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Biochemical and Morphological Characteristics of β-cyclodextrin Assisted Spray dried *Citrus limon* Burm Powder

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Abstract

Lemon is one of the widely used fruit crop in the world and its availability is found throughout the world. In North Eastern region of India, one of the primary cultivar of lemon is Assam lemon or *Citrus limon* burm grown as seedless lemon cultivar and its physiochemical behavior depends on biological changes during processing, storage, and transportation of the raw fruit. To develop a shelf stable juice powder for longer duration of storage and easy handing, this study was carried out by spray drying of Assam lemon juice with maltodextrin and β-cyclodextrin and evaluating its effect on different physiochemical, functional and morphological properties. Central composite design based response surface methodology has been applied to optimized the process parameter. In this study, the inclusion complex of β-CD in spray dried juice powder contain total phenolic content as 5.00±0.12mg/100ml and radical scavenging activity as 44.13±0.33 % and 3.22± 0.19 mg AAE/g in FRAP and significantly maintain ascorbic acid as 26.00±0.41mg/100g. Further the powder was characterized by particle size distribution with respect to its intensity and zeta potential based on agglomeration properties which found -4Mv in optimized lemon powder. Particle morphology was analyzed through field emission scanning electron microscopy-energy dispersive X-Ray with elemental mapping showed stabilized composition of element present in optimized lemon powder. The results showed that maltodextrin act as better binding agent when encapsulated with β-CD that provide stability from thermal degradation and oxidative damage when spray dried and act as potential ingredient for preserving fruit biological properties.

Keywords: Antioxidant, β-cyclodextrin, Preserving, Stability, Encapsulated

Full length article *Corresponding Author, e-mail: sumidas071994@gauhati.ac.in

1. Introduction

Citrus is one of the most widely cultivated fruit crop in the world with high nutritional and functional properties**.** India ranks sixth in the production of citrus fruit, commonly produced mandarins, sweet oranges and limes having 50, 20 and 15% of total area respectively [1]. In NE region of India, citrus is cultivated covering 57.2 thousand hectares, with 300.7 thousand tons of production [2]. *Citrus limon* burm or Assam lemon is a seedless lemon cultivar widely grown in North-Eastern region of India and mostly used for preparing different food products. Physiochemical integrity and storage of this lemon are depends on biological changes occurring in fruit growth, maturation and development stages. This fruit may develop physiological disorder during storage and changing the bioactive quality parameter of the fruit. Hence, processing and encapsulation of this fruit is an essential parameter to maintain its bioactive stability. Encapsulation is a technique widely applied to stabilise and protect bioactive compounds against degradation by creation of barriers around active ingredients which modulate their interactions with the environment [3].

Encapsulation method depend on physiochemical properties, size, biocompatibility and biodegradability of the micro particles, that can be used with different carrier materials. Carbohydrates, esters, gum lipids, proteins and inorganic material are few examples that act as carrier agents used in the food industry. In encapsulation process cyclodextrin (CD) are mostly used encapsulating agent that are cyclic oligosaccharides which can form inclusion complexes with bioactive compounds and increasing their stability, solubility and bioavailability and generally regarded as safe (GRAS), that being widely used in pharmaceuticals and food industry. CD are ring molecule with hollow cylindrical structure, and its external part is hydrophilic and internal part is hydrophobic, which makes CD as a medium for encapsulation of less polar molecules into internal cavity through hydrophobic interaction [4]. Major applications in CD improving physical and chemical flavour stability and elimination of bitter odour in food and beverages extracts, that being non-toxic, heat stable, inexpensive, and mostly hygroscopic in nature [5]. Encapsulation method can be classified as chemical and mechanical process and spray drying being one of the prime methods to produce powder from a slurry by rapidly drying with hot gas. The process involves atomization, diffusion, evaporation and separation of the product widely being used in food and pharmaceutical industries [6,7]. Spray drying is an efficient and widely used method to transform liquid into low moisture powder form with low cost process, wide choice of encapsulation, good product stability and enhanced shelf life by reducing stickiness [8]. For spray drying maltodextrin (MD) with different dextrose equivalent (DE) have been used, for example in watermelon juice 9-DE, 6-DE in blackberry juice [9]. In order to evaluate the optimization condition of concentrated orange juice, the drying conditions were maintained at 155℃ inlet air temperature and 74% MD content which showed significant effect on physical and chemical properties of juice powder allowing free flow properties without masking the original aroma or flavour [10]. Optimization of drying conditions such as inlet air temperature, relative humidity of air, outlet air temperature, and atomizer speed is significant to achieve an effective drying for obtaining an acceptable product [11,12]. The aim of the present study was design to develop a shelf stable Assam lemon juice powder, easier to handle, transportation and storage for longer duration by spray drying at different temperature in respect powder physiochemical and morphological properties.

2. Materials and methods

2.1 Sample collection and plant identification

Fresh and matured lemon samples (*Citrus limon* burm) were collected from local fruit garden, Darrang district of Assam, India during the season between June to July and transported to laboratory in sterile condition for further analysis.

The targeted plant species of the fruit flower was identified and deposit in the Herbarium branch, Department of Botany, Gauhati University, Assam, India for possessing of accession number of the given sample.

Chemicals used were of analytical grade and supplied by Sigma, Loba Chemie Pvt. Ltd., Merck, Qualigens Fine Chemicals, Hi-Media Laboratories Pvt. Ltd., Mumbai.

2.2 *Juice preparation with β-CD*

Juice was extracted from lemon using a screw press juice extractor (Philips) and filtered using whatman no. 1 filter paper to remove any dirt particle or impurities. Further ultrasonic treatment was subjected to lemon juice samples using probe-based ultrasonicator for 60 min. Sonicator was operated with 19mm diameter probe at 20 KHz of constant frequency maintained at fixed pulsation. A 100 ml clean sterile glass beaker was filled with the juice sample and placed into the treatment chamber of the instrument. Further, freshly prepared juice was treated with β-CD at 2.5% (w/v) and mixture was stirred for 60 min at room temperature (24 ± 2℃) for complete solubilization of β-CD. The juice was clarified and filtered by coarse sintered glass buchner funnel under reduced pressure and transferred to a screw capped glass bottles and pasteurization was performed for 2 min at 95℃ and the juice was immediately cooled down and used for further study. All sample preparations were carried out in triplicates.

