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ANTIOXIDANT PROFILE OF POLYPHENOL-LOADED PECTIN-ALGINATE MICROCAPSULES AND THEIR *IN-VITRO* RELEASE STUDY

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ABSTRACT

A combination of sodium alginate and pectin were used to study the effect on the loading efficiency and the radical scavenging activity of the polyphenols extracted from orange (*Citrus sinensis*) and lime (*Citrus aurantiifolia*) peels for the development and characterization of potential gastro-resistant microbeads by ionotropic gelation method. The ratios used were 1:1:1 and 1:1:5 (w/w/w), respectively for alginate, pectin and the polyphenols, separately. Sustain release studies were accomplished using Folin-Ciocalteau assay as well as CUPRAC assay with simulated physiological fluids having pH 1.2, 7.4 and 9.2. Sustain release was maximum for the sample containing orange peel polyphenol at 1:1:5 ratio after 90 minutes interval (*ca*. 1.18 mg/gm), which was about 42% more than the control (*ca*. 0.83 mg/gm) at pH 1.2. Efficient release was achieved for both types of microbeads with higher load of the polyphenols. Data obtained from ABTS and DPPH radical scavenging assays indicated that the gelispheres contained highly polar polyphenols in greater proportion. The microencapsulated particles provided an effective protection to polyphenols against any degradation as was elucidated by their swelling and erosion behaviours. The microcapsules described in this study indicated possible application as food additives for incorporation into functional foods.

KEYWORDS: Orange, Lime, Microencapsulation, sustain release, free radical

INTRODUCTION

Encapsulation is a modern day technology utilized for packaging very small amounts of solid particles, liquid droplets, or gas molecules in a form so that they can be released easily at controlled rates under specific conditions or upon receiving a certain stimulus. It was developed as drug delivery system to adjudicate the efficacy of many drugs which would otherwise be degraded in the physiological system upon ingestion due to activities of systemic proteolytic enzymes or by external factors like light, moisture and oxygen.[1] Microcapsulation is a variant of the above-mentioned technique that has been widely used in the food and pharmaceutical industries. Microcapsules are small vesicles or particulates with diameter of a few milimeters which contain the substances to be delivered to some specific systemic targets. This technique can be used to reduce the cost of production, to increase the stability of compounds, to mask undesirable tastes, and to improve the release properties of compounds in food industries. They are extensively applied in food industries involving beverage, bakery, meat, poultry, and dairy products.[2] The process involves entrapment of small particles of liquids, solids, or gases in one or two polymeric layers. The component to be incorporated is referred as the "core material", and polymers are called variously such as "wall material", "shell", "coating", "carrier", or "encapsulant".[3] The purpose of microencapsulation is to protect the core material from environmental factors to extend shelf-life and to improve the release properties of compounds.[4] Another encapsulation process is the generation of nanoparticles, which is also effective for targeted delivery of molecules of natural or synthetic origin.[5] All these processes are beneficial for providing specific drug delivery in the field of medical sciences, especially in cancer therapy.[6]

Recently, research and application of polyphenols have been areas of great interest in the functional foods, nutraceutical and pharmaceutical industries.[7] Polyphenols constitute one of the most numerous and ubiquitous groups of plant-derived secondary metabolites, and are an integral part of both human and animal diets which possess a high spectrum of biological activities, including antioxidant, anti-inflammatory, antibacterial, and antiviral functions.[8] However, there is an anomaly between the *in vitro* content of the polyphenols in nutraceuticals and their *in vivo* bioavailability, probably due to insufficient gastric residence time, low permeability or solubility in the gut or their instability under the influence of pH gradients, enzyme activities and presence of other detrimental bioactives.[9] Moreover, polyphenols have got unpleasant astringent taste, which also limits its incorporation in

food products. All these pose a great challenge to the formulators and industrialists to obtain optimum systemic oral bioavailability of the substance for appropriate nutraceutical applications in various disease conditions.

The carrier materials commonly used for encapsulation are maltodextrin (MD), acacia gums, semi-synthetic cellulose derivatives and synthetic polymers.[10] Recent studies indicated that components like green tea polyphenols, silkworm purple oil and ethyl butyrate were successfully encapsulated using suitable matrix materials viz. binary polymer[11], soybean protein isolate/β-cyclodextrin[12] and lactose/MD/WPI (whey protein isolate) matrix[13], respectively. However, all the carriers have advantages and disadvantages in terms of properties, cost, and encapsulation efficiency (EE). Several factors like nature and ratio of wall to core materials, their interactions and the kind of encapsulation method employed might affect the overall efficacy on the delivery.

