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Optimization of freeze drying parameters for moringa (*Moringa oleifera*) flower powder by using response surface methodology and principal component analysis

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ABSTRACT

Moringa oleifera **Lam. is an incredible plant because of vital nutrients such as minerals, vitamins and phytochemicals. The present work is focused on studying the optimization and quality attributes retention in moringa flowers in a freeze dryer (FD). Because the conventional drying process takes more time and energy which will affect the product quality and safety. Response surface methodology (RSM) was employed to optimize the effect of drying temperature (- 65 to - 45°C), vacuum pressure (0.5 to 2.5 mmHg) and drying time (18 to 24 h.) on the vitamin C, total antioxidant activity(TAA) and hygroscopicity (HS) of moringa flower. The developed model response R^2 values of vitamin C 0.96, total antioxidant activity 0.97 and hygroscopicity0.95. Based on response surface and desirability (0.74) functions, moringa flower was freeze sdried at - 63.75°C for18 hr under 0.55 vacuum pressure had an optimum level of vitamin C 285.84 mg/100g, TAA 453.20 mg/100g and HS 1.57 percent. Freeze dried moringa flower powder at -55°C had maximum drying characteristics with special reference to high powder recovery (98.75%) and excellent flowability.The first principal component, accounting for 52.15 per cent and two 23.02 per cent of the total variance resolved the different drying temperatures.**

Keywords: Dehydration, Freeze drying, moringa flower, nutraceutical and response surface methodology.

INTRODUCTION

Moringa oleifera lam is also known as the "tree of life" because of its crucial importance and belongs to the solitary genus from the family *Moringaceae* and contains 13 known species (Hedhili *et al.,* 2022). Moringa is frequently regarded as a curative for all health issues or diseases. Leaves, flowers and pods of moringa are used in folk medicine to treat many diseases and they have cardiac and circulatory stimulants, anti-tumor, anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer, anti-spasmodic, diuretic, cholesterol-lowering, anti-oxidant, anti-diabetic, anti-bacterial and anti-fungal compounds. These health-promoting effects have been attributed to its constituent phytochemicals, such as zeatin, quercetin, -sitosterol and kaempferol (Farooq *et al.,* 2022). Moringa flower is unique because of its health benefits, medicinal and therapeutic properties. At present, the demand for moringa parts has increased annually due to off-season availability and increased utilization by people. One-fourth of moringa parts produce is spoiled during storage and transport. To prevent postharvest losses in the moringa flower, there is a need for processing, and it will meet the demand of the market throughout the year (Manju *et al.,* 2021)

Dehydration is a traditional system of preservation; done by various means of methods to reduce food moisture and plant materials. During the drying process, a higher temperature can lower the flavour, colour, heat-sensitive nutrients and bioactive compounds. The quality of the dried products can be improved by reducing the process temperature when compared to a higher temperature. (Kinki *et al.,* 2020).

The conventional drying process takes more time and energy than advanced techniques. Due to prolonged processing time and improper handling, higher temperature and pressure will affect the organoleptic characteristics and product quality. Alternatives to conventional drying, advanced dryers specifically freeze or vacuum-drying was used to dry heat-sensitive materials. (Klungboonkrong *et al.,*2018). Response surface methodology (RSM) is a popular and effective statistical tool for the optimization of interactive processes. (Sifat *et al.,* 2021). It is a technique which empowers the experimenter to determine the interrelationships between one or more responses and factors (Chakraborty *et al.,* 2020). RSM is commonly used to map a response surface over a specified region of interest, optimize responses, or choose operating settings to meet target specifications or customer requirements. (Okpe *et al.,* 2018).

Generally, moringa leaves are more focused than moringa flowers because of its high nutritional benefits and availability but the research on moringa flowers is unexplored. The present study aims to optimize the freeze-drying parameters of moringa flower powder using response surface methodology (RSM).