2.3. Experimental model

One of the extensively used statistical and mathematical experimental technique is response surface methodology (RSM), design to development, improvement and optimization of the processing or extraction methods to evaluate the effects of different process optimization parameters to build effective models [13]. The RSM experiment was carried out in three factor with three levels and central composite design (CCD) used in design expert version 7.0.0 (Stat-Ease Inc, Minneapolis, USA) was used. The optimum independent variables against dependent variables were determined by RSM. The independent variables considered for optimizing lemon powder processing conditions were inlet temperature (X_1 in ℃), Aspirator speed (X_2 in %), and maltodextrin (X_3 in %), while moisture content (MC), water solubility index (WSI), ascorbic acid (AA), total phenolic content (TPC), antioxidant activity through 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant property (FRAP) were the dependent variables of the treatment. A total of 20 experiments were performed including six replicates at center point (Table.1). Data were analyzed to express the independent variable responses using a multiple linear regression model and a second order polynomial equation as mentioned in following equation (E1)
.

$$Y_k = \beta_0 + \sum_{i=1}^{n} \beta_{ki} X_i + \sum_{n=1}^{n} \beta_{kii} {X_i}^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{kij} X_i X_j$$

(E1)

Y_k, is the desired response variables such as Y_1: MC, Y_2: WSI, Y_3: AA, Y_4: TPC, Y_5 : DPPH, Y_6 : FRAP, $\beta_0$is constant, β_{ki} is linear coefficient, β_{kii} is quadratic coefficient, β_{kij} is cross product coefficient, X_i represents the coded independent variables such as X_1isinlet temperature, $X_2$is aspirator speed, X_3isMD). Statistical significant and regression model adequacy was checked by ANOVA, R^2, AdjR^2 and Adeq. Precision for each response.

2.4 Spray drying emulsion preparation

Emulsion for spray drying was prepared using 2.5% of β-CD and MD-20DE to 100 ml juice by maintaining brix at 20. The mixture was constantly stirred in a room temperature for 120 min. Independent variables were taken such as inlet temperature (130-180℃), aspirator speed (40-80%) and MD (10-25%) selected for RSM. Laboratory scale spray drier (LSD-48, JISL, India), with a concurrent flow and 0.7mm diameter nozzle, drying chamber pressure 2.5kg/cm^2 used to operate. Feed flow rate controlled by peristaltic pneumatic pump were set and used for spray drying microencapsulation process. From the cyclone separator the β-CD encapsulated powder was collected and kept in an airtight container and stored at 4ºC temperature until further use.

2.5 Determination of Moisture Content (MC) of the powder

MC was determined according to method mentioned by Shreve et al. [14]. Dried the empty dish and lid, weight 3g of sample with dry dish and placed the dish with sample in oven at 105℃ and 3 hours. The dried powder transferred to desiccator and take weight of the dried sample and calculated the final moisture using equation (E2).

$$\text{Moisture (\%)} = \frac{W1-W2}{W1} \times 100 \qquad (E2)$$

W_1: Weight (g) of sample before drying; W_2: weight (g) of sample after drying

2.6 Water Solubility index (WSI)

WSI was determined according to Santhalakshmy et al. [15] with slight modifications. Samples mixing with distilled water at 1:10 w/v and stirred 60 min at room temperature and centrifuged at 2000 rpm for 10 min. The supernatant was collected in pre-weighed petri dish and dried at 105℃ for 3 hours. Solubility was calculated by weighing the weight difference initial and final weight of the petri dish.

2.7 Determination of ascorbic acid

Ascorbic acid was determined by the titration method based on 2,6-Dicholorophenol indophenol (DCPIP) method [16]. The dye solution was prepared by exactly weighing 50 mg of 2,6-dichlorophenolindophenol in 150 ml of distilled water with 42 mg sodium bicarbonate, the solution was then made up to 200 ml with distilled water. The dye was later standardized by titrating with a 5 ml standard ascorbic acid solution (0.1 mg/ml) added with 5 ml 3% metaphosphoric acid (HPO_3) and titrating till pink colour appears. The dye factor (mg of ascorbic acid per ml of the dye) is determined by using the following formula (F1).

$$\text{Dye Factor} = \frac{5}{\text{titre}} \qquad (F1)$$

For estimation 20 of the powder was made to 100 ml with 3% HPO_3, 3 ml aliquot of the sample titrated with dye till a pink end point persisted for 15s and ascorbic acid in sample determined using equation (E3)

$$\text{mg of ascorbic acid per 100ml} = \frac{\text{titre} \times \text{dye factor} \times \text{Volume made up x 100}}{\text{Aliquot of extract taken for estimation x Volume of sample taken for estimation}}$$

(E2)

2.8 Extraction of polyphenols

Extraction of polyphenols were done with 50% ethanol to water and centrifuged at 3000 rpm for 10 min. the supernatant was collected and analysed.

2.8.1 Total Phenolic Content Estimation (TPC)

Total phenolic content of the juice was estimated using the Folin-Ciocalteau assay with slight modification, Gawałek et al. [17]. A 0.2 ml of the samples were taken in different test tubes and 2 ml water was added to it followed by 0.3 ml of Folin-Ciocalteau's phenol reagent and after 5 min 0.8 ml 20 % sodium carbonate was added and make up the 5ml of volume. Sample incubated for 30 min at room temperature the absorbance was measured at 765 nm in a UV-Vis spectrophotometer (Cary 60 UV-Vis, Agilent). Using a standard solution of gallic acid, a calibration curve was prepared and the absorbance was measured at 765 nm in a UV-Vis spectrophotometer. The results of phenols were expressed in mg of gallic acid equivalent (GAE) per 100 mililitre of sample.

2.8.2 Determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH)

Radical scavenging activity of the samples were measured by determining the inhibition rate of DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical method mentioned by Saikia et al. [18]. Methanolic solution of 0.1 mM DPPH radical was added to 200 µl of extract. The mixture was then incubated at dark for 30 min at room temperature. 1 mM DPPH solution with added 200 µl was used as blank. The absorbance was measured at 517 nm in UV-Vis spectrophotometer and results were expressed in terms of radical scavenging activity using following equation (E4).

$$\text{Radical scavenging activity (\%)} = \frac{A_0-A_s}{A_0} \times 100 \quad (E4)$$

Where, A_o is absorbance of control blank; As is absorbance of samples

2.8.3 Determination of Ferric reducing antioxidant power (FRAP) activity

Ferric reducing antioxidant property of the sample was measured according to method mentioned by kashyap et al. [19] Briefly, a 0.1ml of the samples were diluted using distilled water and mixed with 3ml of FRAP solution prepared using 300Mm acetate buffer at pH 3.6, 0.03mg of TPTZ in 10:1:1 ratio with 40mMHCl and 20mM ferric chloride and incubated in dark for 30 min. The absorbance was measured at 593 nm in UV—Vis spectrophotometer and ascorbic acid was taken as standard and results were expressed as mg AAE/g.

