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ARTICLE

Encapsulation of Plant Oils in Porous Starch Microspheres

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Natural plant products such as essential oils have gained interest for use in pest control in place of synthetic pesticides because of their low environmental impact. Essential oils can be effective in controlling parasitic mites that infest honeybee colonies, but effective encapsulants are needed to provide a sustained and targeted delivery that minimizes the amount of active ingredient used. The present study reports the encapsulation of essential oils in porous microspheres that are within the size range of pollen grains and can be easily dispersed. The microspheres were made by pumping an 8% aqueous high-amylose starch gelatinous melt through an atomizing nozzle. The atomized starch droplets were air-classified into two fractions and collected in ethanol. The size range for each fraction was measured using a particle size analyzer. The mean particle size for the largest fraction was approximately 100 μm with a range from 5 μm to over 300 μm. Part of the reason for the large particle size was attributed to the merging of smaller particles that impinged upon each other before they solidified. The smaller fraction of spheres had a mean particle size of approximately 5 μm. The starch-based porous microspheres were loaded with 16.7% (w/w) essential oils including thymol (5-methyl-2-isopropylphenol), clove, origanum, and camphor white oil. The essential oils appeared to be largely sequestered within the pore structure, since the spheres remained a free-flowing powder and exhibited little if any agglomeration in spite of the high loading rate. Furthermore, SEM micrographs verified that the pore structure was stable, as evidenced by the persistence of pores in spheres that had first been loaded with essential oils and then had the oil removed by solvent extraction. Thermal gravimetric analyses were consistent with a loading rate at predicted levels.

KEYWORDS: Essential oils; thymol; clove; origanum; camphor; *Apis mellifer*; *Varroa jacobsoni*

INTRODUCTION

Starch is one of the most abundant and widely available agricultural commodities in the world (*1*). While starch is widely used in food products and processes, it has also been used for industrial applications for many years (*2*, *3*). For example, starch phosphate esters and cationic starches that have adhesive and film-forming properties are valuable in the manufacture of paper (*2*, *4*), as flocculants in water (*4*), and as sizing in textiles (*5*). Dextrinized starches are effective as adhesives for paper and other substrates, including metal and glass (*6*). Acid-modified, oxidized, hydroxyalkyl, and esterified starches have been used in the textile industry and in the manufacture of paper, paperboard, and drywall (*1*, *3*, *7*).

Another application for starch is in the controlled release of agrochemicals. Starch is a common encapsulating media for food products (*8*, *9*). Starch is also used for encapsulating herbicide and pesticide products because of its biodegradability in many agricultural environments and versatility in processing. Doane (*10*) used an extrusion process to encapsulate an active herbicide in a starch extrudate. Wing et al. (*11*−*13*) used jet cooking to disburse an herbicide in a cornstarch melt that was dried and finally ground to the desired particle size. In yet another approach, McGuire et al. (*14*) controlled the release of an herbicide by chemically linking the active ingredient to pregelatinized starch. The active ingredient was released as the starch component degraded in the field. More recently, there have been concerted efforts to replace herbicides and pesticides with natural control agents. There is a need to develop delivery systems for natural agents, some of which may be volatile.

2-Heptanone is a natural solvent produced by honeybees that is effective in controlling Varroa mites (*15*). Glenn et al. (*16*, *17*) described a process for encapsulating 2-heptanone in a food-grade, starch-based microcellular foam. The foam had small pores, generally smaller than 2 μm, and a large pore volume which enabled the foam to encapsulate several times its weight in liquid (*16*, *17*). Encapsulation of liquids in starch foam occurs by absorption and capillary flow through the network of micropores present within the foam matrix. However, due to the open-cell structure of the foam and the volatility of 2-heptanone, a vapor barrier film was necessary to control the release rate within the desired range (*17*, *18*).

