



WHEY FORTIFIED READY-TO-RECONSTITUTE ELEPHANT APPLE (*DILLENIA INDICA*) JUICE POWDER: METHODOLOGICAL OPTIMIZATION, MICRO-STRUCTURAL AND IN VITRO DIGESTION ANALYSES

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ABSTRACT

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Elephant apple fruit, though bestowed with multifarious medicinal properties and available in many Asian countries, has no known commercial processed product due to its tough fibrous calyces, highly astringent juice and brown discolouration. In this study, the juice was blended with concentrated whey in different proportions (v/v) for assessing organoleptic acceptability. Principal Component Analysis of the sensory scores and subsequent evaluation of blends' antioxidant activity revealed the superiority of 1:1 (v/v) admixture over other counterparts. Further, study focussed on optimization of process variables (maltodextrin concentration and inlet temperature of spray dryer) for recuperating beverage powder from the juice-whey blend (1:1 v/v). Developed powders presented different surface morphologies (wrinkled/shrunk/ellipsoidal/fused particles having 2-30 µm diameters), and were organoleptically favourable with good yield (63.33-88.33%), dispersibility (33.40-56.57%), moderate antioxidative potential (37.45-54.57%), improved sugar, protein and whiteness index. In-vitro digestion release of protein and antioxidant activity from the optimized powder showed increasing trend with time, unlike those of fresh juice and juice-whey blend (1:1 v/v). Thus, ready-to-reconstitute juice powder(s) with appreciable features was developed from an underutilized fruit using methodical statistical approaches, and the resultant product was found to provide protracted release of protein and antioxidants during simulated digestion.

Contribution/Originality: *Dillenia indica* based products are not available commercially till date; in this study, juice processed into a convenient reconstitutable form without affecting much of its bioactive compounds is a “first-of-its-kind” example. Amalgamation of whey masked the astringency of pristine *Dillenia indica* juice; aiding in value added functional drink.

1. INTRODUCTION

Dillenia indica, commonly known as Elephant apple, is an underutilized minor wild fruit, growing abundantly in India, Bangladesh, Nepal, Sri Lanka, China, Indonesia, Vietnam, Thailand, Myanmar and Malaysia. The fruits are large ovoid consisting of imbricate sepals enveloping numerous seeds embedded in a mucilaginous core. Lately, there has been a growing interest for this fruit owing to its several biological activities such as antidiabetic, antioxidative, anti-inflammatory, antimicrobial and wound healing property (Migliato et al., 2011). However, the mature fruits are very astringent to sour in taste, highly fibrous with cumbersome processing and the juice turns dark brown soon after extraction. As such, the fruit is used sporadically in culinary preparation (Abdille, Singh, Jayaprakasha, & Jena, 2005), with no known commercially available processed product reported till date. For balancing out the undesirable astringency, Saikia and Saikia (2002) processed the fruit juice into squash and evaluated its quality changes during storage. In light of the burgeoning attention to functional drinks, it seems prudent to blend the juice with a weak or bland flavoured ingredient possessing other positive attributes. In this milieu, whey, a byproduct of the cheese industry, has been popularly incorporated with different fruits and vegetables, adding nutritional value to conventional juices (Bazaria & Kumar, 2016; Uscategui, Velásquez, & Valencia, 2018). Unfortunately, literature pertaining to blending of *Dillenia indica* juice/pulp with dairy or non-dairy ingredients is currently lacking.

Seasonal availability of *Dillenia indica* fruit creates another bottleneck in its processing. Though Nayak, Basumatary, Chandrasekar, Seth, and Kesavan (2020) determined the effect of thermo-sonication and high pressure processing on quality attributes of the pristine juice, the shelf-life was established as 60 days only at 4 °C. To ensure its availability throughout the year and also to the large sector of urban population even in far-flung areas, production of quality juice powder is a pressing need. Among the drying technologies, spray drying has successfully produced low-cost quality powders from juice and extract of various plants; however, spray dried powders have an inherent sticky nature, owing to the presence of low molecular weight sugars and acids of juice, which is often mitigated by incorporating high molecular weight drying adjunct or carrier agents like gums, maltodextrin (MD), protein, etc. In the same sense, being an excellent source of protein, whey is reported to form protective layer around sugary mass (Shi, Fang, & Bhandari, 2013), thermo-labile bioactive components (Bernard, Regnault, Gendreau, Charbonneau, & Relkin, 2011; Uscategui et al., 2018), and aids in higher product recovery during spray drying (Bhusari, Muzaffar, & Kumar, 2014). For the development of a new product, it is imperative to methodically standardize the blending or formulation, so that an integral balance that translates into an excellent quality and good acceptability can be achieved. This often necessitates the application of multivariate statistical tools like Principal Component Analysis (PCA), Partial Least Square regression, Artificial Neural Networks, etc., which helps in reducing the data set and prioritizing the factors responsible for sample segregation and selection. Following the work of Mwove, Gogo, Chikamai, Omwamba, and Mahungu (2018) and Puri, Khamrui, Khetra, Malhotra, and Devraja (2016) PCA biplot was adopted in this study for sensory characterization of juice-whey mix, which was subsequently used for spray drying. Again, the quality of spray dried powders is influenced by a wide variety of controllable and uncontrollable factors (inlet air temperature; aspiration; feed flow rate; concentration and nature of carrier agent used; dry matter content, glass transition temperature and viscosity of the feed; relative humidity of the incoming air; etc.), and optimizing these process parameters is crucial for achieving the best output possible. The present investigation, therefore, aims to explore the possibility of developing a nutritious ready-to-reconstitute beverage powder from *Dillenia indica* juice-whey mix having optimum techno-functional properties, using Response Surface Methodology (RSM).

2. MATERIALS AND METHODS

2.1. Materials

Mature fruits of *Dillenia indica* were procured from horticulture orchard located within latitude and longitude of 26°72' N and 94°20' E, respectively (Deshmukh, Okram, Angami, Rymbai, & Jha, 2019) and fresh whey were purchased from local market. MD of 12 dextrose equivalent (DE) and all the reagents were purchased from HiMedia®.

2.2. Extraction of *Dillenia Indica* Juice

Physical morphology of the fruit is analysed and discussed in Supplementary Table S1. Calyces of the fruit were separated manually, washed and cut into small pieces of 1-1.5 cm thickness and the inner mucilaginous core was discarded. These pieces were then ground in a kitchen grinder (Havells, Momenta NV 750, India) using deionised water in a ratio of 1:10 (w/v), filtered through muslin cloth to obtain a clear juice of 2% total solids.

Table S1. Physical parameters of *Dillenia indica* fruit.

Physical properties	Experimental values
Length (mm)	108.4±1.27
Width (mm)	96.5±3.19
Thickness (mm)	90.4±1.99
Weight (g)	628±2.63
Volume (cm ³)	528.9±2.18
Geometric mean diameter (mm)	119.5±3.01
Surface Area (mm ²)	37598.9±2.71
Sphericity index (%)	1.15±1.4
Aspect ratio	103.2±1.18
Density (g/cm ³)	1.43±1.75

Note: Values=Mean ± SD (N=10).

2.3. Pre-Treatment of Whey

Procured whey of 5.23±0.82 total solids and pH 4.06±0.58, was immediately pasteurized at 90°C for 5 min (Lim, Benner, & Clark, 2019), followed by concentrating it in a rotary vacuum evaporator (IKA® RV 10 digital, Germany) till the attainment of 30% total solids (hereafter referred to as 'concentrated whey').

2.4. Formulation of *Dillenia Indica* Juice-Whey Blends and their Sensory Evaluation

Pristine juice of *Dillenia indica* and concentrated whey were blended according to the formulations mentioned in Table 1, and were subjected to sensory evaluation using 9-point Hedonic scale, wherein 1 = disliked extremely, 5 = neither like nor dislike and 9 = liked extremely were denoted.

Table 1. Formulation of blends using *Dillenia indica* juice and concentrated whey.

Code	<i>Dillenia indica</i> juice	Concentrated whey
T1	100	0
T2	90	10
T3	80	20
T4	70	30
T5	60	40
T6	50	50
T7	40	60
T8	30	70
T9	20	80
T10	10	90
T11	0	100

The samples were coded randomly in uniform containers and were presented to 20 semi-trained panelists to rate their sensory parameters, namely, appearance, colour, aroma, taste, flavour and overall acceptance (Pal, Khan, & Mohanty, 2008). Sensory scores of the blends were evaluated by PCA in XLSTAT software (version 22.5.1052.0, Addinsoft, New York, USA). A biplot was constructed to help discriminate the samples based on their spatial distribution across few Principal Components (PCs) (Das Purkayastha et al., 2012). Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as described by Vani, Rajani, Sarkar, and Shishoo (2008).

2.5. Response Surface Methodology (RSM)

RSM was adopted to optimize the process conditions for obtaining reconstitutable beverage powder from *Dillenia indica* juice-whey blend, using a lab-scale spray dryer (LSD-48 Mini Spray Dryer, JISL, India), wherein the aspiration (air flow) and feed flow rate were kept constant at 45% and 20%, respectively. Prior to the operation, total solid of feed was set at 20%, and pH was adjusted within 3.8-4.5 (Park, Bastian, Farkas, & Drake, 2014). Inlet air temperature of the dryer (A) and MD concentration as drying adjunct (B) were taken as independent variables for the optimization of four response/dependent variables (powder yield (%), dispersibility (%), antioxidant activity (%) and organoleptic acceptability). The Central Composite Design (CCD) was applied using Design-Expert® software (Version 11.0.1.0, Stat-Ease Inc., Minneapolis, USA), which generated 13 experimental runs at $\alpha = \pm 1.414$ with 5 center points and 3 levels of each variables set as 120, 140 and 160°C of inlet air temperature, and 5%, 15% and 25% of MD Table 2. Analysis of variance (ANOVA) was performed on the experimental data and a difference of $p \leq 0.05$ between the experimental and predicted data was considered statistically significant. Experimental data for the selected responses were fitted into second order polynomial Equation 1 and were optimized by Desirability function Equation 2. High values of coefficient of determination ($R^2 > 0.90$) indicate good fit between the observed and predicted values. Non-significant ($p > 0.05$) lack of fit implies that the model is good and fit for prediction.

$$Y_k = \beta_{k0} + \sum_{i=1}^n \beta_{ki} x_i + \sum_{i=1}^n \beta_{kii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n \beta_{kij} x_i x_j + e_k \quad (1)$$

where, Y_i = response variable.

β_{k0} = Coefficient of fitted response at center point.

β_{ki} = linear coefficient.

β_{kii} = quadratic coefficient.

β_{kij} = interaction coefficient.

x = independent variable.

e_k = error.

$$D = (d_1^{r_1} \times d_2^{r_2} \times d_3^{r_3} \times d_4^{r_4})^{\frac{1}{(r_1+r_2+r_3+r_4)}} \quad (2)$$

where, d_i = desirability index for i^{th} responses.

r_i = relative importance.

2.6. Physicochemical Parameters

Prior to analysis, reconstitution of spray dried powder was performed by dispersing in distilled water at a ratio of 1:10 w/v (Chau, Wang, & Wen, 2007), while the fresh juice and whey were used as-is. Sensory evaluation was done by 9-point Hedonic scale in same manner as described earlier. Viscosity was measured at 25°C using Fungilab viscometer (VISCO BASIC Plus L, Spain), attached with L2 spindle at 100 rpm and expressed in centiPoise (cP). The pH was recorded by a digital pH meter (Eutech Instruments, Singapore). Titratable acidity and moisture content (MC) were determined by AOAC standard methods (AOAC, 2000). Total soluble solid (TSS) was measured by a digital refractometer (MA871 model, ATACO, Tokyo). Yield of powder was calculated using Equation 3 (Suliman, 2014).

$$\text{Yield (\%)} = \frac{\text{weight of powder (g)}}{\text{solid content of the feed (\%)}} \times 100 \quad (3)$$

Solubility was determined according to the method described by [Chau et al. \(2007\)](#), and [Equation 4](#) was used to calculate the percent solubility. Sample was reconstituted as mentioned earlier, stirred for 1 h at room temperature (25°C) and centrifuged at 1500 rpm for 10 min. The supernatant was dried and weighed.

$$\text{Solubility (\%)} = \frac{\text{weight of supernatant after drying (g)}}{\text{weight of sample (g)}} \times 100 \quad (4)$$

Dispersibility was determined by the method proposed by [Aliakbarian, Casazza, Nani, and Perego \(2017\)](#). [Equation 5](#) was employed to calculate the percent dispersibility. Sample was reconstituted in water at a ratio of 1:10 (w/v), and stirred manually for 1 min at room temperature. The suspension was then allowed to stand undisturbed for 30 min, followed by weighing 50 ml of the aliquot.

$$\text{Dispersibility(\%)} = 2 \times \frac{M_2 \text{ (g)} - M_1 \text{ (g)}}{M_0 \text{ (g)}} \times 100 \quad (5)$$

where, M_2 = Weight of 50 ml of aliquot (g).