Very recently, alginates are used for controlled delivery of incorporated materials by many researchers. It is a naturally occurring polysaccharide for marine brown algae which is inoffensive as well as highly biocompatible, mechanically resilient and acid stable.[14] Pectin is another polysaccharide found in the cell wall of plants. It is a high molecular weight heterogalacturonan comprised of D-galacturonic acid units joined to one another in chains by means of α(1 4) glycosidic linkages. Alginates and pectin were used in tandem to produce a number of microcapsules for effective delivery of bioactive substances like aspirin[15], folic acid[16], vitamin E in cosmetic applications[17] and probiotics like *Lactobacillus rhamnosus*.[18] Sodium alginate alone or in tandem with chitosans were used previously to encapsulate yerba mate extracts.[19] Alginates were also used to immobilize resveratrol in therapeutic purposes.[20] In this study, our main aim was to develop a delivery system of microcapsules made up with alginate and pectin, using ionotropic gelation method, for effective delivery of polyphenols isolated from peels of orange (*Citrus aurantium*) and sweet lime (*Citrus limetta*). The objective was to utilize the otherwise waste material, i.e. citrus peels, as the source of pectin as well as the bioactive substance for an effective food application. This would be ascertained with a few *in vitro* release studies along with antioxidant studies to adjudicate the efficacies of the microcapsules as potential food additives.

MATERIALS AND METHODS

Materials

Sodium alginate (food grade, conforming to NF) and pectin (pure) were purchased from Loba Chemie, India. All other chemicals were of AR grade and purchased from Merck, India or SRL, India. All the spectrophotometric determinations were done in a Systronics (India) double beam UV-Vis spectrophotometer (model – 2202).

Extraction of polyphenols from citrus peel

Polyphenol enriched fraction was extracted from orange and sweet lime (Mousambi) peels by solvent extraction. Briefly, 1 gm of sun-dried peel powder, devoid of essential oil, was soaked in 100 ml of methanol-water (1:1 v/v) mixture for 30 minutes at 90±2^{o}C. The mixture was filtered with Whatman#1 filter paper and stored at –4^{o}C for microcapsule preparation. Essential oil was removed by heating the dried peels in a commercial microwave oven at medium power for 5 minutes.

Encapsulation of polyphenol enriched fractions

Microcapsules were prepared using the ionotropic gelation method with some modifications.[21] Firstly, pectin (2.5 g in 100 ml) and sodium alginate (5 g in 100 ml) were dissolved in deionised water and the polyphenol extract from orange and sweet lime peel was dispersed in it at two different weight ratios (*ca*. 1:1:1 and 1:1:5), separately. This mixture was then added drop wise through the needle of an injection syringe on a gently agitated 0.1 M $CaCl_2$ solution. Subsequent microcapsules obtained by the gelation of the pectin and the alginate was held in magnetic stirring for 3 hours. The microcapsules were then separated, washed with distilled water and dried *in vacuo*. The following sample codes were used for rest of the study –

C – control microcapsules prepared with alginate and pectin only.
O1 – Microcapsules containing pectin, alginate and orange polyphenols at 1:1:1 weight ratio.
M1 – Microcapsules containing pectin, alginate and lime polyphenols at 1:1:1 weight ratio.
O5 – Microcapsules containing pectin, alginate and orange polyphenols at 1:1:5 weight ratio.
M5 – Microcapsules containing pectin, alginate and lime polyphenols at 1:1:5 weight ratio.

Release of loaded polyphenols at different pH conditions

The content of extract released from weighed amounts of beads was determined by suspending known amount of capsules in the release media while gently agitated in a shaking water bath. The release media used were *in vitro* model digestion fluids – Simulated Gastrointestinal Fluid, SGF, and Simulated Intestinal Fluid, SIF. Both these fluids were prepared in accordance with a published procedure which in turn is based on United States Pharmacopeia.[22] SGF was prepared from a solution of hydrochloric acid 0.1 M corrected with sodium hydroxide to (pH 1.2), and SIF from phosphate buffer (pH 7.4). An alkaline release medium, ARM, comprised of sodium carbonate-bicarbonate buffer (pH 9.2) was also used to monitor release at higher pH. Total polyphenols content was determined by the Folin-Ciocalteau method and with CUPRAC assay in the model fluids after release at different time intervals, viz.