2.9 Field emission scanning electron microscopy (FESEM)-elemental mapping in energy dispersive X ray(EDX)

Morphology of the optimized lemon powder was analysed through FESEM. The powder particles were mounted on a carbon coated copper grid, and fixation was done with gold coating on the powder particles using focused ion beam. EDX analysis were accomplished by FESEM. The micrographs of the lemon powder were recorded at 2,500x, 5,000X at 3µm and 10,000X at 1µm with or without inclusion of β-CD.

2.10 Particle Size Distribution

The particle size distribution was determined using a particle analyser (Litesizer, 500) A small quantity of powder was suspended in water as a solvent and analysed at 25℃. The particle size and polydispersity index (PDI) of powder were determined using a dynamic light scattering device. The results obtained from equipment software version 2.22.2 were expressed in D_{10}, D_{50} (median size) and D_{90} with respect to its intensity, which are the diameters for the accumulated size distribution of 10, 50 and 90 percentile of particles [20]. The polydispersity index (span) was also determined by following Equation

$$\text{Span}= \frac{D90 - D10}{D50} \qquad (E5)$$

D_{10}, D_{50} and D_{90} correspond to the diameters relative to 10, 50 and 90 percentile of the accumulated size distribution.

2.10.1 Zeta potential

The stability of the powder was analysed by determining the net electrical charge (Litesizer,500). Freshly prepared optimized powder sample of concentration 1mg/ml solution was injected into disposable folded capillary cells and placed in the sample holder. The sample was then equilibrated at 25℃ for 120s before measurement. The analysis was performed under low light conditions.

3. Results and Discussions

3.1 Identification of plant species

The herbarium species was identified as *Citrus limon* (L.),Rutaceae family with accession number GUBH19904 and reference number Herb/GUBH/2021/004.

3.2 Optimization of carrier agent concentration

The optimum conditions for lemon juice processing were determined to retain maximum WSI, TPC, AA and antioxidant activity with minimal moisture content accumulation during processing. Second order polynomial models obtained in the present investigation for dependent variable response in identifying the optimum processing conditions. In this study temperature, aspirator speed and maltodextrin concentration were optimized to 130-180 ℃, 40-80 % and 10-25 % respectively. By applying point prediction method, it was determined that the optimum processing conditions are treatment time of 155℃, 60 % aspirator speed and 17.50 % of MD. At this point, predicted model validation was conducted by using the optimized treatment conditions and experimentally obtaining the value of the responses. The experimental values were mentioned in table 4 and compared with predicted values and found to be close, with low error. Therefore, the model obtained in this study could be used for optimization of powder process.

3.3 Model fitting

In response surface methodology, the response impact on different independent variables were observed on dependent variables such as MC, WSI, TPC, DPPH, FRAP, AA as listed in Table. 1 and calculation were done at 95% confidence level. Central composite design (CCD) with face centered model consisting of 20 experimental trial was designed in response surface methodology. The analysis of predicted values was calculated from regression equation mentioned in Table. 3, which shows a high correlation with the experimental values. These values resulted in calculation of coefficient of determination R^2, which were shown to have high adequacy as presented in table 2. More substantial impact on dependent variables indicated large regression coefficient and small p values. According to ANOVA for dependent variables, the resulting quadratic model adequately reflect the experimental data as represented in Table 4. The results of the current study were able to determine ideal processing condition for lemon juice powder with β-CD inclusion.

3.4 Effect of maltodextrin and β-CD inclusion on Moisture content and water solubility index

The moisture content ranged from 1.320 to 5.060 % with an average of 3.076 respectively as presented in Figure 1. 1A. The increase in temperature moisture drops at an acceptable level as high amount of MD concentration with a low aspirator speed that bind with intermolecular spaces helps in reducing the moisture content. Table.1 represents higher the MD concentration lowers the MC which is also observed in blackberry juice powder where increase MD reduces MC as mentioned by Ferrari et al. [21] MD influenced the solid proportion of feed which further reduces rate of evaporation. The predicted model for calculating the MC was described the polynomial equation as mentioned in Table 3. WSI shows an affective measurement of solubility of the powder, which ranges from 83.50 to 95.56% with an average of 89.41 % that determines repose of a powder product in aqueous phase as well as reconstitution and dissolution quality as represented in Figure1. 2A with significant difference. WSI in directly linked with MC, as lower the MC better the solubility index and higher temperature positively influenced the WSI and lesser time will be required for complete solubilisation of the product. This study supported the processing of jamun fruit juice where processing temperature runs at 140-150℃ as mentioned by Santhakalswamy et al.[15] and in black mulberry juice as processing at temperature 110-150℃ as reported by Fazaeli et al.[22]. WSI of lemon powder 89.41 % which is higher as compared to spray dried tomato powder which ranges and orange flesh sweet potato powder [23, 24]. The rise in MD concentration provides better solubility of the powder, but in optimization process, the predicted values are based on other characteristic properties such as raw materials, feed concentration and rate of drying which influence the WSI property. The temperature may increase the molecular diffusion which also lowers the viscosity of the solvent and lead to solute solubilisation and reduces the surface tension of β-CD. This inclusion complex with CD form within their lipophilic cavities and through non-covalent forces with hydrophobic polyphenols that increases the water solubility. β-CD inclusion showed better effect on WSI, higher rate of entrapped the solubility of the spray dried powder.

Table 1. Representation of effect of independent variables on spray dried Assam lemon powder with MD-β-CD