M_1 = Weight of 50 ml of distilled water (g).

M_0 = Weight of sample (g).

Hygroscopicity of powder was determined by the method explained by [Cai and Corke \(2000\)](#) with minor modifications. Sample (0.5 g) in a pre-weighed dish was placed in a desiccator having $75 \pm 1\%$ relative humidity (RH) and a temperature of 30 °C. During the equilibration, RH and temperature inside the desiccators was constantly maintained and monitored by a digital RH clock (103-CTH, HTC™, China). After 15 days, the sample was re-weighed and the hygroscopicity was expressed as g of moisture absorbed per 100g dry solids.

Bulk density and tap density were determined according to the methodology of [Dantas, Pasquali, Cavalcanti-Mata, Duarte, and Lisboa \(2018\)](#) and [Equation 6 & 7](#) were used for its calculation. Powder was poured into a graduated measuring cylinder and the volume occupied was recorded. Similarly, for tap density, the same sample in the measuring cylinder was tapped until no difference was noticed in the scale of the cylinder.

$$\text{Bulk density } (\rho_b) = \frac{\text{Sample mass (g)}}{\text{Apparent volume of sample (ml)}} \quad (6)$$

$$\text{Tap density } (\rho_{\text{tap}}) = \frac{\text{Sample mass (g)}}{\text{Tap volume of sample (ml)}} \quad (7)$$

Flowability and cohesiveness of the powder were deduced from Carr index (CI) and Hausner ratio (HR), respectively [Equations 8 & 9](#). [Supplementary Tables S2 and S3](#) show the specifications for CI and HR corresponding to their flowability and cohesiveness ([Jinapong, Suphantharika, & Jamnong, 2008](#); [Sidlagatta, Venkata, Rao, Daniel, & Lakshmiopathy, 2020](#)).

$$\text{CI} = \frac{(\rho_{\text{tap}}) - (\rho_b)}{(\rho_{\text{tap}})} \times 100 \quad (8)$$

$$\text{HR} = \frac{(\rho_{\text{tap}})}{(\rho_b)} \quad (9)$$

Table S2. Specification for Hausner ratio.

Hausner ratio	Cohesiveness
<1.2	Low
1.2-1.4	Intermediate
>1.4	High

Total sugars was estimated by phenol-sulphuric acid method as described by [Jain, Karibasappa, Dodamani, and Mali \(2017\)](#). Total soluble protein was estimated by Lowry method ([AOAC, 2000](#)). Micro-Kjeldahl method was

used to determine the total nitrogen content, which was then multiplied by a factor of 6.25 to derive crude protein (AOAC, 2000). Ash (total mineral) content was determined as per the procedure of AOAC (2000). Folin–Ciocalteu reagent assay was employed to determine the total phenol content (TPC) (Singleton & Rossi, 1965; Soong & Barlow, 2004). The total flavonoids content (TFC) was determined following the protocol of Kamtekar, Keer, and Patil (2014).

Table S3. Specification for Carr index.

Carr Index (%)	Flowability
<15	Very good
15-20	Good
20-30	Fair
35-45	Bad
>45	Very bad

Whiteness index (WI) was measured by the means of weighted ordinate method, using an ELISA microplate reader (BioTek® Instruments EPOCH 2NS, USA). The colour parameters L' , a' , b' were calculated (Equations 10, 11, 12) from the transmission spectra between 380 to 760 nm with 10 nm interval, and the recorded readings were converted to XYZ tristimulus values (Saikia, 1999). WI was deduced using Equation 13 (Pathare, Opara, & Al-Said, 2013).

$$L' = 10\sqrt{Y} \quad (10)$$

$$a' = 175 \left(\frac{1.02X}{Y} - 1 \right) \quad (11)$$

$$b' = 70 \left(1 - \frac{0.847Z}{Y} \right) \quad (12)$$

$$WI = \sqrt{(100 - L'^2) + a'^2 + b'^2} \quad (13)$$

2.7. Scanning Electron Microscopy (SEM)

Surface morphology of the spray dried powder was observed under a field emission scanning electron microscope (FESEM) (Carl ZEISS Microscopy, Germany). Prior to the observation, the particles were mounted on carbon stubs fixed with double sided adhesive tape, followed by coating with a thin layer of gold under at an accelerating voltage of 5 kV (Both, Boom, & Schutyser, 2020).

2.8. In-Vitro Digestion

Digestibility of the juice, concentrated whey, juice:whey blend (T6) and reconstituted optimized powder were evaluated using a simulated gastric and intestinal fluid models (Chen, Li, & Tang, 2015; Chen, Liu, & Tang, 2020). Individual sample solution (10 ml) was added to 0.1 M HCl while adjusting the pH to 1.5, followed by pre-incubation for 10 min. For simulating the gastric digestion, 10 mg of pepsin powder was added and incubated at 37 °C for 1 h. Next, 250 mg of bile extract powder was mixed along with the adjustment of pH to 7.0 with 4 M NaOH and incubated for another 10 min. Finally, to simulate intestinal digestion, 20 mg of pancreatin powder was added to the gastric chyme. Finally, digesta (1 ml) were collected after 60, 90, 120 min of intestinal digestion, centrifuged at 10,000 rpm for 30 min at 4 °C, and the supernatant was extracted twice with 95% ethyl acetate (5 ml) to inactivate the enzymes. An aliquot of 500µl was sampled for estimation of liberated soluble protein and antioxidant activity, following the methods explained before.

2.9. Statistical Analysis

ANOVA using Tukey's honest significant difference (HSD) test was performed in SPSS software (version 16.0, IBM® SPSS Statistics, Chicago) and considered significantly different among the samples at 95% confidence interval. Number of replicates taken for each analysis was 3, except for the physical parameters wherein 10 repetitions were performed.

3. RESULTS AND DISCUSSION

3.1. Organoleptic Comparison of *Dillenia Indica* Juice-Whey Blends Using PCA

Average sensory scores assigned to the various blends formulated as per Table 1, are shown in Supplementary Table S4; amongst which T1 and T11, having pristine *Dillenia indica* juice and concentrated whey, respectively, were rated the lowest score. This in-turn indicates that use of lone base ingredient (especially juice), cannot qualify as a satisfactory drink and requires blending with appropriate components.

Table S4. Sensory parameters rating of the juice-whey blends.

Code	Appearance	Colour	Taste	Aroma	Flavour	Overall acceptance
T1	5.11 ± 0.93 ^{ab}	4.89 ± 1.36 ^a	5.22 ± 1.30 ^{ab}	5.22 ± 0.97 ^a	5.33 ± 0.87 ^{ab}	5.44 ± 1.01 ^a
T2	5.22 ± 1.20 ^{ab}	5.33 ± 0.87 ^a	5.33 ± 1.00 ^{ab}	5.67 ± 0.71 ^a	5.00 ± 1.58 ^{ab}	5.67 ± 1.22 ^a
T3	5.11 ± 1.36 ^a	5.33 ± 1.00 ^a	4.44 ± 0.73 ^{ab}	5.44 ± 1.42 ^a	5.11 ± 0.60 ^{ab}	5.56 ± 1.13 ^a
T4	5.78 ± 0.97 ^{ab}	6.67 ± 1.41 ^a	4.56 ± 1.24 ^{ab}	5.00 ± 0.87 ^a	5.11 ± 0.93 ^{ab}	5.11 ± 1.36 ^a
T5	5.89 ± 1.36 ^{ab}	6.44 ± 1.74 ^a	4.89 ± 2.26 ^{ab}	4.33 ± 2.00 ^a	4.56 ± 2.01 ^{ab}	5.67 ± 1.80 ^a
T6	7.11 ± 1.05 ^b	6.78 ± 1.20 ^a	6.22 ± 1.09 ^b	6.00 ± 0.87 ^a	5.89 ± 1.17 ^b	6.33 ± 1.22 ^a
T7	6.33 ± 1.32 ^{ab}	6.67 ± 0.71 ^a	5.67 ± 0.87 ^{ab}	5.44 ± 1.24 ^a	5.33 ± 1.12 ^{ab}	6.11 ± 1.17 ^a
T8	6.56 ± 0.88 ^{ab}	6.22 ± 1.20 ^a	5.22 ± 1.30 ^{ab}	5.11 ± 1.45 ^a	5.11 ± 0.93 ^{ab}	5.78 ± 1.39 ^a
T9	6.33 ± 1.66 ^{ab}	5.78 ± 1.72 ^a	5.56 ± 1.24 ^{ab}	5.89 ± 1.27 ^a	4.89 ± 1.05 ^{ab}	5.33 ± 1.32 ^a
T10	6.22 ± 1.64 ^{ab}	5.44 ± 2.13 ^a	4.00 ± 1.22 ^a	4.67 ± 1.41 ^a	4.33 ± 1.00 ^{ab}	4.67 ± 1.00 ^a
T11	5.89 ± 1.45 ^{ab}	5.33 ± 1.66 ^a	4.00 ± 1.22 ^a	4.44 ± 1.01 ^a	4.00 ± 1.12 ^a	4.89 ± 0.78 ^a

Note: Values = Mean ± SD (N=9), a-d Means in the row with different letters are significantly different (p < 0.05).

So for establishing the most suitable formulation(s), a PCA biplot was constructed Figure 1. Among the six PCs, the first two, namely PC1 and PC2, could satisfactorily account for 84.38% of the total variability in the data set (62.17% and 22.22%, respectively), and both had Eigen values greater than 1.0 (Supplementary Table S5).

Table S5. Eigen value and contribution of the principal components (PCs).

	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	3.730	1.333	0.485	0.250	0.120	0.081
Variability (%)	62.170	22.215	8.089	4.172	1.997	1.357
Cumulative %	62.170	84.385	92.474	96.646	98.643	100.00

The vector representation using first two PCs in Figure 1 clearly depicts the clustering of T6, T7, T8 and T9 in the first and second quadrants, wherein the sensory attributes are either overlying with the variables or in the vicinity of the formulations having significant effect (p < 0.05). The distinction between these formulations against the least acceptable ones was clearly visible as the points were well-dispersed within the biplot. Judging from PCA biplot, the samples T6, T7, T8 and T9 were found to have greater organoleptic acceptability than their counterparts, and were accordingly tested for their antioxidant potential (Supplementary Table S6). The blend T6 possessed highest radical scavenging value (59.16 ± 0.37%), which can be credited to the presence of higher fraction of polyphenol-rich *Dillenia indica* juice than T7, T8 and T9. As T6 was found to be superior over the pristine fruit juice, concentrated whey and other blended formulations, it was selected for further study.

Table S6. Antioxidant activity (%) of the selected juice-whey blends.

T6 (1:1)	T7 (2:3)	T8 (3:7)	T9 (1:4)	CD _{0.05}
59.16 ± 0.37	55.37 ± 0.15	52.87 ± 0.41	47.82 ± 0.66	0.115

3.2. Spray Drying of T6: Optimization of Process Variables By RSM

The blend T6, mixed with different concentration of MD, was spray dried at various inlet air temperatures as per CCD Table 2. These ranges of MD and inlet temperature were selected on the basis of preliminary trials. Low level of MD (<5%) and inlet temperature (<120°C) resulted in poor yield, and hence, were not considered in the study.

Likewise, very high concentration of MD (>25%) caused blocking of the spray atomizer and inlet temperature greater than 160°C resulted in severe loss of antioxidative compounds in the final product.