10, 60 and 90 minutes after suspension. All tests were performed at 37°±2°C.

Swelling and erosion behaviour

Swelling and erosion studies were carried out according to a reported method with minor modifications.[23] About 200 mg of microcapsule was soaked in 5 ml of the model fluids at 37±2ºC. At predetermined time intervals (viz. 1 and 2 hours), microcapsule were taken out and lightly wiped with tissue paper to remove the excess liquid. The swollen microcapsules were then weighed. After that, the microcapsules were dried in the oven at 54±2ºC for 24 hours. The dehydrated microcapsules were weighed again to determine the remaining dry weight. The swelling degree and percentage of erosion were calculated at each time point using a pre-established formula represented in the literature.

***In vitro* antioxidant/radical scavenging assays**

ABTS radical scavenging assay was performed using a previously described procedure.[24] The oxidant was generated by persulfate oxidation of 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid. The oxidant solution was mixed with the sample/standard solutions to adjudicate reduction of radical colour. Measurements were done at 734 nm. DPPH radical scavenging assay was studied by monitoring reduction in radical concentration by mixing radical solution with sample/standard solution.[24] Measurements were done at 517 nm. Total polyphenol content was measured by Folin-Ciocalteau method.[25] CUPRAC or cupric ion reduction assay was performed by measuring reduction power of the samples of cupric ion to cuprous ion, which was then quantitatively estimated using neocuproine.[26] Measurement was done at 455 nm with gallic acid used as positive control. Hydroxyl radical scavenging abilities of the samples were monitored by their prevention of formation of malondialdehyde from 2-deoxy-D-ribose *in vitro*, measured at 532 nm in a spectrophotometer.[27] Gallic acid was used as positive control.

Statistical analyses

Experimental results were expressed as mean ± SD of four individual experiments. Analyses were done using the software 'SPSS Statistics 17.0' (IBM Corporation, USA).

RESULTS AND DISCUSSION

Release of loaded polyphenols at different pH conditions

Foundation of the present study was based on the effectiveness of release of polyphenols loaded in microcapsules by ionotropic gelation method, a method immensely applied in the field of pharmaceutical formulations worldwide. The method utilises the ability of cross-linking of polyelectrolytes like alginate and pectin in presence of counterions like Ca^{2+} to form hydrogel beads.[28] Formation of these gelispheres encapsulate substances, if added in the medium, and release them in appropriate conditions. Different pH conditions in the alimentary system of humans might thus affect release of such encapsulated substances in different manners. In the present study, such release patterns were observed for three typical pH conditions – viz. 1.2, 7.4 and 9.2.

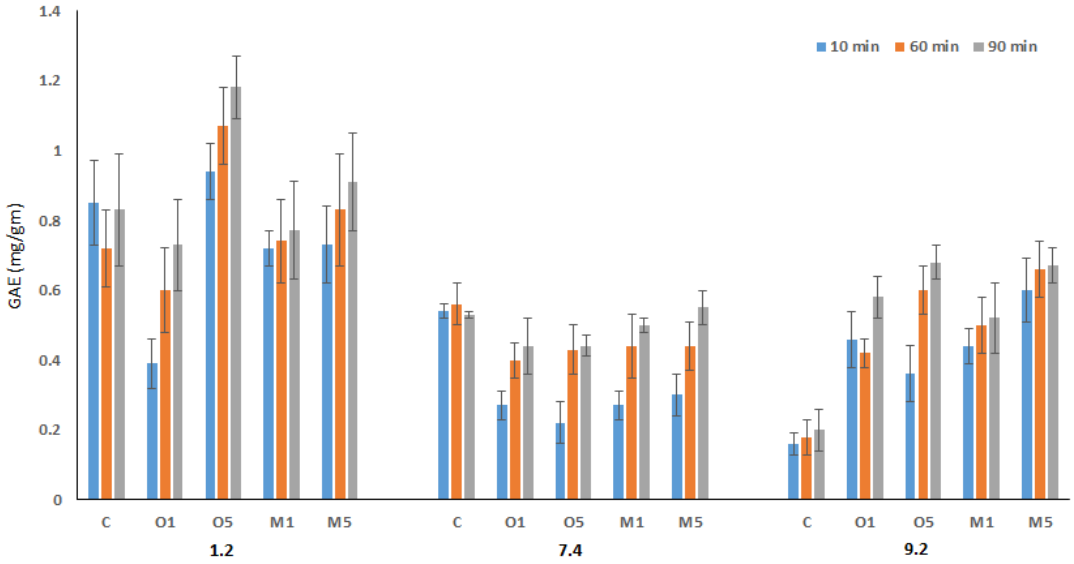

Fig. 1: Folin-Ciocalteau assay for estimation of total phenolic contents of the released bioactives from different microcapsules at pH 1.2, 7.4 and 9.2 at different time intervals. Results are expressed as gallic acid equivalent (GAE)±SD.

