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Sporopollenin exines: A novel natural taste masking material

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ABSTRACT

Sporopollenin exines extracted from the spores of the plant *Lycopodium clavatum* were used to encapsulate water, sunflower oil (0.5 g/g) and differing amounts of cod liver oil (cod liver oil per gram of sporopollenin exines: 0.5 g/g, 1.0 g/g, 2.0 g/g, 4.0 g/g). A double-blind taste trial, involving 20 volunteers, was conducted to compare the products. The encapsulated oils were in the form of a fine powder up to an oil loading of 1/1 (w/w). Blind tasting could not distinguish the cod liver oil preparation up to 1/1 (w/w) loading compared to the sporopollenin exines filled with either water or sunflower oil. At a loading of 2/1 and 4/1, the cod liver oil was uniformly identified. Therefore, sporopollenin exines can be loaded highly, at up to 1 g oil to 1 g of the exines, and still remain as a dry powder and retain flavor masking, thus disguising the contents.

1. Introduction

Human taste discrimination is highly evolved and can distinguish unpalatable flavours at low concentrations (Blakeslee & Salmon, 1935; Chalé-Rush, Burgess, & Mattes, 2007). This then poses a problem for the incorporation of food supplements that have a nutritional value, but are unpalatable. This is exemplified by fish oil preparations, products with important health benefits (Akapede, Omotara, & Ambeet, 1999; Balk et al., 2006; Gruenwald, Graubaum, & Harde, 2002; Klaypradit & Huang, 2008; Von Schacky, 2000;), but that have a strong unpleasant flavor. Cod liver oil, as well as some other fish oils, being rich in polyunsaturated fatty acids, namely eicosapentaenoic acid and docosahexaenoic acid, and in vitamins A and D, may have positive effects on the joints, heart, brain, nervous system and skin (Klaypradit & Huang, 2008). For example, it is reported that cod liver oil may ease stiffness and pain associated with arthritis (Gruenwald et al., 2002), reduce the risks of cardiovascular diseases (Balk et al., 2006; Von Schacky, 2000) and prevent rickets (Akapede et al., 1999). Unfortunately, in a normal oxygen-rich environment, oxidized by-products rapidly develop a pungent flavor that is unacceptable to many people despite their minute quantity. This effect thus limits the use of cod liver oil in food, especially if its incorporation as an ingredient in preparations should expose it to air. Therefore, an inexpensive and renewable technology to mask the pungent fishy flavor of cod liver oil could have extensive applications.

Routinely, fish oil is mixed with a flavoring to disguise its taste such as with a citrus or mint blend. When sold as a food supplement, cod liver oil is available in soft gelatin capsules; however, while such capsules mask the fishy flavor, they do not prevent a "burp-back" effect and cannot be used as an added ingredient in functional food. Therefore, one of the most efficient techniques to make pungent oils palatable and applicable as a food additive is perhaps microencapsulation (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007; Gibbs, Kermasha, Alli, & Mulligan, 1999). In addition to taste masking, encapsulation of ingredients can provide some protective features (e.g. against light, water, aerial oxidation) that makes it attractive in current food science (Weinbeck & Bodnár, 2007). Encapsulation of fish oils has been achieved using different materials and techniques, such as ultrasonic atomization of a chitosan emulsion, (Klaypradit & Huang, 2008) or spray drying of a maltodextrin emulsion (Jafari, Assadpoor, Bhandari, & He, 2008).

The purpose of the present study was to demonstrate that sporopollenin exines (SECs), extracted from readily available and renewable *Lycopodium clavatum* spores, can be used as effective

Abbreviations: IRR, incident rate ratio; SEC, sporopollenin exine capsule; OR, odd ratio; CI, confidence interval.

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food microcapsules to mask the taste of cod liver oil and provide an oil-rich material in a powder form. Exines are the exoskeletal shells of plant spores and pollens, composed of sporopollenin (Brooks, Grant, Muir, Van Gijzel, & Shaw, 1971).