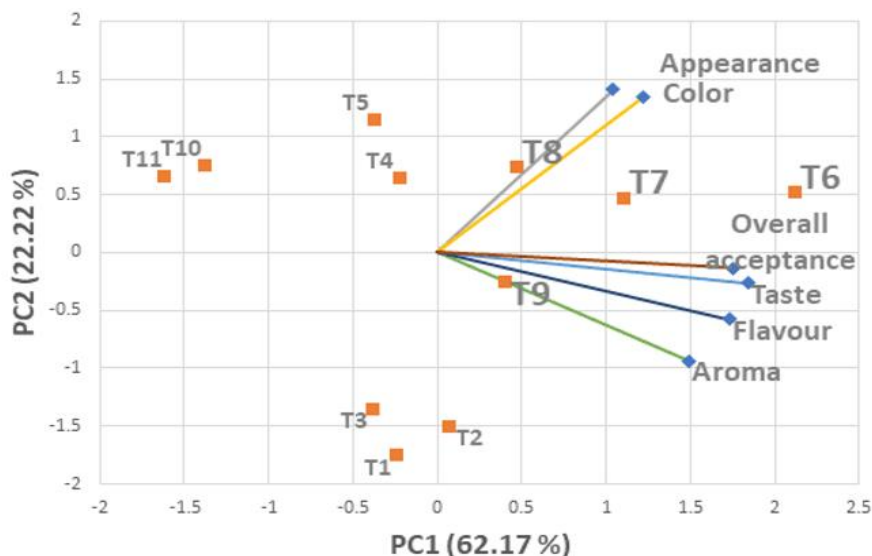


Figure 1. PCA biplot for the sensory ratings of *Dillenia indica* juice-whey blends.

Amongst the several determining factors, yield, dispersibility, antioxidant activity and organoleptic acceptability were chosen as the dependent variables for optimization because of their predominant dependency in regulating industrial output and marketability of the developed powder(s).

Table 2. Central composite design (CCD) with experimental values of dependent variables.

Exp. Run	Actual and coded values		Response values			
	Inlet Temperature (°C) (A)	MD (%) (B)	Yield (%)	Dispersibility (%)	Antioxidant activity (%)	Organoleptic acceptability
1	168.284	15	72.67 ± 1.26 ^d	43.30 ± 0.40 ^f	47.56 ± 0.28 ^{gh}	7.72 ± 0.84 ^f
2	140	15	83.00 ± 2.00 ^f	38.61 ± 0.10 ^d	43.54 ± 0.33 ^d	5.92 ± 0.67 ^{bc}
3	140	0.857864	67.67 ± 1.53 ^b	38.97 ± 0.31 ^d	42.87 ± 0.24 ^c	4.00 ± 0.97 ^a
4	140	15	84.00 ± 1.00 ^f	34.51 ± 0.03 ^b	44.18 ± 0.43 ^e	6.01 ± 0.75 ^{bc}
5	140	15	85.67 ± 0.58 ^g	35.57 ± 0.27 ^c	47.52 ± 0.30 ^g	6.14 ± 0.62 ^{bc}
6	111.716	15	71.50 ± 1.00 ^e	33.40 ± 0.44 ^a	53.58 ± 0.37 ^j	6.35 ± 0.55 ^{cd}
7	120	25	72.33 ± 2.52 ^{cd}	34.44 ± 0.22 ^b	48.14 ± 0.23 ^{hi}	6.84 ± 1.70 ^{de}
8	140	15	88.33 ± 1.53 ^h	38.51 ± 0.25 ^d	48.32 ± 0.45 ⁱ	5.82 ± 0.93 ^{bc}
9	140	15	83.67 ± 1.15 ^f	35.44 ± 0.27 ^c	45.49 ± 0.41 ^f	5.82 ± 0.93 ^{bc}
10	160	25	77.00 ± 2.65 ^e	56.57 ± 0.25 ^h	37.45 ± 0.46 ^a	8.16 ± 0.97 ^f
11	140	29.1421	86.67 ± 0.58 ^g	48.73 ± 0.21 ^g	39.78 ± 0.62 ^b	7.10 ± 0.62 ^e
12	120	5	63.33 ± 2.08 ^a	33.47 ± 0.11 ^a	54.58 ± 0.42 ^k	5.69 ± 0.98 ^b
13	160	5	76.67 ± 1.53 ^e	40.44 ± 0.16 ^e	47.55 ± 0.33 ^{gh}	5.80 ± 0.70 ^{bc}

Note: Value=Mean ± SD. (N=3) **Mean value in a column with different letters are significantly different (p < 0.05).

3.2.1. Yield

Yield of the powder at different temperatures and MD concentration are depicted in Table 2. The highest yield was recorded at 140°C of inlet temperature and 15% MD. The second order polynomial model for yield is given in Equation 14.

$$\text{Yield} = 84.00 + 3.73A + 4.32B - 2.67AB - 4.03A^2 - 4.99B^2 \tag{14}$$

where, A = Inlet temperature
B = MD.

Table S7. ANOVA showing the effects of independent variables as linear, interaction, and quadratic terms on the response variables.

Source	df	Yield				Dispersibility				Antioxidant activity				Organoleptic acceptability			
		β	SS	F value	p-value	β	SS	F value	p-value	β	SS	F value	p-value	β	SS	F value	p-value
Model	5		542.46	33.59	< 0.0001		505.63	15.98	0.0010		230.63	6.71	0.0134		12.60	42.21	< 0.0001
A- Inlet Temp.	1	3.73	111.06	34.38	0.0006	5.39	232.21	36.69	0.0005	-3.28	86.04	12.51	0.0095	0.4209	1.42	23.75	0.0018
B- MD	1	4.32	149.15	46.18	0.0003	3.86	119.45	18.87	0.0034	-2.61	54.60	7.94	0.0259	0.9868	7.79	130.5	< 0.0001
AB	1	-2.67	28.46	8.81	0.0209	3.79	57.51	9.09	0.0195	-0.92	3.36	0.488	0.5074*	0.3025	0.366	6.13	0.0424
A ²	1	-4.03	112.94	34.97	0.0006	0.945	6.21	0.981	0.3550*	2.62	47.91	6.97	0.0335	0.6290	2.75	46.12	0.0003
B ²	1	-4.99	172.99	53.56	0.0002	3.69	94.96	15.00	0.0061	-2.00	27.74	4.03	0.0846*	-0.1135	0.089	1.50	0.2601*
Residual	7		22.61				44.30				48.14				0.417		
Lack of Fit	3		18.70	6.38	0.0527*		29.88	2.76	0.1755*		31.00	2.41	0.2073*		0.346	6.37	0.0528*
Pure Error	4		3.91				14.42				17.14				0.072		
Corrected Total	12		565.07				549.93				278.7				13.01		
R ²		0.960				0.919				0.827				0.9679			
Adjusted R ²		0.931				0.862				0.704				0.9450			
Adeq Precision		16.16				13.69				9.865				23.186			
CV%		2.29				6.39				5.68				3.90			

Note: *Non-significant at 5% level, df: degree of freedom, β : coefficients, SS: sum of squares.

Linear, quadratic and interaction terms were found to have significant positive effect on the process yield ($p < 0.05$) (Supplementary Table S7). MD concentration was found to have higher impact on yield ($\beta = 4.32$) than the inlet temperature ($\beta = 3.73$), as MD is known to improve the handling properties of spray dried products by acting as a bulking agent (Watson, Lea, & Bett-Garber, 2017). Figure 2(A) shows the increase in yield with increasing inlet temperature and MD concentration. Higher temperature ensures faster drying rate for product formation. However, this trend was obliterated beyond 160°C, when the sugars on particle surfaces might have been heated, causing them to stick to the wall of drying chamber (Telang & Thorat, 2010; Wang, Dufour, & Zhou, 2015), and thus reducing the powder yield.

3.2.2. Dispersibility

Dispersibility is a vital reconstititional parameter, which allows the particles to separate (disperse) into individual entity on gentle mixing. Figure 2(B) indicates the increase in dispersibility index with increasing inlet temperature and MD concentration, which is well demonstrated by significant effect of all the linear and interaction terms ($p < 0.05$), along with the quadratic term of MD (Supplementary Table S7). The second order polynomial model for dispersibility is given in Equation 15.

$$\text{Dispersibility} = 36.53 + 5.39A + 3.86B + 3.79AB + 0.9446A^2 + 3.69B^2 \quad (15)$$

where, A = Inlet temperature

B = MD

Low MC of the final product results in greater moisture gradient between the particles and surrounding water. Therefore, when dispersed in water, uptake of water is fast. This contributes to better reconstitution of the powder. Results were in accordance with Aliakbarian et al. (2017) and Giri Saroj, Mangaraj, Sinha Lalan, and Tripathi Manoj (2017), who developed powders from chocolate beverage and soy beverage, respectively.

3.2.3. Antioxidant Activity

Radical scavenging activity of the powders was in the range of 37.45%—54.57% Table 2. The polynomial equation for antioxidant activity of the spray dried powders is given below Equation 16.

$$\text{Antioxidant activity} = 45.81 - 3.28 A - 2.61 B - 0.9159 AB + 2.62 A^2 - 2.00 B^2 \quad (16)$$

where, A = Inlet temperature.

B = MD.

Figure 2(C) clearly depicts the decrease in antioxidant activity with increasing temperature and MD concentration, which could be linked to the degradation of heat-labile phenolics and flavonoids of the juice, and polypeptides of the whey (Saikia, Mahnot, & Mahanta, 2015). Dilution effect by high quantity of MD in feed might also lead to reduced antioxidative capacity of powders (Mishra, Mishra, & Mahanta, 2014). The linear terms as well as quadratic term of inlet temperature were found to have significant effect ($p < 0.05$) on powders' antioxidative potential (Supplementary Table S7).

3.2.4. Organoleptic Acceptability

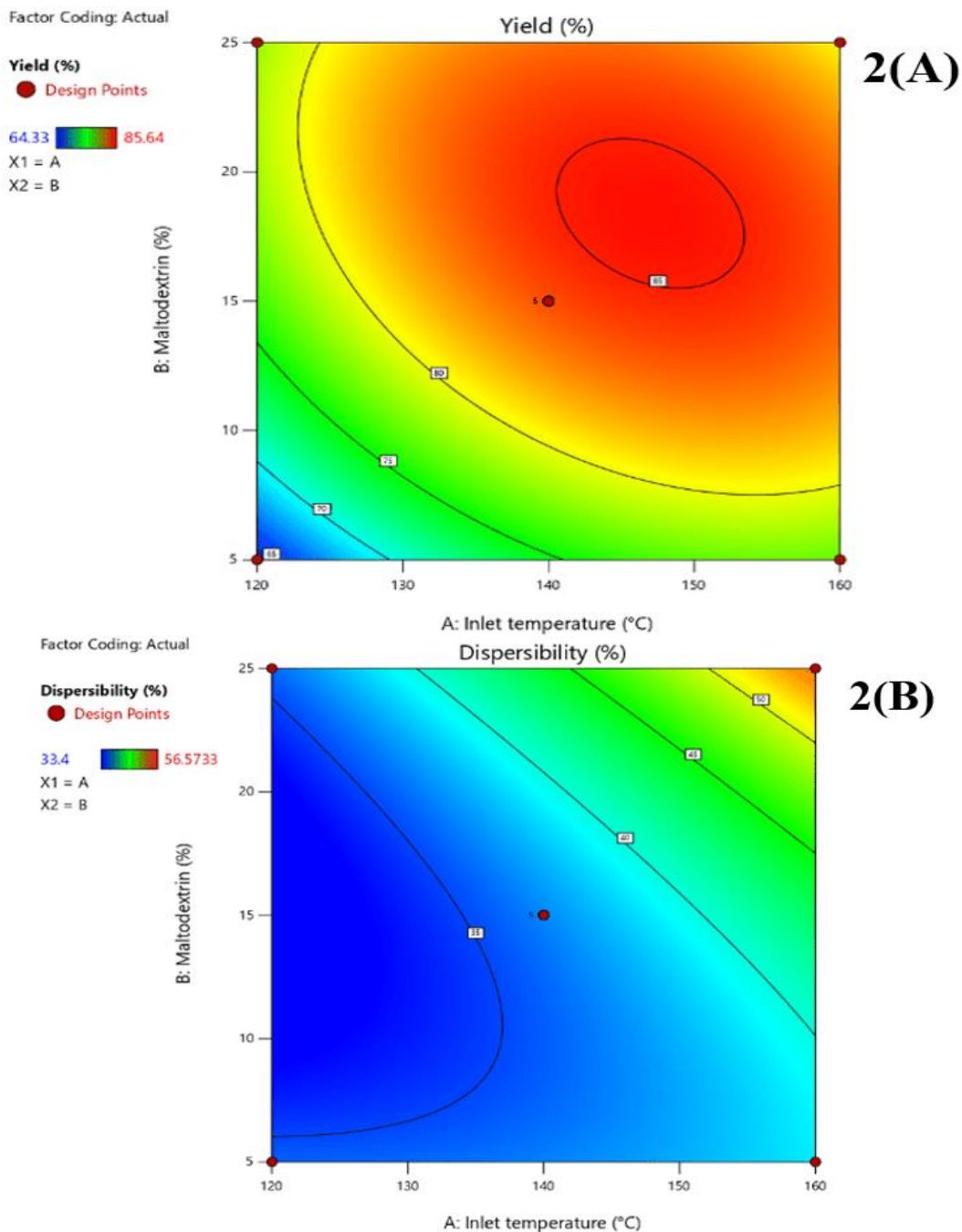
Sensory scores of the reconstituted beverage varied within 4-8.2 Table 2. Sample containing the lowest amount of MD (0.86% and 5%) scored the lowest rating (≥ 5), which could be attributed to the stickiness of the powder obtained at such low MD level; such powders fail to form uniform suspension during mixing, resulting in the formation of tiny agglomerates suspending in the reconstituted drink. This is in line with the observation of better dispersibility in presence of higher MD. The equation for organoleptic acceptability of the reconstituted beverage is given in Equation 17.

$$\text{Organoleptic acceptability} = 5.94 + 0.4209A + 0.9868B + 0.3025AB + 0.6290A^2 - 0.1135B^2 \quad (17)$$

where, A = Inlet temperature.