Run	Inlet temp(℃)	Aspirator Speed (%)	MD (%)	MC (%)	WSI (%)	AA(mg/100g)	TPC(mg/100ml)	% scavenging activity(DPPH)	FRAP (mg AAE/g)
1	155.00	60.00	17.50	2.76 ±0.50	91.11 ±0.17	29.00± 0.28	6.71±0.50	52.00±0.27	3.75±0.26
2	180.00	60.00	17.50	2.55 ±0.44	89.54 ±0.19	21.00± 0.25	3.00±0.22	38.00±0.34	2.87±0.56
3	130.00	40.00	10.00	5.06 ±0.32	90.00 ±0.11	39.77± 0.71	5.12±0.34	54.12±0.27	5.00±0.34
4	180.00	40.00	10.00	3.98 ±0.12	95.56 ±0.46	18.00±0.43	3.01±0.65	39.31±0.77	2.76±0.29
5	155.00	60.00	17.50	2.77 ±0.21	91.67 ±0.54	32.08±0.44	6.11±0.32	51.42±0.44	3.79±0.53
6	130.00	80.00	10.00	5.04 ±0.22	87.77 ±0.76	39.00±0.39	4.87±0.13	50.13±0.54	4.99±0.54
7	180.00	40.00	25.00	1.66 ±0.32	84.12 ±0.65	15.00±0.34	2.78±0.61	34.00±0.30	2.03±0.65
8	155.00	60.00	17.50	2.88 ±0.33	91.32 ±0.76	32.08±0.54	6.00±0.18	51.21±0.55	3.94±0.11
9	130.00	60.00	17.50	3.65 ±0.45	88.64 ±0.34	32.00±0.76	5.98±0.15	49.00±0.33	4.34±0.27
10	180.00	80.00	10.00	3.78 ±0.34	87.42 ±0.43	17.85±0.43	3.00±0.15	37.21±0.19	2.00±0.22
11	155.00	60.00	17.50	2.87 ±0.13	91.51 ±0.12	32.08± 0.10	6.71±0.11	51.19±0.55	3.76±0.14
12	130.00	80.00	25.00	2.76 ±0.43	86.69 ±0.79	23.09± 0.41	4.01±0.54	43.00±0.27	2.87±0.62
13	130.00	40.00	25.00	3.77 ±0.65	83.51 ±0.62	28.09±0.43	4.08±0.46	49.33±0.16	2.34±0.54
14	155.00	60.00	17.50	3.00 ±0.43	94.02 ±0.18	32.08±0.28	7.00±0.57	48.00±0.65	3.15±0.29
15	155.00	60.00	25.00	2.08 ±0.41	87.99 ±0.12	21.00±0.45	5.00±0.11	43.11±0.54	2.87±0.65
16	180.00	80.00	25.00	1.32 ±0.54	84.49 ±0.17	15.44±0.28	3.09±0.71	37.00±0.33	1.98±0.27
17	155.00	60.00	17.50	2.06 ±0.55	91.39 ±0.43	32.00±0.66	6.31±0.32	51.00±0.42	3.67±0.18
18	155.00	40.00	17.50	2.78 ±0.33	90.42 ±0.37	35.65±0.76	6.89±0.42	51.00±0.43	3.78±0.49
19	155.00	80.00	17.50	2.98 ±0.22	87.23 ±0.78	32.00±0.72	6.60±0.51	47.00±0.29	3.00±0.56
20	155.00	60.00	10.00	3.77 ±0.18	93.81 ±0.28	31.21±0.11	5.61±0.19	50.00±0.27	3.89±0.30

Table 2. Representation of the significant levels (p-values) of the dependent variables of spray dried powder with MD

	MC	WSI	TPC	TFC	DPPH	FRAP	AA
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
A-Inlet temp	< 0.0001	0.1891	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
B-Aspirator speed	0.1828	0.011	0.8136	0.1384	0.0449	0.2094	0.0957
C-Maltodextrin	< 0.0001	< 0.0001	0.0653	< 0.0001	0.0017	< 0.0001	< 0.0001
AB	0.5797	0.0125	0.6001	0.5617	0.0553	0.0919	0.2023
AC	0.1879	0.0398	0.1553	0.3891	0.1977	0.0002	0.0005
BC	0.2163	0.0007	0.6716	0.4849	0.5674	0.1103	0.4315
A^2	0.0746	0.011	< 0.0001	0.2233	0.0006	0.6064	0.0006
B^2	0.451	0.0053	0.2103	0.1712	0.8378	0.0803	0.0169
C^2	0.3268	0.8742	0.001	0.9378	0.0619	0.072	0.0004
R-Squared	0.9498	0.9504	0.9623	0.9539	0.9551	0.959	0.9775
Adj R-Squared	0.9047	0.9058	0.9284	0.9124	0.9146	0.922	0.9573
Adeq. Precision	17.194	16.87	14.477	18.716	16.006	17.46	22.881
Lack of fit	0.6947^{ns}	0.6188^{ns}	0.451^{ns}	0.0586^{ns}	0.1742^{ns}	0.6416^{ns}	0.2106^{ns}

Note: ns: non-significant;

Table 3. Response variables and their model equations of the optimized powder

Response variables	Carrier agent maltodextrin
MC	$2.73+0.70A - 0.14B - 1.00C + 0.361A^2 + 0.143B^2 + 0.194C^2 + 0.061AB - 0.156AC - 0.145BC$
WSI	$91.40 + 0.421A - 1.00B - 2.78C +1.91A^2+2.17B^2-0.100C^2-1.091AB - 0.85AC + 1.741BC$
AA	$31.38 - 7.47A - 0.911B - 4.32C- 4.61A^2 + 2.71B^2 + 5.01C^2 + 0.76AB + 0.271AC - 0.45BC$
TPC	$6.45 - 0.92A - 0.031B - 0.27C - 1.93A^2 + 0.33B^2 +1.11C^2 + 0.078AB + 0.221AC +0.063BC$
DPPH	$49.91 - 6.00A - 1.30B - 2.40C - 5.27A^2 + 0.23B^2 - 2.27C^2 + 1.38AB + 0.881AC + 0.38BC$
FRAP	$3.68 + 0.791A - 0.11B - 0.601C+ 0.081A^2 -0.301B^2- 0.31C^2 -0.174AB + 0.503AC + 0.1BC$

[i]**Note:** A, B, C: coded values of treatment temperature, aspirator speed and maltodextrin concentration respectively

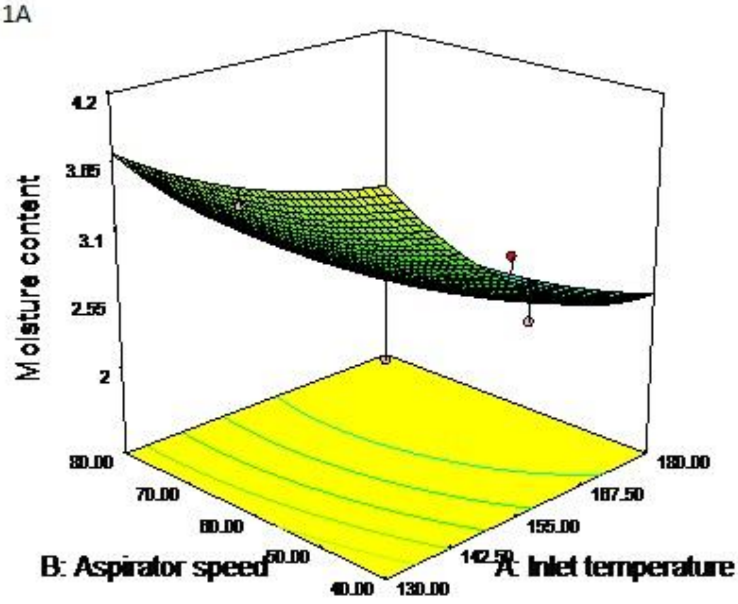
Table 4. Optimized responses analysed of Assam lemon juice powder