The above figure clearly indicated that release of bioactives, mainly polyphenolics, were significantly better in pH 1.2 compared to the other two pH conditions. Maximum release was obtained from the sample containing orange peel extract at 1:1:5 ratio (*ca.* 1.2 mg/gm) after 90 minutes interval. It was also

observed that more was the amount of loaded peel extract, more was the release with time. This was all in accordance with earlier literature as acidic conditions usually helps in better leaching of the loaded substances from these types of gelispheres.[29] It was also observed from the figure that microcapsules prepared with bioactives obtained from orange showed better results than their counterparts prepared with bioactives obtained from lime. However, in the other two pH conditions, results were almost similar.

In an almost identical reaction set, the leachates were estimated for their cupric ion reduction potential by CUPRAC assay. The assay was conducted to ascertain if there were any variation in the probable therapeutic aspects of the leachates as CUPRAC assay conforms radical scavenging potential of almost all types of bioactives close to physiological conditions (~ pH 7).[30] Fig. 2 furnished the outcome of the investigation. It was observed that maximum release was obtained from the sample containing orange peel extract at 1:1:5 ratio (*ca.* 12.5 mg/gm) after 90 minutes interval. The result also conforms to the previous experiment, where microbeads with higher ratio of orange peel extract showed maximum release. The figure clearly illustrated that the reduction abilities of loaded materials, after release from the microbeads, were significantly higher at pH 7.4 in comparison to the other two pH conditions. The result indicated that the loaded polyphenols did not lose their potential on undergoing the loading procedure which would establish their potential use as sustained-release therapeutic agents.

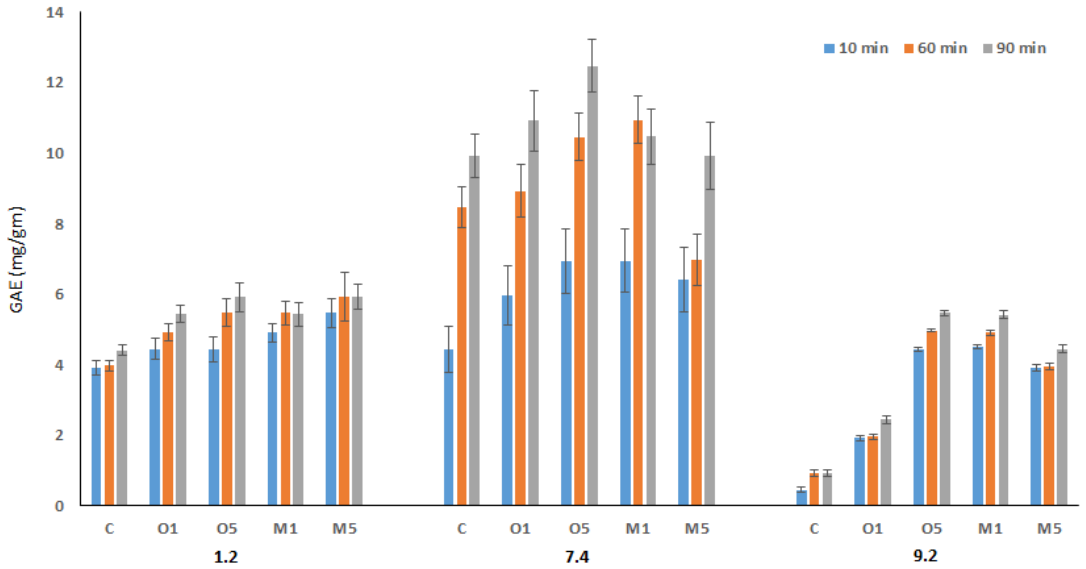

Fig. 2: CUPRAC assay for the released bioactives from different microcapsules at pH 1.2, 7.4 and 9.2 at different time intervals. Results are expressed as gallic acid equivalent (GAE)±SD.

