

Optimization of spray drying parameters of *Amaranthus cruentus* juice using Response Surface Methodology

Gowrishankar L^{*1}, Ragesh S A¹, Sudeeptha V Mohan¹, Raja Rajeshwari E¹, Divyadharshini S¹, Sathanya P S¹, Poojitha P¹, Balakrishnaraja R¹, Sureshkumar J²

¹Department of Food Technology, Bannari Amman Institute of Technology

²Department of Food Technology, Saintgits college of Engineering

ABSTRACT

Amaranthus cruentus is a highly perishable leafy vegetable, possessing enormous phytochemicals and nutrients with potential antioxidant, and anti-inflammatory properties. The edible parts of the plant have been reported as a vital source of water soluble betalain compounds alternate to beet roots. Our present study is carried out to determine and evaluate the physicochemical properties of *Amaranthus cruentus* juice powder with varying concentrations of drying agent (maltodextrin) and inlet temperatures of spray drying. The optimization of juice powder is devised by using Response Surface Methodology (Central Composite Design) which optimizes the drying parameters such as inlet air temperature (IAT) (130–150°C), maltodextrin concentration (MD) (5–10%) and the characterization response are in terms of the Powder Yield, Colour, Water Activity, Betalain Retention (BR), Moisture Content, Bulk Density, Tapped Density, Cohesiveness (HR), Flowability (CI), Water Solubility Index (WSI) and Water Absorption Capacity (WAI). According to Response Surface Methodology, an inlet temperature of 150°C with 10% maltodextrin concentration was observed as the optimum conditions. The powder yield significantly increases with increasing Maltodextrin concentration while the moisture content decreased. The spinach juice powder could increase the use of spinach juice powder in various nutraceutical applications thus helping in its commercial product formulation.

Keywords: Spray drying, optimization, spinach juice powder, betalain, RSM, maltodextrin

1. INTRODUCTION

Plants of the *Amaranthaceae* family have coloured vegetative tissues that contain diverse betalain pigments and are often produced in high biomass, and used as potential substitutes for the betalains instead of beet roots. The findings indicated that *Amaranthus* pigments have a high potential for use as natural food colourants. Betalains are nitrogenous pigments that are water soluble. They are classified into two structural groups: red to red-violet betacyanin and yellow betaxanthins.

Natural pigments have been replacing synthetic colourants in the last 20 years due to their safety and health benefits, as well as strong consumer demand for more natural products (Boyd, 1998; Jackman & Smith, 1996). With naturally increasing markets for natural colourants, it is valuable to develop new or alternative sources. Nowadays, the main commercial source of betalain (concentrates or powders) is beet roots (*Beta vulgaris*).

This project is focused on the optimization of spray dried spinach juice powder, particularly from the *Amaranthus cruentus*. Betalains are primarily made up of betacyanin and betaxanthin, both of which contain betalamic acid as the bioactive unit. The stability of betalains varies with water activity, temperature, oxygen and light exposure. Red beet, *Amaranthus*, and *Hylocereus Polyrrhizus* (Dragon Fruit), are the richest natural sources of betalains. It is difficult to dehydrate *Amaranthus cruentus* Leaves while maintaining a high betalain content.

Spray drying is a popular method for encapsulating sensitive ingredients by using carrier agents that act as a coating material or a wall material to isolate them from the outside environment and protect against oxidation and to produce a high-quality powder with low water activity and ease of transport and storage. The powder produced by spray drying spinach juice without a carrier is highly hygroscopic and unsuitable for food applications due to low quality and stability. To avoid stickiness of the wall chamber and among the dried particles, high molecular weight drying carriers

such as Maltodextrin (MD) are added to juices. It is the most commonly used microencapsulating agent. It yields powders with good reconstitution properties, low water activity, and storage suitability. The physicochemical properties of spray-dried powders are determined by carrier type and concentration and drying conditions. Spray-dried spinach juice powders can be incorporated into food systems to provide a variety of functional benefits which should ideally reconstitute instantly and be used as a betalain-rich additive.