Responses	**Predicted value**	**Experimental value**
Moisture content (%)	3.08 ± 0.06	3.00 ± 0.01
Water solubility index (%)	89.41± 0.01	88.11 ± 0.23
Total phenolic content (mg/100ml)	5.09± 0.04	5.00 ± 0.12
DPPH activity (%)	46.25± 0.11	44.13 ± 0.33
FRAP(mg AAE/g)	3.34± 0.39	3.22 ± 0.19
Ascorbic acid content (mg/100g)	27.92 ± 0.21	26.00 ± 0.41

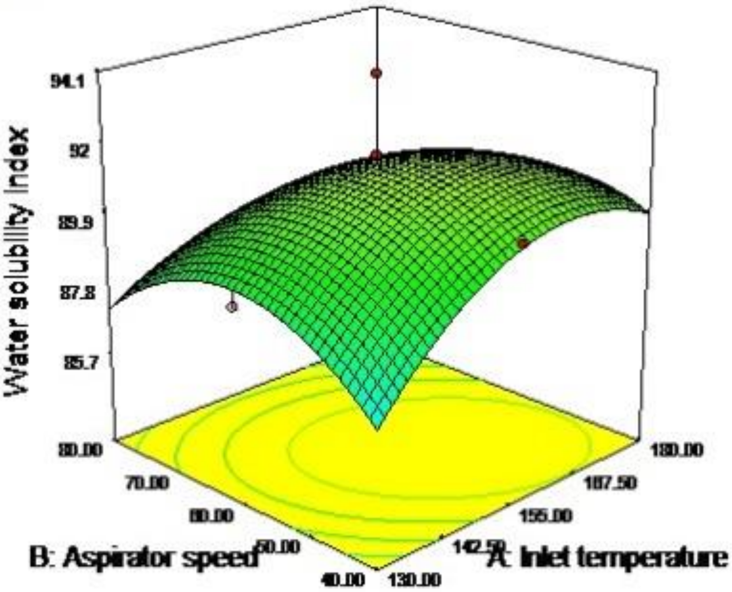
Table 5. Particle morphology of the spray dried lemon powder

Samples	D_{10} (nm)	D_{50} (nm)	D_{90} (nm)	span
MD	263.32	1098.85	3977.56	3.3801
MD+β-CD	385.80	1449.21	4377.64	2.754

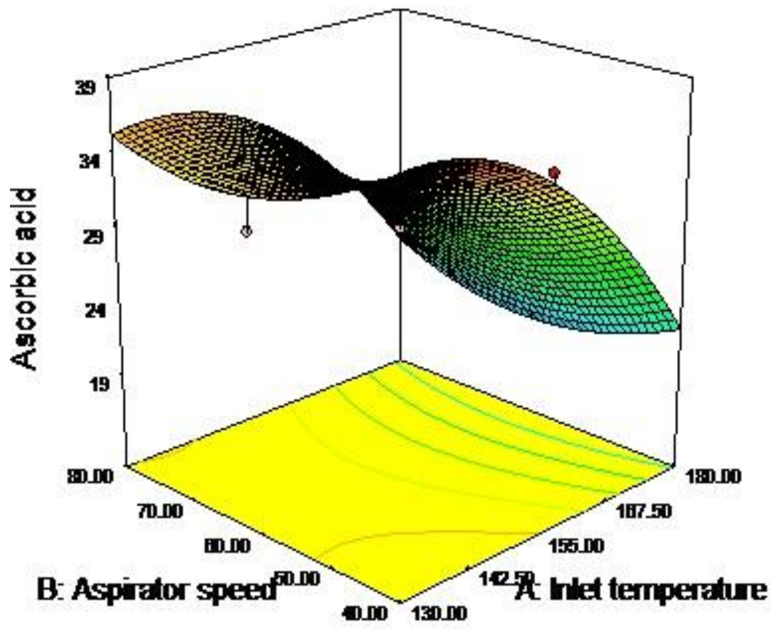
1A


Moisture content
4.2
3.65
3.1
2.55
2
80.00
70.00
60.00
50.00
40.00
B: Aspirator speed
130.00
142.50
155.00
167.50
180.00
A: Inlet temperature

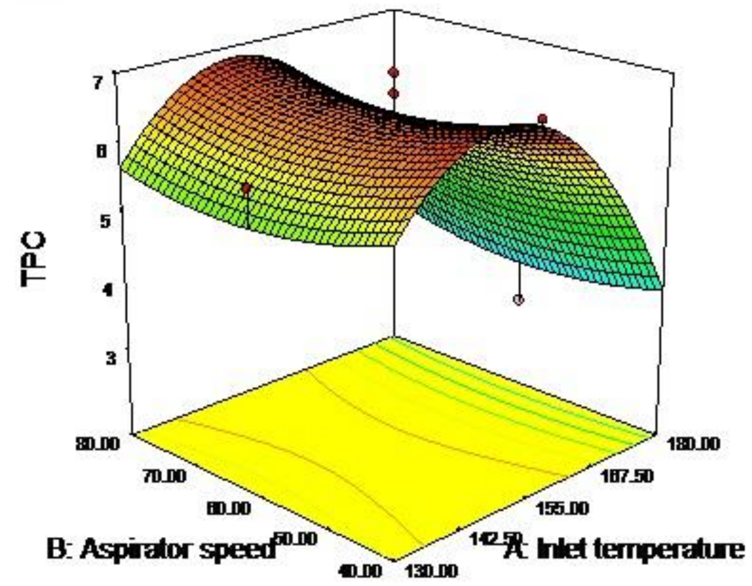
2A


Water solubility index
94.1
92
89.9
87.8
85.7
80.00
70.00
60.00
50.00
40.00
B: Aspirator speed
130.00
142.50
155.00
167.50
180.00
A: Inlet temperature

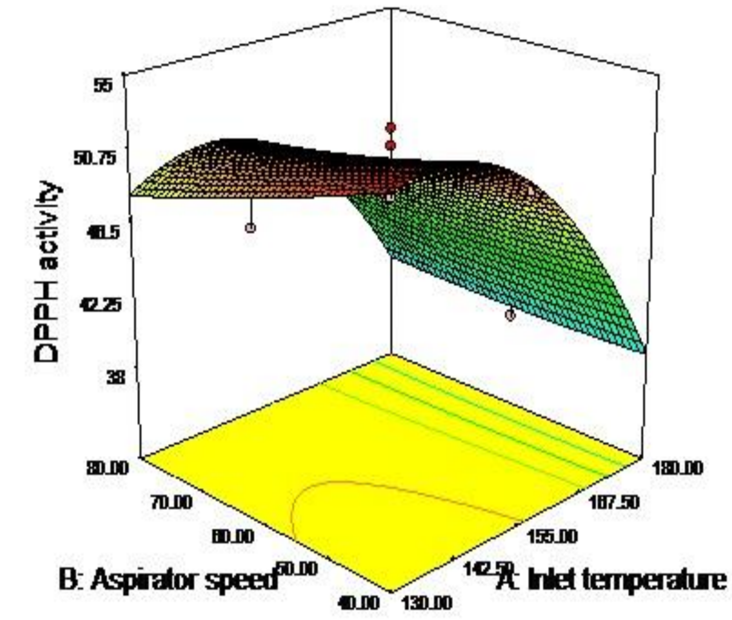
3A


Ascorbic acid
39
34
29
24
19
80.00
70.00
60.00
50.00
40.00
B: Aspirator speed
130.00
142.50
155.00
167.50
180.00
A: Inlet temperature

