Microencapsulation of cinnamon oil to improve its usability as a digestive supplement

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Abstract

Cinnamon oil (CO) when used as a digestive supplement can result in a wide range of beneficial medicinal properties important to human health. However, its direct usage is reported to cause undesirable side effects in certain individuals. Therefore, during this research, CO was microencapsulated using chitosan and gum arabic wall materials via complex coacervation method to mask such unnecessary side effects and to improve the usability of CO. These synthesized CO microcapsules were irregular in shape and contained rough surfaces according to the optical and scanning electron microscopic images. UV-visible analysis confirmed the effective encapsulation of CO with an average loading capacity of 860 μg/g. CO microcapsules displayed a significant antioxidant activity of 2930±74 μg PGE/mg during the Folin-Ciocalteu assay. The brine shrimp lethality assay indicated that the cytotoxic effects of CO are notably masked upon microencapsulation. The CO microcapsules exhibited antibacterial activity against a few selected bacterial strains in the disk diffusion assays. The synthesized CO microcapsules displayed a 47 % and 32% release of the core oil in pH 2 and pH 7.4 solutions mimicking the stomach and intestine conditions respectively. This resulted in about 79% release of the encapsulated oil in a controlled manner from the microcapsules under conditions similar to the gastrointestinal tract.

Keywords: microencapsulation, cinnamon oil, antioxidant activity, antibacterial activity, controlled release , brine shrimp lethality assay.

1. Introduction

Since ancient times, cinnamon is believed to contain medicinally important natural ingredients due to its activity against many diseases including digestion related problems.[1] When considering the importance of cinnamon essential oil overcinnamondried spice, the essential oil is reported to be more effective than the dried spice (bark) since it contains concentrated amounts of active ingredients.[2] Main constituents of cinnamon oil such as eugenol and cinnamaldehyde, are strongly believed to be responsible for its important bioactivities such as antioxidant, antimicrobial, antidiabetic, anticoagulant and carminative properties and are useful in treating various disease conditions.[3]

In addition to cinnamaldehyde and eugenol, there are other compounds such as camphor and linalool which are also reported to possess antioxidant activity.[4] As antioxidants are capable of removing free radicals and reactive oxygen species that are producedduring metabolic processes such as lipid peroxidation and peroxynitrite induced nitration, they reduce the risk of certain diseases such as atherosclerosis and cancers.[5]

Cinnamon oil is used for decades as a well-known food flavouring agent and it is also beneficial to treat many digestive disorders. Due to its carminative properties, cinnamon oil helps to eliminate excess gas that accumulates in the stomach and also acts as a digestive tonic. Cinnamon oil is known to be effective against indigestion, diarrhoea, vomiting, nausea, upset stomach and help to reduce the acidity in stomach and morning sickness as well.[3]

Although cinnamon oil is an excellent cure against numerous gastrointestinal tract related diseases, the direct usage of cinnamon oil has been reported to cause problems to certain individuals.[6] Additionally, cinnamon oil has a strong fragrance which is intolerable to some people. Furthermore, direct usage of cinnamon oil has been reported to cause skin irritations and allergic reactions in some individuals.[7] Even though orally taken cinnamon oil might not show adverse effects in small amounts, higher doses can increase the heart rate, gastric motility and respiration which is followed by sleepiness or depression.[8]Additionally, it has been reported that cinnamon oil can lose its bioactivities with time if not kept under carefully controlled conditions.[9]Therefore,efforts were made to microencapsulate cinnamon oil so that the fore-stated limitations could be overcome and the usability of cinnamon oil could be improved.

"Microencapsulation is defined as a technology of packaging solids, liquids or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under the influence of specific conditions."[10] Coacervation (also called phase separation) used during this research work, is the most common physico-chemical method used for the microencapsulation of essential oils.[11] Coacervation does not require high heat or highly controlled reaction conditions for the microencapsulation process and hence s a less material and energy consuming process compared to other microencapsulation methods. Coacervation is also beneficial due to the formation of true microcapsuleswhere the core material is completely coated by the wall material.[12] Complex coacervation involves the interaction between two oppositely charged biopolymers and is different from simple coacervation where only one polymer is employed as the wall material.[11] During this research chitosan and gum arabic were used as wall materials to synthesize cinnamon oil microcapsules *via* the complex coacervation method.