MATERIALS AND METHODS

Processing for the preparation of dried moringa flower powder (MFP): Healthy and disease free moringa flowers were selected. After that, a dehydration process was performed with freeze drying (Silva *et al.,* 2019) used a Liotop® L101 tabletop freeze drier the capacity of drying temperature ranged from -40°C to -80°C. Freeze drying was started at 0.15 mbar after moringa flowers were placed on unheated shelves. The ambient radiation that reached the samples through the clear glass drying chamber provided the sublimation energy.

RSM modeling experimental Design and analysis: Central Composite Rotatable Design (CCRD) is used to optimize the drying temperature of moringa flowers. Drying temperature (- 65°C, -55°C and - 45°C), vacuum pressure (0.5, 1.5 and 2.5 mm/Hg) and drying time (18, 21 and 24 h.) were taken as the independent variables and vitamin C, total antioxidant activity (TAA) and hygroscopicity (HS) were taken as a dependent variable. The experimental runs for the varied levels of the input variables and 6 number of center points (-55°C, 1.5 mm/Hg and 21 h) were obtained using Design Expert (Version 13.0) software. CCRD as it allows for a larger spread of conditions which helps in prediction where the model involves more complexity the second-order polynomial equation was fitted into each output variable as given in Eqn 1.

$$Y = \beta 0 + \beta 1\, X1 + \beta 2 X2 + \beta 3 X3 + \beta 11\, X1\, X1 + \beta 22 X2\, X2 + \beta 33\, X3\, X3 + \beta 12\, X1\, X2 + \beta 13\, X1\, X3 + \beta 23\, X2\, X3 \ldots \text{Eqn. 1}$$

Where Y is a response factor of the Vitamin C, TAA and HS from freeze dried moringa flower and β_0 is anintercept. Furthermore, β1 and β_2 and $\beta_3$are linear regression coefficients, β_{11}, β_{22} andβ_{33} denote interaction coefficients and β_{12},and $\beta_{13}$and β_{23} represent quadratic coefficients. To show the interactive effects of independent factors on a single dependent variable while keeping the other variables constant then three-dimensional surface plots were created for the optimized drying process. For greater precision, an analysis of variance (ANOVA) was used to determine the coefficients of the final equation. Vitamin C, TAA and HS were taken as the dependent variables. Twenty drying experimental runs were obtained from RSM and presented in Table 1.

The dehydration characteristics of MFP were analysed, *viz.,* ascorbic acid, by the methods given by AOAC (1990), total antioxidant activity (Lim *et al.,* 2007), powder recovery, water solubility index (Grabowski *et al*., 2006), flowability, Hausner ratio, Carr index (Seerangurayar *et al,* 2017), bulk and tap density (Chegini and Ghobadian 2005), hygroscopicity (Cai and Corke 2000), water and oil absorption capacity (Rosario and Flores 1981), rehydration ratio and dehydration ratio (Ranganna, 1986).

Statistical analysis

RSM was analyzed by Design Expert (Version 13.0) software. Data analysis was performed in a completely randomized design (CRD) using SPSS 14.0 for Windows (SPSS, 2005). According to the varied drying temperatures, multivariate analysis (Principal Component Analysis - PCA) was performedon the significant variables.

Table 1 : Optimization of process parameters for moringa flower powder (MFP) by RSM

Run	Independent variable			Dependent variable [a]		
	Temperature (ºC)	Pressure (mm/Hg)	Time (min)	Vitamin C (mg)	TAA (mg/100g)	Hygroscopicity (%)
1	- 55	1.5	21	291.64	457.99	1.68
2	- 65	2.5	24	259.05	434.58	1.51
3	- 65	2.5	18	281.2	451.89	1.56
4	- 55	1.5	21	291.64	457.99	1.68
5	- 65	0.5	18	289.18	456.23	1.59
6	- 45	0.5	18	265.29	438.11	1.71
7	- 55	1.5	21	291.64	457.99	1.68
8	- 45	2.5	18	260.77	434.9	1.73
9	- 55	1.5	24	266.5	439.56	1.67
10	- 55	1.5	18	305.81	460.18	1.68
11	- 45	1.5	21	252.46	427.63	1.75
12	- 65	1.5	21	274.24	449.02	1.54
13	- 55	1.5	21	291.64	457.99	1.68
14	- 55	1.5	21	291.64	457.99	1.68
15	- 45	0.5	24	242.28	417.44	1.77
16	- 45	2.5	24	238.53	409.71	1.78
17	- 65	0.5	24	269.12	443.97	1.53
18	- 55	1.5	21	291.64	457.99	1.68
19	- 55	2.5	21	279.33	446.27	1.67
20	- 55	0.5	21	283.12	452.14	1.67

