Video Article

Extraction of Plant-based Capsules for Microencapsulation Applications

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URL: https://www.jove.com/video/54768
DOI: doi:10.3791/54768

Keywords: Bioengineering, Issue 117, *Lycopodium clavatum*, natural spores, sporopollenin exine capsules, encapsulation, microstructure, microridges

Date Published: 11/9/2016

Citation: Potroz, M.G., Mundargi, R.C., Park, J.H., Tan, E.L., Cho, N.j. Extraction of Plant-based Capsules for Microencapsulation Applications. *J. Vis. Exp.* (117), e54768, doi:10.3791/54768 (2016).

Abstract

Microcapsules derived from plant-based spores or pollen provide a robust platform for a diverse range of microencapsulation applications. Sporopollenin exine capsules (SECs) are obtained when spores or pollen are processed so as to remove the internal sporoplasmic contents. The resulting hollow microcapsules exhibit a high degree of micromeritic uniformity and retain intricate microstructural features related to the particular plant species. Herein, we demonstrate a streamlined process for the production of SECs from *Lycopodium clavatum* spores and for the loading of hydrophilic compounds into these SECs. The current SEC isolation procedure has been recently optimized to significantly reduce the processing requirements which are conventionally used in SEC isolation, and to ensure the production of intact microcapsules. Natural *L. clavatum* spores are defatted with acetone, treated with phosphoric acid, and extensively washed to remove sporoplasmic contents. After acetone defatting, a single processing step using 85% phosphoric acid has been shown to remove all sporoplasmic contents. By limiting the acid processing time to 30 hr, it is possible to isolate clean SECs and avoid SEC fracturing, which has been shown to occur with prolonged processing time. Extensive washing with water, dilute acids, dilute bases, and solvents ensures that all sporoplasmic material and chemical residues are adequately removed. The vacuum loading technique is utilized to load a model protein (Bovine Serum Albumin) as a representative hydrophilic compound. Vacuum loading provides a simple technique to load various compounds without the need for harsh solvents or undesirable chemicals which are often required in other microencapsulation protocols. Based on these isolation and loading protocols, SECs provide a promising material for use in a diverse range of microencapsulation applications, such as, therapeutics, foods, cosmetics, and personal care products.

Video Link

The video component of this article can be found at https://www.jove.com/video/54768/

Introduction

There is significant interest in natural plant-based capsules obtained from plant spores and pollens for use in microencapsulation applications.[1-15] In nature, spores and pollen provide protection for sensitive genetic materials against harsh environmental conditions. The basic structure of plant spores and pollen typically comprises an outer shell layer (exine), an inner shell layer (intine), and the internal cytoplasmic material. The exine is comprised of a chemically robust biopolymer[1,9,10,13,16] referred to as sporopollenin and the intine is comprised primarily of cellulosic materials.[16-18] Empty capsules can be isolated by various processes[7,9] for removing cytoplasmic material, proteins, and the intine layer.[2,12,16] These sporopollenin exine capsules (SECs) provide a compelling alternative to synthetic encapsulants due to their narrow size distribution and uniform morphology.[7,9,13,19,20] The development of standardized processes to obtain SECs from various plant species, such as *Lycopodium clavatum*, opens the potential for a wide range of microencapsulation applications in the fields of drug delivery, foods, and cosmetics.[6,10-13,21]

In order to obtain SECs, researchers first treated spores and pollen with organic solvents and refluxed in alkaline solutions to remove cytoplasmic contents.[22-25] However, the remaining capsule structure was determined to still contain the cellulosic intine layer. In order to remove this, researchers explored the use of prolonged acidic hydrolysis processing using hydrochloric acid, hot sulfuric acid, or hot phosphoric acid over several days,[22-25] with phosphoric acid becoming the preferred method of SEC intine removal.[2] However, ongoing research over the years has shown that various spores and pollens have differing degrees of resilience to the harsh processing methods commonly used.[26,27] Some spores and pollens are completely degraded and lose all structural integrity in strong alkaline solutions, or become heavily damaged in strong acidic solutions.[16] The variability in SEC response to treatment conditions is due to subtle variations in the chemical structure and exine morphology of the sporopollenin exine material between species.[28] Due to the variability in the robustness of sporopollenin exine capsules (SECs), it is necessary to optimize the processing conditions for each species of spore and pollen.

Plant spores from the species *L. clavatum* have become the most widely studied single source of SECs. It is proposed that this is primarily due to its widespread availability, low cost, monodispersity, and chemical robustness.[9,29] The spores can be easily harvested and contain sporoplasmic contents in the form of groupings of 1 - 2 µm cellular organelles and biomolecules.[11] *L. clavatum* spores have been used as a