B = MD.

All linear and interaction terms, as-well-as quadratic term of inlet temperature of the polynomial equation were found to be significant ($p < 0.05$) (Supplementary Table S7). The magnitude of the coefficient of the linear terms of MD evidently indicates its impact on the organoleptic acceptability of powders ($\beta = 0.99$). Likewise, Figure 2(D) shows the rise in organoleptic scores with the increasing quantity of MD. This can be explained by the good dispersibility of powder in presence of MD, as stated earlier. Another reason could be possible masking of the astringent flavour of *Dillenia indica* juice by MD, which has often been reported to mask undesirable flavours and odours during spray drying (Abraham & Mathew, 2013; Alayoubi et al., 2016; Bertelsen, Laursen, Knudsen, Møller, & Kidmose, 2018).



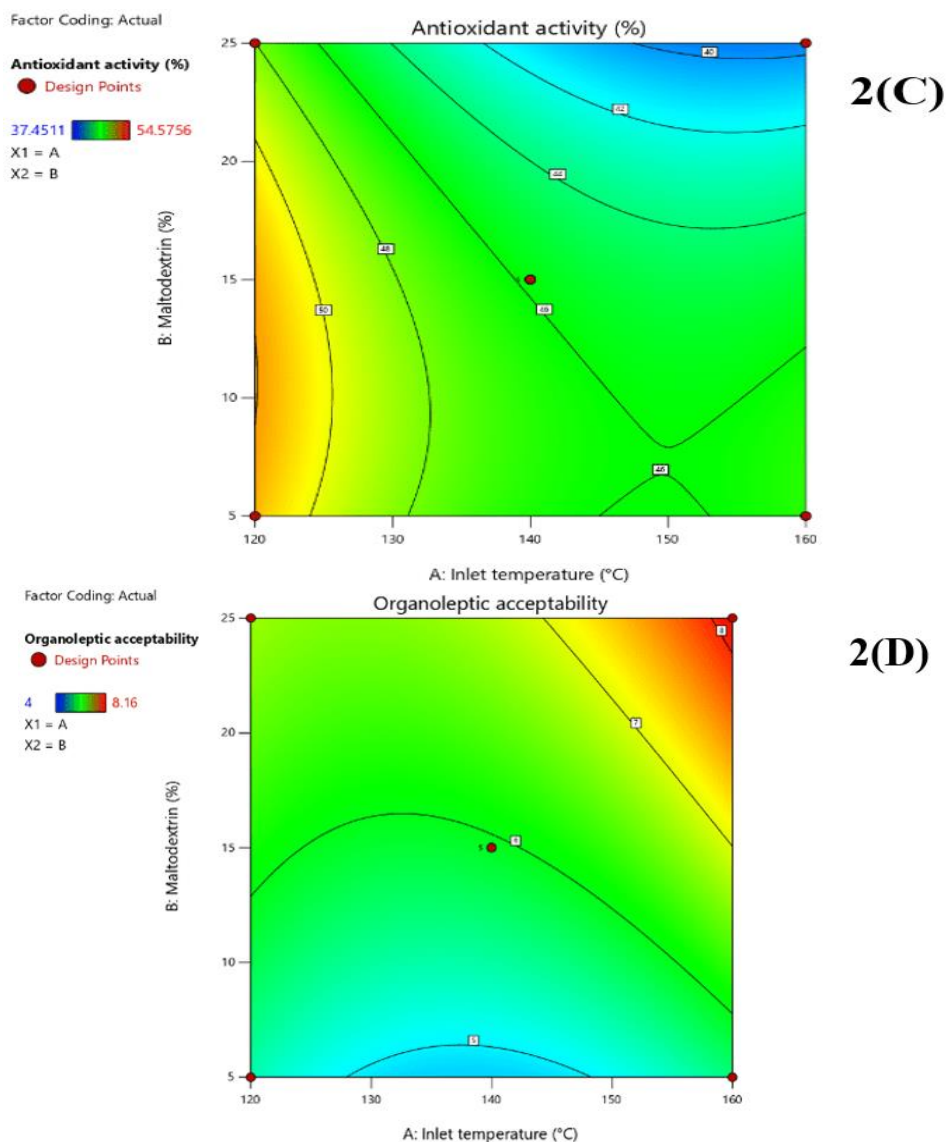


Figure 2. Two-dimensional plots for the effects of process variables on (a) yield, (b) Dispersibility, (c) Antioxidant activity and (d) Organoleptic acceptability.

3.3. Prediction of the Optimum Condition and its Validation

The desired goals for independent variables were set within the range of experimental values, while the response parameters were chosen to maximize (Supplementary Table S8). The optimum values predicted for independent variables were 160°C of inlet air temperature and 19.727% MD (Supplementary Table S8). Experimental runs performed at the stated optimum condition were then used to verify the accuracy of the polynomial models. Table 3 vouches the fact that observed experimental values were reasonably close to the predicted values (p>0.01), which in turn confirms the validity of the models for optimization.

Table S8. Optimum solution of process and response variables at desired goal and criteria.

Parameters	Desired Goal	Criteria		Importance	Predicted
		Lower limit	Upper limit		
Inlet temp. (°C)	In range	120	160	3	160
MD (%)	In range	5	25	3	19.72
Yield (%)	Maximize	64.33	85.64	3	83.35
Dispersibility (%)	Maximize	33.4	56.57	3	47.30
Antioxidant activity (%)	Maximize	37.45	54.57	3	43.04
Organoleptic acceptability	Maximize	4	8.16	3	7.58

Table 3. Validation of the developed models and experimental values of the quality parameters of powder developed at predicted optimum condition.

Validation of models	Predicted		Experimental values			CD _{0.01}
	Inlet	MD (%)	Parameters	Observed [§]	Predicted	
Validation of models	160	19.727	Yield (%)	83.90±0.65	83.35	3.70
			Dispersibility (%)	49.73±0.33	47.30	1.88
			Antioxidant Activity (%)	48.85±1.18	43.04	6.77
			Organoleptic acceptability	8.030±0.04	7.58	0.23
Experimental values of other quality parameters						
			MC (%)	4.84 ± 0.06		
			Solubility (%)	89.02 ± 0.41		
			Hygroscopicity (g/100g)	23.00 ± 0.61		
			Bulk Density (g/ml)	0.29 ± 0.02		
			Tap Density (g/ml)	0.39 ± 0.01		
			HR	1.33 ± 0.05		
			CI (%)	24.80 ± 3.0		
			WI	58.87 ± 0.79		
			Protein (mg/g)	86.71 ± 1.24		
			Sugar(mg/g)	241.19 ± 1.02		
			Crude protein (%)	37.11 ± 0.68		
			Ash (%)	4.44 ± 0.28		
			TPC (mg GAE/g)	1.82 ± 0.03		
			TFC (mg QE/g)	0.38 ± 0.02		

Note: §Value=Mean ± SD. (N=3).

3.4. Quality Attributes of the Powder Obtained at the Predicted Optimum Condition

Physicochemical parameters of the powder obtained at the predicted optimum condition (160°C inlet temperature and 19.7% MD) are described in Table 3. Powders exhibited good solubility and dispersibility with intermediate hygroscopicity, and fair flowability. Its total soluble sugar decreased by 49.04% as compared to that of T6 (Supplementary Table S9). This decrease can be ascribed to the probable formation of sugar-protein complex (Maillard browning) and degradation of free sugars during the drying process. Mitrović, Popović, Miletić, Leposavić, and Korićanac (2019) reported similar observation while drying plum fruits. Crude protein content and polyphenols of the optimized powder decreased by 38.41% and 98% respectively, when compared with those of the feed (T6) (Supplementary Table S9), probably due to thermal degradation of the heat labile proteins and polyphenols. Another reason for this reduction could be the interaction of sugars and polyphenols with the proteins (Papadopoulou & Frazier, 2004; B. Wang, Tchessalov, Warne, & Pikal, 2009). Despite of drastic reduction in phenolic content, the decrease in antioxidant activity of the optimized powder was only 17.42%, with respect to that of T6, although both are expected to be in concurrence with each other.

Table S9. Physicochemical parameters of juice, concentrated whey and T6 blend.

	Viscosity (cP)	pH	Acidity (g/100ml)	Total sugar (g/100g)	Crude protein (%)	Soluble protein (g/100g)	TPC (g GAE/100 g)	Antioxidant activity (%)
Juice	16.57±0.78	3.71±0.95	1.56±0.52*	53.0±0.25	4.12±0.82	1.95±0.81	27.85±0.86	64.0±0.59
Whey	6.20±0.98	4.50±1.03	0.50±0.79 [§]	5.02±0.46	58.84±0.76	24.05±0.69	ND [#]	6.83±0.68
T6 Blend	11.2±1.7	4.10±1.35	1.74±0.82	47.33±0.71	60.25±1.87	19.08±0.93	11.45±0.66	59.16±0.37

Note: Value=Mean ± SD. (N=3), *expressed as citric acid equivalent, [§]expressed as lactic acid equivalent, [#]Not detectable concentration of <0.01gGAE/100g.

This anomaly can be construed from the antioxidative potential of the Maillard products formed between sugars and amino acids of protein at high temperature of spray dryer. Reconstituted beverage from optimized powder showed a WI of 58.87, which was better than that of the fresh juice (33.94), rendering better acceptability to oxidation prone

Dillenia indica based products. Addition of whey and MD, thus, improved the colour of the beverage powder by masking the undesirable brown pigments of the fruit juice; the latter is otherwise besmirched for its dark-coloured astringent juice

3.5. Other Physicochemical Parameters of Spray Dried Powders

Apart from the above mentioned dependent variables, other parameters anticipated to impact the quality of the powder, as influenced by the independent variables, were also analyzed and discussed alongside Table 4. The graphical interpretation of these interactions can be viewed in Supplementary Figure S1.

