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Full Length Research Paper

Encapsulation of cinnamon oil in β-cyclodextrin

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Inclusion complexes between the *Cinnamomum verum* essential oil and β-cyclodextrin were prepared by co-precipitation method with the four oil to β-cyclodextrin ratios of 5:95, 10:90, 15:85 and 20:80 (w/w) in order to determine the effect of the ratio on the inclusion efficiency of β-cyclodextrin for encapsulating oil volatiles. The characterization of the complex involved the analysis of the initial essential oil, the surface and the total extracted oils. The retention of essential oil volatiles reached a maximum of 94.18% at the oil to β-cyclodextrin ratio of 10:90. Though, the maximum inclusion efficiency of β-cyclodextrin was achieved at the ratio of 15:85, in which the complex powder contained 117.2 mg of oil/g of β-cyclodextrin. Thirty-one flavor compounds were detected in the original oil and 21 compounds with the content higher than 0.10% were considered in complexation process. The qualitative and quantitative composition of the volatiles in the total oil extracts was similar to the starting oil. However the chromatographic profile of the surface adsorbed oil was different. The thirteen major flavor compounds, found in the commercial *C. verum* essential oil, were present in all of the extracts, but in the different proportions between the total and surface oil extracts.

Key words: *Cinnamomum verum* oil, β-cyclodextrin, inclusion complex, (*E*)-cinnamaldehyde.

INTRODUCTION

The *Cinnamomum verum* J.S. Presl (syn. *Cinnamomum zeylanicum* Blume), popularly known as cinnamon in the trade, is a small evergreen tree 10 - 15 m tall, belonging to the family Lauraceae, native to Sri Lanka and Southern India. It has been known from remote antiquity, and it was highly prized among ancient nations that it was regarded as a gift for monarchs and other great potentates. *C. verum* bark and leaf are widely used as a spice throughout the world (Seidemann, 2005). It is principally employed in cookery as a condiment and flavoring material, being largely used in the preparation of some kinds of desserts, chocolate, candies, tea, liqueurs and a cinnamon - sugar mixture is even sold separately for such purposes. In medicine it has been used to treat diarrhea and other problems of the digestive system. Cinnamon is high in antioxidant activity and also has antimicrobial properties (Baratta et al., 1998; Jayaprakasha et al., 2003). Its flavor is due to an aromatic essential oil which makes up 0.5 - 1% of its composition. Several chemotypes of *C. zeylanicum* have been reported, based on the chemical composition of leaf oil. Rao et al. (1988) reported two chemical races of *C. zeylanicum* from Bubhaneshwar, India, one rich in eugenol (83.1 - 88.6%) and the other dominated by benzyl benzoate (63.6 - 66.0%). Another chemotype with 85.7% linalool in leaf oil was reported by Jirovetz et al. (2001). Nath et al. (1996) reported a chemotype of *C. verum* growing in the Brahmaputra Valley containing benzyl benzoate as its major component (65.4%) in leaf oil. Two chemotypes of *C. verum* from Brazil were reported by Koketsu et al. (1997); one rich in eugenol (94.14 - 95.09%) and the other predominated by eugenol and safrole (with 55.08 - 58.66% eugenol and 29.57 - 39.52% safrole, respectively). According to Variyar and Bandyopadhyay (1989), eugenoltype is the most commonly occurring chemical race of *C. verum*.

The volatile oil from the stem bark of Madagascar origin was rich in eugenol (Medici et al., 1992). Krishnamoorthy et al. (1996) reported 2.7 - 2.8% volatile oil in the bark of the cinnamon varieties *Navashree* and *Nithyasree*, with 58 - 68% cinnamaldehyde content. Nath

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et al. (1996) recorded a chemotype of *C. verum* with 84.7% benzyl benzoate in bark oil from the Brahmaputra Valley, India.

Thus, cinnamon offers a variety of oils with different aroma characteristics and composition to the flavor industry.

Cyclodextrins have been widely used to prepare inclusion complexes to improve the stability and solubility, modify the release of drugs and turn liquid substances into stable powders (Szejtli, 1982; Nagai and Ueda, 1996; Villaverde et al., 2004). Among various flavor encapsulation techniques, molecular inclusion in a β-cyclodextrin molecule is the most effective one (Hedges et al., 1995). Inclusion complexation of flavors with β-cyclodextrin has been applied to protect oil volatiles against oxidation, heat and light degradation, evaporation and moisture. This protection is due to the fact that the flavor molecules are tightly held within the β-cyclodextrin molecule. The interaction between β-cyclodextrin (host) and flavor molecules (guests) may involve total inclusion or association with only hydrophobic part of the molecule (Shaidi and Han, 1993). The complexing of β-cyclodextrin with flavor molecules can be achieved by using three different methods (Pagington, 1985). In the first method, the cyclodextrin and flavor are stirred in aqueous solution, often in the presence of an amount of solvent. In the second method, comlexation is achieved by bubbling the flavors in vapor form through a solution of cyclodextrin. In the third method, the flavors are mixed with the cyclodextrin paste. During the selection of the preparation method several factors including good yield, simplicity, rapidity, simplicity of scaling up, low cost and characteristics of the end product should be taken into account.