[a]Values observed in the mean value of the three replicates.

RESULTS AND DISCUSSION

RSM model effect of freezedrying on responses of the moringa flower

RSM models for the three response variables statistical summarization (R^2 and ANOVA Estimation) were presented in Table 2 and Eqn 2 to Eqn 4. The model fitting using regression analysis exhibited that the models described the relationship between the input and output variables with regression coefficient (R^2 = Vitamin C - 0.96, TAA - 0.97 and HS - 0.95) indicating that the predicted values were well fitted with the actual values in the experimental conditions. The predicted R^2 values were 0.77, 0.90 and 0.95 and are in reasonable agreement with the adjusted R^2 values of vitamin C - 0.93, TAA - 0.95 and HS - 0.99. The model F value of 29.65 (Vitamin C), 47.99 (TAA) and 360.08 (HS) with p – values < 0.0001 implies that the quadratic model is significant. All response variables were statistically significant based on p - values with 95 per cent level of significance.

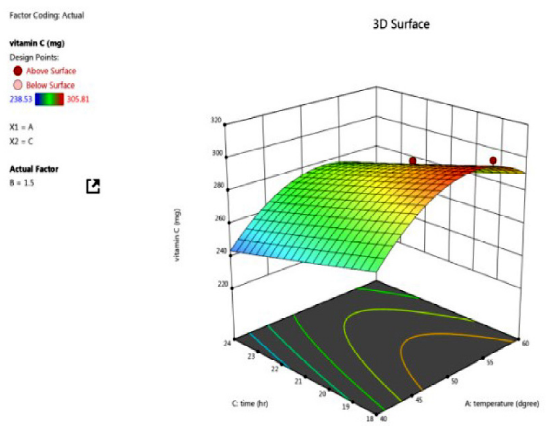
The 3D plots of the group effect of the response variables showed the synergetic effect of drying temperature, vacuum pressure and drying time (Fig.1). The three Independent factors have proven significant combined effects on vitamin C, TAA and HS. In RSM prediction, based on the desirability (0.74), the moringa flower freeze dried at - 63.754°C for 18h under 0.555mm/Hg vacuum pressure had an optimum level of vitamin C - 285.84 mg, TAA - 453.20 mg and HS 1.57 per cent 100 mg, respectively.

Whereas increasing drying temperature (- 45°C), vacuum pressure (2.5mm/Hg) and drying time (24 h)

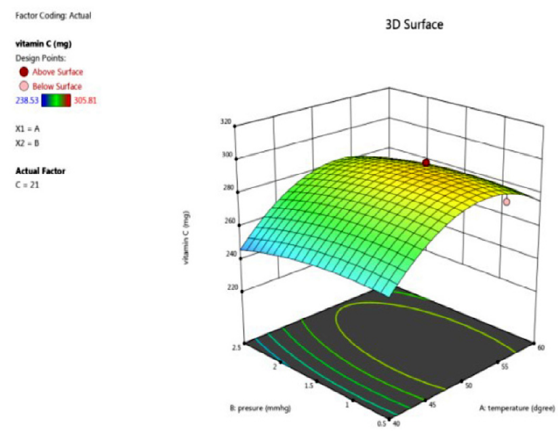
Table 2 : Regression coefficients and ANOVA estimated for response variables of freeze-dried moringa flower