The biological activities of the directly used or unencapsulated cinnamon oil could be reduced due to many reasons such as degradation or inactivation under harsh acidic conditions of the stomach, enzymatic reactions, insufficient residence time, and low permeability in the gastrointestinal (GI) tract. However, microencapsulation of cinnamon oil could help to overcome these problems and the controlled release of the core ingredients from the microcapsules could be achieved bycareful selection of the microcapsule wall materials.[13] Controlled release of the active ingredients of cinnamon oil could also be important to minimize the adverse effects it displays when utilized directlyor at higher doses. Microencapsulation ensures the delivery of the core material to the targeted sites and also enhances the chemical and physical stability of the encapsulated material.[14]The encapsulated core material can be released from the

microcapsules*via*degradation or dissolution of wall material, diffusion of core material through the wall material, pH dependent or enzymatic hydrolysis of wall material and mechanical disruption of wall material.[15]

2. Materials and methods

2.1 Materials

Cinnamon oil (eugenol content 80%) and Muller-Hinton agar were purchased from Royal Surgical, Colombo, Sri Lanka. Gentamicin was purchased from Union chemists, Colombo, Sri Lanka. Solvents (Commercial grade) and other chemicals (analytical grade) were obtained from the Department of Chemistry, University of Colombo, Sri Lanka. The solvents were double distilled prior to being used.

2.2 Preparation of cinnamon oil microcapsules via complex coacervation

A 5 mL portion of cinnamon oil was added to a 50 mL of 2 %(w/v) chitosan solution followed by a drop wise addition of 50 mL of 5 %(w/v) gum arabic solution with stirring. Then the temperature of the mixture was reduced to 5ºC. The resulting solidmicrocapsules were separated by vacuum filtration.[16]Microcapsules were further washed with 70% ethanol to remove any unencapsulated oil and air dried before use.

2.3 Confirmation of encapsulation

In order to recover the encapsulated cinnamon oil, a known amount of microcapsules was crushed well using a glass rod followed by sonication for 15 min and the oil was extracted in to a known volume of 70% ethanol.After the sample was centrifuged at 2200 rpm for 10 minutes, the clear supernatant was collected and analysed by UV-visible spectroscopy.UV-visible spectra of 0.1 g/mL uncrushed and crushed microcapsule samples were compared with that of pure cinnamon oil to confirm the encapsulation of cinnamon oil and to calculate the capsule loading.

A solution of chitosan and gum arabic in a 70% ethanolic medium was used as the blank to obtain the UV-Visible spectra during this experiment.

2.4 Investigation of the morphology of cinnamon oil microcapsules

The cinnamon oil microcapsules were observed using the light microscope (under ×40 magnification) and the Scanning Electron Microscope(Model: 51-ADD0048) (under ×2.50 K magnification) to investigate their morphology.

2.5 Investigation of biological activity of cinnamon oil microcapsules

2.5.1 Folin-Ciocalteu antioxidant assay

A volume of 100 µL of the ethanolic extracts of the 0.2 g/mL crushed and uncrushed microcapsule samples were reacted with100 µL of Folin-Ciocalteu reagent to develop a blue colour complex. Its absorbance was measured at 750 nm. Pyrogallol was used as the standard to calculate the antioxidant capacity (AOC) of the tested samples. Same procedure was repeated with an equivalent amount of unencapsulated cinnamon oil that is similar to the loading of the microcapsule samples. All the tests were carried out in triplicate.

2.5.2 Brine shrimp lethality assay

Brine shrimp eggs were hatched inside a glass container using 3.5% NaCl solution.[17] After about 36 hours, a set of ten nauplii was transferred to a sample vial containing 5 mL of 3.5% NaCl solution. Then 200 μL from 0.1 g/mL of crushed microcapsules in water was added in to the first sample vial. A portion of 200 μL from 0.1 g/mL of uncrushed microcapsules in water was added in to the second sample vial. A volume of 200 μL of an aqueous solution containing an equivalent amount of pure oil to the loading of the crushed microcapsule sample was added in to the third sample vial.A volume of 200 μL of water was added in to the forth sample vial (negative control) and mixed. The samples were observed for a period of 8 hours to monitor the mortality of shrimp nauplii and the percentage mortality was calculated.

Relative mortality percentage of the samples (% M) was calculated using following equation.[18]

% M = Percentage of survival in the control - Percentage of surviving nauplii in the treatment.