Essential oils are one of the most relevant raw materials in food, perfumery and also pharmaceutical industries. Molecular encapsulation of essential oils upgrade their chemical and thermal stability and facilitate handling which could increase the potential uses in new dosage forms (Fernandes et al., 2004; Reineccius et al., 2002). But, a proper balance of the encapsulated flavor volatiles in the final complex powder is of great importance. It was shown that complexing of the essential oil with β-cyclodextrin improves physical and chemical stability mainly of terpenoid and phenylpropane derivatives (Szente and Szejtli, 1986). It opposite, it was found that smaller molecules are less retained than larger molecules (Reineccius and Risch, 1986). As each essential oil is composed of individual flavor compounds in various proportions, the final note offered by the product will depend on maintaining the original flavor composition during processing.

The aim of the present study was to prepare the inclusion complexes of the *C. verum* essential oil with β-cyclodextrin in various ratios, using a co-precipitation method, and to investigate the amount as well as the composition of the surface adsorbed and entrapped oil by GC-MS and compared with the composition of the initial oil.

MATERIALS AND METHODS

Hydrodistilled *C. verum* essential oil, commercially available, was purchased from BeoLab Laboratory (Serbia). β-Cyclodextrin was purchased from Merck (Germany). Other chemicals used in experiments were of analytical grade and were obtained also from Merck (Germany).

Complexation process

A precipitation method was used to prepare the *C. verum* oil - β-cyclodextrin complex (Reineccius, 1989). Five grams of β-cyclodextrin was dissolved in 50 mL of an ethanol/water (1:2) mixture at 55°C (±2°). A predetermined quantity of essential oil dissolved in ethanol (10% w/v) was then slowly added to the warm β-cyclodextrin solution. The following starting ratios of *C. verum* essential oil to β-cyclodextrin were used: 5:95, 10:90, 15:85 and 20:80 (w/w). The mixture was continuously stirred on the magnetic stirrer and the temperature maintained at 55°C. The mixture was stirred for another 4 h, without heating, while its temperature decreased spontaneously to 25°C. The final solution was refrigerated overnight at 7°C. The cold precipitated material was recovered by vacuum filtration. The precipitate was dried in a convection oven at 50°C for 24 h. The powder was then allowed to air-dry at 25°C for an additional 24 h in order for the powder to reach its equilibrium moisture content and weighed after that. The amount of recovered powder (dry basis) was calculated in percentage by deducting its moisture content. The obtained complex was stored in airtight glass containers, at room temperature, prior to further analysis.

Moisture determination

The moisture content of the β-cyclodextrin and the *C. verum* oil powder was analyzed by drying a powder sample (1 g) in a vacuum oven at 70°C for 24 h, under pressure <6.7 kPa (AOAC, 1990). The moisture content of the β-cyclodextrin was 8.18% and the *C. verum* oil powder was 6.34%.

Capillary GC-MS analysis

The standard oil and the concentrated oils extracted from the complexes (total oil and surface oil) were analyzed by GC-MS procedure, using a Agilent Technologies 6890N gas chromatograph interfaced to a Agilent Technologies 5975B mass selective detector operating in the scan mode (m/z 35-500). The column was Agilent 19091S-433, phenylmethylsiloxane fused silica capillary column (30 m × 0.25 mm; film thickness 0.25 μm). For all injections one microliter of the concentrated volatile extracts were used in a split less injection mode together with the helium carrier gas at a constant flow rate of 1 mL/min (column head pressure of 0.606 bar). The column oven was temperature-programmed to rise from 70°C (1 min initial hold) to 225°C at 5°C/min and then held isothermal at 225°C for 10 min. The injector temperature was 250°C and electron impact mass spectral analysis was carried out at ionization energy of 70 eV and an ion source temperature of 230°C. Retention indices were determined by interpolation of the GC-MS retention times to those of n-alkanes (C_8-C_{25} mixture) under identical conditions.

Table 1. Recovery of the powder (complex) at various *C. verum* essential oil to β-cyclodextrin (β-CD) ratios.