Factor		Vitamin C (mg)			TAA (mg)			Hygroscopicity (%)		
	df	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value
Model	9	6253.78	29.65	< 0.0001	3983.75	47.99	< 0.0001	0.1165	360.08	< 0.0001
Linear										
A	1	271.76	11.60	0.0067	028.36	03.07	0.1101	0.0368	1024.95	< 0.0001
B	1	099.97	04.27	0.0658	079.41	08.61	0.0149	0.0004	010.54	0.0088
C	1	1188.00	50.70	< 0.0001	561.79	60.90	< 0.0001	0.0017	047.69	< 0.0001
Interaction										
A^2	1	1400.98	59.79	< 0.0001	571.54	61.96	< 0.0001	0.0022	060.37	< 0.0001
B^2	1	060.64	02.59	0.1388	34.39	03.73	0.0823	2.273	0.0006	0.9804
C^2	1	0.1507	0.0064	0.9377	22.67	02.46	0.1480	2.273	0.0006	0.9804
Quadratic										
AB	1	11.96	0.5102	0.4914	0.9730	0.1055	0.7520	0.0009	024.54	0.0006
AC	1	01.16	0.0493	0.8288	33.17	03.60	0.0872	0.0063	174.50	< 0.0001
BC	1	0.2178	0.0093	0.9251	11.45	01.24	0.2913	0.0000	001.13	0.3134
Mean ±SD	275.84 ± 4.84				445.48 ± 3.04					1.67 ± 0.0060
R^2	0.9639				0.9774					0.9969
Adjusted R^2	0.9314				0.9570					0.9942
Predicted R^2	0.7769				0.9042					0.9584
CV	1.75				0.68					0.3598

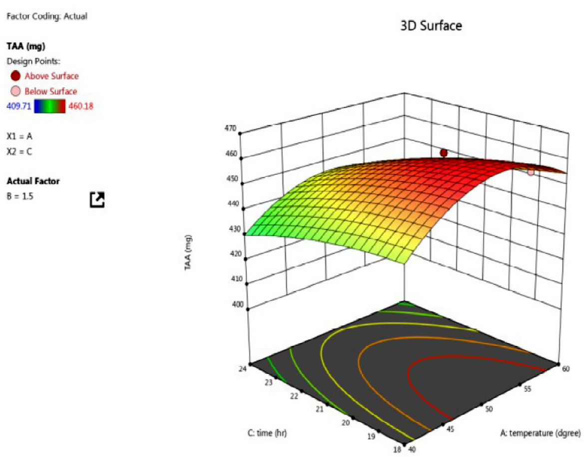
Note: The *p* values indicated that to check the significance level of eachRegression coefficient. A, B and C indicate that drying temperature, pressure and drying time respectively. The pd"0.05.are indicated as a significant effect of an independent factor on the response variable at 5 per cent.(CV, critical value; *df*, degree of freedom; *SD*, standard deviation)



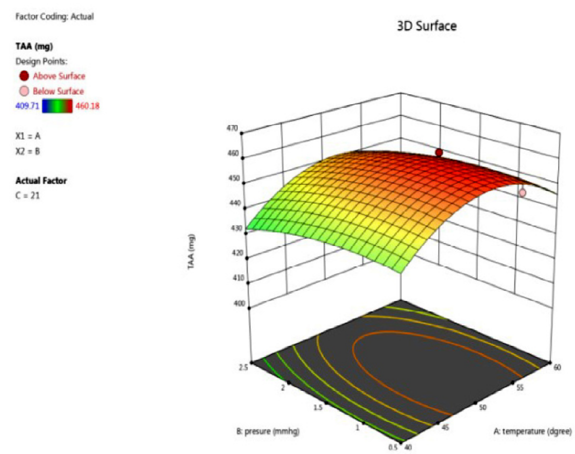
Vitamin C
(Drying temperature Vs drying time)



