Analysis of monoterpenoids in inclusion complexes with β-cyclodextrin and study on ratio effect in these microcapsules

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Abstract

In recent studies, the insecticide activity against some stored products pests of monoterpenoids, such as linalool, *S*-carvone, camphor, geraniol, γ-terpinene and fenchone, and phenylpropanoids, like *E*-anethole and estragole, has been proved. Currently, applications of these volatile compounds are complicated due to their chemical and physical properties. This is one of the major problems for their use as insecticides; therefore, microencapsulation could be the solution to problems of stability, evaporation and release. Microencapsulation of these chemicals was carried out with β-cyclodextrin using a chemical precipitation method at four different ratios (β-cyclodextrin: monoterpenoids), 1.33:1, 3.33:1, 4.66:1 and 6.66:1 (w/w) in order to determine the ratio effect. This study establishes that encapsulation at the ratio of 3.33:1 to linalool and γ-terpinene was higher, whereas *S*-carvone, camphor, *E*-anethole, geraniol, estragole and fenchone showed the greatest encapsulation when the ratio was 6.66:1. Furthermore, the efficiency of encapsulation was estimated by measuring the content of the compounds in the powder by gas chromatography. The maximum inclusion efficiency of β-cyclodextrin was reached by camphor (52%) followed by geraniol (34%) using 10 g of β-cyclodextrin and linalool (31%) using 5 g of this matrix. The present study indicates that natural products such as monoterpenoids or phenylpropanoids could be microencapsulated in an efficient way using an appropriate amount of β-cyclodextrin.

Keywords: Microencapsulation, β-cyclodextrin, Camphor, Geraniol and Linalool

1. Introduction

Essential oils and others phytochemicals have been studied as insecticides to control pests lately due to their particular properties. These natural products show toxic (Don-Pedro, 1996; Clemente et al., 2003), repellent (Pascual-Villalobos and Ballesta-Acosta, 2003), antifeedant and ovicidal effect in insect pests (Regnault-Roger and Hamraoui, 1994; Alvarez-Castellanos et al., 2001). Some monoterpenoids and phenylpropanoids, obtained directly from plant secondary metabolism, have insecticidal activity against stored product pests (Lee et al., 2003; García et al., 2005).

Recently, López et al. (2008) demonstrated that some monoterpenoids such as linalool, *S*-carvone, camphor, geraniol, γ-terpinene and fenchone and phenylpropanoids like *E*-anethole and estragole, could be alternatives to synthetic insecticides against some stored-product pests such as *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) or *Cryptolestes pusillus* (Schönherr) (Coleoptera: Cucujidae). However, applications of these volatiles have turned out to be particularly complicated due to their chemical and physical properties (low stability, high evaporation and release).

Microencapsulation allows immobilization, protection, release and functionalisation of active ingredients. The use of microcapsules in food (Versic, 1988) or pesticides (Fuyama et al., 1984) are generally one of the main applications in the industry since the main purpose of microencapsulation is to entrap sensitive ingredients, such as volatile and flavours into solid carriers to increase their protection, reduce evaporation, promote easier handling, and control their release during storage and applications (Baranauskiené et al., 2007).

Starch matrix is one of the natural materials that are receiving considerable attention because of its great abundance, ease of recovery from plant sources, low cost and its ready conversion chemically, physically and biologically into a broad spectrum of low molecular weight polymers (Doane, 1993). Starch and starch-based ingredients (modified starches, maltodextrins, *β*-cyclodextrins) are widely used to retain and protect volatile compounds (Madene et al., 2006). Cyclodextrins (*α, β* and *γ*) are cyclic oligosaccharides consisting of six, seven and eight glucose units, respectively.

The binding of volatile compounds to starch has been classified into two types. On the one hand, the flavour compound surrounded by the amylase helix through hydrophobic bonding is known as an inclusion complex. On the other hand, polar interactions have been determined which involve hydrogen bonds between the hydroxyl groups of starch and aroma compounds (Arvisenet et al., 2002; Boutboul et al., 2002).