natural powder lubricant,[30,31] a base for cosmetics,[30] and in herbal medicine[32-36] for a wide range of therapeutic applications. The SECs obtained from *L. clavatum* have been shown to be more resilient to processing than SECs from other species of spores and pollen.[2] After processing, the resulting SECs have been shown to retain their intricate microridge structures and high morphological uniformity while providing a large internal cavity for encapsulation.[7] Studies indicate that *L. clavatum* SECs can be used for the encapsulation of drugs,[10,13] vaccines,[11] proteins,[7,14] cells,[8] oils,[5-7,9] and food supplements.[5,15] Observed SEC loading efficiencies are relatively high in comparison to conventional encapsulation materials.[7] There are also a number of reported benefits to SEC encapsulation such as the ability to mask tastes,[6,10] and to provide some degree of natural protection against oxidation.[12] In the existing studies, the most commonly used SEC extraction method for *L. clavatum* is based on four main steps. Step one is solvent refluxing in acetone for up to 12 hr at 50 °C to defat the spores.[11] Step two is alkaline refluxing in 6% potassium hydroxide for up to 12 hr at 120 °C to remove cytoplasmic and proteinaceous materials.[11] Step three is acid refluxing in 85% phosphoric acid for up to 7 days at 180 °C to remove the cellulosic intine material.[11] Step four is a comprehensive washing process using water, solvents, acids, and bases to remove all remaining non-exine material and chemical residues.

The main goals of SEC extraction in relation to encapsulation applications are to produce capsules which are empty of cytoplasmic material, free from potentially allergenic proteins, and morphologically intact.[2,37] However, from an industrial manufacturing perspective, it is also important to consider additional economic and environmental factors, such as, energy efficiency, production duration, safety, and resulting waste. With regards to energy efficiency, both high temperatures and long processing times affect production costs as well as environmental footprint. Production duration and turnaround time are key factors influencing processing profitability. Of particular concern is that high temperature phosphoric acid processing increases safety issues and is known to result in corrosive scaling which leads to significant increases in infrastructure maintenance and delays in batch turnaround times.[38-40] Where possible, minimizing the number of steps required may lead to significantly reducing the waste produced. However, the commonly used four-step process of *L. clavatum* SEC extraction has simply evolved from decades of research and has had little actual process optimization. Recently, Mundargi *et al.*,[41] made a significant contribution to the ongoing work in this field by systematically evaluating and optimizing one of the most commonly reported SEC extraction techniques.

In the first section of this study: spore defatting is demonstrated utilizing acetone processing at 50 °C for 6 hr; sporoplasm and intine removal procedures are demonstrated utilizing 85% phosphoric acid processing at 70 °C for 30 hr; extensive washing with water, solvents, acid, and base is used to demonstrate the removal of residual sporoplasmic contents; and SEC drying is demonstrated utilizing convection drying and vacuum oven drying. In the third section, SEC vacuum loading is demonstrated utilizing vacuum loading of a model protein, bovine serum albumin (BSA), followed by BSA-loaded-SEC washing and lyophilization. In the fourth section, the determination of the BSA encapsulation efficiency is demonstrated utilizing centrifugation, probe sonication, and UV/Vis spectrometry.

Protocol

1. Extraction of Sporopollenin Exine Capsules (SECs) from *L. clavatum* Spores

Note: The SEC extraction process involves a flammable powder (*L. clavatum*), hot corrosive acids, and flammable solvents, hence proper personal protective equipment (goggles, face mask, gloves, lab coat), approved risk assessment on usage, and disposal of chemicals by authorized laboratory personnel is essential.

1. **Spore Defatting**
    1. Prepare a reflux set-up in a fume hood by using a glass condenser, water circulation system, and water bath (**Figure 1**).
    2. Weigh 100 g *L. clavatum* spores without creating dust and away from any ignition source.
    Note: The spores used here were commercially obtained (See Materials List).
    3. Transfer spores slowly to a 1 L polytetrafluoroethylene (PTFE) round bottom flask with a magnetic stirring rod.
    4. Add 500 ml acetone to the spores in the PTFE flask and shake the flask gently to form a homogeneous suspension.
    5. Place the PTFE flask in the water bath set at 50 °C and connect to the reflux condenser.
    6. Perform refluxing in acetone for 6 hr with stirring at 200 rpm and then allow to cool at RT.
    7. Filter the suspension using filter paper under vacuum and collect the defatted spores in large (15 cm diameter) glass petri dishes.
    8. Dry the spores at room temperature (fume hood) by covering with aluminum foil with holes for solvent evaporation.

2. **Acidolysis**
    1. Prepare 85% (v/v) phosphoric acid with deionized (DI) water and transfer defatted dry spores to a 1 L PTFE flask.
    2. Add 500 ml of phosphoric acid (85% v/v) to the defatted spores.
    3. Place the PTFE flask in a water bath set at 70 °C and connect to the reflux condenser.
    4. Perform gentle refluxing (70 °C) in phosphoric acid for 30 hr and then allow to cool at room temperature.
    5. Dilute the suspension by using 500 ml warm DI water and collect the SECs by vacuum filtration using filter paper.
    6. Transfer the SECs to a 3 L glass beaker to perform a series of washings.

3. **SEC Washing**
    1. Place the 3 L glass beaker containing SECs in a fume hood.
    2. Add 800 ml hot (50 °C) DI water to the SECs with gentle stirring for 10 min.
    3. Filter the suspension by using filter paper and a vacuum filtration set-up.
    4. Collect the SECs in a clean 3 L glass beaker.
    5. Repeat steps 1.3.1. to 1.3.4. five times.
    6. Add 600 ml hot (45 °C) acetone to the SECs with gentle stirring for 10 min.
    7. Collect the SECs by filtration under vacuum and transfer the SECs to a clean 3 L glass beaker.
    8. Repeat steps 1.3.6. and 1.3.7. using 600 ml hot (50 °C) 2 M hydrochloric acid.
    9. Repeat steps 1.3.6. and 1.3.7. using 600 ml hot (50 °C) 2 M sodium hydroxide.
    10. Repeat steps 1.3.1 and 1.3.4 eight times.
    11. Repeat steps 1.3.6. and 1.3.7. using 600 ml hot (45 °C) acetone.