C. verum oil: β-CD ratio	Starting material[a] (g, db*)	Recovered powder (g, db*)	Recovery (%)
5:95	4.836 ± 0.04	4.047 ± 0.24	83.68
10:90	5.102 ± 0.01	4.309 ± 0.35	84.46
15:85	5.408 ± 0.03	5.047 ± 0.17	93.32
20:80	5.743 ± 0.02	5.385 ± 0.14	93.77

[a]Total amount of dry β-CD (4.591 g) plus *C. verum* oil used. db* - dry weight basis.

The identification of the compounds was made by computer library search (Wiley7Nist0.5L) based on matching of MS fragments and retention Indices, followed by visual comparison of the mass spectra peaks with published data (Ten Noever de Bravw et al., 1988).

Total oil extraction

The total oil content in the complex powder was determined using a solvent (hexane) extraction method, followed by GC-MS analysis of the concentrated extract (Bhandari et al., 1992; Ayala-Zavala et al., 2008). Distilled water (20 mL), hexane (10 mL) and 0.5 g of the sample powder were put in a glass tube and sealed. The solution was then kept in a ultrasonic bath at 85 °C for 20 min. The organic phase containing the volatile compounds was decanted, and the aqueous phase was exhaustively extracted with hexane 3 times using the above method. The combined hexane extract was dried over anhydrous sodium sulphate and decanted. The final extract was evaporated using a nitrogen stream and the residual oil was weighed and stored at 7°C till the GC-MS analysis. The total oil corresponds to the amount of the complexed molecules in the β-cyclodextrin cavity plus the surface adsorbed oil.

Surface oil extraction

The volatile compounds adsorbed on the surface of the β-cyclodextrin were determined by washing a sample of powder (3 g) with hexane (20 mL) which was gently shaken manually for 20 min (Bhandari et al., 1998). The suspension was then filtered and the residue was further washed with hexane (10 mL). The obtained extract was treated as it was described above. The difference between the total oil and the surface adsorbed oil is the amount complexed in the β-cyclodextrin cavity.

Quantitative analysis of oil volatiles

For the quantitative determination of essential oil components (present in the initial oil, total oil extracted from the powder and surface oil), a calibration curve with initial *C. verum* oil was set up. Precise quantities of initial oil were weighed and dissolved in hexane, to obtain the concentration in the range of 1 - 15 mg/mL. There was a good linear response up to 12 mg/mL.

Statistical analysis

Analysis of variance for the treatments was done using Statistica 6.0 software. Pair comparisons of the investigated parameters between treatments were done using the least significant difference (LSD) test at the 5% level ($P < 0.05$).

RESULTS AND DISCUSSION

All of the treatments produced the powders of light yellow color, different from the white color of the pure β-cyclodextrin powder. This difference in color is due to the interaction of the *C. verum* oil with β-cyclodextrin either as an inclusion of oil pigments into the β-cyclodextrin cavity or adsorption on the powder surface. In the case of the 15:85 and 20:80 ratios, before the filtration of the powder, some droplets of oil were noticed on the surface of the solutions. This observation suggested that some of the essential oil was not included into the β-cyclodextrin molecules.

Table 1 shows the recovery of the powder (at equilibrium state) at various *C. verum* essential oil to β-cyclodextrin ratios. As can be seen, the amount of the powder that was recovered is less than the amount of essential oil and β-cyclodextrin originally used. The material loss can be attributed to the oil, β-cyclodextrin and complex dissipation. There are several factors which may contribute to the loss of *C. verum* oil: retention of the oil in the solution after forming the complex; equilibrium of flavors between the liquid and the complexed state; evaporation of surface oil during the long complexation process and evaporation during the drying step. The loss of the β-cyclodextrin and complex powder is mainly assigned to their solubility in water. Inspection of the data in Table 1 shows that there was significantly large increase ($P < 0.05$) in powder recovery for the 15:85 and 20:80 treatments compared to the 5:95 and 10:90 ratios. Statistical comparison also indicated that there was no significant difference ($P > 0.05$) between the 5:95 and 10:90 treatments, and between the 15:85 and 20:80 treatments. Starting ratios of essential oil to β-cyclodextrin that were greater than 15:85 did not significantly affect ($P > 0.05$) the amount of the recovered powder.

Results in Table 2 show that the amounts of co-crystallized products are not much greater than that for the theoretical products in the case of the 5:85 and 10:90 treatments. However, for the 15:85 and 20:80 treatments, there is a sizable increase in the amount of recovered product relative to theoretical amount of product. It suggests that this increase was not directly proportional to the amounts of essential oil added. As can be seen, there are no significant difference ($P > 0.05$) between the

Table 2. Effect of complexation on co-crystallization of β-cyclodextrin (β-CD).