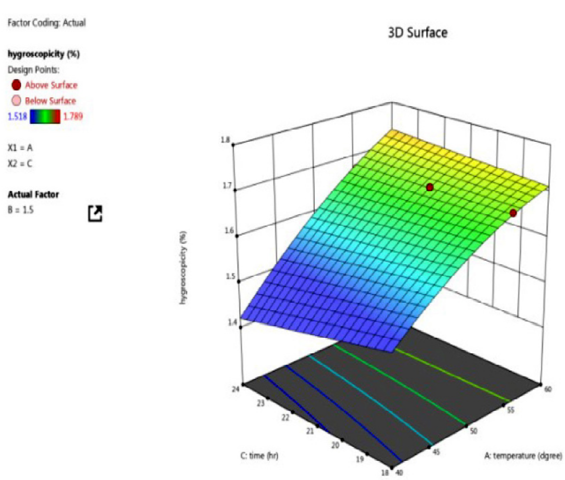
Vitamin C
(Drying temperature Vs Vacuum pressure)



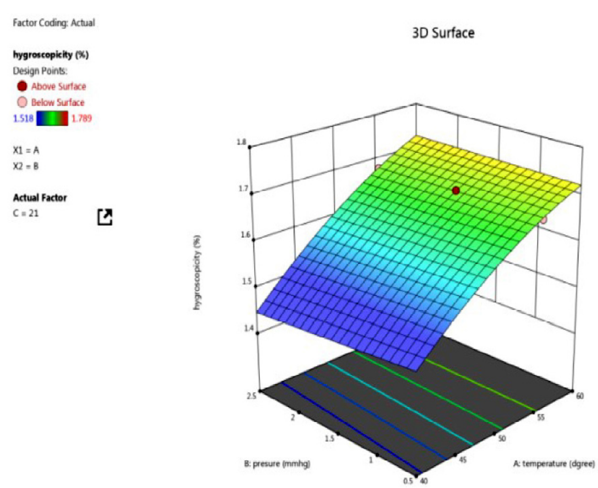
TAA
(Drying temperature Vs drying time)



TAA
(Drying temperature Vs Vacuum pressure)



HS
(Drying temperature Vs drying time)



HS
(Drying temperature Vs Vacuum pressure)

Fig. 1 : RSM 3D plots of the combined effect of independent variables on all the responses in freeze-dried moringa flower

vitamin C (305.81to 238.53 mg) and TAA (460.18 to409.71 mg) were decreased and hygroscopicity (1.51 to 1.78 %) was increased. A similar trend was recorded by Sifat *et al.* (2021) and Ademiluyi *et al.* (2018) in freeze-dried moringa at different temperatures. Due to thermal deterioration and oxidation, raising the drying temperature (- 45°C) resulted in a greater loss of heat sensitivity nutrients such as carotene and ascorbic acids. When drying temperature increase above optimum level, reduction in TAA due to the phenolic compound's high hydrogen atom-donating ability, increased phenolic content was associated with better antioxidant activity at optimum temperature (Ramarao *et al.,* 2022).

$$Y(Vitamin\ C) = +289.38 + 11.22(A) - 3.62(B) - 12.49(C) + 1.22(AB) - 0.3800(AC) - 0.1650(BC) - 22.57(A^2) - 4.70(B^2) + 0.2341(C^2) \ldots \text{Eqn. 2}$$

$$Y(TAA) = +457.68 + 3.63\ (A) - 3.23\ (B) - 8.59\ (C) + 0.3487\ (AB) - 2.04\ (AC) - 1.20\ (BC) - 14.42\ (A^2) - 3.54\ (B^2) - 2.87\ (C^2) \ldots \text{Eqn. 3}$$

$$Y(HS) = +1.62 + 0.1307(A) - 0.007(B) - 0.015(C) + 0.0105(AB) + 0.0280(AC) + 0.0022(BC) - 0.0281(A^2) - 0.0001(B^2) - 0.0001(C^2) \ldots \text{Eqn. 4}$$

Optimization of freezedrying for the production of moringa flower powder by PCA

The results of the principal component analysis (PCA) are presented in Table 3 and Fig. 2 : PCA was applied to describe the relationship between the different dehydration variables and to identify the most important sources of variability *viz.,* different drying temperatures.