2.5.3 Disk-diffusion antibacterial assay

MHA spread plates of *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778) and *Salmonella typhimurium*(ATCC 14028)were prepared according to the previously published protocols.[19],[20],[21]

Sterilized filter paper disks with a diameter of 6 mm were separately loaded with 80 μL of each test sample (crushed and uncrushed microcapsule samples) (0.2g of capsules in 1 mL of ethanol), negative control (70% ethanol), positive control (1 mg mL^{-1} Gentamycin solution.), pure cinnamon oil without dilution and a pure oil sample with an equivalent amount of oil to the loading of the crushed microcapsule sample).Sample loaded disks were allowed to dry at room temperature and were placed on the bacterial spread plates. The plates were then incubated at 37˚C overnight and the average diameters of the resultant inhibition zones were recorded. Each experiment was carried out in triplicate.

2.6 Investigation of the controlled release of cinnamon oil from the microcapsules under the conditions similar to the gastrointestinal tract

A weight of 0.1 g of dry microcapsules was added in to a falcon tube containing 2.0 mL of pH 2 buffer. The sample was kept at 37˚C and 200 rpm in an orbital shaker for an hour. After an hour, the supernatant was collected. To the pellet of the sample, a 2.0 mL portion of pH 7.4 buffer solution was added and kept at 37 ˚C and 200 rpm in an orbital shaker for another hour. After an hour, sample was centrifuged and supernatant was collected. Then the pellet of the sample was crushed and the released core content was extracted to a 2.0 mL portion of water. The UV absorbance at 230 nm wavelength and the AOC was measured for each of the above test solutions. Using the UV absorbance values, the percentage release of the encapsulated oil in each solution was calculated (considering the percentage release of the encapsulated oil in an untreated crushed sample as 100%). Similarly, using the initial AOC of the microcapsules and the AOC of the above test samples, the percentage AOC of the test solutions were calculated. The experiment was carried out in triplicate.

3. Results and discussion

3.1 Preparation of cinnamon oil microcapsules

Cinnamon oil microcapsules were produced using complex coacervation method. Process of Complex coacervation involves deposition of two wall materials on emulsified oil droplets. During the preparation of microcapsules, cinnamon oil was stirred in a chitosan aqueous solution in order to emulsify the oil in water and to deposit chitosan around those oil droplets. Chitosan is a positively charged wall material. When adding this oil in water emulsion drop wise in to negatively charged gum arabic solution, it results in deposition of gum arabic wall around chitosan microcapsules. This process is influenced by the electrostatic interaction between two oppositely charged biopolymers.

3.2 Confirmation of cinnamon oil encapsulation

Encapsulation of cinnamon oil was confirmed by measuring the UV absorbance of crushed and uncrushed microcapsule samples at 230 nm and 279 nm which were the two prominent absorbance peaks shown by pure cinnamon oil. Average absorbance readings obtained for the samples are tabulated in Table 1.

For the crushed microcapsules, a significantly higher UV absorbance was observed due the release of core material during the crushing step. The uncrushed microcapsules did not show any significant absorbance peaks at the relevant wavelengths. Therefore, these results indicate the successful encapsulation of cinnamon oil by the complex coacervation method using chitosan- gum arabic wall materials and the encapsulated cinnamon oil can be released to the exterior upon breakage of the capsule wall.

3.3 Determination of the loading of cinnamon oil microcapsules

Loading of cinnamon oil in the synthesized microcapsules was determined using a cinnamon oil standard curve (Table A1 in Appendix). It was determined that the loading of cinnamon oil in the microcapsules was 860 μg/g. The synthesized microcapsules did not possess a strong or unpleasant odour compared to the unencapsulated oil.

3.4 Investigation of the morphology of cinnamon oil microcapsules

The cinnamon oil microcapsules prepared by the complex coacervation method using chitosan-gum arabic wall combination appeared to be irregular shaped according to the images obtained from the light microscope (Figure 1) and the scanning electron microscope (SEM) (Figure 2).

The SEM images indicated a rough wall on the cinnamon oil microcapsules and the average diameter of the microcapsules varied between 70 μm – 110 μm.