However, little is known about how encapsulating agents and drying conditions affect the physicochemical properties of spinach juice powder. The current study looks into the effects of Inlet Air Temperature (IAT) and Maltodextrin Concentration (MDC) on spray drying spinach juice and assesses the physical properties of the powder produced. There has been a significant amount of work done on spray drying of beetroot juice to date. However, there have been few scientific studies on the drying of *Amaranthus cruentus* juice. Based on the aforementioned, this study is being conducted to investigate the feasibility of spray drying spinach juice and to assess the physicochemical features of the powder produced.

Central Composite Design (CCD) was employed which is a Response Surface Methodology (RSM) module in the Design-Expert software. Important parameters such as Powder Yield, Water Activity, Colour (a^*), Betalain Retention (BR), Moisture Content (MC), Bulk Density (BD), Tapped Density (TD), Flowability (CI), Cohesiveness (HR), Water Solubility Index (WSI), Water Absorption Index (WAI) were empirically determined and successfully optimized via RSM.

2. MATERIALS AND METHODS

2.1 SAMPLE PREPARATION

Fresh spinach (*Amaranthus cruentus*) was acquired from a local market in Sathyamangalam, Tamil Nadu. Maltodextrin (DE 16.5 to 19.5%) was used as a drying agent. The leaves and stalks of spinach were ground, and the pulp was diluted with 1:1.5 (w/v) distilled water. The spinach juice was extracted with muslin cloth and centrifuged at 6000 rpm and 10°C for 10 minutes in a Refrigerated Centrifuge (Remi C24 Plus). The supernatant was then collected. Based on preliminary trials and prior study results, the minimum and maximum values of inlet air temperature (IAT) and maltodextrin concentration (MDC) were chosen to be 130 - 150°C and 5 - 10% respectively (Bindu Bazaría et al., 2016). The drying agent was mixed to the extracted juice using an stirrer at 500 rpm for 5 minutes to obtain a thoroughly mixed solution (Swaminathan Santhalakshmy et al., 2015).

2.2 SPRAY DRYING OF SPINACH JUICE

A laboratory-scale co-current spray dryer was used to perform the spray drying (SM Scientech, Kolkata, India). The feed sample was fed into the spray drying chamber, where the rotating speed of the pump was used to control the feed flow rate. The atomizer nozzle had an inner diameter of 0.5 mm and the feed flow rate for the drying process was kept constant at 315 mL/h with a pressure of 2 bar (Bindu Bazaría et al., 2016). After drying, the obtained powder was collected in an insulated glass bottle connected at the end of cyclone. The outlet temperature was recorded as $80 \pm 10^\circ\text{C}$ and the powders were packed in polyethylene pouches and stored in a desiccator containing silica gel at 25°C until further analysis (Yojhansel Aragüez-Fortes et al., 2019).

2.3 EXPERIMENTAL DESIGN (RSM)

The optimization method was carried out using the Central Composite Design (CCD). The factors include Inlet Air Temperature (IAT) ranging from 130°C to 150°C and Maltodextrin Concentration (MDC) ranging from 5% to 10%. The aforementioned factors produced a total of 13 trials with 5 replicates at the centre point which was used for conducting the spray drying of spinach juice as shown in Table 2.1. The second order polynomial (SOP) equation was used to analyse the responses (Bindu Bazaría, Pradyuman Kumar, 2016).

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k \beta_{ij} x_{ij}$$

Where Y is the desired value of response, β_0 is the constant; β_i , β_{ii} and β_{ij} are linear coefficient, quadratic coefficient, and cross-product coefficients respectively. x_i and x_{ij} are the levels of the independent variables. The analysis of variance (ANOVA) was carried out using SPSS software (IBM SPSS version 26) (Mohammad Rezaul Islam Shishir et al., 2016).

RUNS	FACTOR 1: INLET TEMPERATURE (°C)	FACTOR 2: MALTODEXTRIN CONCENTRATION (%)
1	140	7.5
2	150	5
3	140	7.5
4	140	7.5
5	125.858	7.5
6	130	5
7	140	3.96447
8	150	10
9	140	7.5
10	130	10
11	140	7.5
12	154.142	7.5
13	140	11.0355

Table 2.1 RSM Trials

2.4 POWDER ANALYSIS

2.4.1 POWDER YIELD

The powder yield after spray drying of the samples was determined using the following formula (Swaminathan Santhalakshmy et al., 2015).

$$\text{Powder yield (\%)} = \frac{\text{Obtained spray dried powder (g)}}{\text{Feed sample (g) + Drying agent (g)}} \times 100$$

2.4.2 WATER ACTIVITY (a_w)

The water activity of the powders was determined at 25°C using a water activity analyser (Pre AquaLab).