C. verum oil : β-CD ratio	Theoretical co-crystallized β-CD[a] (g, db*)	*C. verum* oil used (g)	Oil + theoretical co-crystallized β-CD (g, db*)	Recovered powder (g, db*)	Difference (g, db*)
5:95	3.691	0.246	3.937	4.047	+0.110
10:90	3.691	0.512	4.203	4.309	+0.106
15:85	3.691	0.818	4.509	5.047	+0.538
20:80	3.691	1.153	4.844	5.385	+0.541

[a] Amount of co-crystallized β-CD: total amount of dry β-CD (4.591 g) used minus its soluble amount in 50 mL of water (1.8 × 0.5 = 0.9 g). db* - dry weight basis.

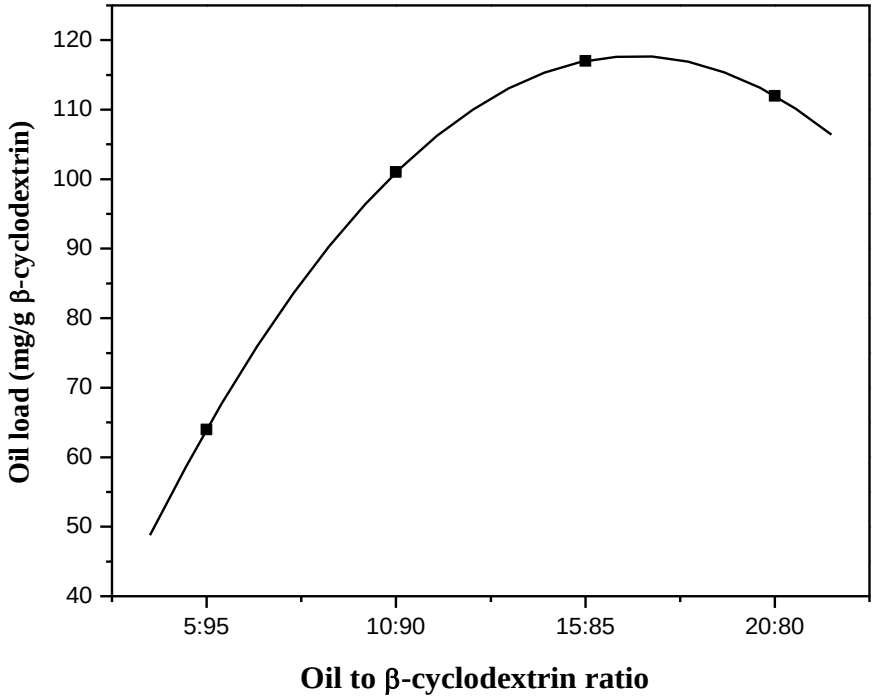

Figure 1. Flavor oil load of β-cyclodextrin as a function of the initial essential oil to β-cyclodextrin ratio.

20:80 treatments. Therefore, the increase in recovered powder is more likely to be due to increasing amounts of β-cyclodextrin co-crystallizing from the solution as the initial ratio of essential oil to β-cyclodextrin is increased, since the co-crystallized product is less soluble than pure β-cyclodextrin. Apparently, the *C. verum* essential oil is being included more strongly by the available β-cyclodextrin at greater concentrations of oil in the starting solution, and the noncomplexed β-cyclodextrin in the solution is at its slightest level.

Statistical comparison of data both in Tables 1 and 2 indicated that there was no significant difference ($P > 0.05$) between the 5:95 and 10:90 treatments and between the 15:85 and 20:80 treatments but there was a significantly large increase ($P < 0.05$) between these two pairs of treatments since the maximum co-crystallization of β-cyclodextrin with essential oil has been reached.

In conclusion, it appears that high starting ratios of the *C. verum* essential oil to β-cyclodextrin produce the maximum recovery of the oil powder, maximum inclusion of essential oil and minimum noncomplexed β-cyclodextrin. An optimum ratio of essential oil to β-cyclodextrin during complexation existed at around 15:85.