3.5 Investigation of biological activity of cinnamon oil microcapsules

3.5.1 Antioxidant activity

The UV absorbance analysis indicated that all the tested samples were in the same cinnamon oil concentration range. However, when consider the AOC of these samples (Table 2), the AOC of unencapsulated cinnamon oil was higher than that of microencapsulated oil.

This could be due to the loss of hydrophilic antioxidants during the microencapsulation process, poor crushing of the microcapsules that did not release its ingredients to the solution completely or destruction of some of the antioxidant active ingredients of cinnamon oil during the microencapsulation process. However, as the UV data indicated that the concentration of cinnamon oil in both encapsulated and unencapsulated samples are compatible, the above difference could be mainly due to the loss of a potent antioxidant agent during the encapsulation process. Still, a significant antioxidant activity of 2930 ±74 μg PGE / mg was retained in the synthesized microcapsules.

3.5.2 Brine shrimp lethality assay

As high doses and direct usage of cinnamon oil was reported to cause toxic effects to some of its users, it was desirable that microencapsulation allows to mask such toxic effects of cinnamon oil. The brine shrimp lethality assay was used to monitor the difference in toxicities of directly used cinnamon oil and microencapsulated cinnamon oil.

According to Figure 3, it was clearly evident that the microencapsulated cinnamon oil had significantly lower toxic effects compared to a similar equivalent of the unencapsulated cinnamon oil. The intact microcapsules clearly indicated a significantly lower relative mortality percentage compared to that displayed by the unencapsulated cinnamon oil throughout the period of study. The crushed microcapsules displayed a higher relative mortality percentage compared to that of the intact microcapsules indicating the release of core ingredients to the exterior when the microcapsule walls are disrupted by crushing. . Therefore, these results clearly indicate that the cytotoxic effects of cinnamon oil can be successfully masked by microencapsulation. Furthermore, it also indicates that the encapsulated oil can be released to the exterior upon breakage of microcapsule wall. The slight rise of relative mortality percentage displayed by the intact microcapsules with time indicated that these cinnamon oil microcapsules can slowly release its ingredients in to an aqueous environment *via* diffusion through wall.

3.5.3 Disk-diffusion assay

Natural products such as eugenol, cinnamaldehyde, and camphor that are resistant to many bacterial strains are present in cinnamon oil.[5]Disk diffusion assays were carried out to study the antibacterial activity of encapsulated and unencapsulated cinnamon oil against a few selected bacterial strains.

According to the results of the antibacterial studies shown in Table 3, pure cinnamon oil has shown a significant antibacterial effect against all four tested bacterial strains to inhibit their growth. Highest inhibition for pure cinnamon oil was observed against *Bacillus cereus* (23±2 mm)and *Staphylococcus aureus* (19±1 mm)strains. Inhibition zones of pure cinnamon oil and the positive control gentamycin (25 μL/mL)suggest that cinnamon oil possess an antibacterial activity compared to that displayed by a 25 μL/mL gentamycin solution.

Inhibition zone diameters of the crushed microcapsules sample and an equivalent concentration of unencapsulated cinnamon oil were comparableagainstallthe tested bacterial strains. Therefore, these results suggest that the encapsulation process does not significantly affect the original antibacterial activity of the pure oil.

3.6 Investigation of the controlled release of cinnamon oil from the microcapsules under conditions similar to the gastrointestinal tract

After subjecting to each GIT conditions, the test solutions were tested to check for the amount of encapsulated oil released under each condition and the AOC of the released oil. (Table 4)

During the GIT mimicking experiment, the reaction mixtures were kept at 37 °C assuming the human body temperature and the orbital shaker was used to mimic the peristaltic movements of gastrointestinal tract. The pH variation inside the GIT was simulated by using a pH 2 buffer to mimic the gastric fluid and a pH 7.4 buffer to mimic the intestinal fluid.[22]

According to the cinnamon oil release percentages shown in Table 4 (Table A2 in Appendix), 47% of encapsulated oil was released under the gastric conditions at pH 2 and 32% of core oil was released under the intestinal pH of 7.4. This indicates that the pH conditions in the GIT can facilitate 79% release of the encapsulated cinnamon oil from the microcapsules. When the microcapsules subjected to both gastric and intestinal conditions were crushed at the end of both treatment steps, another 7% of the encapsulated oil was released to the exterior. Therefore, these results indicated the ability of the synthesized microcapsules to efficiently release the encapsulated core material in a controlled manner inside the GIT. Although the pH dependent release of cinnamon oil was 79%, the release of the remaining 7% of cinnamon oil from the microcapsules could be expected as a result of the digestion by pancreatic enzymes.[23]