4A


TPC
7
6
5
4
3
80.00
70.00
60.00
50.00
40.00
B: Aspirator speed
130.00
142.50
155.00
167.50
180.00
A: Inlet temperature

5A



6A

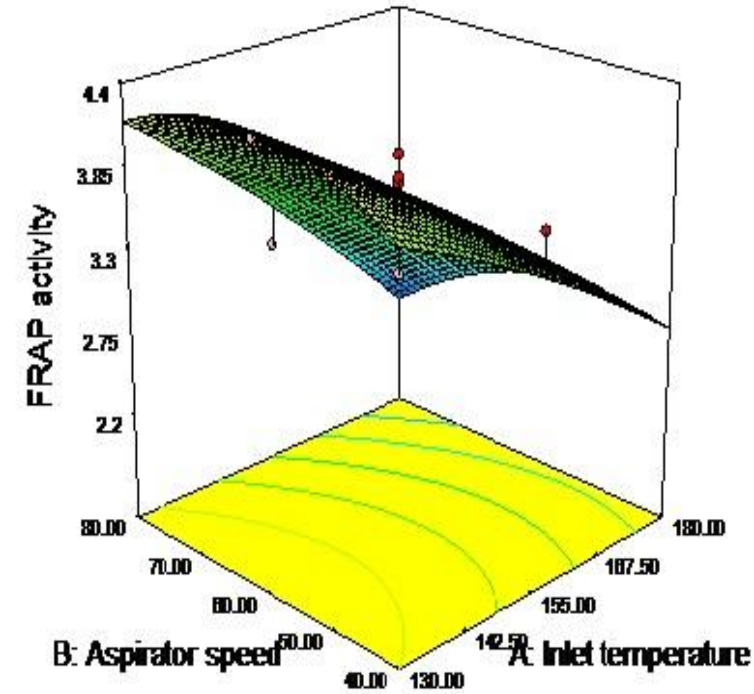


Figure 1. Response surface plot for MC.1.A; WSI: 2. A; AA: 3.A; TPC: 4. A; DPPH: 5.A; FRAP assay: 6.A

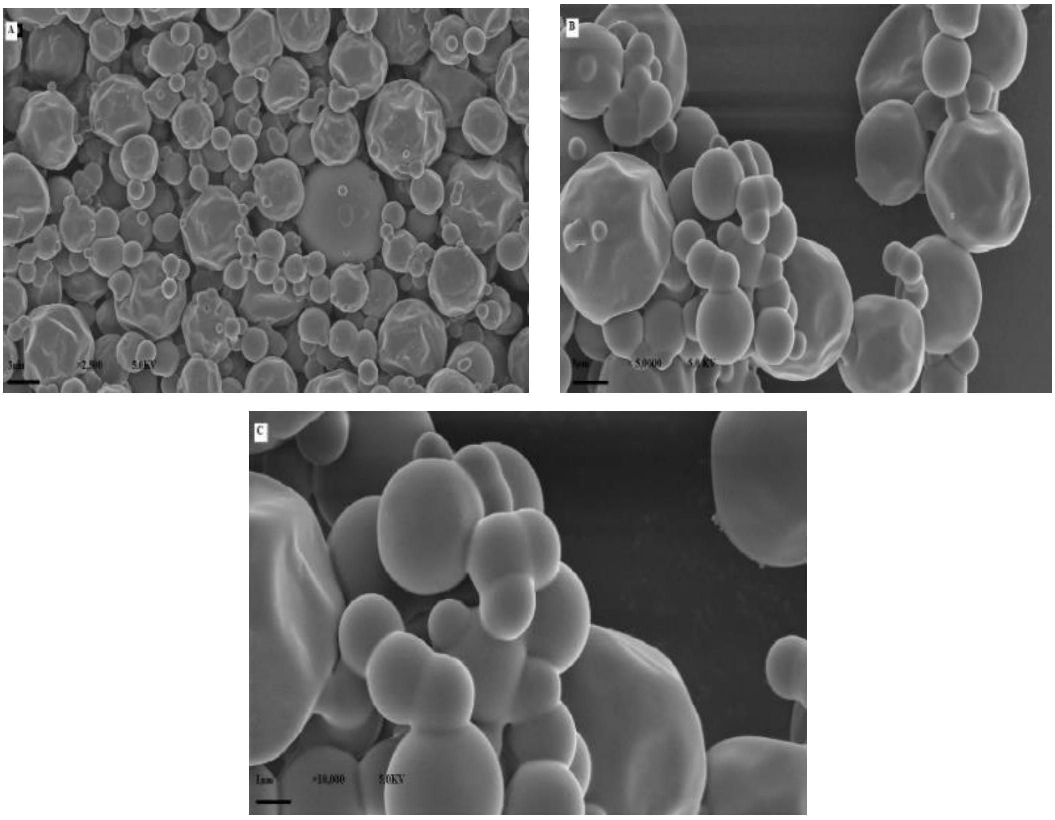


Figure 2. SEM images of spray dried lemon powder with MD at A.2,500x at 3µm B. 5,000X at 3µm C.10,000X at 1µm

