presented in **Figure 2**. One notes that the release occurs as a burst with this type of encapsulation as well. The times to I_{max} are very comparable to those obtained with coacervate capsules, except for the slower release of β-damascenone and perhaps methyl-propanal. A summary of times to maximum intensity is presented in **Figure 4**. This figure also includes data collected when only an equivalent amount of flavored oil (MCT) was added to the vessel (no encapsulation), instead of a dry powder. This figure illustrates that there is no statistical difference between the various samples (i.e., between not-clk and spray dried) but that there is a substantial difference between β-damascenone and the other three compounds. This reinforces the idea that, although volatility and hydrophobicity play a substantial role in the release, they cannot be used as predictors of the release pattern.

The difference in release between β-damascenone and the other compounds is also found in the persistence from spray-dried powder (**Figure 2**): at 0.5 min, about 30% of I_{max} was still being released from the spray-dried powder compared to 15−20% from the coacervate. The persistence for the three other compounds are similar across all encapsulation systems. In comparison to the relative release from coacervate powder and spray-dried powder, it appears that the matrix (process type and structure) did not influence the release kinetics. In addition, neither particle size (average 350 μm particle size for coacervates regardless of the wall/core ratio versus 45 μm for spray-dried powder) nor surface area of the capsules was found to have an effect on the release.

We also evaluated volatile release "without matrix", i.e., using only flavored oil (MCT) with the same four compounds. The release occurred as burst (**Figure 3**), similar to the release from the various systems presented above. The same slight variability between the compounds was also noted, in terms of time to maximum intensity (**Figure 4**). The decay, however, was sharper when only oil was present compared to the encapsulated products. I/I_{max} reached about 10−15% at about 0.25 min for the four compounds, i.e., half the time compared to the relative

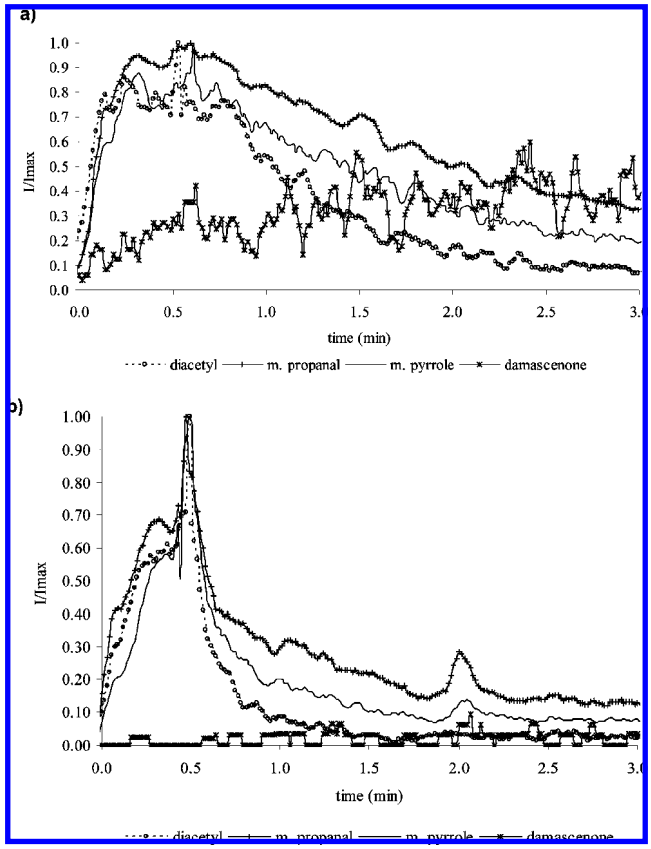

Figure 5. Relative release of volatiles at room temperature (air and water) from (**a**) spray-dried powder and (**b**) coacervates. The time 0 is the moment when water (room temperature) was added. Each curve is an average of triplicate runs.

release from the encapsulated materials. This implies that some additional "reservoir" or controlling system is present when using encapsulated material.

This observation led us to model the overall release system. The amount of a given volatile in the sample headspace is a function of the compound partitioning between water (continuous phase) and air and partitioning between the capsule reservoir and water. Because the water/air partition coefficients are constants (one water/air partition coefficient for each compound at a given temperature), this suggests that the capsule reservoir/water partitioning was similar across the various encapsulants. A mathematical model using a bi-exponential function fit the observed decays very well. The model used was as follows:

$$f = K_1 \exp^{(-K_2 t)} + K_3 \exp^{(-K_4 t)}$$

The parameters extracted (K_2 and K_4) from these models support the hypothesis that there was no effect of the type of encapsulating matrix on the release but that there was a difference with the "oil only" system (which followed a single-exponential decay model). This suggests that the partition coefficients played a significant role in the observed release. To confirm this hypothesis, all samples were run at ambient temperature (25 °C). The underlying reason is that partition coefficients are temperature-dependent, and therefore, varying the environmental temperature should affect the release substantially more than the differences between compounds. Relative releases collected from spray-dried powder and coacervates (clk, intermediate wall thickness) are presented in parts **a** and **b** of **Figure 5**, respectively. In both cases, the release of all compounds at ambient temperature (25 °C) is significantly different from the release observed at the higher temperature (75 °C). In addition, a significant difference exists between the two encapsulation methods, i.e., coacervates and spray-dried powder. One can note that the release from coacervates still occurs in some type of burst (within 1 min) for all compounds, except damascenone (no detectable release until after 1.5 min after the addition of water). In spray-dried powder, the release lasts longer (from 2 to >3.5 min) but is different for each compound. This suggests that the release from an encapsulated complex aroma would not be constant over time and would potentially lead to sensory imbalances.

Castelli et al. (*37*) also found a similar effect of media temperature on drug release from coacervate capsules. However, they were studying the release in biological lipidic membranes, which could explain some of the differences observed in terms of persistence duration.

To conclude, there was no significant difference in volatile release profiles from hot aqueous systems when volatiles were prepared via coacervation versus spray drying. However, a significant difference in release profiles occurred when the release was studied at room temperature. Coacervate capsules offered a uniform burst release for hydrophilic to slight hydrophobic compounds, whereas spray-dried powder presented a long- lasting, non-burst release but was non-uniform across aroma compounds.

In summary, this study investigated the volatile release from microcapsules prepared by complex coacervation and spray drying. Coacervate wall thickness and chemical cross-linking were manufacturing variables. No effect of the cross-linking or wall thickness on volatile release was found. Furthermore, within the study limits, no correlation between the physical/chemical properties of the test volatile compounds and their release was observed. Finally, no significant differences in volatile release were observed between coacervates and spray-dried powders under high-temperature release conditions. However, testing temperature had a highly significant effect on the overall release most likely by altering the partition coefficients (oil/water and water/air).

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