2. Materials and methods

2.1. Sporopollenin exine capsules

L. clavatum L. (club moss) spores were purchased from Tibrewala International (Nepal), acetone from Aldrich UK, and potassium hydroxide, ethanol, orthophosphoric acid, hydrochloric acid, and sodium hydroxide from Fisher Scientific UK Ltd.

Sporopollenin exine capsules (SECs) were extracted from *L. clavatum* L. Spores as follows. Spores (300 g) were stirred in acetone (900 mL) under reflux for 4 h, filtered and dried overnight in open air. They were stirred under reflux for 12 h in an aqueous solution of potassium hydroxide (54 g in 900 mL), the solution being renewed after 6 h, filtered, washed with hot water (5×300 mL) and hot ethanol (5×300 mL), and dried overnight in open air. The particles were stirred under reflux for 7 days in orthophosphoric acid (900 mL), filtered, washed with water (5×300 mL), acetone, 2 mol/L hydrochloric acid, 2 mol/L sodium hydroxide (each 300 mL), water (5×300 mL), acetone and ethanol (each 300 mL), and dried at 60 °C until constant weight.

Typical elemental analysis of sporopollenin (g/100 g) was: carbon 68.90, hydrogen 7.90, nitrogen 0.00, as determined on a Fisons Instruments Carlo Erba EA 100 C H N S analyzer.

2.2. Scanning electron microscopy (SEM)

Scanning electron micrographs were obtained using a Leica Cambridge Stereoscan 360 Scanning Electron Microscope (SEM). Samples were mounted on a metal stub with an adhesive and coated under vacuum with carbon before being coated with a 200 Å gold film.

2.3. Laser scanning confocal microscopy (LSCM)

Confocal images were obtained using a Bio-Rad Radiance 2100 laser scanning microscope equipped with Ar (488 nm), Green HeNe (563 nm) and Red diode (637 nm) laser lines and connected to a Nikon TE-2000E inverted microscope from Nikon, Japan. Images were collected using LaserSharp2000. Samples were spread onto a glass microscope slide and sealed under a cover slip using nail varnish.

2.4. Encapsulated oils

Sunflower oil was purchased from Tesco plc (UK) and cod liver oil from Seven Seas (UK).

Four samples were prepared with fish oil encapsulated in SECs and one with sunflower oil. Oil (respectively cod liver oil: 0.5 g, 1.0 g, 2.0 g, 4.0 g; and sunflower oil: 0.5 g) was poured over loose SEC powder (1.0 g). The mixture was gently stirred until a homogeneous paste resulted that was then subjected to a vacuum (ca. 10hPa) for 2 h to facilitate passive loading of oil into the particles through the porous sporopollenin walls. Water (5 mL) was mixed with SECs (1.0 g) to constitute a blank of similar texture as the other samples.

Samples were randomly labelled by an independent worker who drew them blindly out of an opaque box randomly labeled samples. Here, they are named after the oil they contain followed by the ratio oil/SECs (w/w).

2.5. Taste trial

Ten women and ten men participated in the taste trial. Ages of the volunteers were equally distributed into four categories (20–30, 30–40, 40–50 and 50–60), which contained no less than two men and two women each. The tasters had not been trained and were not blinded during the test. Each one of the twenty volunteers was presented the six freshly prepared samples in clear glass containers, which all had the same aspect of a brown paste. They tasted, in a random order, ca. 10 milligrams of each sample, using a clean plastic spatula. Between each sample tasting, the volunteers rinsed their mouth with water. The samples consisted of SECS loaded with water, sunflower oil and cod liver oil respectively, in amounts identified in the previous section (Encapsulated oils). Firstly, the volunteers were informed that the samples possibly contained water, sunflower oil, cod liver oil, olive oil or cocoa butter. Secondly, they were challenged to identify which product was encapsulated in each sample on the basis of taste. Thirdly, they scored the samples on a scale one, for the mildest or most palatable, to five, for the strongest or most pungent.

2.6. Statistical methods

The data for taste strength consisted of counts of observations. This data was thus analyzed by Poisson regression. Overdispersion was examined using methods developed elsewhere (Dean, 1992; Dean & Lawless, 1989).

