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ENCAPSULATION OPTIMIZATION OF TAMARIND SEED ANTIOXIDANTS IN ALGINATE HYDROGELS USING RESPONSE SURFACE METHODOLOGY: STUDY OF CONTROLLED RELEASE BEHAVIOR IN SIMULATED GASTROINTESTINAL CONDITIONS

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Keywords:

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ABSTRACT: *Tamarindus indica* seeds - a byproduct of the food industry, are generated in huge amounts during tamarind pulp processing. These seeds are a source of a wide range of polyphenols with natural antioxidant content. In this study, we have encapsulated the polyphenolic components extracted from the seeds in an optimized condition. Statistical optimization of the encapsulation parameters was done using the central composite design of response surface methodology**.** The concentrations of sodium alginate (m:v, g ml $^{-1}$), calcium chloride (m:v, g ml^{-1}) and tamarind seed polyphenolic extract (m:v, g ml $^{-1}$) were the independent variables whose direct and interaction effects were studied on the dependent variable or response of encapsulation efficiency. Further, the encapsulated beads were characterized by physical, chemical, morphological, radical quenching properties as well as textural characteristics. A higher percentage of polyphenols were recovered from beads in simulated gastric fluid, and the rest contents of polyphenols remaining inside the beads were found to be released in simulated intestinal fluid. The study was concluded with the remark that encapsulation of tamarind seed extracts in alginate could be a promising technique for supplementation in food with natural antioxidants.

INTRODUCTION: A sizeable number of preclinical and epidemiological research findings suggest that polyphenols from the seeds of *Tamarindus indica* have therapeutic effects. It has been found that the use of this seed extract can effectively decelerate the progression of certain cancers by regulating cell apoptosis [1] and also reduce the risk of disease development due to free radical load [2].

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The bioactive components so far isolated from tamarind seed extract are mainly catechin and epicatechin [3]. These are potent antioxidants and a part of the family of flavonoids. These compounds are easily oxidized involving their catechol and resorcinol groups, and the oxidation process is pH dependent; hence, they are unstable during food processing and storage methods involving temperature, oxygen, light *etc*. To overcome this drawback of instability and in addition to improve the bioavailability of the bio active components, the consumption of encapsulated polyphenols is preferred over free compound intake.

In our preceding study, we had extracted antioxidant-rich polyphenolic compounds from *T. indica* seeds in optimized extraction condition

using generally recognized as safe (GRAS) status solvents [4]. To ensure easy handling and better usability, the extract could be encapsulated. Reports are plentiful, where antioxidant from other seeds have been encapsulated using cross-linking biopolymers such as alginate, chitosan, pectin. Alginate is the most abundant polysaccharide in nature and also the most widely used material in hydrogel formation for encapsulation. Owing to its easy gelation, low cost, and non-toxic nature, alginate was selected as encapsulating material in this study. Alginate solution forms gels by reacting with divalent ions, for example, calcium ions. The efficiency of gelation is, therefore, dependant on the properties of alginate and divalent calcium solutions as well as another gelation parameter. Thus, optimization of gelation parameters is a necessary prerequisite to achieving successful encapsulation of tamarind seed polyphenols (TSP).

In the current investigation, response surface methodology (RSM) was used in the evaluation of the effects of multiple parameters, namely concentrations of sodium alginate solution, calcium chloride solution, and tamarind seed extract, alone or combination, on the response of encapsulation efficiency. Further, the beads produced in optimized condition was characterized, and their extract release in simulated gastric conditions have been evaluated to study the efficacy of encapsulation, which has not been done before, to the best of our knowledge.

MATERIALS AND METHODS:

Chemicals: The chemicals used for experimental purposes were Gallic Acid (S.d fine chem Ltd., Mumbai, India), 2, 4, 6 – Tri (2 - pyridyl)-S - triazine (Hi-media, Mumbai, India). Sodium Carbonate (Na_2CO_3), Iron (III) chloride 6-hydrate ($FeCl_3$, $6H_2O$), Iron (II) sulfate 7-hydrate (Fe_2SO_4), acetic acid, sodium acetate, hexane, hydrochloric acid, folin-ciocalteu's phenol, sodium alginate, calcium chloride were supplied by Merck (Germany). The chemicals used were of analytical grade.