This conclusion is supported by the data presented in Figure 1 where maximum load of *C. verum* essential oil occurred at the 15:85 treatment (117.2 mg of oil/g of β-cyclodextrin). This value was similar to that observed for ratio 20:80. However for the rest of applied ratios loading

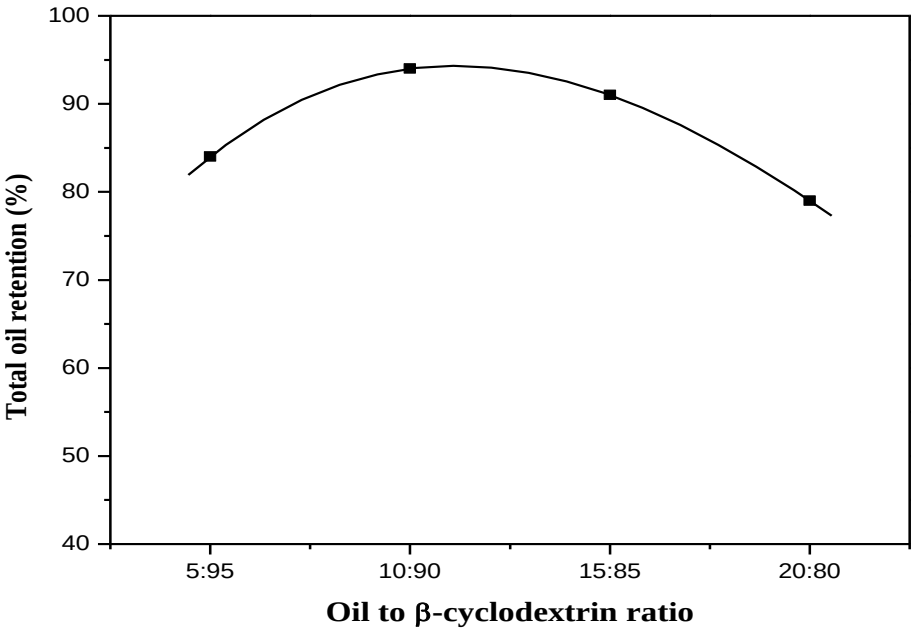

Figure 2. Retention of total oil volatiles as a function of the initial essential oil to β-cyclodextrin ratio.

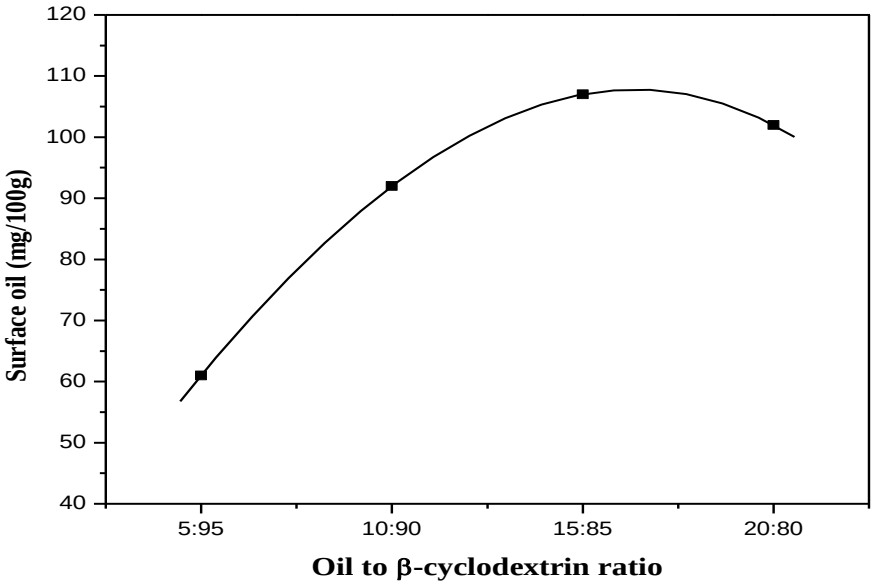

Figure 3. Surface retention of flavor volatiles as a function of the initial essential oil to β-cyclodextrin ratio.

of oil was significantly lower ($P < 0.05$). This inclusion efficiency is in the range of the theoretical maximum loading for β-cyclodextrin with essential oil of 8 - 12% (Pagington, 1986).

The retention of total oil volatiles is determined as a percentage of total extracted volatiles to the volatile content of the essential oil used (as determined by GC). The results in Figure 2 show that the retention of *C. verum* essential oil volatiles reached the maximum of 94.18% for the 10:90 treatments. This result was significantly different ($P < 0.05$) from the retention found for the other three treatments.

The amount of surface oil volatiles as determined by GC analysis of the powder washed with hexane, lie down in the range from 61 - 107 mg/100 g of dried powder (Figure 3). The highest amount of surface oil was found

Table 3. Composition (%) of initial *Cinnamomum verum* essential oil, total and surface oil extracted from the β-cyclodextrin complex.