2.4.3 BETALAIN RETENTION

The betalains were evaluated by mixing 0.1g of powder sample with 10ml of 50% ethanol and agitating for 10 seconds. The homogenate was centrifuged at 6000rpm for 10 minutes using a refrigerated centrifuge and this was repeated twice. The concentration of betalains (betacyanins and betaxanthins) in the samples was evaluated using UV-Visible Spectrophotometer (Shimadzu UV 1800) at 538nm (betaxanthins) and 480nm (betacyanins). For juice samples, 1 mL of juice was diluted with 9mL of distilled water, and the concentration of betalains was determined using the absorbance

method described above. The absorbance was noted for each wavelength and the concentration was determined using the formula.

$$\text{Betain Concentration } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{A} \times \text{DF} \times \text{MW} \times 1000}{\text{e} \times \text{l}}$$

where A is the absorbance, DF the dilution factor and l is the path length (1cm) of the cuvette. For measurement of betacyanins and betaxanthins, the subatomic weights (MW) and molar elimination coefficients (e) (MW=550g/mol; e=60,000 L/mol cm in H₂O and MW=308 g/mol; e=48,000 L/mol cm in H₂O) were used(Gaurav Pandey et al., 2018).

The percentage of betalain retention was calculated from the concentration of betalains in juice and its respective powder sample.

2.4.4 COLOUR

The colour of the powders was determined by using Hunter colour lab (ColorFlex EZ). The CIE values were obtained in terms of L* (light/dark), a* (red/green) and b* (yellow/blue). The a* value was considered as a measure of the pigment present in *Amaranthus cruentus* juice powder (Bindu Bazaria, Pradyuman Kumar, 2016).

2.4.5 MOISTURE CONTENT

The moisture content analysis was conducted using a moisture analyser (Mettler Toledo HE 53) which works on the principle of the AOAC method of moisture determination. 3g of sample was measured and dried at 105°C with halogen lamp as the heating element until constant weight was obtained. The final moisture content was recorded.

2.4.6 BULK DENSITY AND TAPPED DENSITY

3g of the sample was loaded into a 10 mL graduated cylinder and the volume occupied by the cylinder was measured. The bulk density (ρ_B) was calculated by the formula (Hira Yuksel & Safiye Nur Dirim, 2020).

$$\rho_B = \frac{\text{Mass of the sample (g)}}{\text{Volume accupied in the cylinder (mL)}}$$

The tapped density (ρ_T) was calculated by tapping the cylinder manually 350±10 times (70 taps per minute for 5 minutes) with a displacement amplitude of 2±0.5 cm from the base height and the final volume occupied by the cylinder after tapping was recorded. The tapped density (ρ_T) was calculated by the formula (Hira Yuksel & Safiye Nur Dirim, 2020).

$$\rho_T = \frac{\text{Mass of the sample (g)}}{\text{Volume accupied in the cylinder after tapping (mL)}}$$

2.4.7 FLOWABILITY (CARR INDEX) AND COHESIVENESS (HAUSNER RATIO)

The flowability of the powders was evaluated in terms of Carr index (CI) (Table 2.2) and the cohesiveness of the powders was evaluated in terms of Hausner ratio (HR) (Table 2.3), which was calculated using the following formulae from the respective values of bulk density (ρ_B) and tapped density (ρ_T) (Swaminathan Santhalakshmy et al., 2015).

$$\text{CI} = \frac{\rho_T - \rho_B}{\rho_T} \times 100$$

$$\text{HR} = \frac{\rho_T}{\rho_B}$$

CI (%)	Flowability
< 15	Very good

15-20	Good
20-35	Fair
35-45	Bad
>45	Very bad

Table 2.2 Classification of powder flowability based on Carr index (CI)

HR	Cohesiveness
<1.2	Low
1.2-1.4	Intermediate
>1.4	High

Table 2.3 Classification of powder cohesiveness based on Hausner’s ratio (HR)