12. Repeat steps 1.3.6. and 1.3.7. using 600 ml hot (45 °C) ethanol.
13. Repeat steps 1.3.6. and 1.3.7. using 800 ml hot (50 °C) DI water.
14. Transfer the clean SECs to six large glass petri dishes and dry in a fume hood at RT for 24 hr to remove all water content.
15. Dry the SECs in a vacuum oven at 60 °C and 1 mbar vacuum for 10 hr or until constant weight.
16. Collect the dried SECs and transfer to a polypropylene bottle.
17. Store the SECs in a dry cabinet.

2. Characterization of SECs

1. Perform scanning electron microscopic analysis[41] using spores and SECs produced at different stages.
2. Perform elemental analysis[41] using spores and SECs produced at different stages. Dry all samples at 60 °C for at least 1 hr before elemental analysis and calculate the protein content using percent nitrogen with a multiplication factor of 6.25.[11] Obtain results using triplicate measurements for each sample.
3. Conduct micromeritic properties analysis using a dynamic image particle analyzer.[41]
4. Scan unprocessed, processed, and FITC-BSA-loaded SECs using confocal laser scanning microscopy.[41]

3. Biomacromolecule Encapsulation by Vacuum Loading Technique

1. Prepare 1.2 ml of 125 mg/ml bovine serum albumin (BSA) solution using DI water and transfer to a 50 ml polypropylene tube.
2. Weigh 150 mg of SECs and transfer to the BSA solution.
3. Vortex the tube for 10 min to form a homogeneous solution.
4. Cover the tube using filter paper.
5. Transfer the tube to a freeze dryer flask and dry for 4 hr with vacuum at 1 mbar.
6. Perform steps 3.1 to 3.5. for three independent batches.
7. Collect the BSA-loaded SECs and remove the agglomerates by using a mortar and pestle.
8. Transfer the BSA-loaded SECs to a 50 ml tube and add 2 ml DI water to remove the residual BSA.
9. Collect the SECs by centrifugation at 4,704 x g for 5 min and discard the supernatant.
10. Repeat the washing with DI water once.
11. Cover the tube containing BSA-loaded SECs using filter paper.
12. Transfer the SECs to a freezer (-20 °C) and freeze for 1 hr.
13. Freeze dry the SECs for 24 hr and store in a freezer until further characterization.
14. Prepare the placebo SECs without BSA using the same procedure as in steps 3.1. to 3.13.
15. Prepare the FITC-BSA loaded SECs using the same procedure as in steps 3.1. to 3.13.

4. Determination of Encapsulation Efficiency

1. Weigh 5 mg of BSA-loaded SECs in 2 ml polypropylene tubes.
2. Add 1.4 ml phosphate buffered saline pH 7.4 (PBS) and vortex for 5 min.
3. Probe sonicate the suspension at 40% amplitude for 3 cycles of 10 sec.
4. Filter the solution using a 0.45 µm Polyethersulfone (PES) filter.
5. Perform the same procedure as in steps 4.1. to 4.4. using placebo SECs.
6. Measure the absorbance at 280 nm using placebo as a blank.
7. Quantify the amount of BSA encapsulated using a BSA standard curve (200 to 1,000 µg/ml) in PBS.[42]

Representative Results

Streamlined Extraction Process for Sporopollenin Exine Capsules

The *L. clavatum* SEC extraction was achieved by three main steps: (1) Defatting using acetone; (2) Acidolysis using phosphoric acid 85% (v/v); and (3) Extensive SEC washing using solvents. The flow of the streamlined SEC extraction process is presented in **Figure 1 A - I**. Briefly, the process involves a defatting step with acetone refluxing for 6 hr at 50 °C and the defatted spores were then dried overnight in a fume hood to remove residual acetone. The defatted spores were subjected to acidolysis using phosphoric acid for 30 hr. During acidolysis the majority of the sporoplasmic materials are dissolved and then removed during the vacuum filtration step. In order to remove residual sporoplasmic materials an extensive series of washing steps using solvents, acid, base, and water is performed. The SECs were initially dried at RT for 24 hr and further dried at 60 °C and 1 mbar vacuum for 10 hr to remove residual solvents and moisture.

As shown in **Figure 1,** all SEC extraction steps were performed in a fume hood with a hazard notice. During SEC extraction, approximately 70% mass loss is observed due to the removal of sporoplasmic materials and our data is consistent with existing reports of the production of clean and intact SECs.[7, 19,20,29] The streamlined process from the initial defatting step to the final product is completed in approximately 5 days and the process can be easily scaled up to a 1 kg lab scale batch with proper safety controls.