Peak	Compound	T_R (min)	Initial oil	Surface oil	Total oil
1	Tricyclene	3.89	0.14	0	0
2	α-Pinene	4.06	0.20	0	0.16
3	Camphene	4.30	0.83	0.48	0.66
4	Benzaldehyde	4.46	3.74	2.89	3.48
5	α-Phellandrene	5.20	1.91	0	1.17
6	1-Hexyl acetate	5.25	0.24	0	0.16
7	*p*-Cymene	5.58	0.71	0.37	0.49
8	Limonene	5.66	2.26	1.77	1.92
9	Benzyl alcohol	5.74	2.63	1.93	2.19
10	Linalool	7.10	1.58	1.39	1.63
11	(*Z*)-Cinnamaldehyde	10.05	0.66	8.98	7.15
12	Benzenepropanol	10.29	2.46	1.68	1.92
13	Carvone	10.65	0.99	0.77	1.01
14	Linalyl acetate	10.85	0.48	0	0.52
15	(*E*)-Cinnamaldehyde	11.64	70.67	65.46	67.78
16	Eugenol	13.56	8.60	6.86	7.47
17	β-Caryophyllene	15.09	0.67	0.47	0.82
18	Cinnamyl acetate	15.62	0.13	0	0.23
19	Eugenyl acetate	17.58	0.12	0.21	0.13
20	Asarone	19.76	0.23	1.08	0.29
21	Benzyl benzoate	22.99	0.30	0.63	0.54

for the 15:85 treatments. The content of surface oil volatiles significantly increased ($P < 0.05$) from 5:95 treatments to the 15:85 treatments. On the other hand the value of surface oil volatiles in the 20:80 treatments was slightly decreased compared to the 15:85 treatments but the decrease was not significant ($P > 0.05$).

Identification of the flavor compounds in the initial oil, essential oil powder and on the surface of this powder was accomplished by GC-MS analysis. The composition of *C. verum* standard oil was similar to that reported in the literature (Raina et al., 2001; Senanayake et al., 1978; Mollenbeck et al., 1997). Thirty-one flavor compounds were detected in the initial oil. Among the detected compounds 15 were monoterpenoids, 5 were sesquiterpenoids, 7 phenylpropanoids and 2 esters. Only the compounds with the content higher than 0.10 % in the initial oil were considered in complexation process.

Table 3 shows the compounds related to their chromatographic peaks and their amount in the initial, surface-adsorbed and total oils. The qualitative composition of the volatiles in the total oil extracts (Figure 4) was similar to the initial oil (Figure 5. On the other hand the chromatographic profile of the surface adsorbed oil (Figure 6) was different from the initial oil. All the flavor compounds in the starting oil were included into β-cyclodextrin, except for tricyclene which was not detected in all the total oil extracts. In addition, a number of compounds were absent in all surface oil extracts, namely: tricyclene, α-pinene, α-phellandrene, 1-hexyl acetate, linalyl acetate and cinnamyl acetate. This fact might result from a loss of certain volatiles during the oven drying process or due to a concentration effect, or both. The thirteen major flavor compounds, with the content higher than 0.50% of the initial oil, found in the commercial *C. verum* essential oil, were present in all of the extracts but in the different proportions between the total and surface oil extracts. One exception was α-phellandrene which was not detected in surface oil extracts.

Although the content of the most compounds in total oil extracts is significantly different from the content of these compounds in the starting oil their proportion is almost the same in both oils. Statistical analysis confirmed that the content of the main flavor volatile, (*E)*-cinnamaldehyde, and linalool and carvone in the total oil extracts were not significantly different ($P > 0.05$) compared with the content of these volatiles in the initial oil. The contents of other compounds in total oil extracts were significantly different ($P < 0.05$) compared with those for the starting oil. The possible explanation of this fact might be a concentration effect.

On the other hand, the quantitative composition of the surface oil extracts were significantly different ($P < 0.05$) from composition of the starting oil. Disregarding minor components whose content in oil are less than 0.50% of the total oil, the molecular mass of the thirteen