2.4.8 WATER SOLUBILITY INDEX AND WATER ABSORPTION INDEX

In a 50 mL centrifuge tube, 2g of samples were added to 40 mL of distilled water, agitated intermittently for 2 minutes and centrifuged at 3000rpm and 10°C for 10 minutes. The supernatant was carefully filtered, then poured off into a petri dish and dried in a hot air oven at 105°C overnight. The solid content of the dried supernatant was recorded, and the water solubility index (WSI) was calculated using the formula,

$$WSI = \frac{\text{Weight of dry solid in supernatant (g)}}{\text{Weight of the sample (g)}} \times 100$$

After centrifugation, the remaining weight of the wet solids were recorded, and the water absorption index (WAI) was calculated using the formula,

$$WAI = \frac{\text{Weight of wet solid in supernatant (g)}}{\text{Weight of the sample (g) – Weight of dry solids in supernatant (g)}} \times 100$$

3. RESULTS AND DISCUSSION

The physicochemical properties of the powders were examined, and the results were obtained as below (Table 3.1).

Response 8: Flowability (CI)	Response 9: Cohesiveness (HR)	Response 10: Water Solubility Index (%)	Response 11: Water Absorption Index (WAI)
38.558	1.627	87.6	3.258
41.671	1.714	73.15	2.839
36.093	1.564	88.31	3.653
36.642	1.578	86.59	3.733
39.513	1.734	82.5	5.517
37.209	1.592	10.65	1.293
39.766	1.6	67.75	2.51
35.29	1.545	80.25	2.362
38.7989	1.633	87.42	3.231
31.243	1.455	71.65	2.707
35.046	1.538	88.29	3.176
17.724	1.215	78.6	3.105
36.114	1.565	74.45	2.747

Run	Factor 1: Inlet Air temperatur (°C)	Factor 2: Maltodextrin Concentratio (%)	Response 1: Powder Yield (%)	Response 2: Water Activity	Response 3: Colour (a*)	Response 4: Betalain Retention (%)	Response 5: Moisture content (%)	Response 6: Bulk density (g/cm ³)	Response 7: Tapped density (g/cm ³)
1	140	7.5	3.543	0.346	8.55	6.994	6.03	0.3614	0.5882
2	150	5	1.416	0.358	9.87	5.361	7.94	0.277	0.4761
3	140	7.5	3.795	0.351	8.83	7.035	6.23	0.3805	0.5954
4	140	7.5	3.648	0.348	8.79	9.704	6.12	0.374	0.5903
5	125.858	7.5	3.768	0.281	9.76	3.702	4.75	0.3703	0.6122
6	130	5	2.806	0.32	8.84	7.713	6.24	0.3488	0.5555
7	140	3.96447	2.34	0.241	8.21	4.031	5.33	0.375	0.6
8	150	10	4.518	0.33	15.38	11.552	6.61	0.3529	0.5454
9	140	7.5	3.367	0.326	7.56	4.144	5.87	0.3251	0.5312
10	130	10	4.598	0.298	5.98	8.603	4.7	0.375	0.5454
11	140	7.5	3.585	0.359	9.28	5.369	6.29	0.3915	0.6022
12	154.142	7.5	3.337	0.368	7.57	4.943	4.22	0.3797	0.4615
13	140	11.0355	4.274	0.276	21.52	10.486	4.09	0.4166	0.6529

Table 3.1 Effect of different physicochemical properties on IAT and MDC

3.1 POWDER YIELD

The yield of the powder was calculated for all the trials. The powder yield ranged from 1.416% to 4.598%. There was a significant difference ($p < 0.05$) in the linear effect of IAT and MDC on the yield of the powder samples. As

the concentration of maltodextrin addition increased, the yield showed a positive outcome, while there was a negative effect of IAT on yield (Fig 3.1). Maltodextrin adds to the total soluble solids of the sample thus increasing the powder yield. The results were in accordance with the studies by Ki-Chang Lee et al., 2017 where the inlet temperature had a negative effect on yield which they predicted was due to the melting of powder on the walls of the chamber at higher temperatures but in contradiction with the studies made by Bindu Bazaria et al., 2016 where the increase in inlet temperature of beetroot juice increased its yield.