Swelling and erosion behaviour

The polyphenol-loaded microbeads showed a good swelling behavior when sodden into a solution having pH 1.2 for 1 hour. The control beads, having only alginate and pectin, found to swell the highest among all experimental beads. It swelled about 92%. In case of the polyphenol-loaded microbeads, swelling diminished as the loading of polyphenols increased. The results are provided in Fig. 3. In case of orange-polyphenol loaded beads, swelling reduced from *ca.* 78% to *ca.* 46% in a dose-dependent manner. In case of lime-polyphenol loaded beads, swelling reduced from *ca.* 67% to 20% as the dose increased. After 2 hours soaking at pH 1.2, the beads released some of their loaded polyphenol into the leaching solution and that is why, weight decreased. On the other hand, when the gelispheres were soaked for 2 hours at pH 9.2, all the beads were deformed or melted, probably due of the harsh pH condition. That is why the release study was performed within a time limit of one hour. We might conclude that since the SGF has a pH of about 1.2 to 1.6, a suitable sustained-release of the loaded nutraceuticals could be achieved for such polyphenol incorporated microbeads.

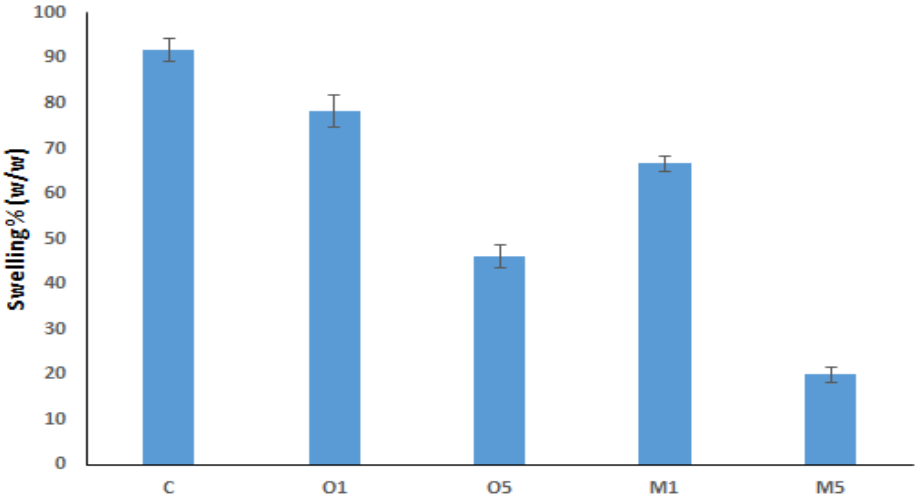

Fig. 3: Swelling pattern of the different microcapsules at pH 1.2.

The experimental microbeads showed encouraging results in case of their erosion behavior. All the microbeads lose their weight about 97% after drying in a hot air oven at 54±2ºC for 24 hours. The erosion study reflects the amount of polymer dissolved in different media during dissolution process, which is strongly pH dependent.[23] Since erosion strongly depends upon pH of the release medium and higher pH leads to higher erosion, we only chose to perform the study at the lowest pH, i.e. 1.2. Results of this study was provided in Fig. 4. Since the percentage of erosion was well at par with the control, we can conclude that the gelispheres survived their erosion-resisting behaviour despite the fact that they are loaded with highly acidic polyphenols of citrus peel.

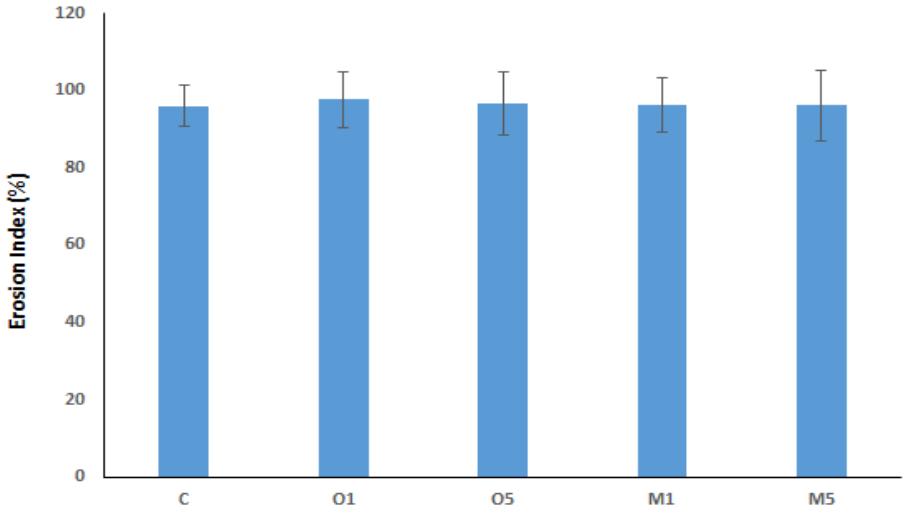

Fig. 4: Erosion indices of the different microcapsules at pH 1.2.