The AOCs of the core cinnamon oil released under each step of the GIT mimicking experiment (Table A3 in Appendix) also indicated comparable results to that observed during the UV data analyses. According to the results tabulated in Table 4, a 42% AOC was observed for the oil released under gastric conditions and a 29% AOC was observed for the oil released under the intestinal conditions. Therefore, compared to the original AOC of the microcapsules, a 71% of AOC was resultant from the core oil released under the GIT conditions (in stomach and intestine). The 7% of core oil that remained within the capsules after being treated at both pH conditions displayed a 16% AOC compared to the original AOC of the microcapsules. As previously stated, these remaining antioxidants can also be harvested with the aid of pancreatic enzyme digestion of the wall materials.

4. Conclusion

Cinnamon oil microcapsules can be successfully prepared by complex coacervation method using chitosan and gum arabic wall materials. The synthesized cinnamon oil microcapsules exhibited considerable antioxidant and antibacterial activitiesand did not possess any strong odour as the unencapsulated oil. Cytotoxic effect displayed by the unencapsulated cinnamon oil was successfully masked *via* microencapsulation.The GIT mimicking experimental data demonstrated the ability of microencapsulated cinnamon oil to be efficiently released in a pH dependent and controlled manner. The results of this research indicate that the usability of cinnamon oil can be improved by microencapsulation and the synthesized microcapsules are more appropriate to be used as a digestive supplement compared to the direct usage of cinnamon oil.

Acknowledgement

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References

[1] J. Gruenwald, J. Freder and N. Armbruester, *Cinnamon and health.*, Crit. Rev. Food Sci. Nutr. 50 (2010), pp. 822–834.

[2] F. Nazzaro, F. Fratianni and L. De Martino, *Effect of Essential Oils on Pathogenic Bacteria*, Pharmaceuticals 6 (2013), pp. 1451–1474.

[3] R.K. Maheshwari, A.K. Chauhan, A. Gupta and S. Sharma, *Cinnomon: An imperative spice for human comfort*, Int. J. Pharm. Res. Bio Sci. 2 (2013), pp. 131–145.

[4] F. Bakkali, S. Averbeck, D. Averbeck and M. Idaomar, *Biological effects of essential oils - A review*, Food Chem. Toxicol. 46 (2008), pp. 446–475.

[5] P.V. Rao and S.H. Gan, *Cinnamon : A Multifaceted Medicinal Plant*, Hindawi (2014), pp. 1–12.

[6] *Cinnamon bark: Uses, Side Effects, Interactions and Warnings*. Available at http://www.webmd.com/vitamins-supplements/ingredientmono-330-cinnamon bark.aspx?activeingredientid=330&activeingredientname=cinnamon bark.

[7] C. Ulbricht, E. Seamon, R.C. Windsor, N. Armbruester and Bryan, *An Evidence-Based Systematic Review of Cinnamon (Cinnamomum spp .) by the Natural Standard Research Collaboration*, J. Diet. Suppl. 8 (2011), pp. 378–454.

[8] N. Martini, *Continuing professional development*, J. Prim. Health Care 7 (2015), pp. 77.

[9] *Shelf Life of Essential Oils*. Available at http://www.usingeossafely.com/shelf-life-of-essential-oils-and-how-to-make-them-last-longer/.

[10] A. Kumar and H. Singh, *Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery*, Trends Food Sci. Technol. 18 (2007), pp. 240–251.

[11] C.G. De Kruif, F. Weinbreck and R. De Vries, *Complex coacervation of proteins and anionic polysaccharides*, Curr. Opin. Colloid Interface Sci. 9 (2004), pp. 340–349.

[12] L.F. Siow, *Effect of pH on Garlic Oil Encapsulation by Complex Coacervation*, J. Food Process. Technol. 4 (2013), pp. 1–5.

[13] S. Jyothi, A. Seethadevi, K.S. Prabha, P. Muthuprasanna and P. Pavitra, *Microencapsulation*, Int. J. Pharma Bio Sci. 3 (2012), pp. 509–531.