Encapsulation steps utilizing SECs are presented in **Figure 1 J - M,** these are advantageous due to being simple steps without involving harsh organic solvents or requiring mechanical shear forces.[7] Our encapsulation experiments involve 150 mg of SECs and a loading solution volume sufficient for uptake by the SECs. The loading in these SECs is limited by compound solubility in selected aqueous or organic solvents, and loading can be achieved by any of three loading methods: passive, compression, and vacuum loading. However, vacuum loading provides relatively higher encapsulation of compounds.[7, 19,20]

Physicochemical Characterizations of Sporopollenin Exine Capsules

To determine the cleanliness of SECs produced by using the streamlined process presented in **Figure 1**, the surface and cross-sectional morphology, micromeritic properties, and elemental composition were examined at different stages of the SEC extraction process. As shown in **Figure 2**, the SEM images indicate intact spores with a unique microstructure, and before processing, micron-scale sporoplasmic organelles were observed in the cross-sectional image. With spore defatting and alkali treatment no morphological and structural changes were observed apart from the rupturing of the sporoplasmic organelles.

Figure 3 shows SEM images of SECs after acidolysis using phosphoric acid at various time points from 5 hr to 30 hr. These results support that acidolysis for up to 30 hr provides intact and clean SECs devoid of sporoplasmic materials. In order to confirm residual proteinaceous material, CHN elemental analysis[29] was performed and data is presented in **Figure 4.** These results provide a guideline for choosing the best processing condition to achieve clean and intact SECs. In the case of defatted and alkali treated SECs, no substantial proteinaceous material removal is observed. However, with acidolysis using phosphoric acid, the majority of proteinaceous materials is removed in 30 hr and no further reduction is observed with increasing process time up to 120 hr. The elemental data is consistent with previous reports on extended processing of SECs[7,11] and with commercial SECs.

Micromeritic properties were analyzed by dynamic image particle analysis (DIPA) and results are presented in **Figure 5.** These results support the uniform size distribution of SECs after acidolysis for up to 30 hr, with a size of 31.0 ± 2.2 µm. In contrast, SEC structural integrity decreases with prolonged acidolysis and begins to cause significant fragmentation of the SECs beyond 30 hr treatment durations.

Figure 6 shows the SEM and DIPA data for SECs produced with only acidolysis processing using phosphoric acid for 30 hr. These results confirm that after the defatting step, acidolysis produced clean and intact SECs with a uniform size distribution of 32.5 ± 2.7 µm. Acidolysis-only SECs have a residual protein content of only 0.15 ± 0.0%, as determined relative to % nitrogen content,[11] which is comparable to SECs produced by alkali and acidolysis treatment as discussed above and with previous reports.[7]

Microencapsulation using Sporopollenin Exine Capsules

In order to demonstrate the use of *L. clavatum* SECs produced using the acidolysis-only process, microencapsulation experiments were performed using BSA as a model protein. The loading into SECs was achieved by using a vacuum loading technique using 150 mg SECs and 1.2 ml of 125 mg/ml BSA. **Figure 7** shows the CLSM images of SECs before and after acidolysis-only processing and with FITC-BSA encapsulation. The data indicates that untreated SECs exhibit autofluorescence due to the presence of sporoplasm constituents inside the spore cavity. However, after acidolysis-only processing, all sporoplasmic contents are removed resulting in an empty inner cavity. Strikingly, after encapsulation of FITC-BSA, green fluorescence is observed inside the SECs, confirming the encapsulation of the model protein into SECs.[19,20,41]

Further, to investigate the morphological changes and micromeritic properties of BSA-loaded SECs, SEM and DIPA analysis were conducted and the data is presented in **Figure 8**. The SEM images confirm the presence of intact and clean microridges after BSA loading. In addition, no substantial changes were observed in diameter, circularity, and aspect ratio after BSA loading into SECs. These data are consistent with previous reports on the encapsulation of drugs using SECs.[7,10]

To quantify the amount of encapsulated BSA, BSA was extracted from 5 mg of BSA-loaded SECs, and the data is presented in **Table 1**. The data indicates 0.170 ± 0.01 g of BSA loading per gram of SECs. Overall, the SEM, DIPA, CLSM, and quantification of BSA by spectrographic analysis, confirm the successful encapsulation of BSA in *L. clavatum* SECs and SEC morphological stability.