Extract Preparation: Raw tamarind seeds were collected from the fruit sellers of Jadavpur market, adjacent to Jadavpur University and were kept frozen at -20 °C till further study. Polyphenolic compounds with high antioxidant activity were extracted from the seeds in their optimized extraction conditions as described by the authors in previous experiment [4]. Briefly, 4.9 mg of tamarind seed powder was extracted in 10 ml of 50% ethanol-water mixture in a water bath shaker regulated at 60 strokes per minute for 194 min. The extract obtained was frozen at -80 ºC for 24 h, followed by lyophilization in a freeze dryer (made: Eyela FDU 1200, Japan). The lyophilized extract was stored in zip lock pouches in desiccators.

Estimation of Phenolic and Antioxidant Content of Lyophilized Extract: Total polyphenol content (TPC) of the extract was determined according to the protocol described by Malik and Singh (1980) [5] with some changes. Briefly, 0.75 ml of different concentrations of the extracts were taken, to which 3 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent (diluted to 1:1 with water) and 1 ml of 20% Na_2CO_3 were added. The absorbance was read after 2 hours of incubation in dark room, by spectrophotometer (made: Hitachi U- 2000) at 760 nm wavelength and plotted in a standard calibration curve of Gallic Acid. These results were expressed as milligram Gallic Acid Equivalents per gram of dry sample (mg GAE g^{-1}).

Estimation of Antioxidant Property of Lyophilized Extract: FRAP assay was carried out according to the method of Benzie & Strain (1996) [6] with minor modifications. Briefly, sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (dissolved in 40 mM HCl) and 20 mM iron(III) chloride solution were mixed in a ratio (v/v) of 10:1:1, respectively to prepare the FRAP reagent. After that, the freshly prepared FRAP reagent was warmed to 37 °C in a water bath before use. 100 µl of the sample solution was added to 3 ml of the freshly prepared FRAP reagent. The absorbance was measured at 593 nm using spectrophotometer after 4 min and plotted in a standard calibration curve of $FeSO_4$ solution. The results are expressed as µmol Fe (II) g^{-1} dry sample (µmol Fe (II)/g).

For DPPH* assay, 0.1 ml of the sample extracts of varying concentrations were added to 3.9 ml (0.025 g/l) of DPPH* solution. The mixture was incubated at room temperature for 60 min, after that measured with a spectrophotometer at 515 nm. The remaining concentration of DPPH* in the reaction medium was calculated from a calibration curve and the

percentage of remaining DPPH* was calculated as described in equation 1:

$$\text{Remaining \% DPPH*} = DPPH_T \times 100 / DPPH_{T0}$$

Where, $DPPH_T$ and $DPPH_{T0}$ are the concentrations of DPPH* after 60 min and at zero time respectively. The percentage of remaining DPPH* was plotted against the sample concentration to obtain the amount of antioxidant necessary to decrease the initial concentration of DPPH* by 50% (EC_{50}) [7]. The results were expressed in terms of mg dry matter of sample g^{-1} DPPH* (mg sample g^{-1} DPPH*).

Optimization Study Design for Encapsulation of TSP Extract: A canonical study for optimization of reaction conditions was carried out to obtain maximum encapsulation efficiency (% EE) of TSP extract in alginate beads employing the central composite design (CCD) of RSM. For this purpose, a design matrix was initially generated by Design expert Software version 8.0.6 (Stat-Ease, Inc., Minneapolis). The design consisted of 20 experimental runs comprising 8 factorial points, 6 axial points, and 6 replicates at the center points. The concentrations of sodium alginate (m:v, g ml^{-1}; X_1), calcium chloride (m:v, g ml^{-1}; X_2) and TSP extracts (m:v, g ml^{-1}; X_3) were the independent variables whose direct and interaction effects were studied on the dependent variable or response of encapsulation efficiency (% EE).

For extract encapsulation, external gelation method was employed, and calcium alginate hydrogels from sodium alginate and calcium chloride solutions were prepared. The basic protocol followed for preparation of alginate hydrogels with TSP extract (Test beads) was- at first, different concentrations of sodium alginate were dissolved in TSP extract of varying concentrations as per the combinations indicated in CCD design matrix **Table 2**. To allow complete hydration of alginate, the solution was homogenized and kept overnight. After that, the alginate solution was passed through as droplets by a 23 gauge metal needle attached to a 5 ml syringe (made: BD Emerald, USA) into calcium chloride solution of different concentrations to form hydrogel beads. The beads were kept in calcium chloride solution at ambient temperature for 15 min (in preliminary trials it was found that hardening time below 15 min resulted in unstable bead, whereas hardening above 15 min gave no better result). Control beads were formed in the same process, except that they were devoid of TSP extract. The beads were filtered followed by subsequent washing with acetate buffer (pH 5.5), freeze-dried and sealed inside zip lock pouches for storage under 4 ºC until further analysis.