[14] P.S. Lee, S.G. Yim, Y. Choi, T. Van Anh Ha and S. Ko, *Physiochemical properties and prolonged release behaviours of chitosan-denatured??-lactoglobulin microcapsules for potential food applications*, Food Chem. 134 (2012), pp. 992–998.

[15] H. Umer, H. Nigam, A.M. Tamboli and M.S.M. Nainar, *Microencapsulation : Process , Techniques and Applications*, Int. J. Res. Pharm. Biomed. Sci. 2 (2011), pp. 474–481.

[16] M. Amid, Y. Manap and N.K. Zohdi, *Microencapsulation of Purified Amylase Enzyme from Pitaya (Hylocereus polyrhizus) Peel in Arabic Gum-Chitosan using Freeze Drying*, Molecules 19 (2014), pp. 3731–3743.

[17] B.. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, *Brine Shrimp : A Convinient General Bioassay for Active Plant Consyituents*, J. Med. Plant Res. 45 (1982), pp. 31–34.

[18] J.L. Carballo, Z.L. Hernández-Inda, P. Pérez and M.D. García-Grávalos, *A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products.*, BMC Biotechnol. 2 (2002), pp. 17.

[19] P.W.N.N. Teklani and B.G.K. Perera, *The important biological activities and phytochemistry of Acalypha indica*, 6 (2016), pp. 30–35.

[20] W.K.O. V. Weeratunge and B.G.K. Perera, *Formulation of a fish feed for goldfish with natural astaxanthin extracted from shrimp waste*, Chem. Cent. J. 10 (2016), pp. 44.

[21] W.M.A.N.K. Wijetunge and B.G.K. Perera, *Preparation of Liquid Medicinal Soap Products Using Adhatoda Vasica (Adhatoda) Leaf Extracts*, Int. J. Multidiscip. Stud. 2 (2015), .

[22] B.N.P. Kumar, K.K. Reddy and P.V.R. Mallikarjuna, *Characterisation of blended sodium alginate microcapsules for controlled release of animal feed supplements in the GIT*, Int. J. Food, Agric. Vet. Sci. 2 (2012), pp. 161–166.

[23] N. Ahmed, M. Dawson, C. Smith and E. Wood, *Disorders of the gastrointestinal tract , pancreas , liver and gall bladder*, in *Biology of Disease*, 2007, pp. 277–314.

Figures

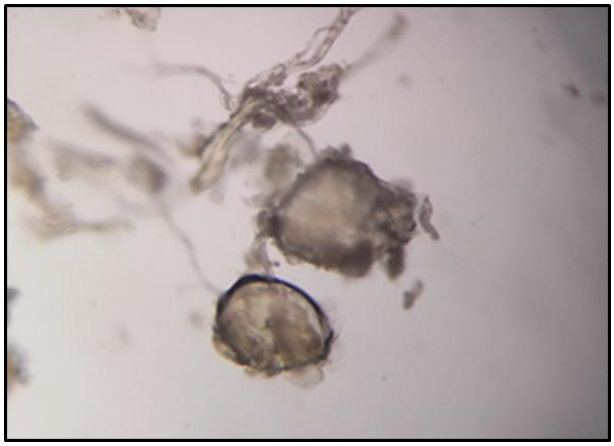

Figure 1- Optical microscopic image of the cinnamon oil microcapsules (×40)