The data for oil recognition consisted of binary observations (i.e. taste and strength correctly identified or not). This data

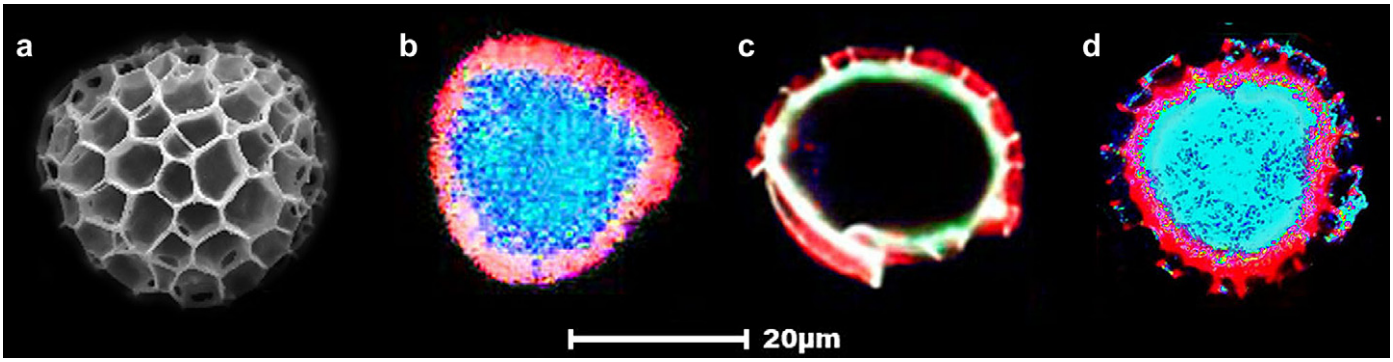

Fig. 1. SEM image of empty SEC (a); LSCM images of a *L. clavatum* spore with cytoplasm *(blue)* and exine *(red)* (b), empty SEC showing the exine *(red and white)* (c), and SEC *(red)* filled with cod liver oil *(blue)* (d). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Taste strength of the SEC preparations according to the 20 volunteers (given as average per taster and total sum of the scores over the 20 people): probability for each sample to be scored higher than the blank (IRR).

Sample	Taste score		Incident rate ratio (IRR)	95% CI
	Average	Total sum		
blank	1.8 ± 1.0	36	1.0	
cod liver oil/SECs (0.5/1)	2.2 ± 1.2	44	1.2	0.8, 1.9
cod liver oil/SECs (1/1)	2.0 ± 1.3	40	1.1	0.7, 1.7
cod liver oil/SECs (2/1)	**3.5 ± 1.1**	**70**	**1.9**	**1.3, 2.9**
cod liver oil/SECs (4/1)	**3.3 ± 1.6**	**65**	**1.8**	**1.2, 2.7**
sunflower oil/SECs (0.5/1)	1.9 ± 0.9	38	1.1	0.7, 1.7

Notes: 1. Each of the volunteers scored each sample on a scale one, for the mildest or most palatable, to five, for the strongest or most pungent. 2. Example interpretation: volunteers were 1.8 times as likely to score 4/1 cod liver oil/SECs high compared to the blank. 3. Bold type indicates significantly different from the blank. 4. Calculations subject to rounding errors.

followed a binomial distribution. Overdispersion was investigated using the approach of Williams (1982).

The taste strength and the oil recognition groups were considered each as a factor at six levels (blank, fish oil strengths 0.5/1, 1/1, 2/1, 4/1, and sunflower oil).

The blank was taken as the reference group against which all comparisons were made. Results from the first approach are presented as incident rate ratio (IRR) and that of the second one as odds ratio (ORs), both with 95% confidence intervals (CIs). There were no a priori reasons to expect differences for either age or sex, so data were not adjusted for these two variables. A nominal level of 5% statistical significance was assumed (two-tailed). The GLIM4 statistical computer package (Francis, Green, & Payne, 1994) was used to analyse the data.