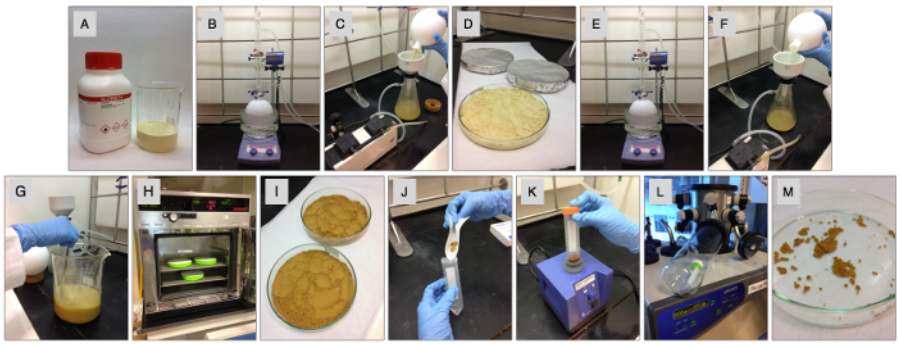

Figure 1. Streamlined Extraction Process for *L. clavatum* Sporopollenin Exine Capsules (SECs). (A) Untreated spores. **(B)** Spore defatting using a refluxing set-up at 50 °C for 6 hr. **(C)** Solvent filtration and collection of defatted spores. **(D)** Drying of defatted spores at room temperature. **(E)** Spore acidolysis in PTFE flask using 85% (v/v) phosphoric acid at 70 °C for 30 hr. **(F)** Acid filtration using vacuum filtration set-up. **(G)** SEC washing using a series of water, acid, base, and solvents washing steps. **(H)** SEC drying using vacuum oven at 60 °C and 1 mbar vacuum for 10 hr. **(I)** Intact, clean, and dried SECs as a final product. **(J)** Mixing of SECs and BSA solution. **(K)** Homogenization of SECs and BSA solution. **(L)** Vacuum loading of BSA into SECs. **(M)** BSA-loaded SECs. Please click here to view a larger version of this figure.

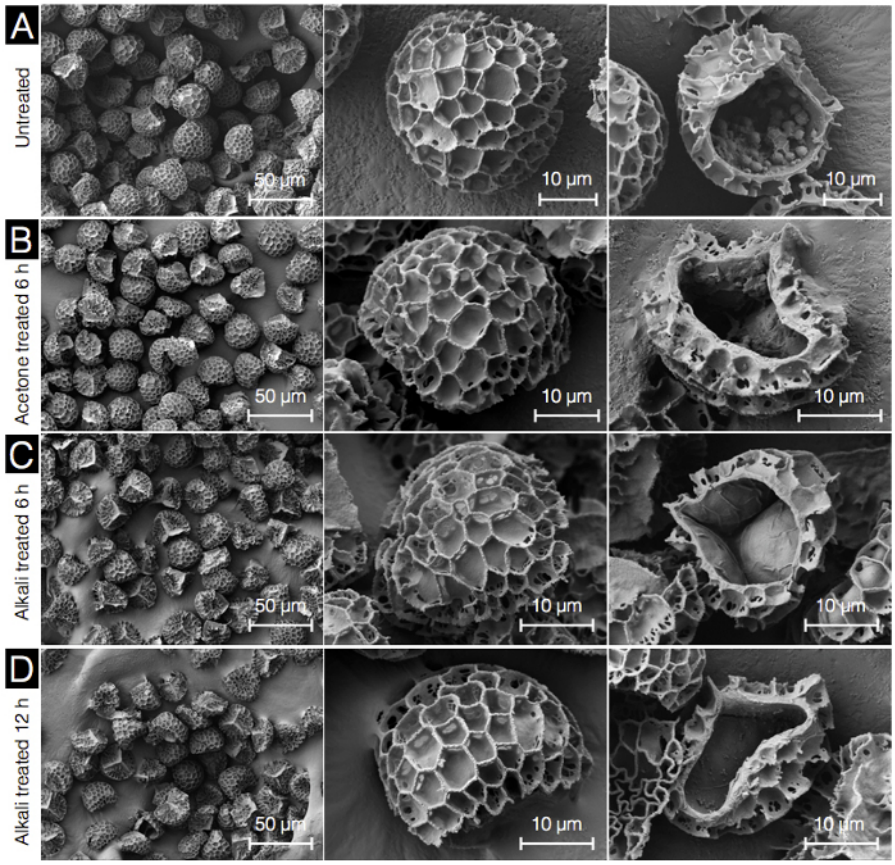

Figure 2. Scanning Electron Microscopic Analysis of *L. clavatum* Spores during Different Stages of Pre-acid Treatment. (A) Untreated spores showing sporoplasmic cellular organelles and biomolecules. **(B)** Acetone treated spores showing disrupted sporoplasmic contents. **(C)** & **(D)** Alkali treated spores showing removal of sporoplasmic contents and wrinkled internal cellulosic intine layer. Adapted from [41] and used with permission from Scientific Reports. Please click here to view a larger version of this figure.