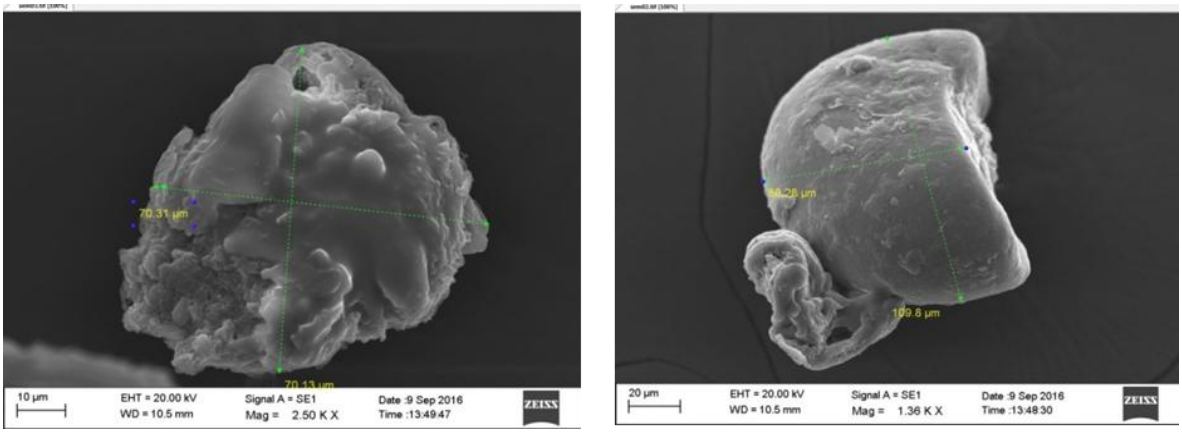

Figure 2- Scanning electron microscopic (SEM) images of the cinnamon oil microcapsules (×2.50 K)

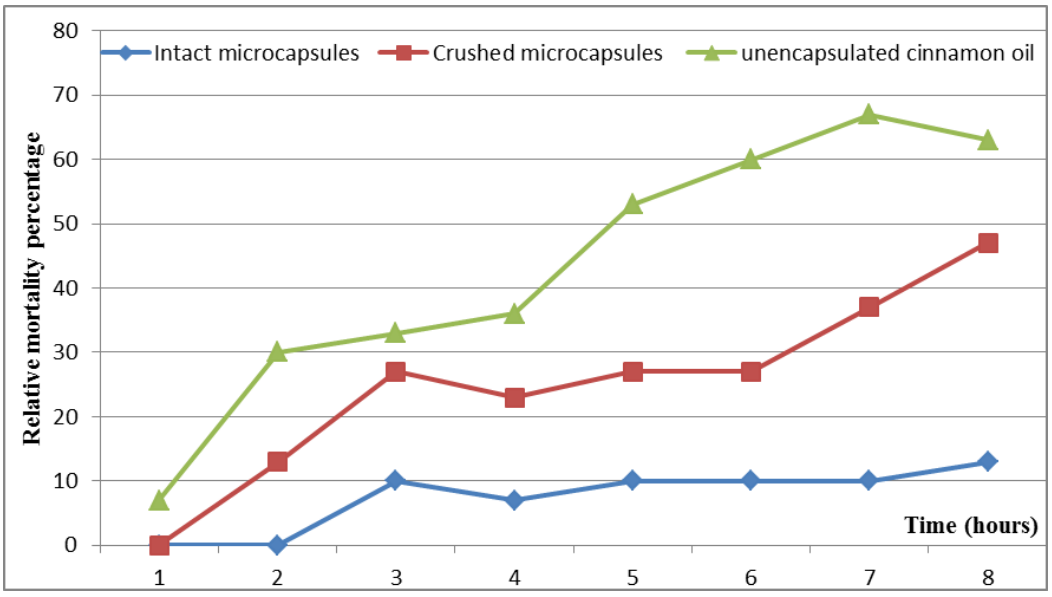

Figure 3– Relative mortality percentage of brine shrimps over time

Tables

Table 1- UV absorbance of the microcapsule samples at 230 nm and 279 nm wavelengths

	Microcapsules	
	Uncrushed	Crushed
Absorbance at 230 nm	0.142 ± 0	2.378 ± 0
Absorbance at 279 nm	0.058 ± 0	1.552 ± 0

Table 2– AOC of the tested cinnamon oil samples

Sample	AOC [µg PGE / mg]
Crushed microcapsules (encapsulated cinnamon oil)	2930 ±74
Unencapsulated cinnamon oil (equivalent to the encapsulated amount)	4810 ± 64

Table 3-Antibacterial activity of cinnamon oil

Sample	Diameter of the inhibition zone (mm)			
	E. coli	*B. cereus*	*S. typhimurium*	*S. aureus*
Pure oil	13±0	23±2	14±0	19±1
Unencapsulated oil (a similar amount to the encapsulated sample)	7±0	7±0	10±1	8±1
Crushed sample	8±2	7±0	8 ±0	7±0
Uncrushed sample	NI	NI	NI	NI
Positive control	8±1	16±0	11±0	8±0
Negative control	NI	NI	NI	NI

NI = No inhibition

Table 4- Percentage release of cinnamon oil (CO) from microcapsules to the supernatant solutions and percentage AOC of cinnamon oil released from microcapsules under GIT mimicking conditions