3. Results

3.1. Encapsulation of oil inside SECs

The integrity of an extracted sporopollenin exine in the form of a round microcapsule with a continuous surface can be seen by SEM (Fig. 1a). Exines are robust with consistent morphology and size (ca. 27 μm in diameter in the case of those obtained from *L. clavatum* spores). A comparison between a spore filled with its natural sporoplasm (Fig. 1b) and an empty SEC (Fig. 1c) can be seen by LSCM. Exines have a large internal volume vacant for encapsulation. The LSCM image (Fig. 1d) shows that an SEC can be efficiently loaded at 1/1 (w/w) with the inner cavity appearing full but with very little oil remaining on its surface.

3.2. Flavor strength

The relationship between flavor strength and taste sample is presented in Table 1. There was no evidence of overdispersion in the Poisson regression model (Dean, 1992; Dean et al., 1989) so this component of variation was not considered further. There was a significant difference in flavor strength between the blank and the samples containing cod liver oil at 2/1 and 4/1 ratios, with volunteers being notably more likely to score these two compounds higher. There was no evidence of dose-response within the fish oil compounds. This datum was also analyzed under a binomial model (r/n, with r being the actual score given; n being the maximum achievable score of 5). Samples containing 2/1 and 4/1 cod liver oil/SECs (respectively) were thus more likely than the blank to have higher scores (OR = 4.2, 95% CI = 2.3,6.5; OR = 3.3, 95% CI = 1.8,5.9 respectively). The odds ratios for the other taste groups were similar to those from the Poisson regression, and in the same order of size.

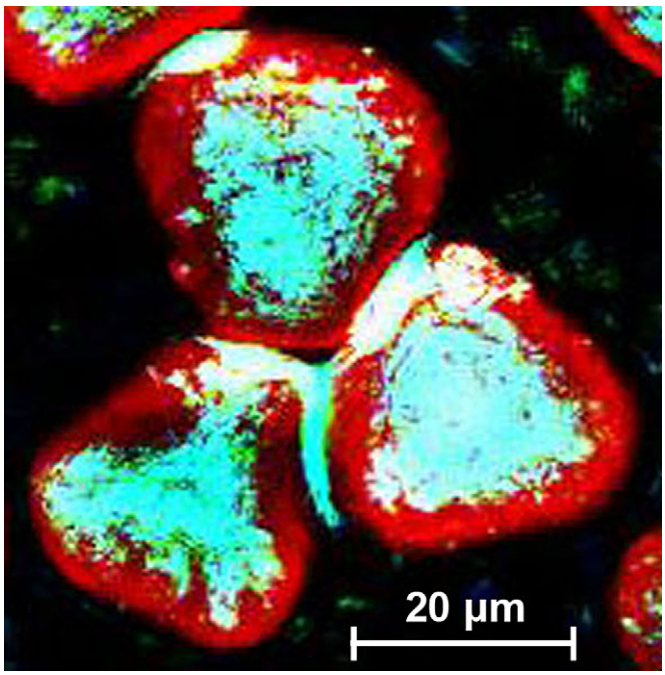

Fig. 2. LSCM image of SECs *(red)* overfilled with cod liver oil *(blue)*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Taste recognition

The relationship between flavor recognition and taste group is presented in Table 2. There was no evidence of overdispersion in the Binomial regression model (Williams, 1982) so this component of variation was not considered further. There was a significant difference between the blank and the samples containing cod liver oil at 2/1 and 4/1 ratios. These two tastes were significantly more likely to be identified correctly compared to the blank. There was no evidence of dose-response within the cod liver oil compounds. Volunteers were one-third less likely to identify sunflower oil mixed with SECs when compared to the blank.

Table 2
Number of positive identifications of the samples by the 20 volunteers: probability for each sample to be recognized (OR).

Taste group	Positive recognitions (out of 20)	Odds ratio (OR)	95% CI
blank	10	1.0	
cod liver oil/SECs (0.5/1)	8	0.7	0.2, 2.3
cod liver oil/SECs (1/1)	7	0.5	0.2, 1.9
cod liver oil/SECs (2/1)	**17**	**5.7**	**1.3, 25.9**
cod liver oil/SECs (4/1)	**16**	**4.0**	**1.0, 16.3**
sunflower oil/SECs (0.5/1)	4	0.3	0.06, 1.0

Notes: 1. Example interpretation: volunteers were four times as likely to identify correctly the 4/1 cod liver oil/SECs sample compared to the blank. 2. Bold type indicates significantly different from the blank. 3. Calculations subject to rounding errors.