Encapsulation efficiency [8] of TSP encapsulated beads was calculated as per equation 2:

$$EE\ \% = W_e \times 100 / W_t$$

Where, W_e are the amount of polyphenols encapsulated in alginate beads. It was determined by shaking 1 g of beads in 5% (w/v) sodium citrate solution in an incubator for 3 h and TPC was determined by the Folin-Ciocalteau method. W_t is the amount of polyphenols in TSP extract.

TABLE 1: CODED AND ACTUAL VALUES FOR CENTRAL COMPOSITE DESIGN (CCD)

Independent variables	Coded levels				
	-α	-1	0	1	+α
Concentration of sodium alginate solution (m:v, g ml^{-1}, X_1),	3.16	3.50	4.00	4.50	4.84
Concentration of calcium chloride extracts (m:v, g ml^{-1}, X_2)	1.16	1.5	2	2.5	2.84
Concentration of TSP extract (m:v, g ml^{-1}, X_3)	0.36	0.7	1.2	1.7	2.04

Table 1 gives the levels of design variables, and the CCD design matrix is given in **Table 2**.

The response surface regression was studied and analyzed by Design expert Software version 8.0.6 (Stat-Ease, Inc., Minneapolis) and the second order polynomial model (equation 3) was used to fit the experimental data:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{11}X_12 + \beta_{22}X_2^2 + \beta_{33}X_3^2$$

Where Y is the predicted response, $\beta_1, \beta_2, \beta_3$ are the coefficients for the linear terms, $\beta_{11}, \beta_{22}, \beta_{33}$ are the coefficients for the quadratic and $\beta_{12}, \beta_{13}, \beta_{23}$ are the coefficients for interaction terms respectively. X represents the coded values for the independent process variables.

Characterization of the Beads:

Moisture Content (%) of Beads: The beads were desiccated in a hot air oven operated at 80 ºC until

a constant weight was achieved and the moisture content was determined using equation 4:

$$\text{Moisture Content (\%)} = W_2 - W_1 \times 100 / W_2$$

Where W_2 is the wet weight of hydrogels and W_1 is the weight of the beads after drying.

The Bulk Density of Beads: This was determined by discharging a measured quantity of beads into a graduated cylinder. The bulk density was calculated by dividing the mass of the beads by their bulk volume [9].

Antioxidant Property of Test Beads: 1 g of beads were placed into 10 ml of distilled water and incubated in shaking condition overnight. This released encapsulated extract with antioxidant potential, which was then measured by using DPPH* and FRAP methods as previously described.

Texture Analysis of Test Beads: Texture analyzer (made: TA.XT Express Enhanced Stable Microsystems, USA) equipped with Exponent Lite Express software was utilized for texture profile analysis (TPA) of the hydrogel bead. The experiment was executed in threefold, where three different hydrogel beads were analyzed in two consecutive cycles. In the first cycle, compression testing was done using a 2 mm cylinder probe with a 5 kg load cell run at a speed of 0.5 mm/s over 2 mm distance.

The hardness of the sample was denoted in terms of the maximum force (N) required for compression, which is, again, equivalent to the maximum resistance offered by the hydrogel surface against the compression of the probe. Springiness and resilience were measured in terms of the sample's recovery capacity after the first compression. At the second cycle, cohesiveness was measured by calculating the ratio of the areas under second and first compression. Chewiness, described as the product of hardness, cohesiveness and elasticity [10], was also considered for textural characterization of the beads.

Bead Porosity and Diameter: Average pore size, surface area and total pore volume of test and control hydrogel beads were measured by Autosorb iQ Station 1 instrument following the Brunauer, Emmett, Teller (BET) method from linear nitrogen adsorption isotherm. Lyophilised test and control beads were degassed at 90 ºC for 12 h under reduced pressure. The total pore volume was given at P/P_0 of 0.9975. Screw gauge was utilized to get the average diameter and radius of the hydrogels.