Plant essential oils are less volatile compared to 2-heptanone and have also been reported to effectively control Varroa mite in honeybee colonies (*19*, *20*). Essential oils may be much more viscous than 2-heptanone but can be easily absorbed into the internal pore structure of microcellular foam when dissolved in a

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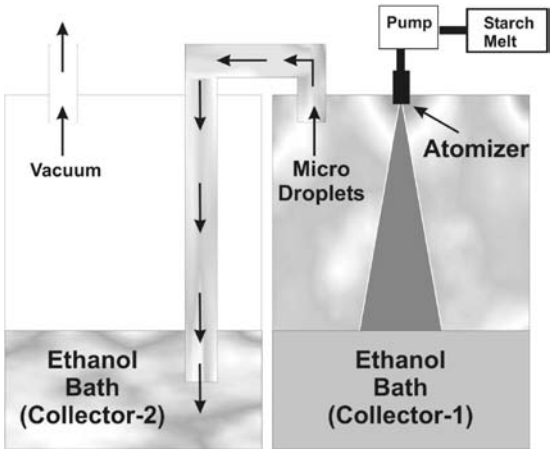

Figure 1. Diagram of system used for collecting microdroplets from an atomized starch melt. The first collector recovered the microdroplets directly from the atomizing nozzle (collector-1). The second collector used vacuum to draw suspended microdroplets from the turbulent air stream into an ethanol bath (collector-2).

solvent blend. A vapor barrier may not be necessary for foam-encapsulated essential oils because of their relatively low volatility. The starch microcellular foam encapsulant could be made into panels that could simply be placed within the colony. However, a more desirable delivery system would be to make foam spheres in the size range of pollen grains (< 45 μm) that could attach or adhere to the hairs on the body of honeybee (*21*) and protect them from varroa mites. Foam spheres containing active agent that are smaller than 45 μm could also be ingested by honeybees (*22*) and possibly provide some systemic control of parasites.

The objective of the current study was to develop a method of forming starch-based foam microspheres less than 45 μm in diameter that can encapsulate essential plant oils and yet remain an easily dispersed powder.

MATERIALS AND METHODS

Materials. Unmodified wheat starch (Midsol 50) was obtained from Midwest Grain (Topeka, KS). Unmodified, high-amylose corn starch (Hylon VII) was purchased from National Starch Inc. (Bridgewater, NJ). Thymol (5-methyl-2-isopropylphenol, CAS registry no. 89-83-8), origanum (CAS registry no. 8007-11-2), clove (CAS registry no. 8000-34-8), and camphor white oil (CAS registry no. 8008-51-3) were purchased from the Sigma-Aldrich Co. (St. Louis, MO).

Wheat Starch Microcellular Foam Panels. Aqueous wheat starch (8%, w/w) gels were made by adding 2.65 g of starch to 30.35 g of water in a sample vial of a Rapid Viscoamylograph (RVA, Newport Scientific, Warriewood, Australia). The RVA was programmed to ramp from 25 °C at a rate of 10 °C/min to 95 °C and then hold at that temperature for 5 min. Following the temperature treatment, the starch melt was immediately transferred to cylindrical molds (2 cm diameter, 3 cm length), covered, and stored overnight in a refrigerator (5 °C). The gelled starch was removed from the molds and equilibrated in a graded ethanol series (40, 70, 90% and three changes of 100% ethanol) to displace the water. Wheat starch MCF was made by simply evaporating the ethanol from the samples under a stream of dry nitrogen gas. The MCF was stored in plastic bags for later use.

Porous Microspheres. High-amylose corn starch has a much higher gelatinization temperature than wheat starch and was cooked using a 1 L pressure reactor (Paar Instrument Co., Moline, IL) equipped with a mixer and heat controller (Model 4843). Aqueous starch suspensions (8%, w/w) were heated at 4 °C min^{-1} and stirred continuously (330 rpm) while heating to 140 °C. The temperature was held for 10 min before cooling the starch melt to 85 °C. The starch melt was pumped through an atomizing nozzle at a rate of 100 mL/min. The air pressure supplied to the nozzle was maintained at 0.55 MPa. The atomized starch was air classified into two fractions that were collected in two separate ethanol (95%) baths (**Figure 1**). The first fraction was collected directly from the spray stream and contained the largest particle size range. The second fraction was collected from droplets suspended by the air turbulence and drawn via vacuum into a second ethanol bath. Both baths were stirred constantly to provide agitation and help minimize particle-to-particle interaction. The samples that were collected were stored in ethanol.