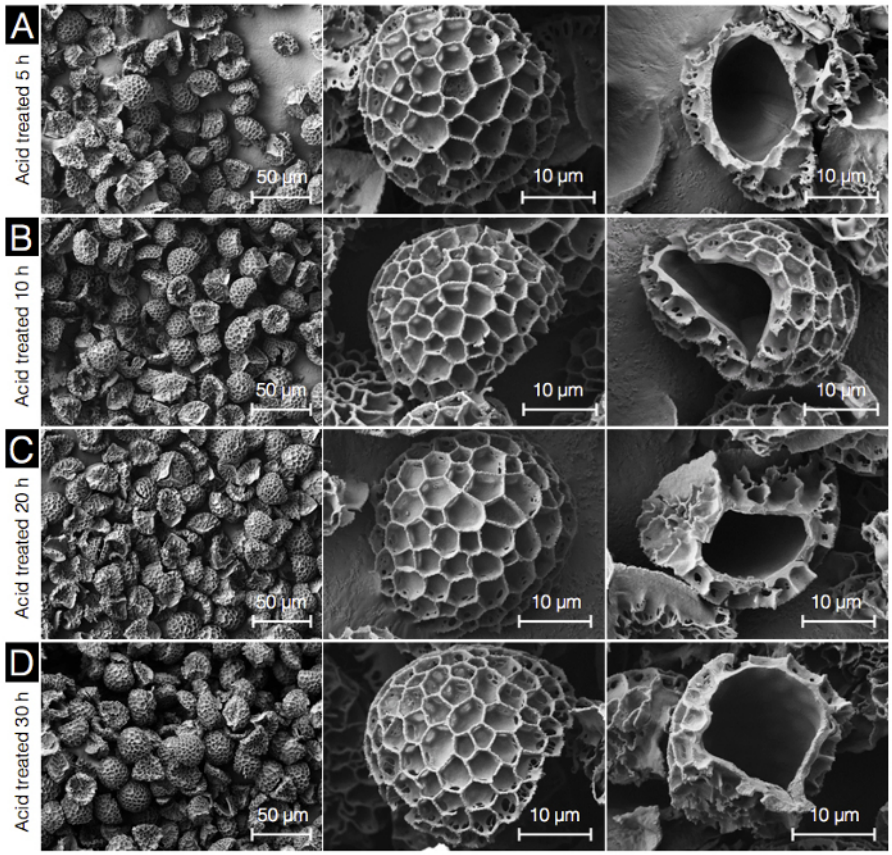

Figure 3. Scanning Electron Microscopic Analysis of *L. clavatum* Spores during Different Stages of Acid Treatment from 5 to 30 hr. (A - D) Respectively, 5, 10, 20, and 30 hr acid treated spores showing intact external microstructure and clean internal cavity. Adapted from [41] and used with permission from Scientific Reports. Please click here to view a larger version of this figure.

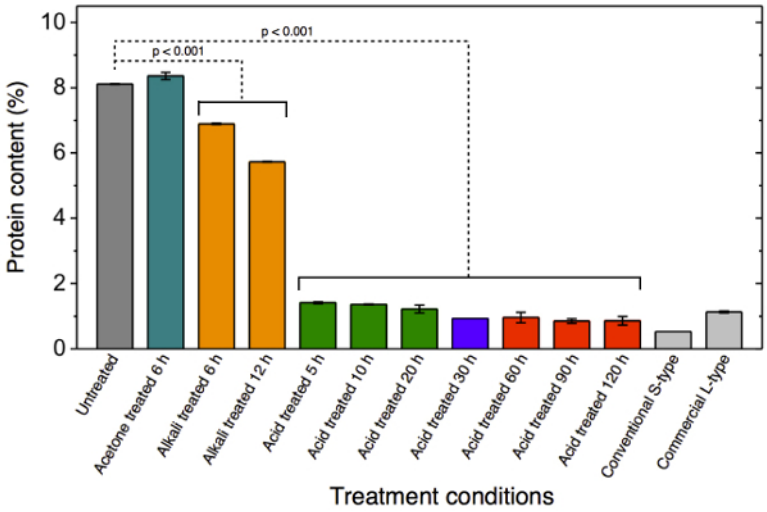

Figure 4. Protein Content of Spores and Sporopollenin Exine Capsules (SECs). Protein content after various stages of treatment. Data represented is an average of triplicate measurements with standard deviation (n = 3). Adapted from [41] and used with permission from Scientific Reports. Please click here to view a larger version of this figure.

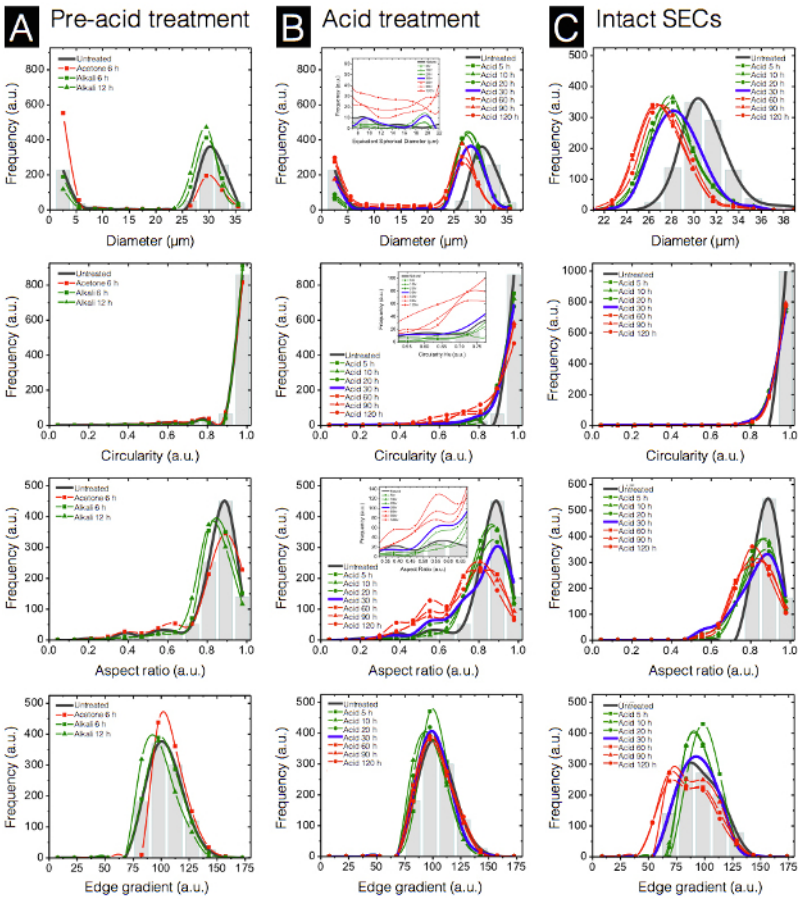

Figure 5. Dynamic Imaging Particle Analysis (DIPA) of Spores and Sporopollenin Exine Capsules (SECs) at Various Stages of Treatment. Columns **(A - C)** are respectively, pre-acid treatment, acid treatment, and intact SECs. Plots are representative graphs of SEC diameter, circularity, aspect ratio, and edge gradient. Adapted from [41] and used with permission from Scientific Reports. Please click here to view a larger version of this figure.