Microscopic Image Capture: Microscopic information related to the structural characteristics of the cross-sectional fractions of both the test and control beads were obtained using optical microscope (made: Lawrence and Mayo, Lynx LM- 52- 1704) and the pictures were captured by Nikon Coolpix A 10 camera. The structures were observed and captured immediately after hydrogel formation.

SEM and EDAX Analysis: Quanta FEC 250 Scanning Electron Microscope equipped with Everhart- Thornley detector was utilized for the elemental (EDAX) and surface morphology analysis of test and control hydrogel beads. Lyophilised beads were mounted and attached to stubs and using double-sided adhesive tape and examined under electron beam with 10 kV acceleration voltage.

Release Assessment: Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were used to assess the release behavior of polyphenols from test beads in simulated gastrointestinal condition [11]. SGF was prepared using 0.1 M HCl with pH 2.0 adjusted with NaOH. 1 g test beads were mixed with 2 ml SGF, after that, incubated at 37 °C under regular agitation of 60 rpm for 3 h. The remaining beads after SGF digestion were filtered and subjected to SIF digestion at 37 °C for 3 h. Phosphate buffer with pH 6.8 was used as SIF. The total polyphenol content of the extractives was determined as previously described. The release percentage of polyphenol content was calculated as equation 5:

$$\text{Released extract \%} = M_c \times 100 / M_e$$

Where M_c is the polyphenol content of released extract and M_e is the encapsulated polyphenols in hydrogel beads which was already determined in sodium citrate solution [9].

RESULTS AND DISCUSSION:

Analysis of the Model: Table 2 shows the experimental data of encapsulation efficiency

obtained from the experimental runs. It was found that encapsulation efficiency of TSP polyphenols in alginate beads ranged from 34.9 to 55.9%.

Statistical significance of the quadratic model was analyzed by Analysis of Variance (ANOVA), and the results are indicated in **Table 3**.

TABLE 2: CCD DESIGN MATRIX WITH EXPERIMENTAL RESPONSES

Run		Independent Variables			Response[a]
Std. Order	Run no.	X_1	X_2	X_3	Y
18	1	4	2	1.2	42.9
16	2	4	2	1.2	42.51
11	3	4	1.16	1.2	46.87
8	4	4.5	2.5	1.7	44.98
20	5	4	2	1.2	41.88
14	6	4	2	2.04	34.9
4	7	4.5	2.5	0.7	47.67
2	8	4.5	1.5	0.7	50.9
3	9	3.5	2.5	0.7	41.51
7	10	3.5	2.5	1.7	39.62
1	11	3.5	1.5	0.7	41.78
17	12	4	2	1.2	41.81
19	13	4	2	1.2	41.76
12	14	4	2.84	1.2	43.99
5	15	3.5	1.5	1.7	40.43
15	16	4	2	1.2	42.87
6	17	4.5	1.5	1.7	47.62
10	18	4.84	2	1.2	55.9
9	19	3.16	2	1.2	44.97
13	20	4	2	0.36	38.67

[a]Experiments were run in triplicate; X_1=concentration of sodium alginate solution (m:v, g ml $^{-1}$), X_2= concentration of calcium chloride solution (m:v, g ml $^{-1}$) and X_3= concentration of TSP extracts (m:v, g ml $^{-1}$) and Y= encapsulation efficiency (% EE)

TABLE 3: ANOVA FOR THE QUADRATIC POLYNOMIAL RSM MODEL

Source	Sum of Squares	Df[b]	Mean Square	F Value	p-value Prob > F
Model	395.3619	9	43.9291	247.5037	< 0.0001**
X_1	156.3719	1	156.3719	881.0248	< 0.0001**
X_2	10.18449	1	10.18449	57.38109	< 0.0001**
X_3	17.7064	1	17.7064	99.76071	< 0.0001**
$X_1 X_2$	2.868012	1	2.868012	16.15885	0.0024*
$X_1 X_3$	0.931612	1	0.931612	5.248856	0.0449*
$X_2 X_3$	0.000313	1	0.000313	0.001761	0.9674^{NS}
X_1^2	119.2082	1	119.2082	671.6384	< 0.0001**
X_2^2	17.64594	1	17.64594	99.42008	< 0.0001**
X_3^2	54.7944	1	54.7944	308.7206	< 0.0001**
Residual	1.774887	10	0.177489		
Lack of Fit	0.338603	5	0.067721	0.23575	0.9306^{NS}
Pure Error	1.436283	5	0.287257		
Cor Total	397.1368	19			

[b] Degrees of Freedom; standard deviation: 0.421; Mean: 43.68
** significance (values of "Prob > F" less than 0.0001); * significance (values of "Prob > F" less than 0.05), ^{NS}non significant

Two important parameters of statistics, The F-value and p-value were used to indicate the significance of the quadratic model and the significance of each coefficient, respectively. A moderately good F-value and its associated p-value indicated the significance of the model.