Particle Size Determination. Aliquots (5 mL) from samples stored in ethanol were added to 400 mL of water and thoroughly mixed using sonication coupled with agitation. Particle size distribution was measured using a particle size analyzer (Horiba Model L-900, Tokyo, Japan).

Encapsulation. Porous starch microspheres stored in ethanol as previously described were combined from both collectors, dried under dry nitrogen, and mechanically agitated to form a free-flowing powder. Thymol, clove, origanum, and camphor (0.2 g) were weighed separately in aluminum sample dishes. Each was solubilized by adding 4.8 g of a 2:1 ethanol−acetone mixture, covering the dish, and gently swirling. Dissolving the active ingredient in a solvent mixture was done to reduce viscosity and facilitate absorption into the pore structure of the foam. Dry starch porous microsphere powders (1 g) were added to the sample dish, which was promptly covered, weighed, and allowed to equilibrate for 15 min. The starch powders essentially absorbed all of the solvent mixture.

The solvent component was allowed to evaporate from the loaded samples, leaving an oil residue within the pore structure of the microspheres. This was accomplished by placing samples loaded with the oil/solvent mixture in a desiccator containing anhydrous $CaSO_4$ to maintain low-moisture conditions while the solvent component was allowed to evaporate. A continuous flow of dry nitrogen passed through the desiccator at a rate of 250−300 cm^3 min^{-1} to prevent the air in the desiccator from becoming saturated with the solvent vapor. The sample was weighed to 0.1 mg (Sartorius, Model MP8-1, Bradford, MA) at periodic intervals to monitor the rate of weight loss. Measurements were taken until the weight loss was less than 1% per day.

Thermogravimetric Analysis (TGA). TGA (Model 2950, TA Instruments, New Castle, DE) was performed by first equilibrating each sample for 1 min at 20 °C and then heating at a rate of 10 °C/min up to 800 °C. The percent weight change as a function of temperature was monitored for each sample.

Scanning Electron Microscopy. *Wheat Starch Samples.* Wheat starch gels equilibrated in 100% ethanol as described previously were placed in liquid nitrogen and cryofractured as per Humphreys et al. (*23*). Briefly, foam samples were cut into rectangular cubes of approximately 5 mm on the longest side using a sharp razor blade. The cubes were soaked in two exchanges of 100% ethanol for 1 h. The samples were removed from ethanol using tweezers and dropped immediately into liquid nitrogen and fractured with a razor blade held in a clamp. The razor blade and clamp were equilibrated to liquid nitrogen temperatures prior to the fracture. The pieces were then collected using liquid nitrogen chilled tweezers and dropped back into ethanol. The fractured samples were critical point dried in a Tousimis Autosamdri 815 critical point dryer (Tousimis, Rockville, MD) and mounted onto aluminum specimen stubs with the fractured surface facing up. The adhesive used for mounting was a two-component epoxy (Extra Time, Loctite Corp.) and water-based carbon graphite (Ted Pella, Inc., Redding, CA) mixed 1:1:1. The samples were sputter-coated with gold−palladium in a Denton Desk II Sputter Coating Unit (Denton Vacuum, Inc., Moorestown, NJ) and viewed and photographed with a Hitachi S4700 field emission scanning electron microscope (Hitachi, Japan).

High-Amylose Corn Starch Samples. Aliquots (5 mL) of samples stored in ethanol were drawn through a Millipore filter to collect the starch microspheres prepared by atomization. The filter containing the starch microspheres was covered with a second filter to sandwich the microspheres before placing the filter sandwich in a 100 mL bath containing 100% ethanol. After it was equilibrated for a minimum of 6 h, the filter sandwich was critical point dried. The filter cover was removed, and the filter surface containing the starch microsphere deposits was sputter-coated and viewed by SEM as previously described.