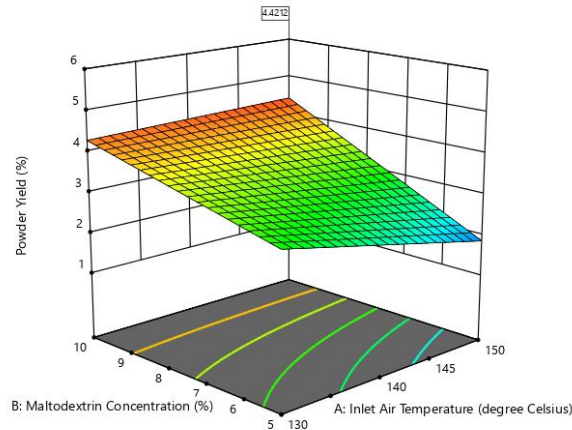


Fig 3.1Effect of IAT and MDC on powder yield

3.2 WATER ACTIVITY

Water activity is the measure of free water in a sample. It is an important factor responsible for the stability of the powders as higher water activity increases the microbial activity. The water activity of our powder samples ranged from 0.241 to 0.368 which is considered to be optimum for juice powders. There was a significant difference ($p < 0.05$) between the linear effect of IAT and MDC on the water activity. While the increase in IAT increased the water activity of the samples, the increasing concentration of the addition of maltodextrin decreased the water activity of the samples (Fig 3.2).

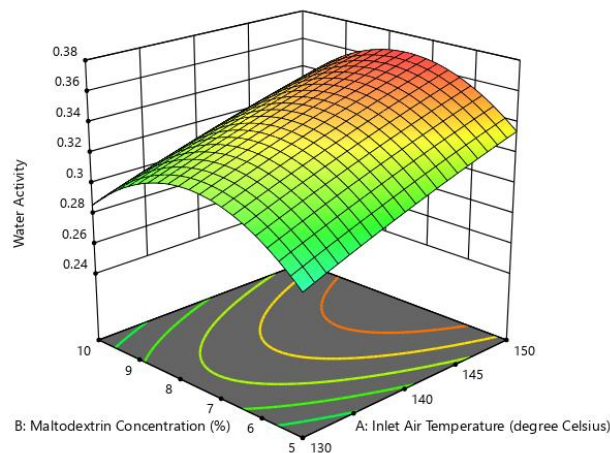


Fig 3.2 Effect of IAT and MDC on Water activity

3.3 BETALAIN RETENTION

The betalains are one of the most important characteristic features of *Amaranthus cruentus* that gives it the pink to violet colour on its leaves and stalks. Maltodextrin, which is added as a drying agent, also helps in the encapsulation

of the pigment thus making it available in the juice powder. Since betalains are known to denature at higher temperatures, the effect of IAT and MDC on the retention of betalains in the juice powders are studied. Analysis of variance shows that the effect of IAT and MDC on the retention of betalains is significant ($p < 0.05$). The range of retention of betalains is from 3.702% to 11.552%. The highest rate of retention of betalains (11.552%) is at the highest IAT and MDC combination (150°C and 10%). This clearly shows that the addition of Maltodextrin has encapsulated the pigment efficiently (Fig 3.3).