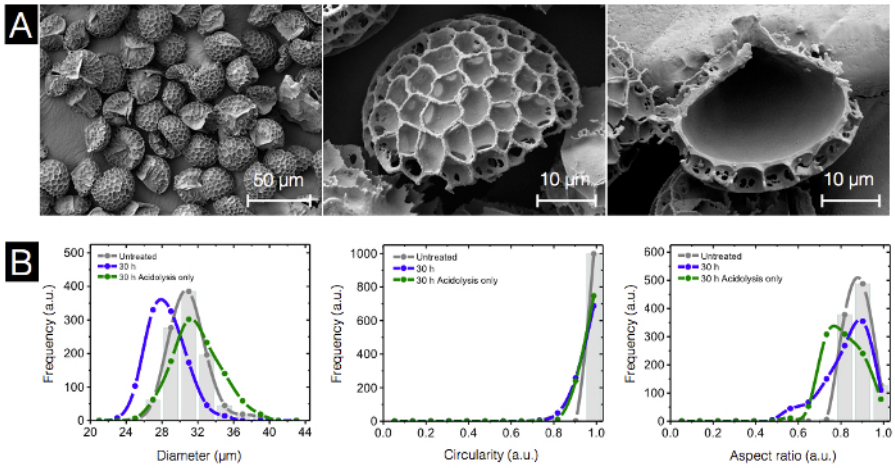

Figure 6. Scanning Electron Microscopy (SEM) and Dynamic Imaging Particle Analysis (DIPA) of 30 hr Acidolysis-only Sporopollenin Exine Capsules (SECs). (A) SECs showing intact external microstructure and clean internal cavity. **(B)** Representative graphs of SEC diameter, circularity, and aspect ratio. Adapted from [41] and used with permission from Scientific Reports. Please click here to view a larger version of this figure.

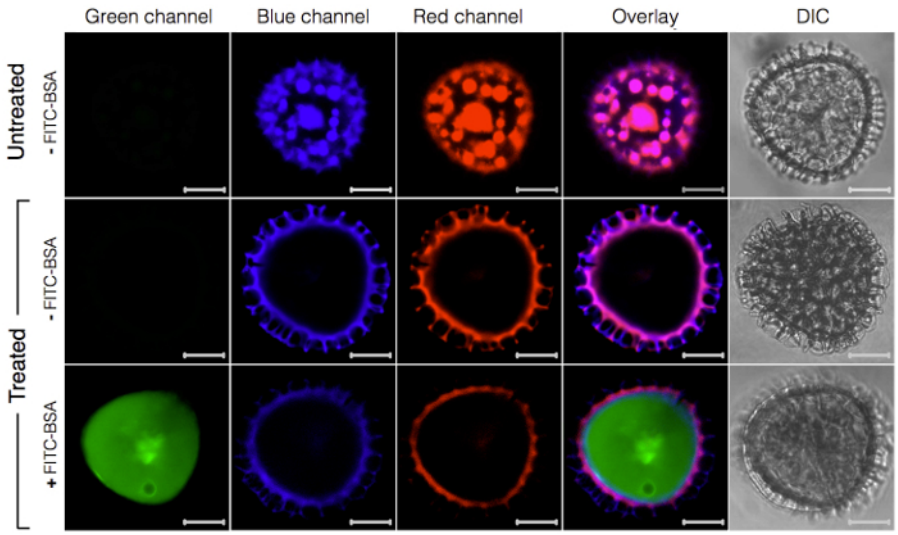

Figure 7. Confocal Laser Scanning Microscopy (CLSM) Analysis of Sporopollenin Exine Capsules (SECs) before and after Treatment and BSA Encapsulation. The first row depicts untreated spores showing sporoplasmic cellular organelle and biomolecule autofluorescence. The second row depicts 30 hr acidolysis-only SECs with an empty inner cavity. The third row depicts FITC-BSA loaded into 30 hr acidolysis-only SECs. (Scale bars are 10 µm). Adapted from [41] and used with permission from Scientific Reports. Please click here to view a larger version of this figure.