Factor Coding: Actual

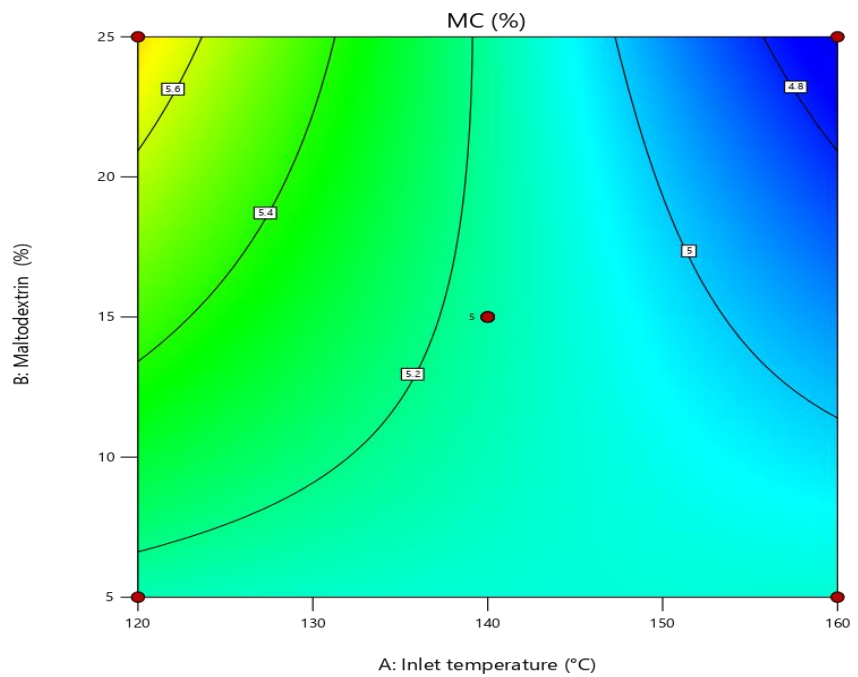
MC (%)

● Design Points

4.76 5.96

X1 = A: Inlet temperature

X2 = B: Maltodextrin



(A)

Factor Coding: Actual

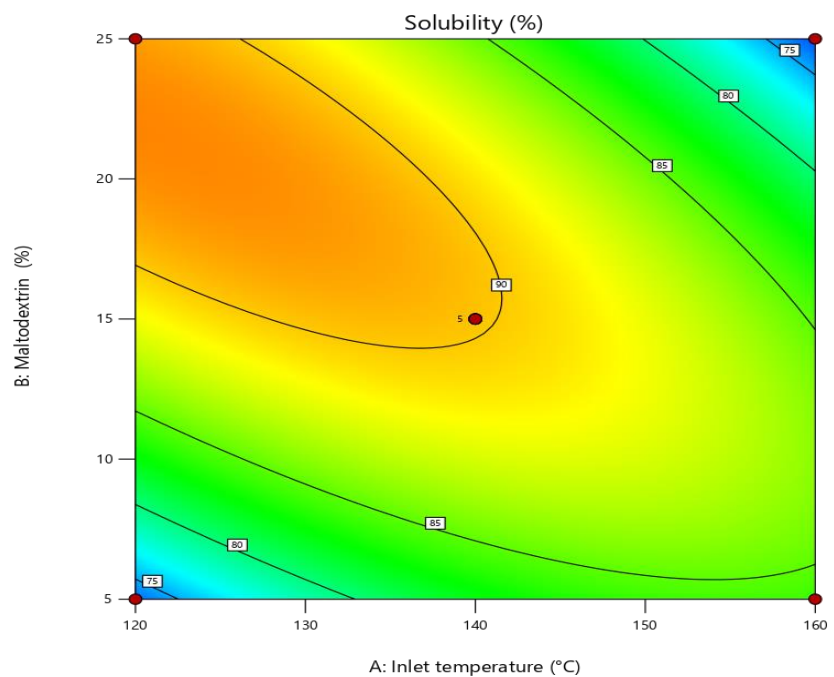
Solubility (%)

● Design Points

71 94.67

X1 = A: Inlet temperature

X2 = B: Maltodextrin




(B)

Factor Coding: Actual

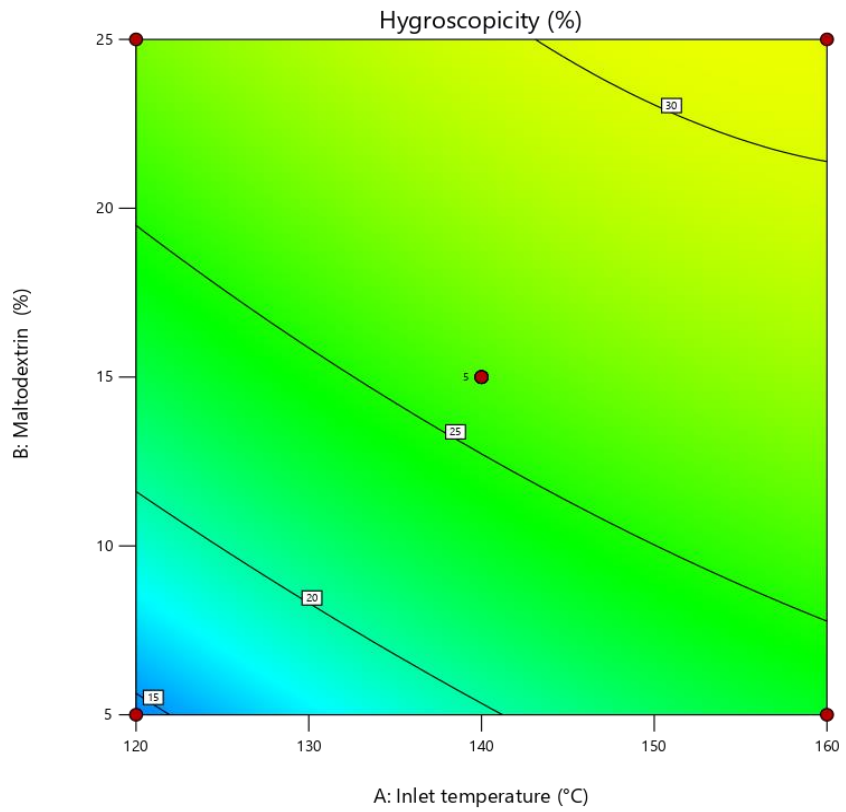
Hygroscopicity (%)

● Design Points

11  37.54

X1 = A: Inlet temperature

X2 = B: Maltodextrin




(C)

Factor Coding: Actual

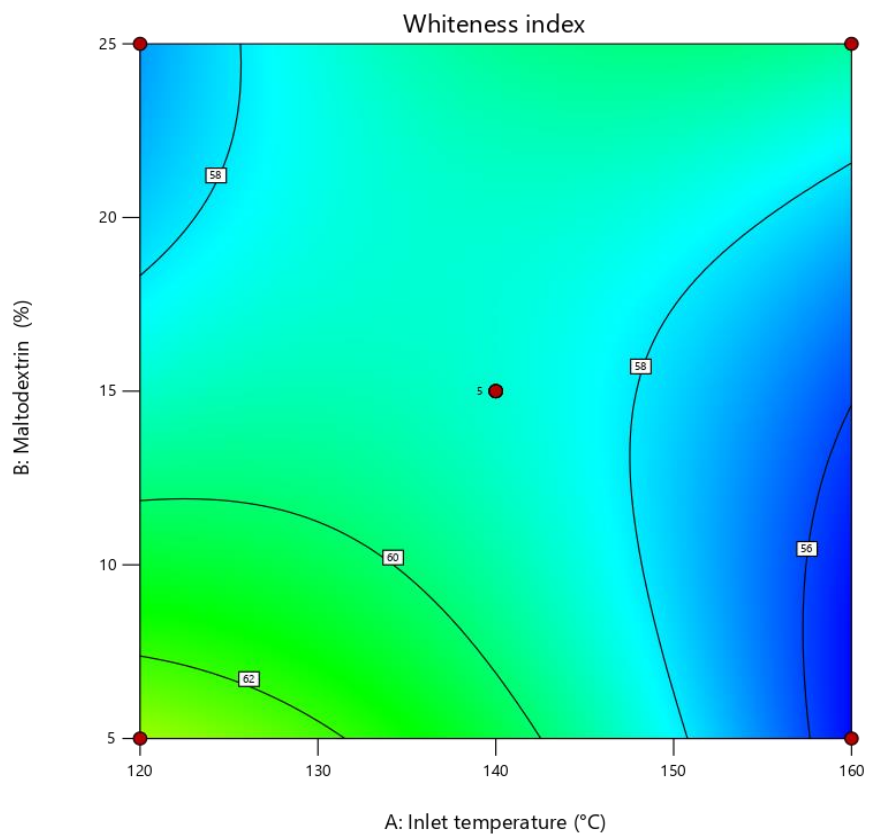
Whiteness index

● Design Points

55.13  67.56

X1 = A: Inlet temperature

X2 = B: Maltodextrin




(D)

Factor Coding: Actual

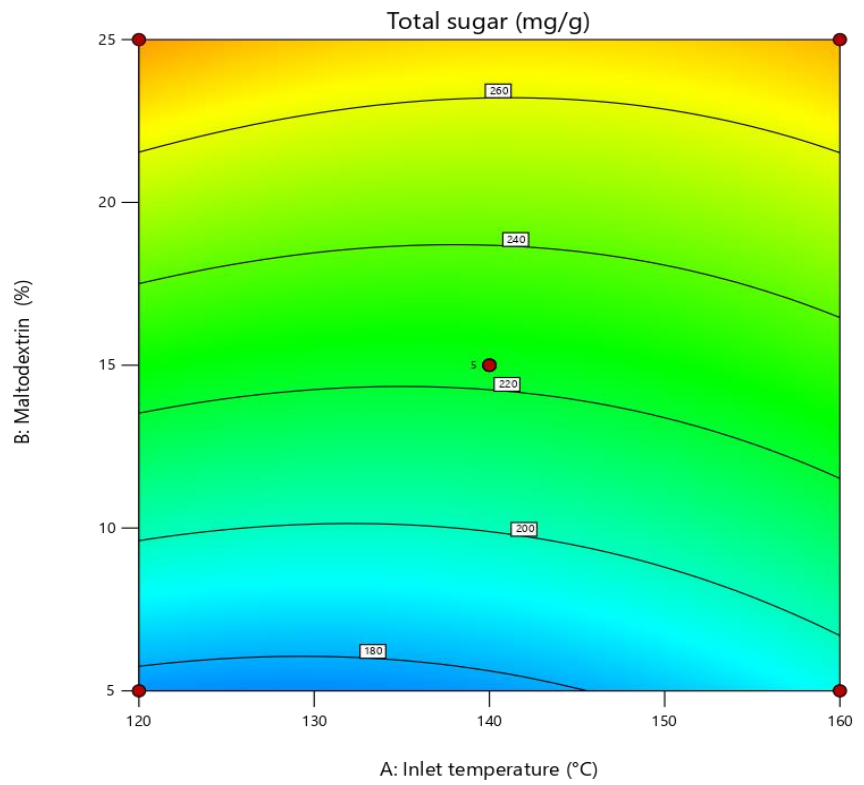
Total sugar (mg/g)

● Design Points

155.1  298.82

X1 = A: Inlet temperature

X2 = B: Maltodextrin



(E)

Factor Coding: Actual

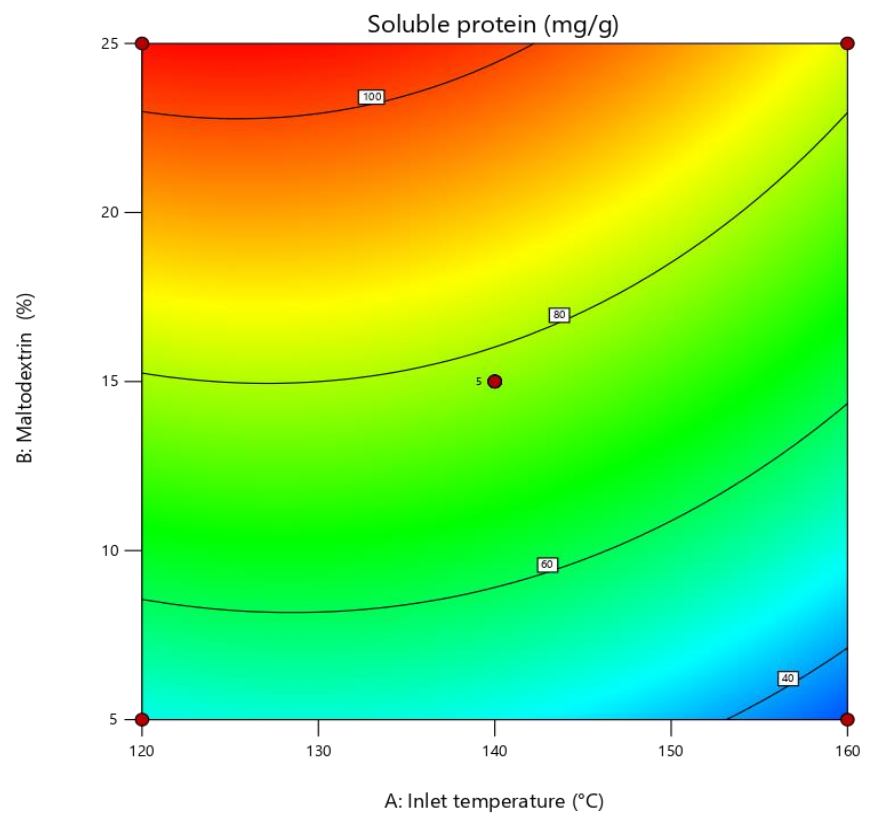
Soluble protein (mg/g)