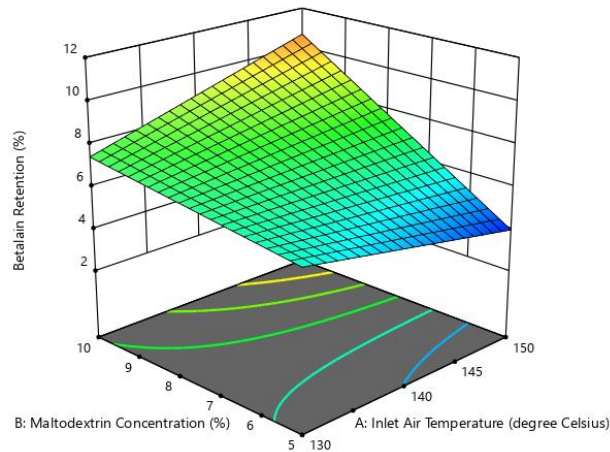


Fig 3.3 Effect of IAT and MDC on Betalain Retention

3.4 COLOUR

Colour is a significant quality factor since it represents the sensory appeal and quality of powders. The L^* , a^* , b^* values represent the lightness/darkness, redness/greenness and yellowness/blueness of the powders respectively. The present study takes the a^* (redness) values into consideration as the powders are known to have betalains thus making the redness of the powders an important parameter. The analysis of variance showed no significant difference between the effect of inlet temperature and maltodextrin concentration on the color of the powders but the presence of betalains did have an effect on the redness of the samples. The sample (140°C and 11.0355%) with the highest betalain concentration (9.2491 mg/L) had the highest a^* value (21.52) thus proving that the increased concentration of betalains led to a significant rise in the a^* value. The redness of a powder sample also corresponds to the L^* and b^* values of the samples thus predominantly showing a particular colour (Fig 3.4).

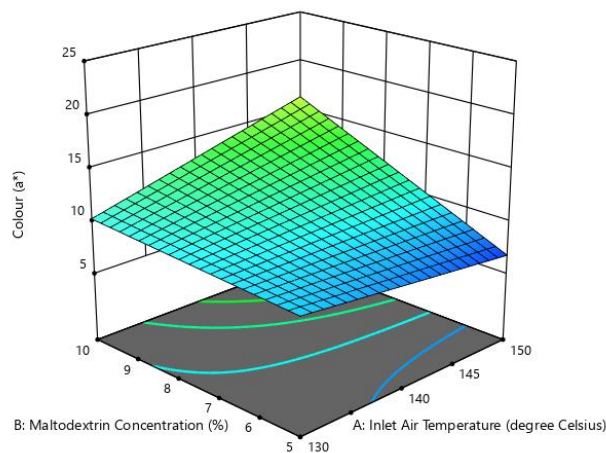


Fig 3.4 Effect of IAT and MDC on Colour (a^*)

3.5 MOISTURE CONTENT

The moisture content is one of the most important parameters required to study the stability and characteristics of juice powders. Moisture content has its own effect on every physicochemical property of the powders. The moisture content of our powders ranged from 4.09% to 7.94% which is considered to be optimum for juice powders. There is a significant difference ($p < 0.05$) between the IAT and MDC and the moisture content of the powders. The addition of increasing concentration of maltodextrin had a negative effect on the moisture content as maltodextrin addition leads to reduced water evaporation due to high TSS (*Mohammad Rezaul Islam Shishir et al., 2016*). This was in accordance with the studies made by Arasb Dabbagh Moghaddam et al., 2017 and Ki-Chang Lee et al., 2016 but our studies on the effect of inlet temperature on the moisture content were contradictory as IAT had a positive effect on the moisture content of our samples (Fig 3.5).

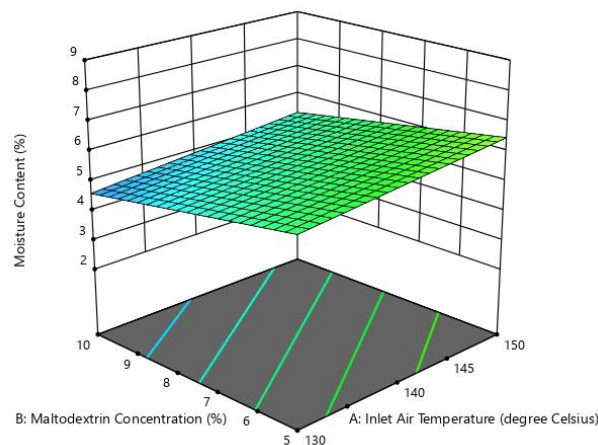


Fig 3.5 Effect of IAT and MDC on Moisture Content

3.6 BULK DENSITY AND TAPPED DENSITY

The density of material when packed or piled in bulk is referred to as its bulk density. According to the table, bulk density ranges from 0.2777 to 0.4166 g/mL. The influence of the carrier agents and their addition rate on the bulk density (BD) is highly significant ($p < 0.05$) according to the analysis of variance. The bulk density decreased as the MDC decreased which was in contradiction with the studies made by Arasb Dabbagh Moghaddam et al., 2017. This may be because of the heaviness of the juice powder and occupying less space thus leading to increased bulk density values (*Swaminathan Santhalakshmy et al., 2015*). The increase in IAT however had a negative effect on the bulk density which was in accordance with the studies made by Arasb Dabbagh Moghaddam et al., 2017. The tapped density values of the powders also had a similar negative effect with IAT and a positive effect with MDC which was in accordance with the studies made by Swaminathan Santhalakshmy et al., 2015 (Fig 3.6).