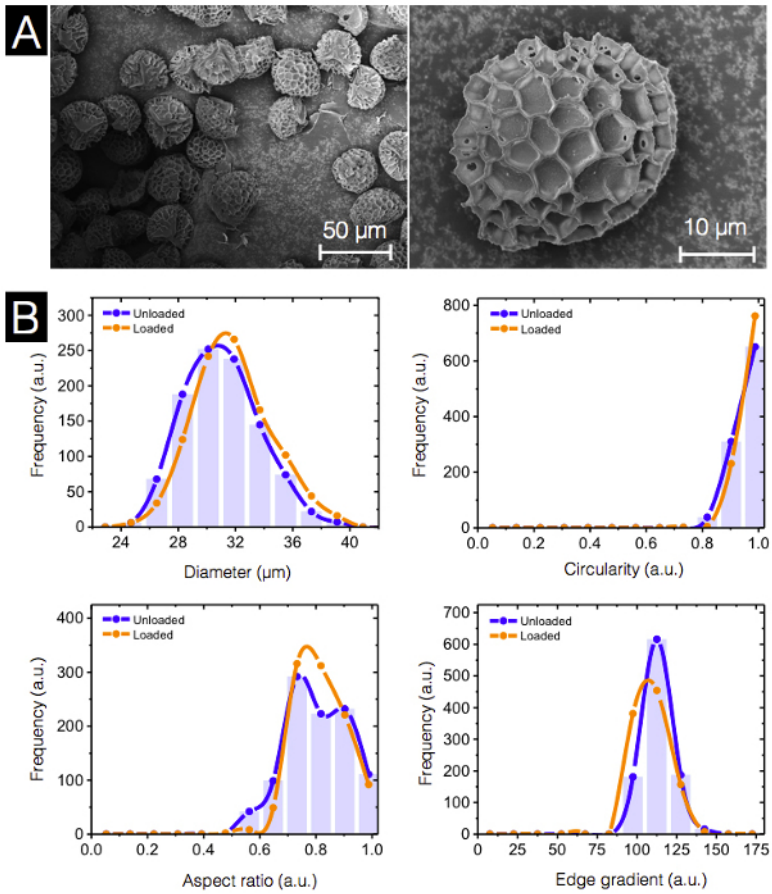

Figure 8. Scanning Electron Microscopy (SEM) and Dynamic Imaging Particle Analysis (DIPA) Characterization of BSA-loaded Sporopollenin Exine Capsules (SECs). (A) SEM images of BSA-loaded SECs. **(B)** Representative graphs of BSA-loaded SEC diameter, circularity, aspect ratio, and edge gradient. Adapted from [41] and used with permission from Scientific Reports. Please click here to view a larger version of this figure.

Material	BSA loading per batch (125 mg/ml)	BSA in 5 mg SECs (mg)	Amount of BSA loaded (g per g of SECs)
Untreated spores	0.6 ml	0.654 ± 0.05	0.131 ± 0.01
SECs	1.2 ml	0.831 ± 0.05	0.170 ± 0.01

Table 1. Encapsulation of Bovine Serum Albumin (BSA) into Untreated Spores and SECs. Volume of BSA solution used for loading per 150 mg batch of 'untreated spores' or 'SECs'. Data represented is an average of triplicate batches with standard deviation (n = 3). Adapted from [41] and used with permission from Scientific Reports.

Discussion

In this work, a systematic analysis of SEC extraction from *L. clavatum* spores is presented and this report shows that it is possible to produce higher quality capsules while also achieving a significant streamlining of the pre-existing commonly used protocol.[11] In contrast to the existing protocol requiring a high process temperature (180 °C) and a long process duration (7 days),[11] the current SEC extraction processing optimization was primarily focused on reducing the temperature and duration of the acidolysis step.

The defatting and alkali treatments provide minimal removal of sporoplasmic material from spores. However, acidolysis from 5 hr to 120 hr removed the maximum amount of the sporoplasmic material. The results indicated that the total processing duration and temperatures could be drastically reduced from conventional techniques,[11] and that only 30 hr in moderate temperature (70 °C) phosphoric acid provided the optimum quality capsules. Also, beyond 30 hr acidolysis, it was found that the SECs began to fracture and a significant increase in particle fragments was observed, suggesting that the entire batch of SECs may be exhibiting a reduction in overall structural integrity. Additionally, it should be noted that *L. clavatum* is considered to comprise comparatively robust sporopollenin,[2] and that the specific processing conditions involved in this protocol, such as, acid concentration, temperature, and/or hydrolysis duration, may need to be adjusted for other pollen species.

It was shown that the production of SECs by acidolysis-only using phosphoric acid (85% v/v) for 30 hr yields clean and intact SECs.[41] The data confirms that acidolysis is the crucial step in SEC production. Finally, the acid-only SECs were used for the encapsulation of BSA and a high degree of loading was observed. The loading was achieved with the application of a vacuum, whereby the internal SEC cavity pressure is altered to forcefully draw the BSA solution into the empty capsules. Nanochannels (dia. 15 - 20 nm), which have been previously identified in the exine walls of *L. clavatum*,[7] allow the solution to enter into the large internal cavity. After loading, the capsules were washed and dried in a freeze-drier to yield a free-flowing powder.

Size uniformity and morphological characteristics are important factors for evaluating the quality of a successful encapsulation material. The observed size uniformity and intact morphology of BSA-loaded SECs confirmed the potential usage of acidolysis-only processed SECs for microencapsulation applications.

These results are important for the large-scale industrial production of SECs and for stimulating greater interest in the potential applications of SECs in the microencapsulation of drugs, proteins, peptides, food supplements, nutraceuticals, and cosmetics.

Disclosures

The authors have nothing to disclose.

Acknowledgements

This work was supported by the National Research Foundation (NRF-NRFF2011-01) and the National Medical Research Council (NMRC/CBRG/0005/2012).

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