● Design Points

27.47  105.58

X1 = A: Inlet temperature

X2 = B: Maltodextrin




(F)

Factor Coding: Actual

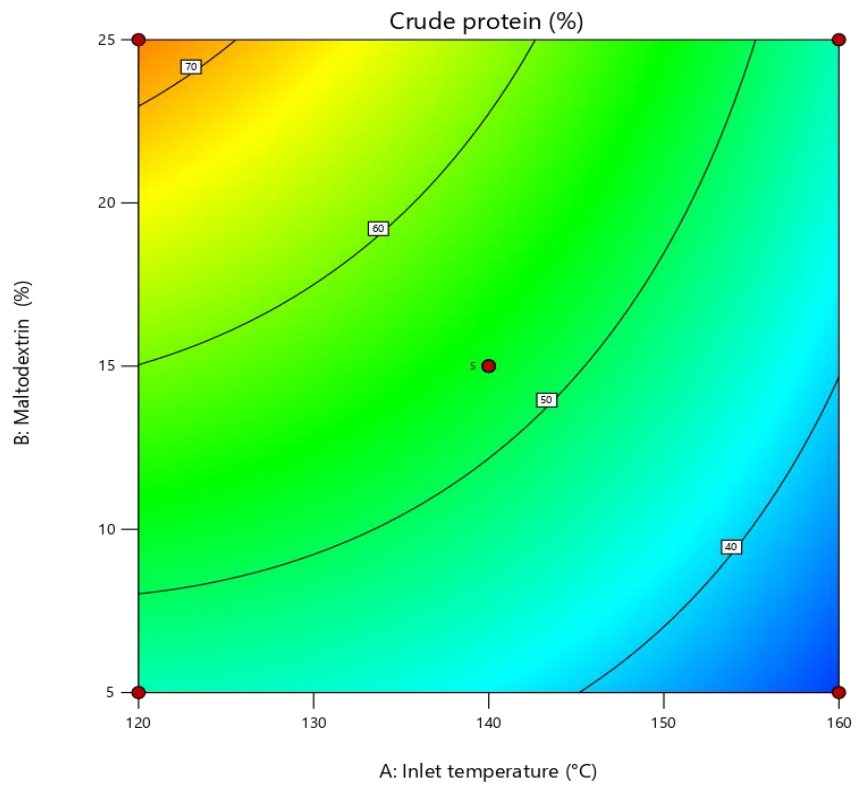
Crude protein (%)

● Design Points

29.75  78.75

X1 = A: Inlet temperature

X2 = B: Maltodextrin



(G)

Factor Coding: Actual

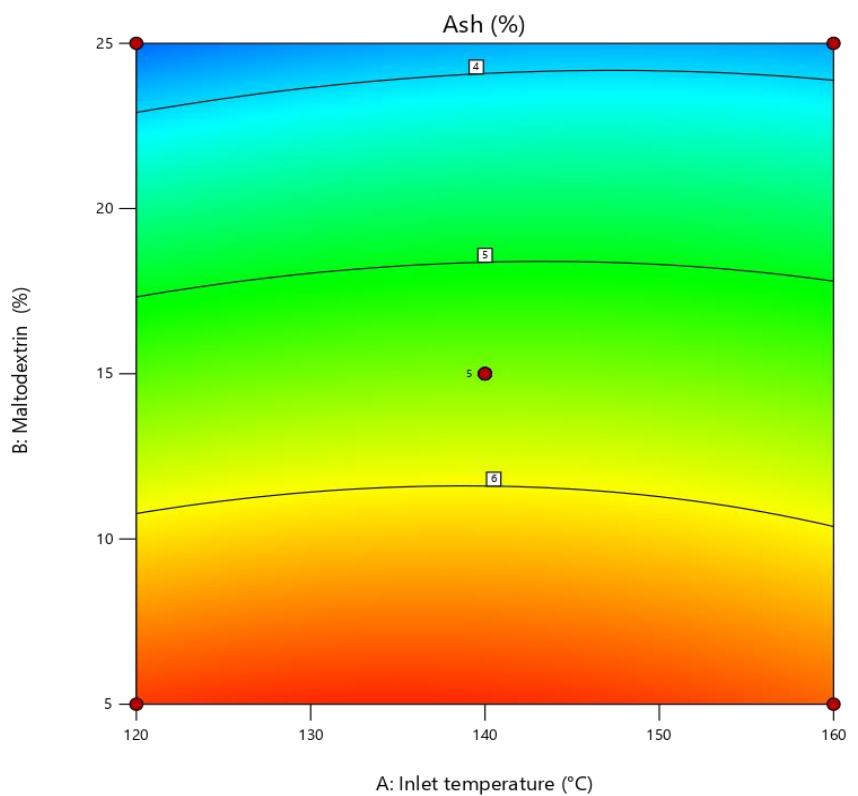
Ash (%)

● Design Points

3.2  6.93

X1 = A: Inlet temperature

X2 = B: Maltodextrin



(H)

Factor Coding: Actual

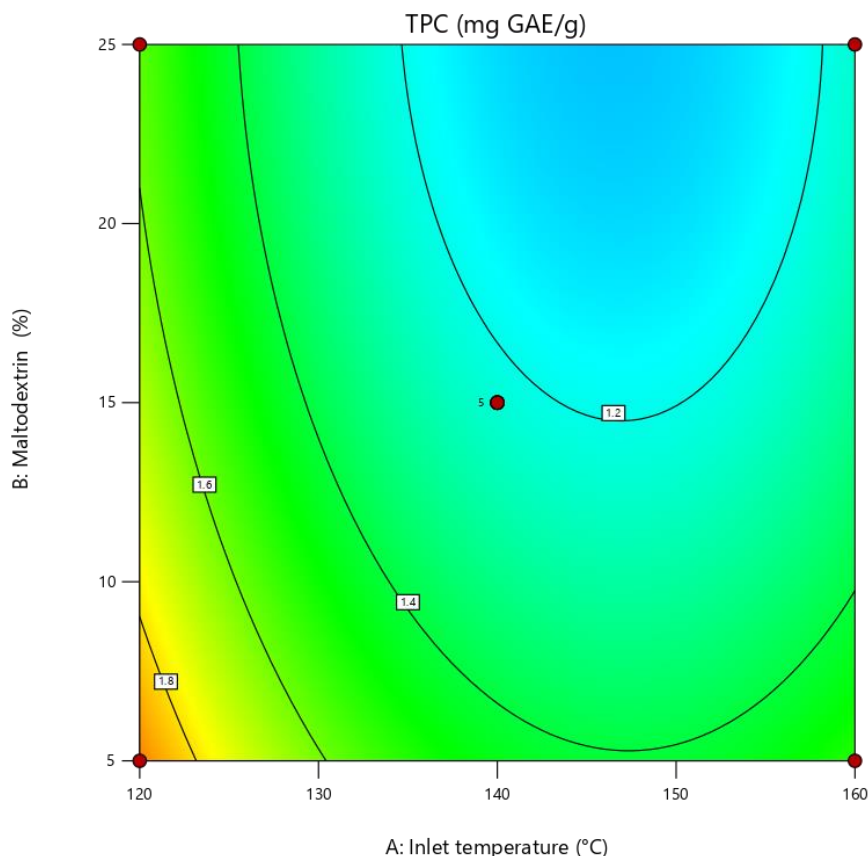
TPC (mg GAE/g)

● Design Points

0.88  2.06

X1 = A: Inlet temperature

X2 = B: Maltodextrin



(I)

Figure S1. 2-D plot for (A) MC, (B) Solubility, (C) Hygroscopicity, (D) WI, (E) Total sugar, (F) Soluble protein, (G) Crude protein, (H) Ash content and (I) TPC.

All desiccated powders had some residual moisture, which was found to vary significantly ($p < 0.05$) from 4.76% to 5.96%. The lowest MC was recorded at inlet temperature of 160°C and MD concentration of 25%. High inlet temperature ensures faster rate of moisture evaporation in the drying chamber and the high MD concentration aids the drying process by increasing the total solute in the feed, thereby, reducing the total moisture to be evaporated (Fazaeli, Emam-Djomeh, Kalbasi Ashtari, & Omid, 2012).

All the spray dried samples were readily miscible in water at room temperature. Increasing inlet temperature and MD concentration significantly ($p < 0.05$) increased the solubility of the spray dried powders. High inlet temperatures usually lead to higher moisture gradient, which in turn increases the particles' solubility (Cao et al., 2020). Presence of simple sugars in MD might have contributed to the high solubility of the developed powders. The results were consistent with the findings reported by Saikia et al. (2015).

Hygroscopicity was found to increase with the increasing inlet temperature and MD concentration. Steep moisture gradient between the low MC particles and the surrounding air results in greater moisture absorption upon exposure (Manickavasagan et al., 2015). Moreover, presence of MD aided the hygroscopicity of powders, given the inherent hygroscopic nature of its sugars (Manickavasagan et al., 2015; Nishad, Selvan, Mir, & Bosco, 2017).

WI of the reconstituted beverage ranged from 55.32 to 67.09 ($p < 0.05$). Probable occurrence of browning (Maillard reaction compounds) and/or caramelization of sugars, especially at high temperatures might have caused a decreased WI. Some published articles also found an increase in WI with increasing MD concentration (Michalska-Ciechanowska, Majerska, Brzezowska, Wojdyło, & Figiel, 2020; Srinivas, Vinoda, & Lingathoti, 2018), which is in congruence to the present investigation. Total soluble sugars of the powders were highly influenced by MD concentration ($p < 0.05$), and the highest value was recorded in powder developed at 120°C inlet temperature and 25% MD. Increase in sugar content of the powders with increasing MD concentration, was invariably credited to the

carbohydrates of MD. However, when compared to that of the feed (T6), soluble sugars of the powders reduced by 36.87% to 60.94%. This could be explained by their interaction with phenols and proteins in presence of high temperature, thus making them insoluble/unavailable for estimation (Mitrović et al., 2019).

In terms of soluble protein content, powders recuperated under different spray drying conditions showed significant differences ($p < 0.05$). It decreased by 44.66–85.60%, as compared to that of feed. Highest soluble protein was found in powder dried at 140°C with MD concentration of ~29%. High amount of MD perhaps averted the thermal denaturation of protein by providing an insulating barrier around them (Wang & Zhong, 2014). Concurrent to the soluble protein, crude protein level presented a decreasing trend with increasing inlet temperature; while an increase was noted with the rising MD concentration. The observed results can be justified similarly as for soluble protein (Wang & Zhong, 2014). Crude protein of the powders decreased in the range of 2.4–50.62%, when compared to that of feed. Ash content of the powders significantly ($p < 0.05$) decreased with increasing MD, probably because of the dilution of minerals in the feed by high amount of MD. Inlet temperature however, did not present any specific trend on the mineral content. Similar results were found by Siacor et al. (2020) while spray drying mango extracts. It is worth mentioning here that TPC of the feed was 11.45 ± 0.66 g GAE/100 g, which reduced by 58.88% as compared to that of fresh juice (Supplementary Table S9) and in the powders, it decreased by 98.20–99.23%, as compared to that of feed. Plausible reason for this reduction may be the interaction of whey protein with the juice phenols (Zhang et al., 2013). Table 4 depicts the decrease in TPC of the developed powders with increasing inlet temperature and MD concentration, which can be ascribed to their thermal degradation and increasing thickness of the carrier material (MD), leading to lesser retention of polyphenols in the particle (Siacor et al., 2020). Similar trend was observed for TFC. Maximum TFC was observed in the sample sprayed at 120°C and 5% MD, whilst the minimum value was recorded at 140°C and 29% MD. Nonetheless, no significant variation ($p > 0.05$) was apparent along the different inlet temperature and MD concentration.