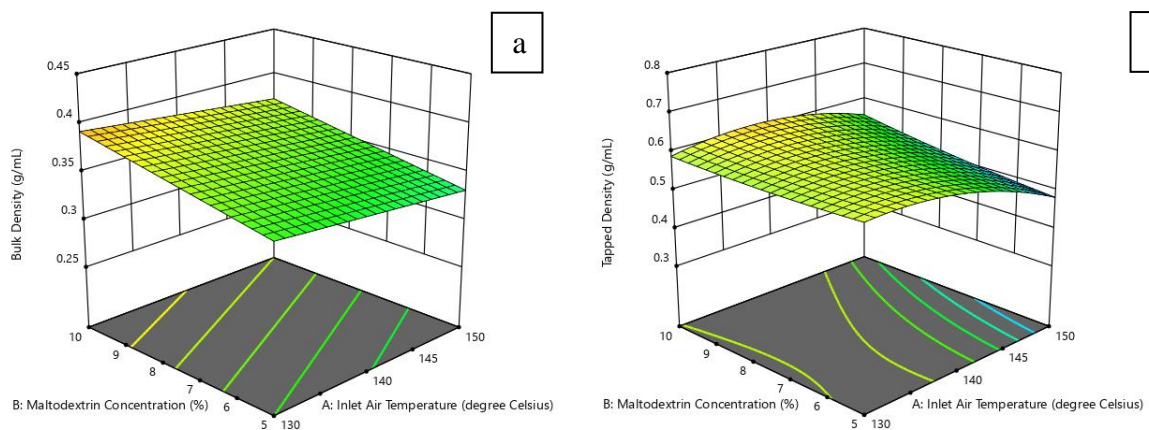


Fig 3.6 Effect of IAT and MDC on (a) Bulk Density and (b) Tapped Density

3.7 FLOWABILITY AND COHESIVENESS

Flowability of powders and granular materials can be calculated from their apparent and bulk density, which shows that values of Hausner’s ratio (HR) and Carr compressibility index (CI) ranges from 1.215 - 1.7342 and 17.7248-42.6719% respectively. Higher values of CI indicate poor flowability and lower values of HR indicates poor cohesiveness. The cohesiveness and flowability are dependent on each other, the lesser the cohesiveness, the better the flowability of the powder is. The effect of the carrier agent and IAT on the Hausner ratio (HR) and Carr's compressibility index (CI) was highly significant ($p < 0.05$) according to the analysis of variance. Our study implies that the IAT and MDC had a negative effect on the values of CI. All powders were difficult to flow except the powder with IAT of 154°C, which showed medium flowing character, which was in accordance with the study by Swaminathan Santhalakshmy et al., 2015. The cohesiveness of the powders also had the same effect as Flowability with a negative relationship with IAT and MDC (Fig 3.7).