***In vitro* antioxidant/radical scavenging assays**

In the following set of experiments for elucidation of the antioxidant profile of the microcapsules, it was observed that the gel beads showed excellent antioxidant capacities. As the release of the bioactives from the microbeads were better at pH 1.2, we further tested the radical scavenging abilities of the microbeads in this pH only. Fig. 5 represented the result of the experiments. In all the three experimental protocols, microcapsules prepared with orange peel extracts showed better antioxidative capacities. It could also be concluded from the experiments that the gelispheres contain more polar bioactives as the antioxidant potential of the leachates were more pronounced in ABTS assay system than the DPPH assay system. The gelisphere containing orange peel extract at 1:1:5 ratio was the best in these two assay systems. It can also be seen from the diagram that ABTS radical scavenging potential of the sample was almost 7 times to that of DPPH radical scavenging ability (gallic acid equivalent values were *ca.* 75 mg/gm and *ca.* 11 mg/gm, respectively). DPPH assay system is based on alcohols as solvents in contrast to water used in ABTS assay, which means that only less polar bioactives would produce better result in this protocol.[31] We can presume from the outcome of the results that the microcapsules would work efficiently in water-based physiological systems.

On the other hand, the microbeads showed significantly better hydroxyl radical scavenging abilities compared to the control (Fig. 5). Likewise in this assay also, maximum release was obtained from the sample

containing orange peel extract at 1:1:5 ratio (*ca*. 109.6 mg/gm) after 90 minutes interval. Hydroxyl radical is supposed to be the most deleterious oxidant for any living system as it has got very short half life.[32] Effective scavenging of the radical by the microbeads thus lent credence for development of an efficient sustained-release nutraceutical in the future.

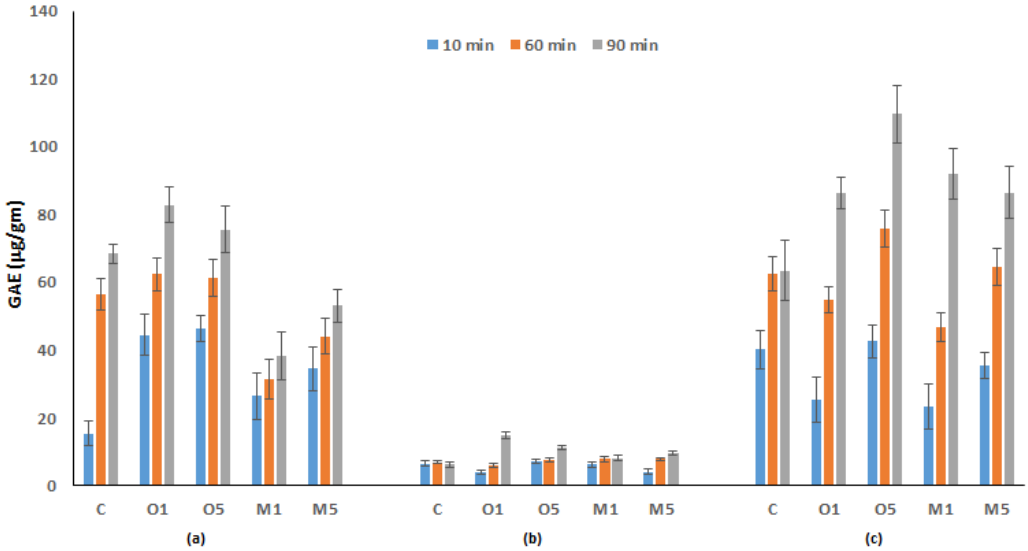

Fig. 5:Antioxidant profiles of the released bioactives from different microcapsules at pH 1.2, 7.4 and 9.2 at different time intervals. Results are expressed as gallic acid equivalent (GAE)±SD. (a) ABTS assay; (b) DPPH assay and (c) Hydroxyl radical scavenging assay.