The aim of this study is to investigate if 6 monoterpenoids (linalool, camphor, geraniol, *S*-carvone, *γ*-terpinene, and fenchone) and 2 phenylpropanoids (*E*-anethole and estragole) could be microencapsulated in an efficient way using different ratios of *β*-cyclodextrin to monoterpenoids during the complexation process.

2. Materials and methods

2.1. Chemicals

Six monoterpenoids, (-)-linalool (97%), camphor (96%), *γ*-terpinene (98%), geraniol (99%), *S*-carvone (98%), fenchone (98%) and 2 phenylpropanoids, *E*-anethole (99%) and estragole (98%) were used as guest molecules and were obtained from ACROS Organics BUBA/SPRL. To carry out the complexation process, the matrix chosen was *β*-cyclodextrin (98%) which was purchased from Sigma-Aldrich.

2.2. Preparation of microcapsules

A chemical precipitation method based on Reineccius (1989) was used to prepare monoterpenoid or phenylpropanoid-*β*-cyclodextrin complexes. Different amounts of *β*-cyclodextrin (2, 5, 7 and 10 g) were dissolved in 500 mLof an ethanol to water (1:2) mixture and were maintained at 50ºC on a stirring hot plate. 1.5 g of monoterpenoid or phenylpropanoid were dissolved in ethanol (10% w/v) and were slowly added to the *β*-cyclodextrin solution with continuous stirring. After this addition, the heating was stopped and was maintained at room temperature and stirred for 4 h. This solution was maintained at 4ºC overnight and the precipitated *β*-cyclodextrin-monoterpenoid or phenylpropanoid complex was recovered by filtration and was dried in a oven at 50ºC for 24 h. Finally, the powder was allowed to air-dry at 25ºC for one day in order to reach its equilibrium moisture content. The powder was observed by optical microscopy (40x) to check the microcapsules formation. This process was carried out for each isolated monoterpenoid and phenylpropanoid. Four starting ratios of *β*-cyclodextrin to core material (monoterpenoids and phenylpropanoids) were used: 1.33:1, 3.33:1, 4.66:1 and 6.66:1. Each ratio was replicated twice; both results (first and second replication) were very similar.All samples were stored at 25ºC in airtight vials.

2.3. Total monoterpenoids and phenylpropanoids extraction

To determine the total amount of volatile compounds in the powder an extraction method was used. 0.5 g of powder (for each monoterpenoid, each phenylpropanoid and each ratios studied) were mixed with distilled water (8 mL) and hexane (4 mL) in glass vials (15 mL) and were sealed.

This solution was heated and stirred in a hot plate at 75ºC for 20 min. The organic phase containing the volatile compounds was decanted, and the aqueous phase was exhaustively extracted with hexane 3 times (4 x 4 mL). These 4 phases were combined. For each treatment the hexane was removed by using a nitrogen stream. Finally the concentrated extracts were transferred to insert vials and stored at 4ºC until required for GC/MS analysis.

2.4. Efficiency of encapsulation

The identification of each monoterpenoid or phenylpropanoid was accomplished by comparing the mass spectra and retention times of compounds with standards. Quantitative analysis of the volatiles extracted from the powder was carried out using GC-MS and internal standards for each monoterpenoid and phenylpropanoid in different range of concentrations. The quantitative analysis of each compound was carried out using a model 5890 Series II equipped with a DB-Waxetr 30 m x 0.32 mm capillary column coated with a polyethylene glycol film (1 μm thick). The chromatographic conditions were as follows: an Agilent model 5972 inert mass spectrometry (MS) detector (Agilent, Palo Alto, CA). The initial oven temperature was held at 60ºC for 1 min. Afterwards, it was increased by 3ºC min^{-1} to 225ºC, injector at 250ºC, column head pressure at 8.00 psi, helium carrier gas, flow rate 2.6 mL min^{-1}, splitless with 2 μL of sample injected. Content of monoterpenoids and phenylpropanoids was calculated according to the area of the chromatographic peaks. A linear regression model was computed and was obtained using standard dilution techniques to quantify components.