Experimental Procedure. An estimate of the amount of plant oils lost during the evaporation step was made by monitoring the weight loss of the oils (0.2 g) placed in open aluminum sample dishes over a 48 h period in a desiccator flushed continuously with dry nitrogen. A second measure of the volatile loss of the oils was done using a mixture of the oils with the

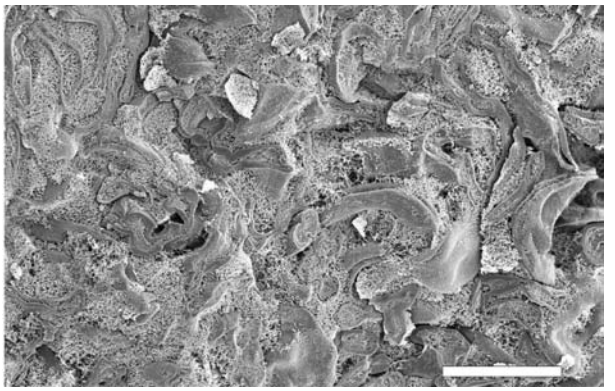

Figure 2. Wheat starch cooked for 5 min. Note the starch granule remnants interspersed throughout the matrix (scale bar 25 μm).

solvent mixture to determine whether the solvent affected oil volatility. Samples were made by mixing each of the oils (0.2 g) with 4.8 g of the 2:1 ethanol−acetone mixture in open aluminum sample dishes. The samples were placed in a desiccator with a continuous flow of dry nitrogen for 48 h. The volatilization loss (percent) of the oil from the oil/solvent mixture was determined from the difference between the initial weight of the oil component (0.2 g) and the weight of the residue after 48 h.

The initial loading rate for each of the oils was calculated on the basis of 0.2 g of oil in 1 g of starch microspheres. The predicted load rate after the 48 h evaporation step was calculated by accounting for the volatilization loss (percent) of the oil from the oil/solvent mixture. The predicted load rate was compared to the actual load rate as measured by TGA. The actual load rate was determined by measuring the weight loss of samples at 260 °C, which is beyond the boiling point of the oils but below the decomposition temperature of the starch. The actual load rate was determined by subtracting the percent weight loss of the starch control (due to moisture) from the measured percent weight loss of each sample. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Starch foam panels made from wheat starch consisted of a porous matrix of starch interspersed with granule remnants that appeared relatively nonporous (**Figure 2**). The presence of starch granule remnants were not of particular concern when making large products such as foam panels (**Figure 2**). However, when making porous starch microspheres that are in the same size range as the native starch granules themselves, it is important to have a homogeneous starch matrix devoid of any granule remnants. Glenn et al. (*24*) showed that both wheat and corn starch granules are very persistent during heating and that increasing the heating time did not dissolve the remnants in starch slurries. However, the conditions used to process the HACS completely solubilized the starch granules and produced a homogeneous foam matrix well suited for making porous microspheres (**Figure 3**).

Porous Microspheres. The porous microspheres from the first collector (**Figure 1**) consisted of spheres ranging in size from approximately 10 μm to over 300 μm with a mean size of over 100 μm (**Figure 4**). It was somewhat surprising that the particle size had such a large range and that there were spheres as large as 300 μm. Initially, it was believed that some of the microspheres were simply aggregating into clusters that resulted in large, loosely associated particles. However, sonication of the sample to minimize particle aggregation did not affect the particle size range. Further examination of the spheres showed that some aggregates were present in the samples but they were tightly bound together. It is most likely that these aggregates formed when multiple spheres impinged upon each other while yet in a molten or partially molten state.