The ANOVA analysis also revealed that all the independent variables in their linear and second order terms significantly affected the response.

However, the interaction effects of the concentrations of sodium alginate (m:v, g ml $^{-1}$; X_1) with calcium chloride (m:v, g ml $^{-1}$; X_2)and the concentrations of sodium alginate (m:v, g ml $^{-1}$; X_1) with TSP extracts (m:v, g ml $^{-1}$; X_3) were significant terms.

Optimization of the Extraction Conditions: The response (Y, EE%) was predicted by the model from the following equation 6:

$Y = 186.2963 - 78.8391X_1 - 9.8819X^2 + 21.8519X_3 - 2.395X_1X_2 - 1.365X_1X_3 + 0.025X_2X_3 + 11.50434X_1^2 + 4.4262X_2^2 - 7.7997X_3^2$

The 3D response surfaces with a contour plot in **Fig. 1a** and **1b** showed that encapsulation efficiency increased with increasing concentration of sodium alginate solution. This phenomenon can be explained by the fact that a highly concentrated alginate solution forms a thicker outer membrane network on the beads which prevents leaching out of the internalized matter, hence better encapsulation is achieved [12].

In this study, we observed that each of the linear, square, and interaction terms of this variable affected the response significantly ($p<0.05$).

The formation of alginate hydrogel by Ca^{++} or by the presence of any other divalent ion is known to be an external gelation process as Ca^{++}, at first, forms a highly crosslinked hydrogel surface by pulling the alginate chains together on the exterior of a hydrogel. Therefore, optimization of calcium chloride concentration is a determinant of successful gelation process; hence, the encapsulation efficiency. In the present study, it was observed that increasing the calcium chloride concentration above the optimal level decreased the encapsulation efficiency. This may be due to the formation of an increased amount of cross-linkages making a densely packed, thus less porous bead which could accommodate less amount of TSP extract inside its structure [13].

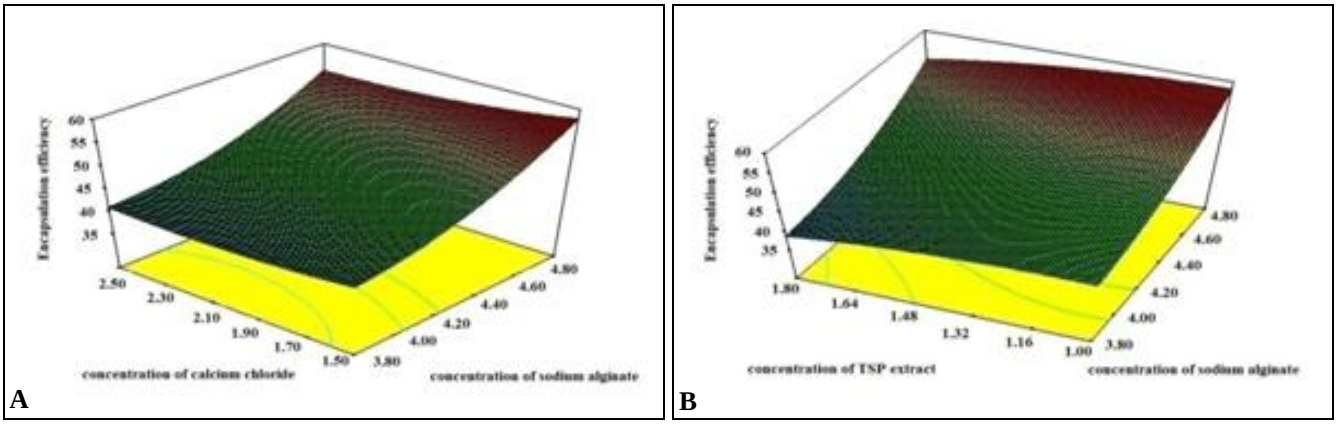

FIG. 1: 3D RESPONSE SURFACE GRAPHS SHOWING THE INTERACTION EFFECTS OF (A) CONCENTRATIONS OF SODIUM ALGINATE AND CALCIUM CHLORIDE SOLUTIONS AND (B) CONCENTRATIONS OF SODIUM ALGINATE AND TSP EXTRACT FOR THE RESPONSE ENCAPSULATION EFFICIENCY (%)