2.5. Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using SPSS. There were four ratios of *β*-cyclodextrin to monoterpenoids. Differences among treatments were determined by Duncan's multiple test at the 5% level ($P<0.05$).

3. Results

For the great majority of components studied, we observed that the higher the ratio of β-cyclodextrin, the greater the encapsulation (Table 1), although there were some exceptions, such as linalool at 3.33:1 ratio (0.4680 g) and *γ*-terpinene at the same ratio (0.0195 g), but in the latter case there was no statistically significant difference. The rest of monoterpenoids and phenylpropanoids showed clearly the highest values for the ratio 6.66:1 (eg. S-carvone: 0.1678 g, camphor: 0.8105 g, E-anethole: 0.4791 g or geraniol: 0.5311 g).

Table 1 Amount of natural insecticides, monoterpenoid or phenylpropanoid, microencapsulated at different *β*-cyclodextrin to monoterpenoid or phenylpropanoid ratios.

Efficiency of encapsulation	Ratio[1]	*β*-cyclodextrin[2] (g)	Natural insecticiede (g)	Natural insecticides recovered from 1.5 g microencapsulated formulation[3]							
				Camphor	Geraniol	Linalool	*E*-Anethole	S-Carvone	Estragole	Fenchone	Terpinene
Insecticide	1.33:1	2	1.5	0.0088 a	0.0171 a	0.0021 a	0.0000 a	0.0136 a	0.0000 a	0.0006 a	0.0004 a
Encapsulated	3.33:1	5	1.5	0.5001 c	0.1862 b	0.4680 c	0.0941 a	0.1135 b	0.1051 c	0.0130 a	0.0195 b
(g)	4.66:1	7	1.5	0.2036 b	0.0424 ab	0.0162 a	0.0325 a	0.0140 a	0.0172 b	0.0037 a	0.0043 a
	6.66:1	10	1.5	0.8105 d	0.5311 c	0.2363 b	0.4791 b	0.1678 c	0.1410 d	0.0515 b	0.0162 b
Insecticide	1.33:1	2	1.5	0.6	1.2	0.1	0.0	0.8	0.0	0.1	0.1
Encapsulated	3.33:1	5	1.5	33.4	12.4	31.3	6.3	7.6	7.0	0.9	1.3
(%)[4]	4.66:1	7	1.5	13.5	2.7	1.1	2.1	0.9	1.1	0.2	0.3
	6.66:1	10	1.5	52.3	34.2	15.2	30.9	10.8	9.1	3.3	1.0
Vapour pressures (mmHg at 20 °C)				--	0.20	0.17	0.05	0.40	0.11	--	0.70
Boiling Point (°C)				204	229	198	235	229	215	--	174

[1]Ratio *β*-cyclodextrin : monoterpenoid or phenylpropanoid. [2]Dry weight basis when 1.5 g of monoterpenoid or phenylpropanoid is added. [3]Treatments having the same letter are not significantly different, Duncan's multiple range test, $P<0.05$, (column comparison), n=2. [4]Insecticides varied from 1.4927 to 1.6337 g.

At the ratio 1.33:1 (2 g of β-cyclodextrin), all monoterpenoids studied (S-carvone, linalool, camphor, γ-terpinene, fenchone and geraniol) exhibited the lowest values of encapsulation or were not encapsulated at all in the case of phenylpropanoids (estragole and E-anethole). This indicated that more amount of matrix was necessary to improve or even achieve the encapsulation.

On the other hand we also observed that adding more matrix (β-cyclodextrin) did not always cause more encapsulation. Microencapsulation values for ratio 4.66:1 (7 g of β-cyclodextrin), were less than 5 g of β-cyclodextrin (3.33:1 ratio). Even though, in some cases, statistical comparison indicated there was no significant difference ($P>0.05$) between 3.33:1 and 4.66:1 ratios (eg. fenchona, E-anethole and geraniol).