The second collector (**Figure 1**, collector-2) used a vacuum to draw suspended starch microdroplets from the turbulent air flow

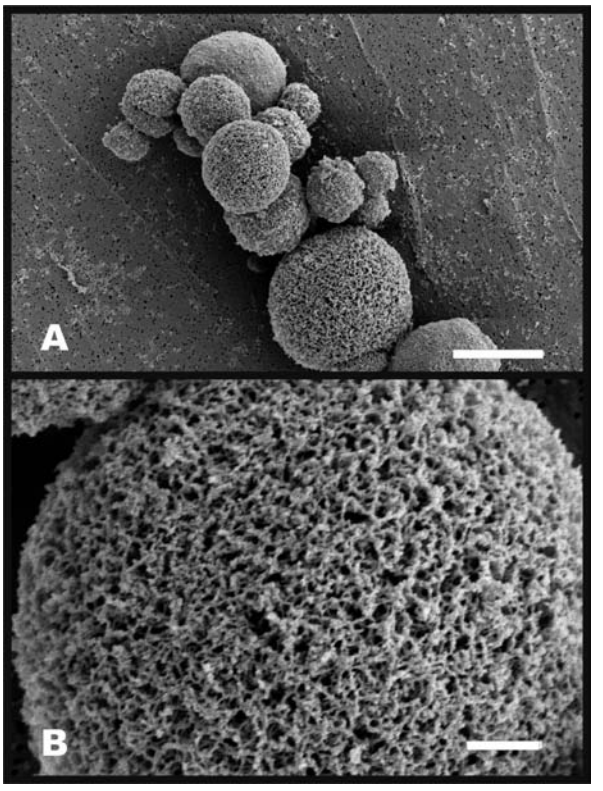

Figure 3. High-amylose corn starch (HACS) microspheres made via atomization and air classification. Note the fine porous structure of the microsphere that was devoid of starch granule remnants. Scale bar: (A) 10 μm; (B) 2 μm.

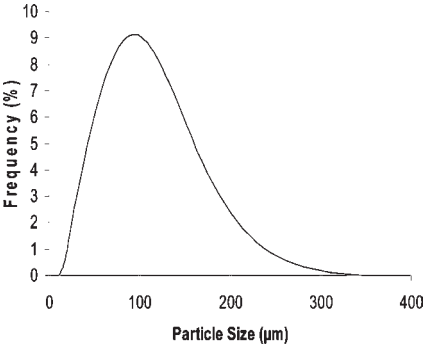

Figure 4. Particle size distribution for high-amylose corn starch microspheres from collector-1 (see **Figure 1**).

of the atomized spray into an ethanol bath. The sample consisted of very fine microspheres ranging in size from approximately 2 μm to about 15 μm with a mean size of 5 μm (**Figure 5**). These porous microspheres are well within the size range of pollen grains.

It was important to sufficiently dehydrate the starch microspheres in ethanol before air drying in order to preserve the pore structure of the microspheres. Scanning electron micrographs of samples that were only partially dehydrated (70% ethanol) before air drying revealed that the pore structure was not preserved and that the microspheres coalesced into aggregates (**Figure 6**). The collapse of the pore structure most likely occurred due to the high surface tension created by evaporating the water trapped within the pores (*25*). Microspheres dehydrated in 100% ethanol did not coalesce with other microspheres, and their pore structure was preserved during air drying (**Figure 3**).

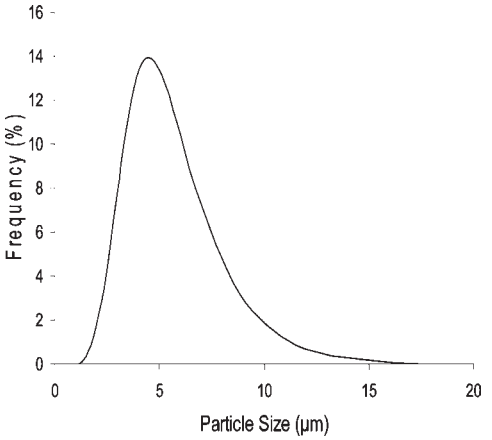

Figure 5. Particle size distribution for high-amylose corn starch microspheres from collector-2 (see **Figure 1**).