Sample	pH 2 (mimicked gastric fluid)				pH 7.4 (mimicked intestinal fluid)				Total % release under GIT conditions	Total % AOC under GIT condition
	Before crushing		After crushing		Before crushing		After crushing			
	% release	% AOC	% release	% AOC	% release	% AOC	% release	% AOC		
Untreated sample	-	-	100±0	100±1	-	-	-	-	-	-
Treated sample	47±1	42±0	-	-	32±1	29±0	7±1	16±1	79±1	71±0

Appendix

Table A1- Absorbance readings for the cinnamon oil standard series

cinnamon oil concentration (ppm)	Absorbance at 230 nm
20	0.671
40	1.256
60	1.830
80	2.318
100	2.827

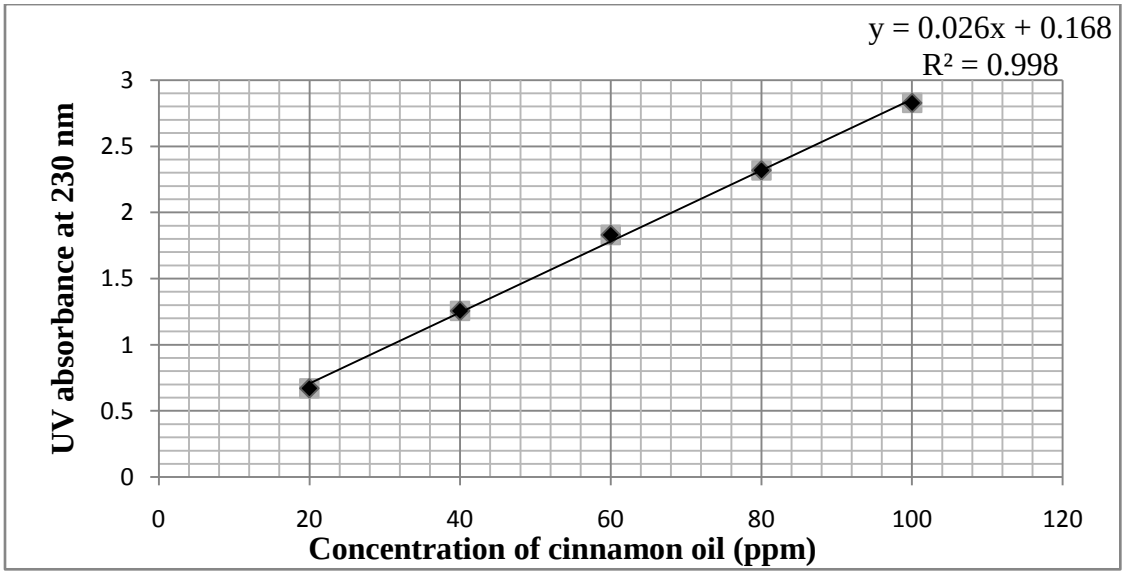

Figure A1- Standard curve for Cinnamon oil

Appendix

Table A2- Percentage release and concentration of released cinnamon oil from microcapsules to the supernatant solutions of GIT mimicking experiment

Sample	% release and concentration of released cinnamon oil from microcapsules							
	pH 2 (mimicked gastric fluid)				pH 7.4 (mimicked intestinal fluid)			
	Before crushing		After crushing		Before crushing		After crushing	
	CO concentration (µg /mL)	% release	CO concentration (µg /mL)	% release	CO concentration (µg /mL)	% release	CO concentration (µg /mL)	% release
Untreated sample	-	-	74±0	100±0		-	-	-
Treated sample	35±1	47±1	-	-	24±1	32±1	6±1	7±1

Table A3- AOC displayed by cinnamon oil microcapsules under different GIT conditions

Sample	AOC and %AOC of released cinnamon oil from microcapsules							
	pH 2 (mimicked gastric fluid)				pH 7.4 (mimicked intestinal fluid)			
	Before crushing		After crushing		Before crushing		After crushing	
	AOC (µg PGE/ mg)	% AOC	AOC (µg PGE/ mg)	% AOC	AOC (µg PGE/ mg)	% AOC	AOC (µg PGE/ mg)	% AOC
Untreated sample	-	-	4659±53	100±1		-	-	-
Treated sample	1952±12	42±0	-	-	1342±9	29±0	736±49	16±1