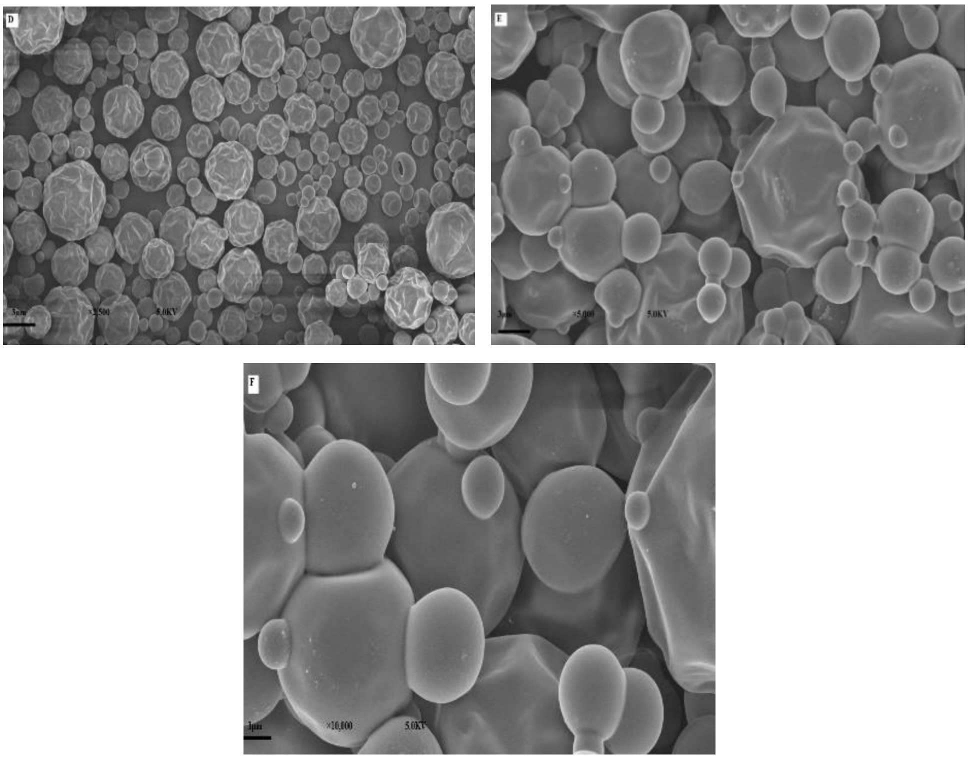


Figure 3. SEM images of spray dried lemon powder with MD-β-CD at A.2,500x at 3µm B. 5,000X at 3µm C.10,000X at 1µm

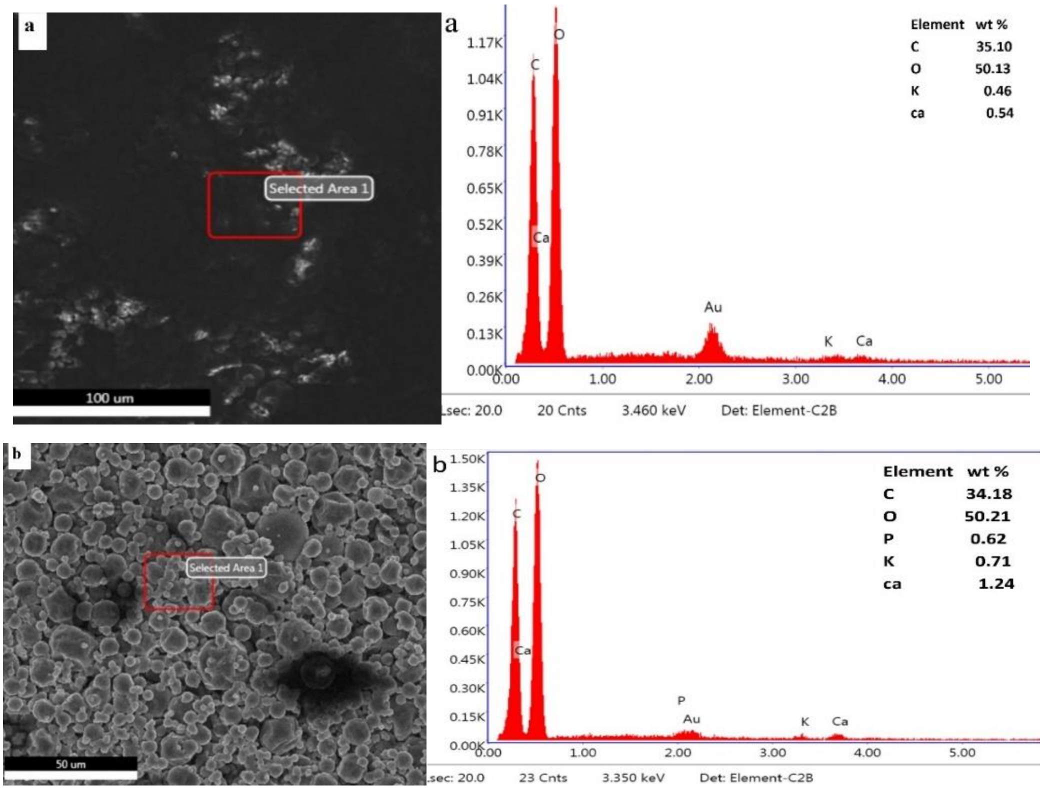


Figure 4. Elemental mapping of spray dried lemon powder a. powder with MD b. powder with MD-β-CD

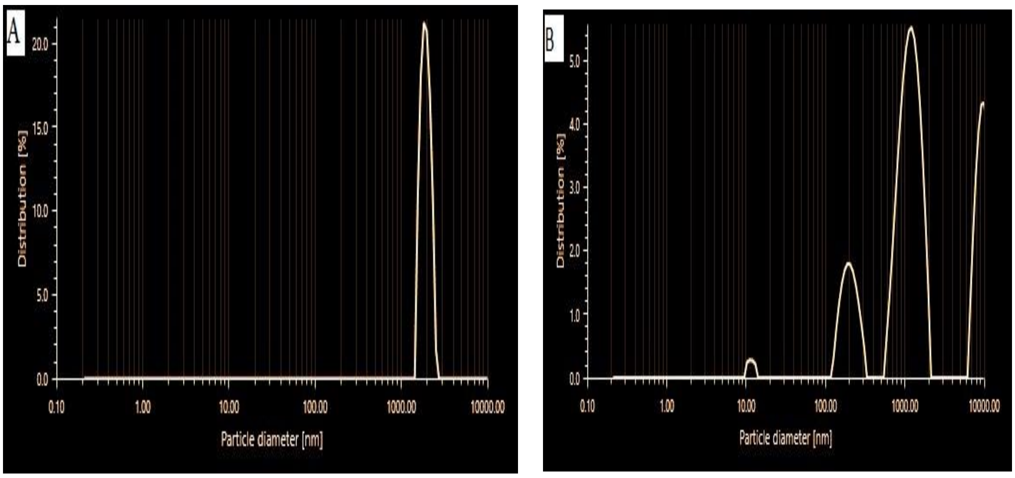


Figure 5. Particle size distribution of spray dried lemon powder (A) powder with MD (B) powder with MD-β-CD

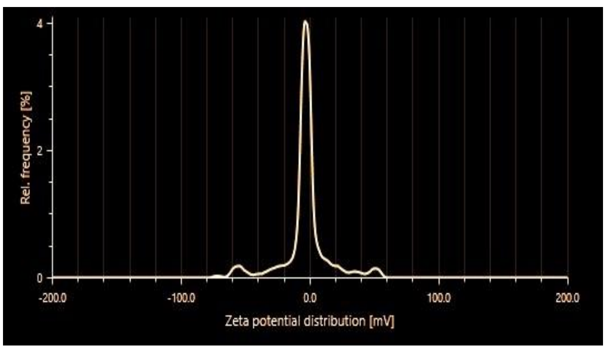


Figure 6. Zeta potential of optimized spray dried lemon powder with MD-β-CD

3.5 Effect of maltodextrin and β-CD inclusion on ascorbic acid content

Ascorbic acid content decreases with increase drying temperature and it was found to be higher at lower temperature with effective encapsulation which protects it from higher rate of degradation as presented in Figure1. 3A. Entrapment of polyphenols in CD cavities stabilizes the guest host complex from oxidation, light and degradation from heat. Ethanolic extraction increasing the vitamin C concentration as it helps in extraction of compounds present in the sample solution. Similar finding observed in increasing vitamin C concentration in peach pomace contain β-CD ethanolic extract which stabilizes the ascorbic acid content and maintain its quality rather than quantity [25].