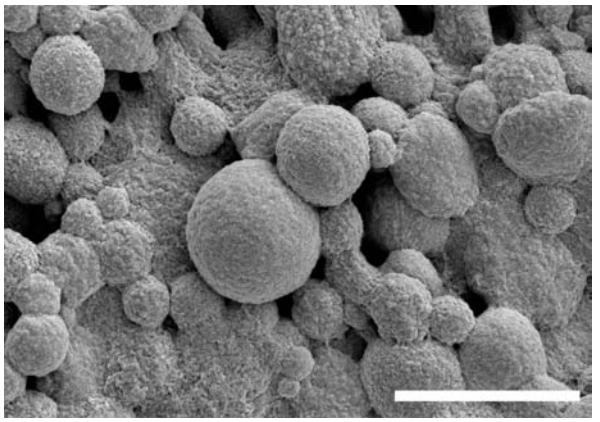

Figure 6. Scanning electron micrographs of starch microspheres that had been equilibrated in 70% ethanol before air drying. Moisture within micropores caused the pore structure to collapse and the microspheres to coalescence during drying process (scale bar 5 μm).

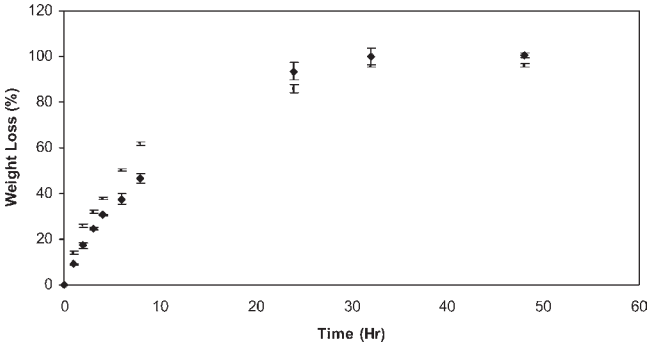

Figure 7. Percent weight loss of control (solvent/oil mixture alone, small box) and starch microspheres (diamonds) loaded with solvent/oil mixture (error bars indicate standard deviation).

Encapsulation. Mixing and dissolving the plant oils in a solvent mixture was done to reduce viscosity, facilitate absorption, and help evenly distribute the oil within the microspheres. The open pore structure of the microspheres had both benefits and drawbacks. While it helped facilitate absorption of the oils, it also provided little resistance to evaporation. This was apparent in solvent evaporation data from loaded starch microspheres versus evaporation of solvent from an open container (**Figure 7**). The weight loss curve was slightly less for the microspheres, but the difference was negligible (**Figure 7**).

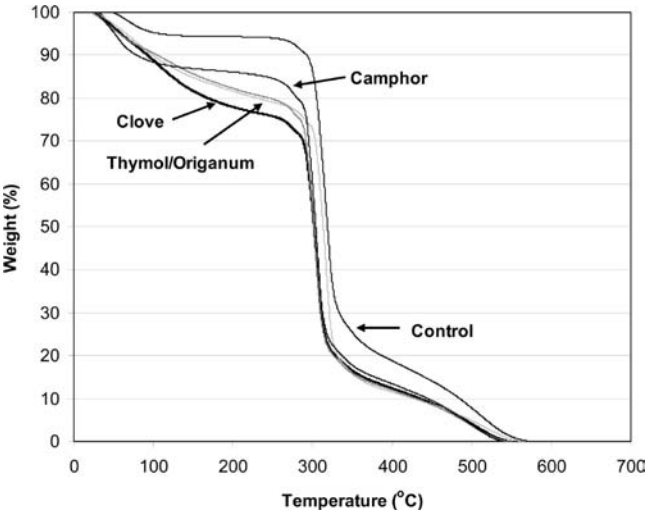

Figure 8. Thermogravimetric data for starch microspheres without oils (control) or with camphor, clove, thymol, or origanum oil.