The PCA results (75.17 %) clearly showed the variances among A (- 65°C), B (- 55°C) and C (- 45°C) of the different drying temperatures. The first principal component, accounting for 52.15 per cent and PC2 accounted for an additional 23.02 per cent of the total variance resolved the different drying temperatures according to the dehydration characteristics. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy for the PCA is 0.61. To further investigate the contributors to the principal components, the factor loadings in PC1 and PC2 were compared (Table 4). The eigenvalues for the F_1, F_2 and F_3 (Factor) were 3.56, 1.98 and 0.801. In factor loading F_1, water solubility (0.98), rehydration ratio (0.90) and water absorption capacity (0.86) were positively correlated and bulk density (- 0.87), carr index (- 0.68) and hygroscopicity (- 0.72) were negatively correlated. Similarly, in F_2 and F_3, oil absorption capacity (0.91) and tap density (0.69) had a positive correlation whereas other variances had a negative correlation. These results suggested that the reasonable score range of the principal components could be used for excellent sample selection according to the correlations between the original three variables and these two principal components.

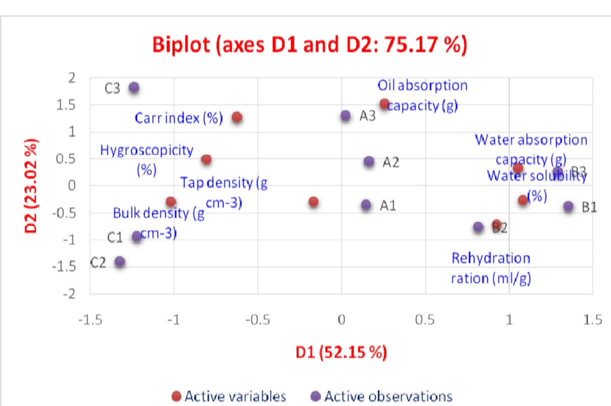


Fig. 2 : Dehydration characteristics variables as a function of first (PC1) and second (PC2) principal components

The moringa flowers were freeze-dried at - 55°C (B) and had 98.75% powder recovery, whereas increasing the temperature (- 45°C) led to a lower process yield. The water solubility index of the MFP increased (65.53 to 78.75 %) with increasing drying temperature. The bulk density and tap density of the FD- MFP varied from 0.43 - 0.48 and 0.30 - 0.45g cm^{-3} respectively. During the drying process, a higher drying temperature (- 55°C and - 45°C) will reduce the density of the powder to rapidly remove moisture. The effects of functional characteristics such as water absorption capacity (WAC) and oil absorption capacity (OAC) in dried drumstick powder were analysed. The WAC was 1.96 g at - 65°C (A), 2.09g at - 55°C (B) and 2.22 g at - 45°C(C). The OAC ranged between 4.10 g and 4.67 g at different temperatures. The WAC was higher at 65°C, and the OAC was higher at - 55°C, which may be due to the protein concentration where the binding of the hydrocarbon chains of oil to the non-polar side chains of the amino acids (Wang *et al.,* 2020). The values of WAC (23.2%) and OAC (18.5%) compared favourably with the results of spinach (*Amaranthus hybridus*) by Adeyey and Omolayo (2011).WAC is an important dehydration characteristic that correlates to the function of hydrophilic molecules such as proteins, carbohydrates and dietary fibre. However, the OAC of palmyra palm flours facilitates the improvement of flavour and mouthfeel during food preparation (Abe – Inge *et al.,* 2018).