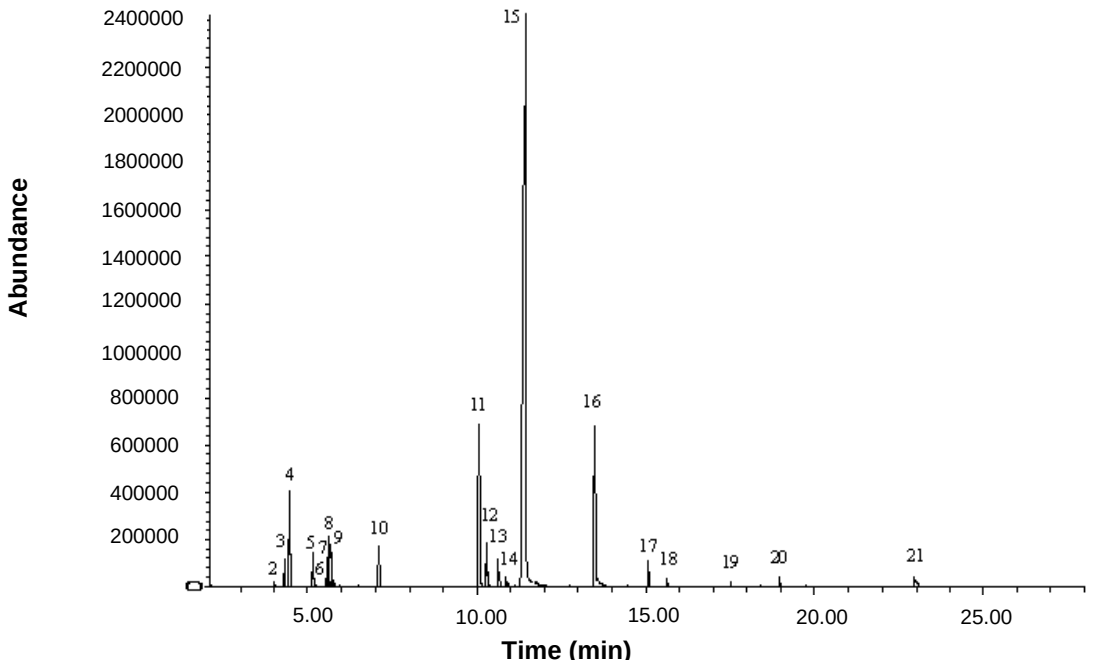

Figure 4. Total ion chromatogram (TIC) of the total oil extracted from the complex powder.

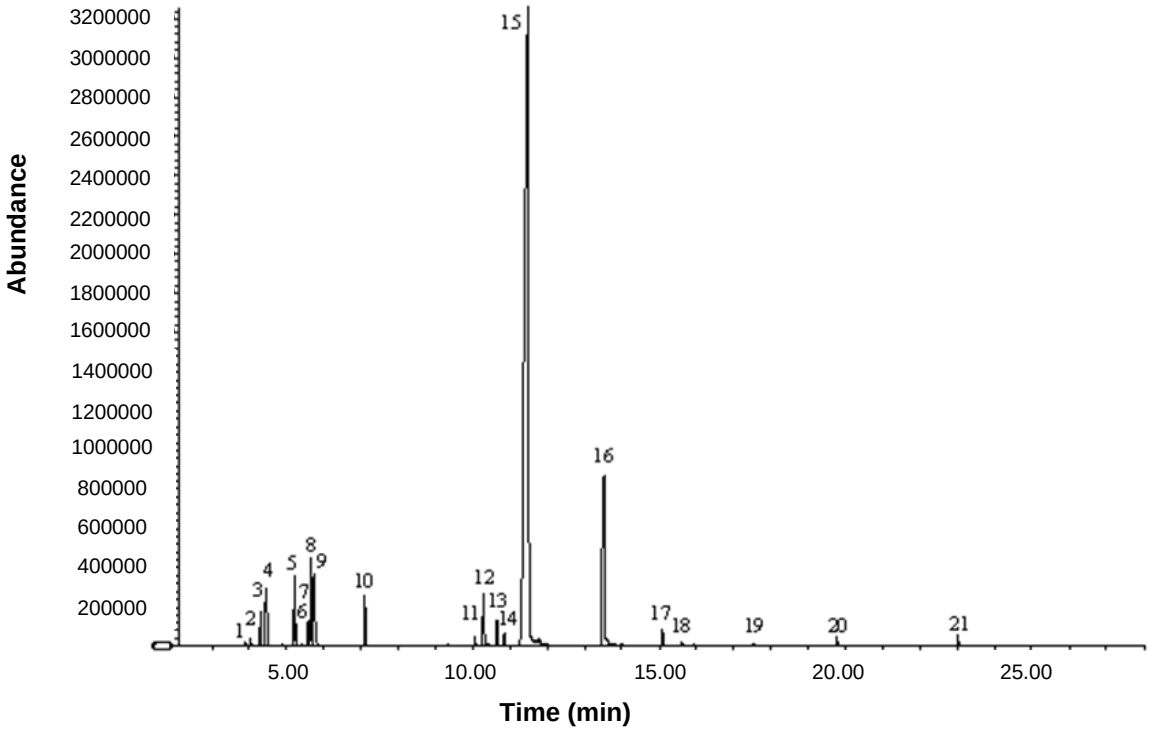

Figure 5. Total ion chromatogram (TIC) of initial *Cinnamomum verum* essential oil.