There was a significant influence of the concentration of TSP extract loaded inside the alginate bead on EE%. It was observed that this factor alone and in interaction with increasing alginate concentration increase EE%. This effect remains true up to a TSP concentration of 1.02 g ml^{-1}, after that; further, increase in TSP concentration lowers the EE%. A similar phenomenon was observed by previous researchers [8], where a decline in EE% was noticeable above extract concentration of 1 g ml^{-1}.

Finally, the sodium alginate solution concentration at 4.50g ml^{-1}, calcium chloride solution concentration at 1.5g ml^{-1} and TSP extract concentration at 1.02 g ml^{-1} were predicted to be the optimal condition by this model, where the maximum encapsulation efficiency of TSP extract in alginate hydrogel was predicted as 50.97%. Experiments according to the predicted condition were done in triplicate, and the achieved response was 51.23 ± 0.53%, which falls within a 95% mean confidence interval of the predicted value. These results confirm the validity and predictability of the model to predict EE% of the process.

Characterization of Alginate Hydrogel Beads: Water content, of alginate test beads and control beads, were 94.81 ± 0.47% and 97.32 ± 0.23% respectively. The high water content of control beads may be attributed to the polymeric mesh-like the structure of alginate which is, in some extent occupied by TSP extract in test bead resulting in lower water content in the later. Such a high amount of water is retained in both the hydrogel beads through hydrogen bonds. The bulk density of the controlled beads and test beads were 0.61 g/cm^3 and 0.83 g/cm^3, respectively.

Since, bulk density holds an inversely proportional association with a porosity of a matter, the greater bulk density of test beads loaded with TSP extract indicates the packing up of macropores in the alginate matrix by TSP extractives.

EC_{50} values, representing the concentration of the extract required to obtain a 50% antioxidant effect, were considered for anti-radical activity assessment by DPPH method. The lower the EC_{50}, the greater is the antioxidant power. EC_{50} and reducing antioxidant power (FRAP) for lyophilized and encapsulated extracts are listed in **Table 4**. The difference in antioxidant power between the lyophilized and encapsulated beads can be justified considering almost a 50% encapsulation efficiency of the alginate beads.

TABLE 4: ANTIOXIDANT CAPACITY OF TSP EXTRACT IN LYOPHILISED AND ENCAPSULATED STATE

	EC_{50} (mg sample /g DPPH*)	FRAP µmol Fe (II)/g
Lyophilised TPC extract	48 ± 1.33	220 ± 2.57
Encapsulated TPC extract	78 ± 2.95	114 ± 2.37

The texture profile analysis of the test beads is shown in **Table 5**. The results are in agreement with previous research findings where polyphenols were successfully encapsulated in alginate beads purposed for application in the food matrix and direct delivery in gut [14].

TABLE 5: TPA OF TEST BEADS

	Hardness (N)	Cohesiveness (ratio)	Resilience (ratio)	Springiness (ratio)	Chewiness
Test bead	2.13 ± 0.35	0.55 ± 0.05	0.25 ± 0.01	0.82 ± 0.07	0.92 ± 0.12

The diameter of the native test and control hydrogel beads ranged from ≈ 2.55 ± 0.2 to 2.85 ± 0.4 mm. The difference in average diameter between the test and control beads was found to be statistically not significant ($p>0.05$). The porosity measurement data indicated encapsulation of polyphenols inside alginate pores as supported by the reduced porosity and surface area in test beads as compared to control beads. The average pore radius and specific surface area in control and test beads were 12 nm, 1.358 $m^2 g^{-1}$and 3.8 nm, 0.236 $m^2 g^{-1}$ respectively. In the optical microscope images, a homogenous layer of alginate surrounding the encapsulated polyphenolic extract was visible **Fig. 2**.