Efficiency of encapsulation depends on monoterpenoids and phenylpropanoids studied and the different amount of matrix in dry weight basis used (Table 1).

The percentage of encapsulation for each compound, working with 2 g of β-cyclodextrin, is lower than when we assayed other amounts of β-cyclodextrin (5, 7 and 10 g), indicating that with the lesser amount of matrix, there was less encapsulation and consequently less recovery.

The data from 5 and 10 g of *β*-cyclodextrin pointed out that some monoterpenoids such as linalool (31.3% to 5 g of *β*-cyclodextrin), camphor (33.4% and 52.3% to 5 and 10 g of *β*-cyclodextrin, respectively). *E*-anethole (30.9% to 10 g of *β*-cyclodextrin) and geraniol (34.3% to 10 g of matrix) had reached a remarkable recovery.

We also conclude that some monoterpenoids are difficult to encapsulate using our methodology: low values for γ-terpinene (recovery: 0.1, 1.3, 0.3 and 1.0% to 2, 5, 7 and 10 g of β-cyclodextrin, respectively) and fenchone (recovery: 0.1, 0.9, 0.2 and 3.3% to 2, 5, 7 and 10 g of matrix, respectively) were obtained (Table 1). We should take into account that other aspects may contribute to the low recovery of these chemicals such as operational loss, evaporation and so on.

4. Discussion

The β-cyclodextrin inclusion complexes with terpenoids have been reported to be effective in preventing oxidation, retaining volatile substances, in masking undesired tastes and odours, and in solubilising water-insoluble substances (Donze and Coleman, 1993). In fact, in recent works the behaviour as modulated release, of volatile compounds within starch matrixes which are precursors of dextrins (Yilmaz et al., 2002; Yilmaz et al., 2004) have been examined. Other authors (Bertolini et al., 2001) have studied the stability of some monoterpenes encapsulated (β-pinene, citral, limonene, β-myrcene and linalool) with gum Arabic as a matrix, indicating that oxidative processes occurred for some core materials and concluded that gum Arabic was not efficient as a wall material.

In our study we have established how different ratios of β-cyclodextrin to monoterpenoids or phenylpropanoids can determine the encapsulated amount of guest molecule. As a result, the major ratio (6.66:1) presents the higher values, although occasionally lesser ratios (3.33:1) gave better results.

Bhandari et al. (1998), investigated the characteristics, including the profile of flavour volatiles, of the complex as affected by the ratio of lemon oil to β-cyclodextrin used during the complexation process. They found that a lemon oil powder could be successfully produced by a microencapsulation technique using β-cyclodextrin: lemon oil with a ratio to 0.4:1 treatment, which was not the highest ratio. In our assay the same occurs with monoterpenoids linalool and γ-terpinene. However, we cannot completely compare our results since we have just worked with isolated compounds instead of essential oils.

The highest rates of encapsulation were between 30 and 50% for camphor, E-anethole, geraniol and linalool. The remaining compounds only had rates of incorporation between 1 and 11%. These rates of incorporation are in agreement with Adamiec and Kalemba (2006) who analyzed two essential oils (elemi and peppermint) using maltrodextrin as carrier. Kim et al. (2006) accomplished a percentage of efficiency of encapsulation of isoflavone from 50 to 90%, although they assayed other coating materials, polyglycerol monostearate and triacylglycerol.

Our results show that a microencapsulation of monoterpenoids and phenylpropanoids using β-cyclodextrin, is possible, but optimum ratios have to be established for each compound and encapsulation method. Besides relating these results to our previous studies about insecticidal activity of monoterpenoids and phenylpropanoids against stored product pests, we have moved forward with regard to the use of these more appropriate and cleaner natural products in integrated pest management. Although these formulations also need to be studied in depth and assayed for toxicity to other stored product insects, with regards to the efficacy and duration of activity.

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