Bulk and tap densities of the spray dried powders were not affected by the varying inlet temperature and MD ($p > 0.05$). This observation corroborates with the findings of Goula and Adamopoulos (2008), who developed spray dried tomato powder. HR ranged within 1.24 to 1.50, which classify the powders' nature to be moderate to highly cohesive. CI varied from 19.35% to 33.50%, signifying that the powders possessed a fair to poor flowability. These postulations agree with the inference from SEM images.

3.6. Particle Morphology

SEM images of the spray dried particles revealed their diameters ranging from 2 to 30 μm . Particles formed at high inlet temperature (140°C, 160°C or 168°C) appeared to have shrunk and wrinkled surfaces Figures 3 (A), (B), (C). On the other hand, the powder formed from low concentration of MD (%) featured fused and clumped appearance (Figure 3 (F)). The latter could be due to cohesiveness among the entities formed with low MD, a phenomenon commonly observed in case of spray dried products (Molina, Kaialy, Chen, Commandeur, & Nokhodchi, 2018) and is also explained earlier in dispersibility and hygroscopicity. Akin to our observation, Du et al. (2014) also stated that spray dried Persimmon powder produced with MD had smooth surface and spherical shape, and that produced with whey protein concentrate or egg albumin was more agglomerated and shriveled. Occurrence of hygroscopic simple sugars and organic acids in both *Dillenia indica* juice and whey might be the contributing factor to the surface instabilities like shrinkage, clumping and wrinkle formation in our samples (Shrestha, Ua-arak, Adhikari, Howes, & Bhandari, 2007; Siemons, Politiek, Boom, Sman, & Schutyser, 2020). Powder recuperated under optimum condition also showed shriveled and wrinkled morphology with size varying from 3 to 9 μm Figure 3 (G), which substantiates its intermediate cohesiveness and flowability Table 3.

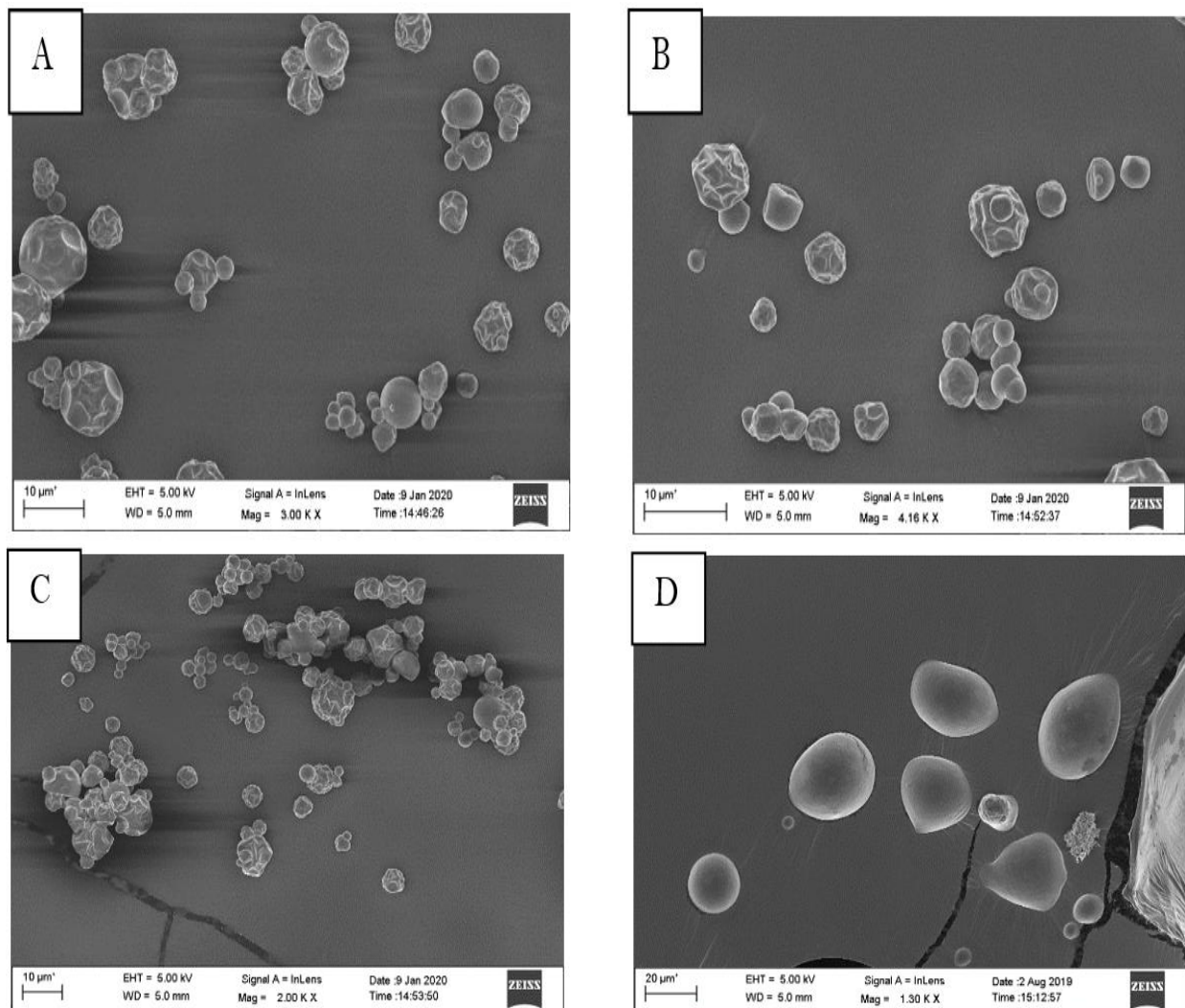
Table 4. Experimental values of the physicochemical properties of spray dried *Dillenia indica* and whey powder.

Inlet temperature (°C)	MD (%)	MC (%)	Solubility (%)	Hygroscopicity (g/100g)	Bulk Density (g/ml)	Tap Density (g/ml)	HR	CI (%)	WI	Soluble Protein (mg/g)	Sugar (mg/g)	Crude protein (%)	Ash (%)	TPC (mg GAE/g)	TFC (mg QE/g)
120	5	5.25±0.08 ^a	75.33 ± 1.53 ^b	15.70±0.15 ^b	0.38±0.07 ^a	0.50±0.15 ^a	1.30±0.10 ^a	23.08±0.31 ^c	67.56 ±0.46 ^h	36.64±0.27 ^b	184.86±1.03 ^b	57.75±0.29 ⁱ	6.90±0.07 ^d	2.06 ± 0.36 ^d	0.55 ± 0.09 ^a
160	5	5.01±0.14 ^a	80.67 ± 2.52 ^e	28.80±0.32 ^b	0.30±0.11 ^a	0.43±0.06 ^a	1.43±0.16 ^a	30.30±0.14 ^g	57.54 ±0.35 ^e	27.47±0.50 ^a	193.97±1.35 ^c	31.50±0.16 ^b	6.75±0.09 ^d	1.48 ± 0.26 ^{bcd}	0.50 ± 0.21 ^a
120	25	5.96±0.21 ^b	94.67 ± 3.06 ^k	20.00±0.45 ^c	0.38±0.15 ^a	0.53±0.14 ^a	1.37±0.35 ^a	26.92±0.34 ^e	55.81 ±0.29 ^b	103.57±0.44 ^j	298.82±1.14 ^m	58.75±0.34 ^m	3.45±0.08 ^a	1.89 ± 0.72 ^{cd}	0.54 ± 0.03 ^a
160	25	4.76±0.05 ^a	71.00 ± 2.65 ^a	27.19±0.62 ^f	0.26±0.05 ^a	0.38±0.25 ^a	1.50±0.28 ^a	33.50±0.12 ⁱ	56.19 ±0.64 ^b	88.65±0.07 ⁱ	287.69±0.42 ^l	38.50±0.03 ^c	3.66±0.14 ^a	1.36 ± 0.22 ^{abc}	0.47 ± 0.14 ^a
111.72	15	5.35±0.11 ^a	82.00 ± 1.00 ^f	24.20±0.37 ^d	0.36±0.20 ^a	0.50±0.31 ^a	1.40±0.23 ^a	28.57±0.16 ^f	56.27 ±0.94 ^{bc}	83.66±0.09 ^h	216.43±0.82 ^c	49.00±0.11 ^f	5.22±0.10 ^b	1.75 ± 0.04 ^{bcd}	0.53 ± 0.14 ^a
168.28	15	4.91±0.42 ^a	84.33 ± 0.58 ^b	26.70±0.18 ^f	0.31±0.09 ^a	0.43±0.27 ^a	1.39±0.27 ^a	28.13±0.17 ^f	55.13 ±0.49 ^a	50.81±0.48 ^d	236.78±0.16 ⁱ	40.25±0.35 ^d	5.26±0.08 ^b	1.49 ± 0.18 ^{bcd}	0.50 ± 0.03 ^a
140	0.86	5.11±0.19 ^a	76.67 ± 1.15 ^c	11.00±0.41 ^a	0.42±0.05 ^a	0.63±0.21 ^a	1.50±0.09 ^a	33.33±0.21 ⁱ	57.57 ±0.43 ^e	45.04±0.15 ^c	155.10±0.18 ^a	29.75±0.06 ^a	6.93±0.18 ^d	1.55 ± 0.31 ^{bcd}	0.52 ± 0.04 ^a
140	29.14	4.98±0.47 ^a	78.33 ± 3.15 ^d	37.54±0.19 ⁱ	0.31±0.19 ^a	0.45±0.09 ^a	1.45±0.16 ^a	31.25±0.27 ^h	63.95 ±0.81 ^g	105.58±0.49 ^k	264.78±0.36 ^k	56.50±0.27 ^l	3.20±0.08 ^a	0.88 ± 0.03 ^a	0.18 ± 0.05 ^a
140	15	5.21±0.28 ^a	83.33 ± 2.52 ^g	28.00±0.38 ^g	0.31±0.23 ^a	0.45±0.19 ^a	1.45±0.31 ^a	31.25±0.28 ^h	57.28 ±0.51 ^{de}	77.97±0.14 ^f	212.83±0.31 ^d	54.25±0.33 ⁱ	5.58±0.10 ^{bc}	1.25 ± 0.45 ^{ab}	0.43 ± 0.11 ^a
140	15	5.19±0.15 ^a	87.67 ± 2.08 ⁱ	23.83±0.27 ^d	0.32±0.25 ^a	0.40±0.23 ^a	1.24±0.26 ^a	19.35±0.22 ^a	61.72 ±0.75 ^f	79.11±0.06 ^g	224.68±0.58 ^g	45.50±0.31 ^e	5.18±0.08 ^b	1.21 ± 0.20 ^{ab}	0.34 ± 0.29 ^a
140	15	5.22±0.36 ^a	93.00 ± 2.00 ^j	24.00±0.12 ^d	0.34±0.14 ^a	0.45±0.08 ^a	1.32±0.17 ^a	24.14±0.19 ^d	56.82 ±0.40 ^{cd}	75.47±0.40 ^e	233.88±1.16 ⁱ	52.50±0.24 ^h	5.47±0.08 ^{bc}	1.19 ± 0.59 ^{ab}	0.36 ± 0.05 ^a
140	15	5.16±0.32 ^a	88.00 ± 4.36 ^j	30.06±0.09 ⁱ	0.36±0.23 ^a	0.50±0.25 ^a	1.40±0.19 ^a	28.57±0.24 ^f	61.86 ±0.77 ^f	75.96±0.15 ^e	227.65±0.82 ^h	55.25±0.10 ^k	5.50±0.14 ^{bc}	1.24 ± 0.35 ^{ab}	0.38 ± 0.06 ^a
140	15	5.13±0.09 ^a	92.67 ± 3.21 ^j	25.20±0.19 ^e	0.36±0.26 ^a	0.45±0.28 ^a	1.27±0.21 ^a	21.43±0.31 ^b	56.06 ±0.17 ^b	78.01 ±0.21 ^f	218.02±0.83 ^f	51.05±0.32 ^g	5.89±0.07 ^c	1.23 ± 0.32 ^{ab}	0.38 ± 0.03 ^a

Note: Value=Mean ± SD (N=3); ^{a-m} Means in the column with different letters are significantly different (p < 0.05).