Table 3 : Dehydration characteristics of freeze-dried moringa flower powder

Particular	Different drying temperature		
	- 65°C	-55°C	-45°C
Powder recovery (%)97.02±2.26[c]	98.75±1.08[a]	98.12±2.23[b]	
Water solubility (%)65.53±1.88[b]	78.75±1.64[a]	72.30±0.32[c]	
Bulk density (g cm^{-3})0.48±0.03[a]	0.43±0.08[a]	0.44±0.02[a]	
Tap density (g cm^{-3})0.45±0.09[a]	0.30±0.04[a]	0.42±0.04[a]	
Dehydration ratio (ml/g)	20.05±0.13[b]	21.69±0.50[c]	19.22±0.02[a]
Rehydration ration (ml/g)	3.92±0.06[a]	4.12±0.04[a]	4.38±0.12[b]
Water absorption capacity (g)	1.96±0.06[a]	2.09±0.01[a]	2.22±0.05[a]
Oil absorption capacity (g)	4.10±0.16[b]	4.52±0.11[b]	4.67±0.16[b]
Hausner's ratio 1.18±0.01[a]	1.28±0.01[a]	1.31±0.02[a]	
Carr index (%) 19.20±0.30[c]	19.51±0.58[c]	19.90±0.17[b]	
Flowability Good	Excellent	Excellent	
Hygroscopicity (%)1.52±0.03[a]	1.68±0.04[a]	1.71±0.02[a]	

Table 4 : Principal Component Analysis, Eigenvalues and factor loadings

Variance	Different factors				
	F_1	F_2	F_3	F_4	F_5
Eigen value	03.56	01.98	0.80	0.53	0.20
Variability (%)	54.21	25.42	9.96	6.74	2.60
Cumulative (%)	54.21	79.63	89.59	96.33	98.94
Factor (F) loadings					
Water solubility (%)	0.98	0.07	-0.03	-0.10	-0.04
Bulk density (g cm^{-3})	-0.87	-0.38	-0.05	0.10	0.27
Tap density (g cm^{-3})	-0.20	-0.65	0.69	0.19	-0.11
Rehydration ration (ml/g)	0.90	-0.18	-0.19	0.30	-0.10
Water absorption capacity (g)	0.86	0.28	0.27	0.23	0.11
Oil absorption capacity (g)	0.05	0.91	0.50	0.11	0.17
Carr index (%)	-0.68	0.63	0.18	-0.18	-0.22
Hygroscopicity (%)	-0.72	0.32	-0.25	0.53	-0.12

The dehydration ratio of FD-MFP ranged between 19.22 and 21.69 ml/g at different drying temperatures. The dehydration ratio was observed to be higher at - 55°C (B) because of the incomplete removal of moisture and lower at - 45°C due to the complete removal of moisture (heat air treatment). Similarly, the rehydration ratio ranged between 3.92 and 4.38 ml/g. The rehydration ratio was observed to be lower at - 65°C (A) due to incomplete reabsorption.

Manju *et al.* (2021) found comparable patterns in freeze-dried moringa, expressing outstanding flowability (Hauser's ratio and carr index) hygroscopicity (1.50 – 1.87), dehydration ratio (17.98 – 23.89) and rehydration ratio (3.37 – 3.72). Potisate *et al.* (2015) also reported the same trend in FD-MFP its having Excellent flowability, hygroscopicity (1.25–2.30) and rehydration ratio

(3.82 – 4.22). As a result, the loss of water and heat causes stress in the product's cell structure, causing the powder to become hygroscopic and dehydration will reduce the dimensions of freeze-dried moringa flower powder.

CONCLUSION

In RSM, the developed model response R^2 values were 0.96 (Vitamin C), 0.97 (TAA) and 0.95(HS) of freeze dried moringa flower powder. Based on response surface and desirability (0.74) functions, moringa flower freeze-dried at - 63.75°C for18 h under 0.55 vacuum pressure had an optimum level of vitamin C 285.84 mg/100g, TAA 453.20 mg/100gand HS 1.57 percent 100 mg. The optimal drying temperature of - 55°C results in improved dehydration features and a high powder recovery (98.75%) as well as great flowability. Based on the findings, freeze drying can be considered as one of the best drying techniques to preserve the nutritional quality features of moringa while also being an efficient and effectively utilized for the entire food processing industry**.** Moringa flower powder, which has been freeze dried, can be used to make novel functional foods. Moringa-infused food products will be ideal for commercialization and will help to reduce the nutritional deficit in the community's most vulnerable residents.

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