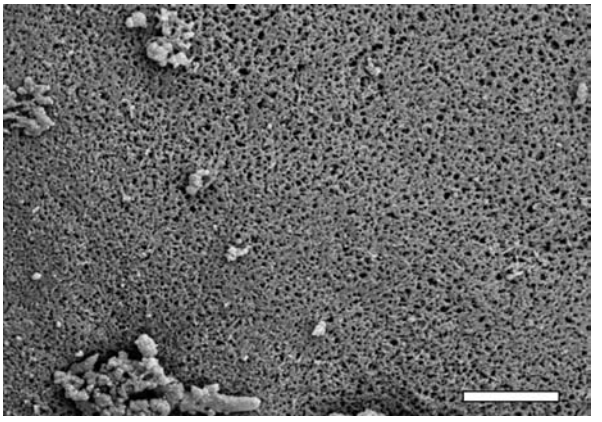

Figure 9. Scanning electron micrograph of the surface of starch microspheres that had been loaded with an oil/solvent mixture (scale bar 2 μm).

The solvent component of the oil/solvent mixture evaporated in less than 40 h (**Figure 7**). Dissolving the oils in a solvent mixture had little apparent effect on the volatilization loss of the oil itself. Percent weight loss data (48 h) for the oils were comparable regardless of whether the oils were dissolved in solvent (**Table 1**).

The percent weight loss in 48 h due to volatilization varied among the oils tested (**Table 1**). Camphor and origanum oils lost 36% and 16%, respectively (**Table 1**). The data for the thymol and clove samples indicate there may be a slight weight gain over a 48 h period (**Table 1**). This could be due to error or to absorption of volatiles from adjacent samples during the drying step. Predicted final loading rates were calculated on the basis of a 16.7% initial loading rate and on accounting for the percent losses after 48 h (**Table 1**). The measured loading rates based on TGA data (**Figure 8**) were in close agreement with the predicted values. The results indicate that the microspheres were successfully loaded with the plant oils and that the loading rate could be accurately predicted.

A further indication that the porous structure of the microspheres was preserved during the sample loading was provided by SEM micrographs of microspheres that had previously been loaded with essential oils (**Figure 9**). Although the surface of the microspheres appeared somewhat compressed, the pore

Table 1. Boiling Points and Evaporation Losses of Select Plant Oils Alone or Dissolved in a 2:1 Ethanol−Acetone Mixture over a 48 h Period[a]

	origanum	thymol	clove	camphor
boiling point (°C)	239	232	251−256	204
volatilization loss (%) 48 h				
oil	16.0 ± 2.6	−4.6 ± 0.93	−4.9 ± 1.7	36.0 ± 3.0
solvent + oil	18.1 ± 1.75	−8.7 ± 1.94	−0.78 ± 0.55	38.6 ± 1.35
initial loading (%, w/w)	16.7	16.7	16.7	16.7
predicted final loading (%, w/w)	14.1	16.7	16.7	10.9
final loading (%, at 260 °C)	14.0 ± 0.87	15.0 ± 0.90	18.0 ± 0.90	9.1 ± 0.77

[a] The predicted final loading was estimated on the basis of an initial loading of 16.7% and a 48 h time period to allow the solvent mixture to evaporate (see **Figure 6**). The measured final loading on the basis of TGA data was in general agreement with the predicted final loading.

structure was still evident, providing support that the pore structure is stable enough to withstand loading of essential oils. A stable pore structure is critical to providing a high amount of surface area through out the interior regions relative to the outer surface area of the microspheres. The high amount of internal surface area created by the pores ensures that much of the essential oil is sequestered within the microspheres. Sequestering the oils largely within the microsphere interior region helps minimize the degree of particle agglomeration and facilitates particle dispersion. The microspheres remained a free-flowing powder that was lightweight and could easily be dispersed. Future studies will focus on field trials of microspheres loaded with plant oils for controlling agricultural pests, including Varroa mites in honeybee colonies.

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