CONCLUSION

The microbeads containing polyphenols extracted from orange and sweet lime peels and formed by ionic gelation method with different combinations of sodium alginate, pectin and the extracted polyphenols were very responsive to every assessment performed. The beads released antioxidative polyphenols in simulated physiological fluids in a controlled manner. However, the microbeads showed excellent results at pH 1.2 in both the compositions and doses. The sustain releases were superior in that particular pH. On the other hand, superior result in ABTS assay over DPPH assay indicated that the gelispheres were ideal for aqueous physiological systems. Hydroxyl radical scavenging abilities indicated potential beneficial effect if ingested in physiological systems.

These microbeads were found to entrap polyphenols in sufficient amount and also in effective manner to protect their antioxidant activity. This was indicated by the swelling and erosion behavior studies. We might conclude that it is possible to develop an effective, cheap and nontoxic microbeads as was described in the present study. Although it was an *in vitro* study, but the methodology, results and the overall success of this study could be the first step towards a signal regarding the probable outcome of their effects when applied *in vivo*. They can be used as food supplements, as nutraceuticals, as drug supplements, or as functional food delivery system in future without any hesitation of unfamiliar non-edible substance ingestion.

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REFERENCES

1. Munin A, Edwards-Lévy F. Encapsulation of Natural Polyphenolic Compounds; A Review. Pharmaceutics, 2011; 3: 793-829.
2. Jackson LS, Lee K. Microencapsulation and the Food Industry. Lebensmittel – Wissenchaft und – Technologie, 1991; 24: 289-97.
3. Krishnasailaja A, Jyothika M. A Review on Microcapsules. CIBTech Journal of Pharmaceutical Sciences, 2015; 4(2): 26-33.
4. Venkatesan P, Manavalan R, Valliappan K. Microcapsulation – A Vital Technique in Novel Drug Delivery System. Journal of Pharmaceutical Sciences and Research, 2009; 1(4): 26-35.
5. Pratyusha M, Rohitha M, Meghanadevi M, Madhuri K, Rajitha B, Bhavani CT, Madhumitha S, Ushakiran G. Scope of Nanotechnology in Drug Delivery System. European Journal of Pharmaceutical and Medical Research, 2020; 7(2): 261-9.
6. Agnihotri N, Mishra R, Goda C, Arora M. Microencapsulation – A Novel Approach in Drug Delivery – A Review. Indo Global Journal of Pharmaceutical Sciences, 2012; 2(1): 1-20.
7. Rustaiyan A, Faridchehr A, Fard MA. Constituents and Biological Activities of Iranian Achillea Species. European Journal of Pharmaceutical and Medical Research, 2017; 4(4): 128-34.