3.7. In-Vitro Digestion

Digesta obtained from simulated gastric and intestinal digestion phases of T6 blend and its powder were compared with those of fresh *D. indica* juice and concentrated whey. It is apparent from Figure 4(A) that the percent protein released from the optimized powder, T6 blend and concentrated whey were lesser than that of fresh juice, possibly due to the presence of β -lactoglobulin in them, which is reported to be resistant to gastric digestion (Fu, Abbott, & Hatzos, 2002; Meena, Prasad, Khamrui, Mandal, & Bhat, 2021). As anticipated, the liberated protein from all the samples was seen to increase with time towards the intestinal digestion. However, the percent release from the optimized powder and T6 in both the phases of digestion was lower when compared to concentrated whey. This observation could be backed by the probable complexation/interaction of proteins with polyphenols, leading to lower leaching (Francielli et al., 2020).



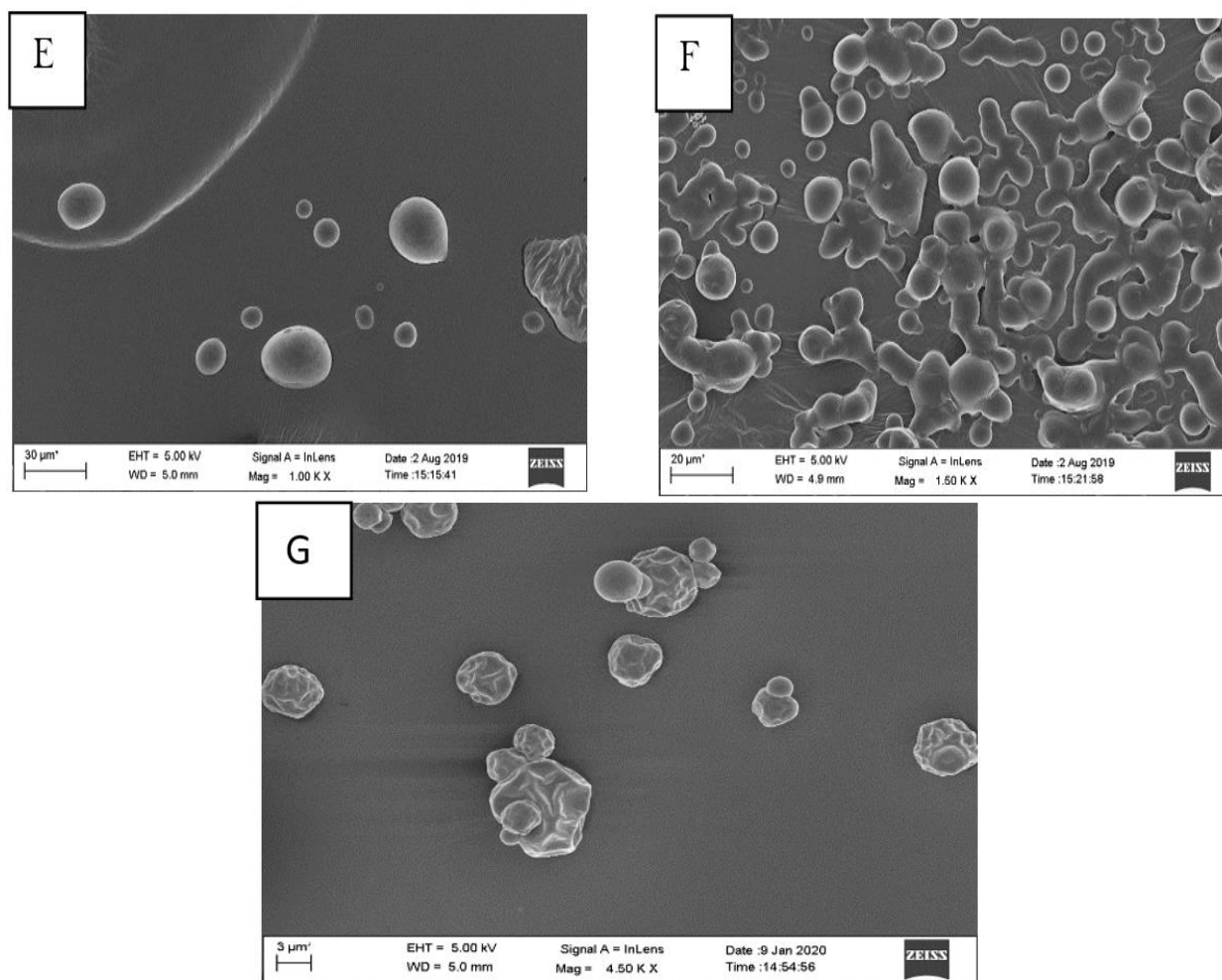


Figure 3. SEM images of beverage powder obtained at (A) 168°C and 15% MD (B) 140°C and 15% MD, (C) 140°C and 29% MD, (D) 111.7°C and 15% MD, (E) 120°C and 2.5% MD, (F) 140°C and 0.89%MD, (G) 160 °C and 19.7% MD.

Furthermore, the antioxidant activity of the digesta obtained at different phases of gastrointestinal digestion is shown in [Figure 4\(B\)](#). The overall DPPH scavenging activity of the optimised powder, fresh juice, T6 blend, and whey, was lower after digestion than that prior to digestion ($p < 0.05$). It reduced by 85% in the optimized powder and that of whey, T6 blend and fresh juice reduced by 72%, 90% and 95% respectively. Similar observation of drastic reduction in antioxidant activities after digestion is reported by numerous authors ([Desseva & Mihaylova, 2020](#); [Mihaylova et al., 2021](#)).

The polyphenols were partly stable under low pH of gastric period and hence were available for radical scavenging ([Mihaylova et al., 2021](#)). In the gastric phase, both the juice and T6 blend exhibited high antioxidant activity, while whey showed the lowest activity. It is interesting to mark that the antioxidant activity of whey digesta showed a persistent increase, which could be explained by the release of bioactive peptides under proteolytic condition ([Francielli et al., 2020](#)). Conversely, antioxidant features of the juice and blend started to decline with time in the intestinal phase. This observation could be explained by the sensitive nature of polyphenols under alkaline condition of the latter. Similar observation has been reported by [Mihaylova et al. \(2021\)](#). In case of the optimized powder, its scavenging activity seemed to increase during the digestion transit ($p > 0.05$). Development of protective layers by drying adjunct as granular matter could illustrate the stability of polyphenols within the powder and the likely formation of active peptides from its constituent, can relate to its antioxidant potential. Lesser reduction of scavenging activity in optimized powder and T6 as compared to that of fresh juice again vouches the significance of whey and spray drying in preserving the bioactive ingredients against the harsh gut condition.

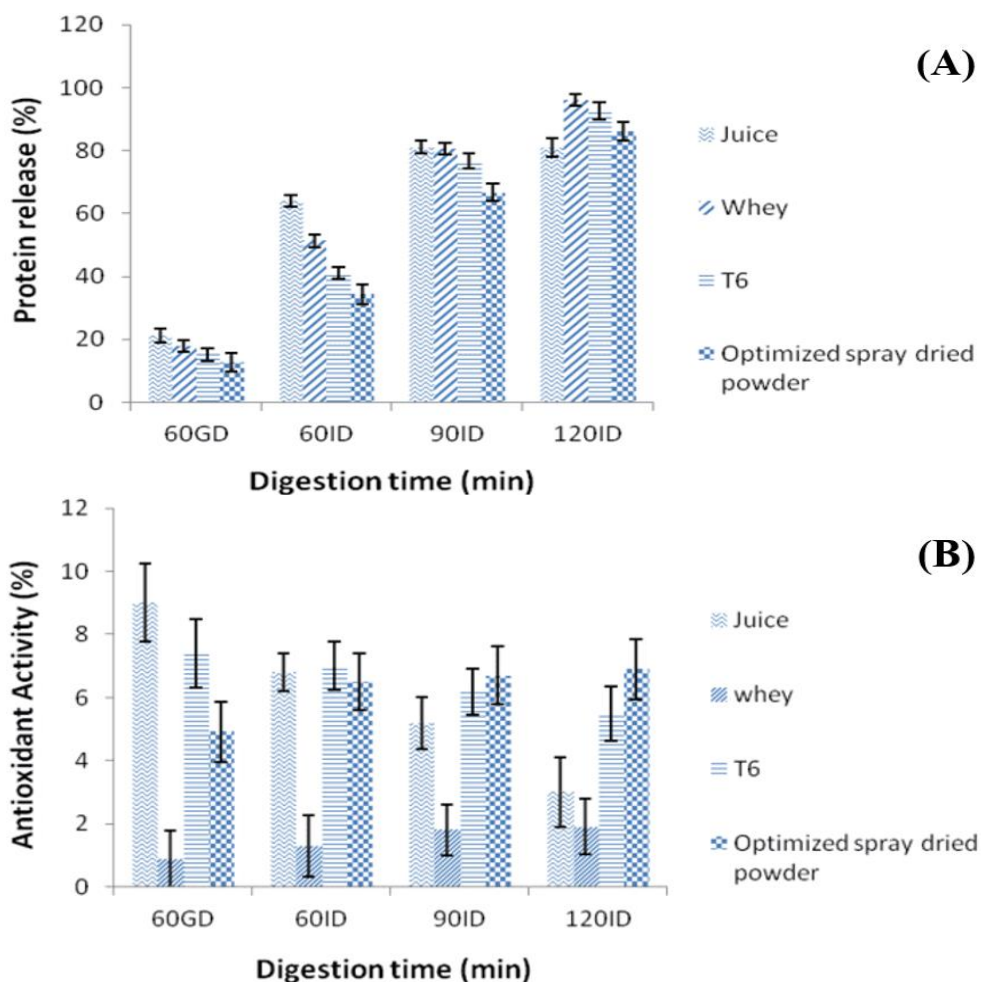


Figure 4. (A) Percent release of protein from *Dillenia indica* juice, concentrated whey, T6 blend and optimized powder during simulated Gastric digestion (GD) and Intestinal digestion (ID); (B) Antioxidant activity of *Dillenia indica* juice, T6 blend, concentrated whey and optimized powder during simulated Gastric digestion (GD) and Intestinal digestion (ID).

4. CONCLUSION

Results of the present investigation paved a novel methodology for processing an underutilized fruit using a protein-rich industrial byproduct (whey), into a beverage powder which could be reconstituted readily without compromising much on its antioxidant as-well-as techno-functional properties. Quadratic polynomial models derived for the responses (powders' yield, dispersibility, antioxidant activity and organoleptic acceptability) were found to be significant ($p \leq 0.05$) and adequate for prediction, which was validated by conducting confirmatory trials at the predicted optimum condition (160°C inlet temperature and 19.727% MD). Effect of the independent variables on additional quality parameters (MC, solubility, hygroscopicity, WI, Hausner and Carr indices, bulk and tap densities, total sugar, protein, minerals, phenols and flavonoids) of the powders were also assessed, wherein maximum retention of polyphenols, flavonoids and antioxidant activity was observed at 120°C inlet temperature and 5% MD, while the highest protein content was noticed at 140°C inlet temperature and 29% MD. Although the spray dried powders showed a drastic reduction in their phenolic content due to high temperature processing, nevertheless possible formation of antioxidative Maillard reaction products might have preserved the powders' antioxidant activity to a considerable extent. Moreover, the reconstituted beverage rendered better WI when compared to the fresh juice, indicating probable masking of the undesirable brown pigments by the amalgamation of whey and MD. Powder obtained under the optimized condition exhibited good solubility and dispersibility, intermediate hygroscopicity, fair flowability and retained high sugar, protein, WI and antioxidant potential when compared to the feed (T6). In vitro digestion of the optimized powder was compared to those of T6, fresh juice and concentrated whey. Lower release of

soluble protein was witnessed in optimized powder in contrast to T6 and whey. Furthermore, the antioxidant activity of digesta from optimized powder showed a gradual increase with advancing phases of digestion ($p < 0.05$), while those of fresh juice and T6 declined progressively due to probable degradation of polyphenols under alkaline intestinal environment. Therefore, the study proved that systematically optimized condition can help in creating a low-cost nutrient-dense beverage powder from an otherwise astringent minor fruit, without the addition of any exogenous sweetening agent.

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