major flavor volatiles is between 106 and 204 g mol^{-1}. It suggests that one volatile molecule could be included into one β-cyclodextrin molecule (Reineccius, 1989; Bhandari et al., 1998; Pagington, 1986). The proportion of these compounds is similar in the starting oil and in the oil powder despite the molecular weights are different (Table

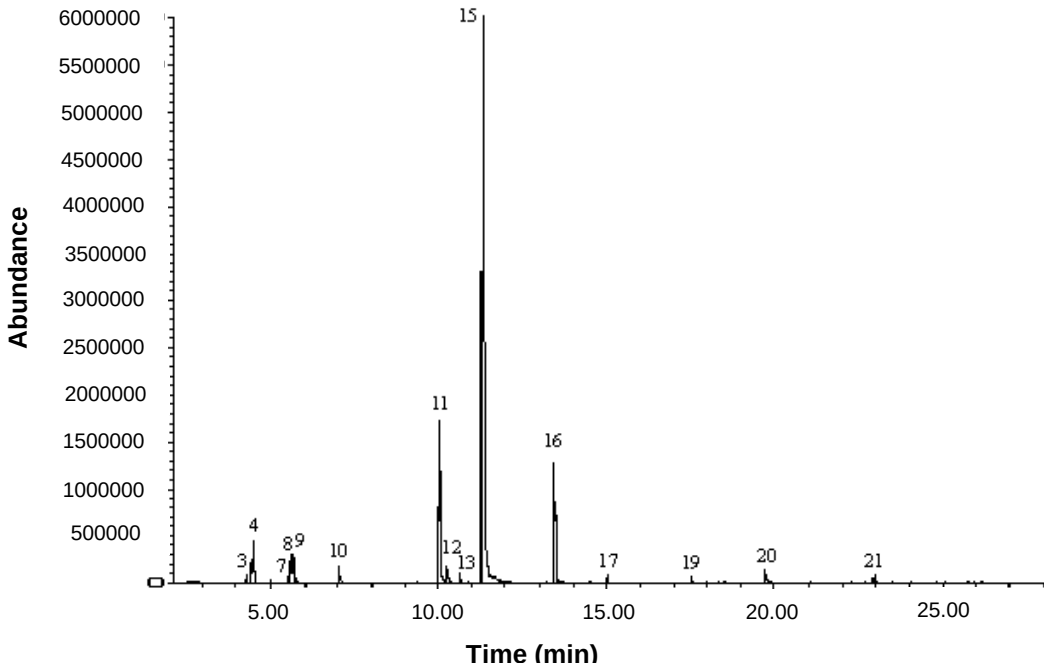

Figure 6. Total ion chromatogram (TIC) of the surface oil extracted from the complex powder.

3). It seems that most of oil constituents are small enough to fit the β-cyclodextrin cavity. In addition, the polarity of the compounds seems to play an important role in the complexation process, since the less polar (*Z*)-cinnamaldehyde was complexed much more than the main component (*E*)-cinnamaldehyde. Obtained results indicated that flavor profile of the oil microencapsulated in β-cyclodextrin is similar to that of initial oil. So, microincapsulation could be used to protect oil of possible degradation during storage.

Conclusions

It was found that *C. verum* essential oil can be successfully complexed with β-cyclodextrin by the co-precipitation method with a 93.77% powder recovery for a starting ratio of oil to β-cyclodextrin of 20:80. The composition of oil extracted from complex was similar in qualitative and not too much different in quantitative composition comparing to the composition of initial oil. The volatile flavor composition of the surface adsorbed oil was different from the starting *C. verum* essential oil in both the profile and compound proportions. Retention of oil volatiles was the highest (94.18%) for a starting ratio of oil to β-cyclodextrin of 10:90 (fresh weight basis). Maximum load of *C. verum* essential oil (117.2 mg of oil/g of β-cyclodextrin) occurred at the 15:85 treatment.

These results suggests that high starting ratio of the *C. verum* essential oil to β-cyclodextrin produce the maximum recovery of the oil powder, maximum inclusion of essential oil and minimum non- complexed β-cyclodextrin. With respect to the cost, the inclusion capacity of β-cyclodextrin is more important than the retention of the volatiles in the product. It implies that an optimum ratio of essential oil to β-cyclodextrin during complexation exists at around 15:85 and the product processed from this starting ratio is commercially acceptable.

Since there is no significant difference between the chromatographic profile of the starting oil and the total oil extracted from the complexed powder, it can be supposed that encapsulated *C. verum* essential oil maintains its organoleptic properties as well as its pharmacological activity. This justifies the use of β-cyclodextrin as complexing agent for cinnamon oil in the food and pharmaceutical industries.

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