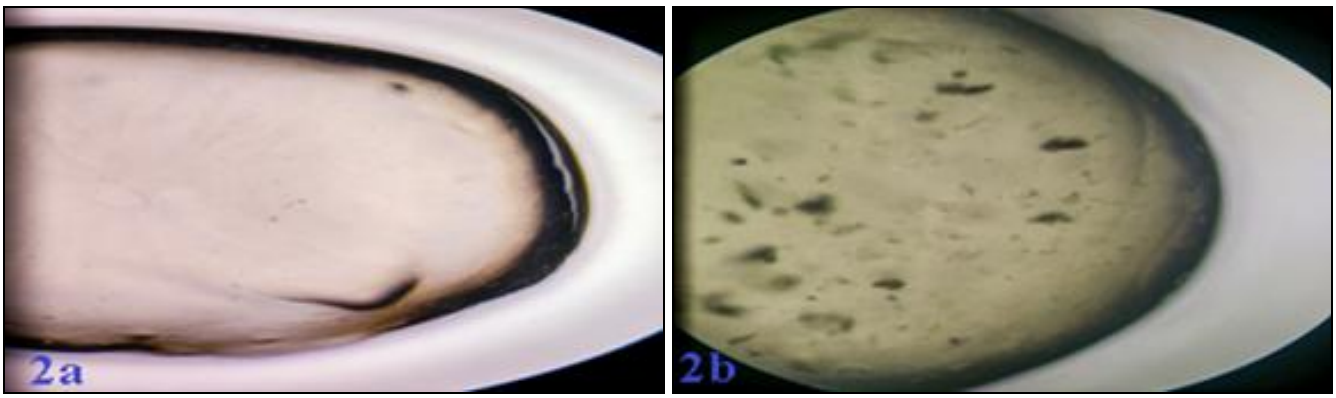

FIG. 2: MICROGRAPHS OF (A) CONTROL BEAD AND (B) TEST BEADS WITH ENCAPSULATED TSP INSIDE

Scanning electron microscopic images **Fig. 3** were used to investigate the morphologic characteristics of alginate beads. Two prominently noticeable characteristics were observed, firstly, the presence of crystals on the surface of the beads which is of sodium chloride formed due to the release and subsequent ionic bonding of sodium ion with chloride ions during the process of calcium alginate development. These sodium chloride crystals survived and retained its structure on the surface of the beads after drying. Secondly, pores were observed in the structure of freeze-dried control beads, which were evidence of the entrapment of water molecule inside alginate mesh before drying. In the SEM images of the test beads, unlike the control beads, the surface is found to be deposited with polyphenols as the pores seen in the control beads are found to be fulfilled by crystals imparting roughness to the surface of the test beads [15].