3.6 Effect of maltodextrin and β-CD inclusion on antioxidant activity and total phenolic content

The antioxidant activity was determined using DPPH and FRAP to see the impact on spray dried lemon power. β-CD addition shows the effect on the antioxidant activity of the encapsulated powder which provide protection against heat and moisture by preserving antioxidant compound and regulate the release rate of the compound present and provide synergistic effects. Total antioxidant activity of the powder sample under different conditions is shown in Table. 1 which signifies that higher MD concentration causing more total solids that reduces the antioxidant activity in both DPPH and FRAP assay with increasing temperature as presented in Figure1. 5.A in DPPH assay and figure 1.6A in FRAP assay. To count the bioactivity of spray dried lemon powder, antioxidant activity measurement is one of the crucial factors. Similar results were found in DPPH and FRAP assay in plums as mentioned by Michalska et al. [26], black garlic, Widyaningsih et al. [27] and in blueberry powder where drying temperature significantly affect the antioxidant properties [28]. Antioxidants activity is attributed to formation of novel compounds such as products of Maillard reaction, which possess antioxidant properties. Improvement of antioxidant activity on inclusion with CD improved the phenolic compound which also reported in thymol essential oil and CD inclusion with chitosan nanoparticles as reported in Alizadeh [29] Therefore, it can be suggested at temperature 155℃ inclusion complesex stabilised the polyphenolic compound as presented in Figure 1. 4A. In the diffusion process mixture time and ultrasonication plays a significant positive effect on role in CD internal diffusion to the solvent and solubilization of target molecule to the particle surface for extraction of polyphenols. β-CD concentration plays a major role in stabilizing the polyphenols instead of increasing the quantity and his might be due to exposure to higher temperature which led to oxidation of polyphenols for longer duration. Ethanol affects the extraction of polyphenols by improving its ability to dissolve lipophilic polyphenols. Ethanol can also change biological membrane chemically by affecting cell permeability and facilitates molecular diffusion. Oxidation of polyphenols observed in powder with MD, affecting polyphenols than powder mixed with β-CD containing higher polyphenol yield. This result supported with Ranjha et al. [30]in an experiment conducted in vine shoot cultivars, where higher retention of polyphenols observed in β-CD inclusion with ethanolic extracts. Hence, from the Figure 1.6A it can be suggesting that β-CD assisted juice powder show's higher polyphenolic extraction with high radical scavenging activity.

3.7 FESEM-EDX and elemental mapping

FESEM is relatively a developing method for the surface morphology as well as their elemental composition analysis. It analyses the smaller regions while using energy dispersible spectroscopy (EDS) and electron accelerating voltages. FESEM provides highs quality, low voltages and minimal electrical charging in samples in contrast with other samples. For the microstructure study of the β-CD inclusion lemon powder, SEM data was obtained for surface morphology presented in Figure.2. SEM micrographs of 17.50 % MD at 20-DE with inlet temperature 155℃ showing smooth spherical particles with less shrinkage when observed at 2,500x, 5,000X at 3µm and 10,000X at 1µm. SEM morphology on powder with MD shows agglomeration which cause shrinkage and less binding ability as observed in Figure 3. Additionally, guest host inclusion complexes in CD can cause aggregation and absence of pores may be an indicator of preservative of active compound (Ibrahim et al. [31]; Thurein et al. [32]. Overall, smaller size of particle was observed with addition of β-cd which indicates that carrier agents or wall materials provide better protection against oxidation and degradation of phenolic compounds present in the juice. Analysis of optimized samples reveals elements present in sample in particular calcium, oxygen, carbon, phosphorous potassium that were identified by the EDS method as shown in figure.4. β-CD provides a barrier to heat and act as stronger binding agent that protects the elements present in the juices as shown in Figure 4. The inclusion of β-CD and without inclusion shows different elemental composition and the powder particle did not include any toxic element such as arsenic, cadmium, lead or mercury.

3.8 Particle size distribution

The size distribution is an important parameter to determine micro particle size, because small particles can influence by larger particles that perceived sensorial such as sandiness and lumps which cause the rejection of product [33]. Table 5, represents the size distribution of the particles with D_{10}, D_{50} and D_{90} values with respect to its intensity. Procopio et al. [34] obtained results between 8.42 ± 1.4 and 72.21 ± 11.27 µm for liquid microparticles containing cinnamon oleoresin. Sartori et al. [35] produced liquid microparticle loaded with ascorbic acid with D_{50} values between 17 ± 1 and 35 ± 11 µm. Figure 5.A represents a single peak which showed powder only with MD and figure 5.B represents particle with different size as inclusion with β-CD. In relation to polydispersity index (span) in Table.5 presented higher value in powder with MD than the powder with MD-β-CD indicating greater range of variation that define less polydisperse behaviour. Span value in the range 1.4 to 5.0 was observed in liquid microparticle as reported by Fadini et al. [33], liquid microparticle containing ascorbic acid has a span of 1.45 for their sample [34].

3.9 Zeta potential

The zeta potential of optimized spray dried lemon powder is shown in figure 6. The average zeta potential of was for β-CD inclusion (− 4 mv). It was observed that the average zeta potential was higher than powder with MD. The high negative zeta potential value inferred the long period stability of powder due to negative–negative charge repulsion [35,36].

4. **Conclusions**

Cyclodextrins gains importance as an important functional component and contributed to different food formulations because of its bioavailability. Inclusion of β-CD in processing of spray dried lemon powder shows a stable moisture content and physiochemical behaviour which provide better storage and transportation at room temperature and in refrigerated condition by maintaining its bioactive properties and enhanced the solubility in water. According to the authors this is the first report worked on β-CD assisted spray dried Assam lemon juice to provide stability of the powdered product that can act as a better potential ingredient by preserving their biological properties and restrict from spoilage.

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