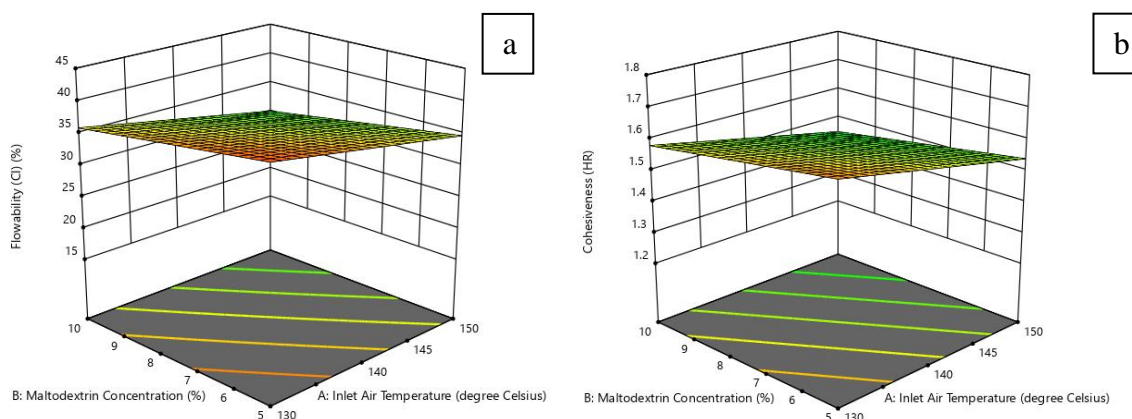


Fig 3.7Effect of IAT and MDC on (a) Flowability (CI) and (b) Cohesiveness (HR)

3.8 WATER SOLUBILITY INDEX AND WATER ABSORPTION INDEX

Water Solubility Index (WSI) and Water Absorption Index (WAI) are very important properties of a powder that measures the extent of solubility and absorption of the powders in the water which determines the overall acceptability of the powder properties as a possible product. The values of WSI ranged from 10.65% to 87.6% and the values of WAI ranged from 1.293 to 5.517. The effect of IAT and MDC on WSI and WAI were highly significant ($p < 0.05$). The WSI had a positive relationship with IAT and MDC and WAI had a negative relationship with IAT and a positive relationship with MDC. The effect of IAT on WSI is in accordance with the study conducted by Swaminathan Santhalakshmy et al., 2015 but in contradiction with the study made by Ki-Chang Lee et al., 2017 where the increase in inlet air temperature decreased the solubility of their powders (Fig 3.8).

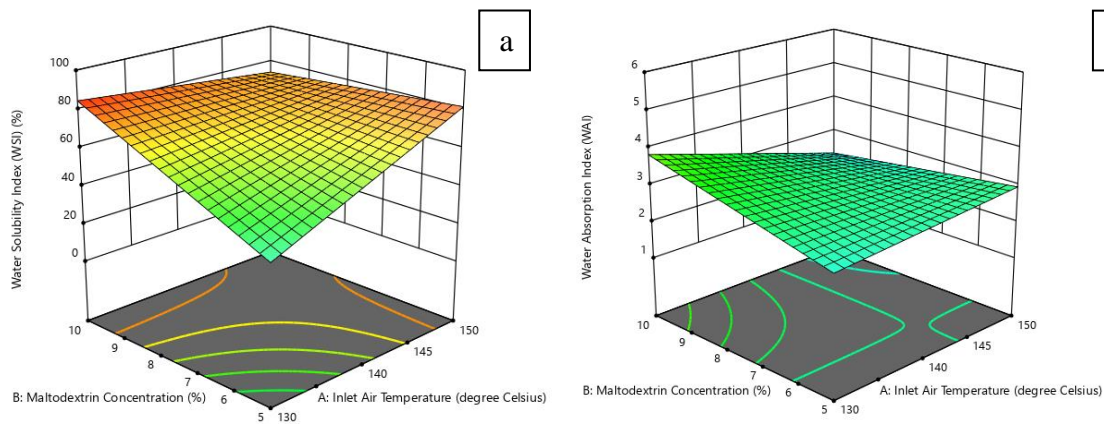


Fig 3.8Effect of IAT and MDC on (a) Water Solubility Index (WSI) and (b) Water Absorption Index (WAI)

4. CONCLUSION

This study looked at the effects of carrier addition rate, and inlet air temperature on the physicochemical parameters of spinach juice powder. The moisture content of the samples with higher IAT was higher while the yield of the powders was lower at higher IAT. The retention of betalain was directly proportional to the concentration of maltodextrin. Powders with high IAT also resulted in better flowability and lesser cohesiveness. The RSM optimization was studied while setting the optimum parameters of the samples yielding strong product recovery, lower moisture content, high betalain retention and acceptable flowability. The Design Expert Software suggested 150°C IAT and 10% MDC as the optimum combination with a desirability of 0.568. The findings of this study show that spray drying can generate high-quality powders with optimal moisture content and water activity, indicating that such powders have a lot of promise for usage in the food industry.

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