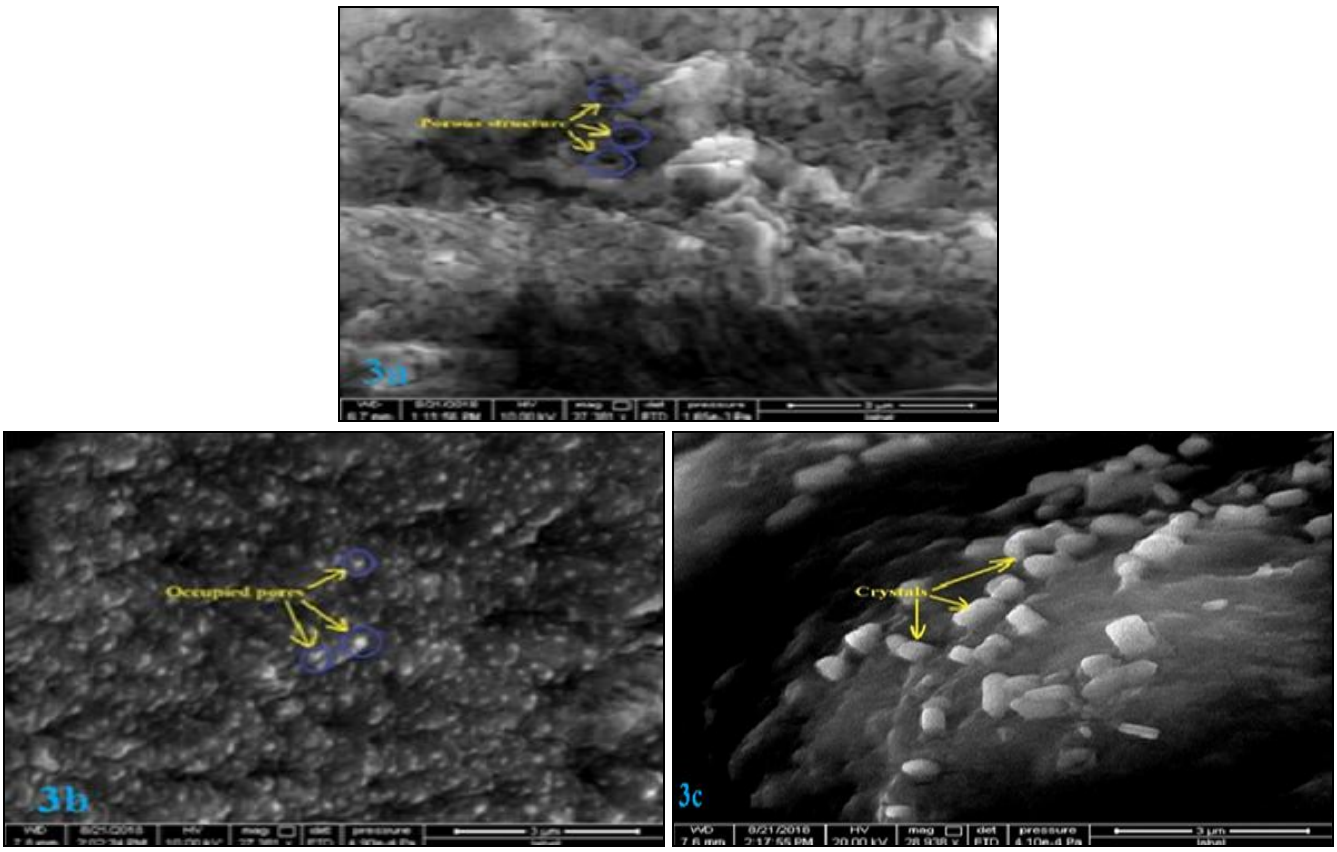

FIG. 3: SEM PHOTOGRAPHS SHOWING (A) THE PRESENCE OF PORES IN CONTROL BEADS WHICH WERE (B) OCCUPIED BY TSP EXTRACTS IN TEST BEADS; (C) PRESENCE OF CRYSTALS ON BEAD SURFACE

Apart from carbon, nitrogen, and oxygen, the EDAX analysis showed the presence of sodium, calcium, and magnesium, which may be present as a component of sodium alginate- calcium chloride system.

A higher percentage of TSP was recovered from hydrogel beads in SGF than SIF, which accounts for 73.65 ± 1.43% and 26.54 ± 0.45% released extract respectively. Therefore, it can be presumed that the encapsulated polyphenols of TSP extract could get to the gut intact. In SGF, the beads showed shrinkage in their structure presumably due to the protonation of free carboxylic group, hence allowing the alginate chains to come closer and contract. After placing the SGF treated beads in SIF, they started to swell, followed by complete disintegration. The swelling behavior may be attributed to the interaction of acidic groups of alginate with electrostatic repulsive forces at pH 6.8; disintegration was presumably due to the electrostatic action of monovalent sodium and potassium ion in SIF on the free carboxyl groups of the alginate molecule that had disrupted three-dimensional mesh structures of alginate beads [11].

CONCLUSION: Considering the undeniable health hazards owing to the usage of synthetic antioxidants in foods, the trials of encapsulated natural antioxidant in the food industry is the recent trend in food research. Tamarind seed polyphenol- a natural source of antioxidants was successfully encapsulated by sodium alginate hydrogel beads, and the process was optimized using RSM. It was observed that optimum levels of reaction variables, which are, alginate solution concentration at 4.50 g ml^{-1}, calcium chloride solution concentration at 1.5 g ml^{-1}, and TSP extract concentration at 1.02 g ml^{-1} could be applied to achieve maximum encapsulation efficiency. The results of the polyphenol release assessment showed a greater amount of extract release in the simulated gastric fluid as compared to the simulated intestinal fluid. This result, together with textural and elemental analysis, presented alginate encapsulated tamarind seed polyphenol extract to be a potent carrier of natural antioxidant which can be explored for functional food formulation.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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