8. Tsao R. Chemistry and Biochemistry of Dietary Polyphenols. Nutrients, 2010; 2: 1231-46.
9. Fang Z, Bhandari B. Encapsulation of Polyphenols – A Review. Trends in Food Science and Technology, 2010; 21: 510-23.
10. Sawale PD, Patil GR, Hussain SA, Singh AK, Singh RRB. Release Characteristics of Polyphenols from Microencapsulated *Terminalia arjuna* Extract: Effects of Simulated Gastric Fluid. International Journal of Food Properties, 2017; 20(12): 3170-8.
11. Belščak-Cvitanović A, Jurić S, Đorđević V, Barišić L, Komes D, Ježek D, Bugarski B, Nedović V. Chemometric Evaluation of Binary Mixtures of Alginate and Polysaccharide Biopolymers as Carriers for Microencapsulation of Green Tea Polyphenols. International Journal of Food Properties, 2017; 20(9): 1971-86.
12. Liu T, Pan W, Zhang J, Wei Z. Microencapsulation and Properties of the Silkworm Pupal Oil with Soybean Protein Isolate/β-Cyclodextrin. Journal of Chemical and Pharmaceutical Research, 2014; 6(7): 295–301.
13. Li R, Roos YH, Miao S. Characterization of Mechanical and Encapsulation Properties of Lactose/Maltodextrin/WPI Matrix. Food Hydrocolloids 2017; 63: 149-59.
14. Suganya V, Anuradha V, Ali MS, Sangeetha P, Bhuvana P. Microencapsulation and Characterization of Astaxanthin prepared using Different Agents. Research Journal of Chemical Sciences, 2017; 7(12): 1-10.
15. Jaya S, Durance TD, Wang R. Effect of Alginate-Pectin Composition on Drug Release Characteristics of Microcapsules. Journal of Microencapsulation, 2009; 26(2): 143-53.
16. Madziva H, Kailasapathy K, Phillips M. Evaluation of Alginate-Pectin Capsules in Cheddar Cheese as a Food Carrier for the Delivery of Folic Acid. LWT, 2006; 39: 146-51.
17. Robert P, Gorena T, Romero N, Sepulveda E, Chavez J, Saenz C. Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying. International Journal of Food Science and Technology, 2010; 45: 1386-94.
18. Lopes S, Bueno L, Junior FD, Finkler C. Preparation and characterization of alginate and gelatin microcapsules containing *Lactobacillus rhamnosus*. Annals of the Brazilian Academy of Sciences, 2017; 89(3): 1601-13.
19. Anbinder PS, Deladino L, Navarro AS, Amalvy JI, Martino MN. Yerba Mate Extract Encapsulation with Alginate and Chitosan Systems: Interactions between Active Compound Encapsulation Polymers. Journal of Encapsulation and Adsorption Sciences, 2011; 1: 80-7.
20. Gartziandia O, Lasa A, Pedraz JL, Miranda J, Portillo MP, Igartua M, Hernandez RM. Preparation and Characterization of Resveratrol Loaded Pectin/Alginate Blend Gastro-Resistant Microparticles. Molecules, 2018; 23: 1-10.
21. Zam W, Bashour G, Abdelwahed W, Khayata W. Formulation and *in vitro* Release of Pomegranate Peels' Polyphenols Microbeads. International Journal of Pharmaceutical Sciences and Rsearch, 2013; 4(9): 3536-40.
22. Chotiko A, Sathivel S. Releasing characteristics of anthocyanins extract in pectin–whey protein complex microcapsules coated with zein. Journal of Food Science and Technology, 2017; 54(7): 2059-66.
23. Ling HW, Lin NK. *In Vitro* Release Study of Freeze-Dried and Oven-Dried Microencapsulated Kenaf Seed Oil. Malaysian Journal of Nutrition, 2017; 23(1): 139-49.
24. Chakrobarty A, Bhattacharyya S. Thermal processing effects on *in vitro* antioxidant activities of five common Indian Pulses. Journal of Applied Pharmaceutical Science, 2014; 4(5): 65-70.
25. Banik I, Som M, Bhattacharjee S, Bhattacharyya S, Rai C. Bacteriostatic Activities of Antioxidant Silver Nanoparticles Prepared from Leaves of *Azadirachta indica* and *Psidium guajava*. European Journal of Pharmaceutical and Medical Research, 2017; 4(10): 223-8.
26. Karasakal A. Evaluation of Antioxidant activities of *Brassica napus's* seeds by CUPRAC, ABTS/Persulphate and DMPD methods. Marmara Pharmaceutical Journal, 2015; 19: 153-8.
27. Aktar N, Rai C, Bhattacharjee S, Bhattacharyya S. Effect of Thermal Processing on Synergistic Antioxidant and Antimicrobial Activities of Turmeric (*Curcuma longa*) and Red Chili Pepper (*Capsicum annuum*). International Journal of Food and Nutritional Science, 2016; 5(3): 19-30.
28. Patil P, Chavanke D, Wagh M. A Review on Ionotropic Gelation Method: Novel Approach for Controlled Gastroretentive Gelispheres. International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4(4): 27-32.
29. Singh AP, Siddiqui J, Diosady LL. Characterizing the pH-Dependent Release Kinetics of Food-Grade Spray Drying Encapsulated Iron Microcapsules for Food Fortification. Food and Bioprocess Technology, 2018; 11(2): 435-46.
30. Ozyurek M, Guclu K, Tutem E, Baskan KS, Ercag E, Celik SE, Baki S, Yildiz L, Karaman S, Apak R. A comprehensive review of CUPRAC methodology. Analytical Methods, 2011; 3: 2439-53.
31. Chakraborty K, Bhattacharjee S, Pal TK, Bhattacharyya S. Evaluation of *in vitro* antioxidant potential of Tea (*Camelia sinensis*) leaves obtained from different heights of Darjeeling Hill, West Bengal. Journal of Applied Pharmaceutical Science, 2015; 5 (1): 63-8.
32. Mitra K, Saha I, Bhattacharjee S, Rai C, Bhattacharyya S. Evaluation of Antioxidant and Antimicrobial Properties of Silver Nanoparticles Prepared from Different Phenotypes of Cabbage (*Brassica oleracea*). Asian Journal of Biochemical and Pharmaceutical Research, 2018; 1(8): 57-66.