4. Discussion

The efficacy of taste masking of the exines for the cod liver oil was apparent that a 1/1 (w/w) loading level was no more likely to be identified than the blank water-loaded exines (40 versus 36). Similarly, sunflower oil, a non-pungent product, was not recognizable, but only 20% of the tasters were able to identify it; in addition, they scored its flavor hardly higher than that of the blank (38).

Nevertheless, the volunteers were able to recognize a fishy flavor in highly loaded sporopollenin when the loading was 2/1 and 4/1. This can be explained by some residual oil being present on the outside of the sporopollenin capsules (Fig. 2) that was immediately detected as such by tasters.

These data are novel by the extent of the loading achieved by the SECs and the degree of taste masking that resulted. In addition, the process of filling was simple, requiring no sophisticated equipment and as such is likely to be appropriate for scaling up to industrial quantities. A remarkable property of the loaded exines at 1/1 (w/w) was that they retained a free flowing powder consistency, potentially important for mixing in with other components. A double encapsulation (e.g. with wax) or use of more traditional methods such as flavoring (e.g. limonene) could enhance the flavor masking properties further.

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References

Akpede, G. O., Omotara, B. A., & Ambeet, J. P. (1999). Rickets and deprivation: a Nigerian study. *Journal of the Royal Society for the Promotion of Health, 119*, 216–222.

Balk, E. M., Lichtenstein, A. H., Chung, M., Kupelnick, B., Chew, P., & Lau, J. (2006). Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis, 189*, 19–30.

Blakeslee, A. F., & Salmon, T. N. (1935). Genetics of sensory thresholds: individual taste reactions for different substances. *Proceedings of the National Academy of Sciences of the USA, 21*, 84–90.

Brooks, J., Grant, P. R., Muir, M., Van Guizel, P., & Shaw, G. (1971). *Sporopollenin*. London/New York: Academic Press.

Chalé-Rush, A., Burgess, J. R., & Mattes, R. D. (2007). Evidence for human orosensory (taste?) sensitivity to free fatty acids. *Chemical Senses, 32*, 423–431.

Dean, C. (1992). Testing for over dispersion in Poisson and binomial regression models. *Journal of the American Statistical Association, 87*, 451–457.

Dean, C., & Lawless, J. F. (1989). Tests for over dispersion in Poisson regression models. *Journal of the American Statistical Association, 84*. 467–472.

Francis, B., Green, M., & Payne, C. (1994). *The GLIM system release 4 manuals*. Oxford: Clarendon Press.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray drying in micro encapsulation of food ingredients: an overview. *Food Research International, 40*, 1107–1121.

Gibbs, B. F., Kermasha, S., Alli, I., & Mulligan, C. N. (1999). Encapsulation in the food industry: a review. *International Journal of Food Sciences and Nutrition, 50*(3), 213–224.

Gruenwald, J., Graubaum, H. J., & Harde, A. (2002). Effect of cod liver oil on symptoms of rheumatoid arthritis. *Advances in Therapy, 19*, 101–107.

Jafari, S. M., Assadpoor, E., Bhandari, B., & He, Y. (2008). Nano-particle encapsulation of fish oil by spray drying. *Food Research International, 41*, 172–183.

Klaypradit, W., & Huang, Y.-W. (2008). Fish oil encapsulation with chitosan using ultrasonic atomizer. *LWT – Food Science and Technology, 41*, 1133–1139.

Von Schacky, C. (2000). N–3 fatty acids and the prevention of coronary atherosclerosis. *American Journal of Clinical Nutrition, 71*, 224–227.

Weinbeck, F., & Bodnár, I. (2007). Improve your ingredient efficiency: encapsulate it!. *Food Manufacturing Efficiency, 1*(3), 33–37.

Williams, D. A. (1982). Extra-binomial variation in logistic linear models. *Applied Statistics, 31*, 144–148.