Optimization and Comparison of Drying Methods of Ultrafiltered Soy Protein Solution Using Response Surface Methodology

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Abstract: The escalating significance of plant-based protein sources, particularly soy proteins, mirrors the evolving health perspectives of individuals. As protein powders continue to be embraced for direct consumption or incorporation into diverse food matrices, the present study addresses the pivotal process of drying soy protein solutions. Employing the ultrafiltration membrane technique, we explored the efficacy of both spray drying and oven drying methods, meticulously optimizing the drying parameters. In our investigation, we sought to gauge the overall protein yield, benchmarking against the protein content of the raw material, defatted soy flour (DSF). The comparative analysis with the freezedried counterpart revealed distinctive outcomes. The freeze dryer exhibited an overall protein yield of 54%, surpassing its counterparts. The oven dryer yielded a respectable 48%, while the spray dryer, though yielding 33%, presented an alternative perspective. These findings underscore the meticulous considerations in selecting drying methods for soy protein solutions, acknowledging the trade-off between different techniques.

Key words: Soy protein, spray drying, freeze drying, response surface technology.

Membrane technology is an innovative technology making a major penetration into food and bioprocessing industries as a routine processing tool. Among pressure driven membrane separation processes, ultrafiltration technique is widely adopted for protein concentration and purification. Ultrafiltration process can be considered as a potential alternative to conventional acid precipitation because of its mild operating conditions and high selectivity during purification and fractionation (John *et al.*, 2021). UF is recognized as an effective method for producing protein isolates and concentrates with low phytic acid content. In addition, UF helps to recover almost solubilized protein and avoids the generation of whey like products, resulting in an increased protein recovery.

Different drying methods used to dry obtained the protein solutions through ultrafiltration also influence the physicochemical and functional properties of protein powder. Hence different drying methods and the drying conditions have to be optimized in to get the maximum protein yield with optimum moisture content. A number of studies have been carried out on the effect of various drying methods viz. spray drying, freeze drying and vacuum drying on functional properties of different protein isolates (Hu et al., 2010; Joshi et al., 2011, John et al., 2018). Drying method chosen and type of protein dried determine the protein functionality (Hu et al., 2010). In general, plant protein solutions are dried using a freeze dryer, spray dryer or vacuum dryer (Joshi et al., 2011; Liao et al., 2013). Liapis and Bruttini (2020) reviewed that freeze drying is generally used at laboratory level to dry protein isolates. According to Hu et al. (2010), freeze-drying influences the morphology, size and surface hydrophobicity of proteins. Also, Freeze-drying is expensive and hence not economical to use for drying all protein solutions (Reyes et al., 2011). Therefore, spray drying technique came into picture for large scale drying of solutions (Niamnuy and Devahastin, 2022). Vacuum drying is also a low-cost process that is performed at low temperatures (Joshi et al., 2011). He compared spray drying and vacuum drying of lentil protein isolate and found that spray drying causes a reduction in solubility. He concluded that the drying method used for preparation of lupin protein isolate (LPI) can significantly affect the physicochemical properties, which in turn adversely affect the functionality of proteins. Ghribi et al. (2015) conducted a study to find out the effect of drying methods on physico chemical properties of chickpea protein concentrate. They observed that the bulk density of chickpea protein concentrate powders was significantly affected by the drying process and temperature at a level of significance p <0.05. Ghribi et al. (2015) also explained the effect of drying methods on color values and observed highest lightness and yellowness values for freeze dried samples compared to the other chickpea protein concentrates. They also reported that the convective drying at 40°C concentrate produces a darkened final product. Researchers conducted studies on convective air drying of chickpea protein and reported

molecular level disruption of protein happened (Bhandari and Roos, 2012). According to Joshi *et al.* (2011), due to longer drying time in convective air drying, denaturation of heatlabile proteins happened and the disappearance of some of the protein bands in SDS-PAGE was observed.

As plant-based proteins become increasingly integral to dietary choices, our study contributes valuable insights into optimizing the processing of soy proteins, catering to both the health-conscious consumer and the food industry seeking innovative applications. Keeping these in view, a study has been conducted to optimize spray drying and oven drying conditions to dry ultrafiltered soy protein solution and compare its properties with freeze dried counterpart.

Materials and Methods

Preparation of soy protein solution using ultrafiltration

The detailed procedure of preparation of soy protein solution using a hollow fiber ultrafiltration cartridge is explained by John and Sinha (2019).

Drying of soy protein solution

Three types of dryers viz., spray dryer (Yamato mini spray dryer, ADL 31),hot air oven dryer (Oric, reliable instruments, India) and freeze dryer (Bio Sync Technology, New Delhi) were used to study the influence of various drying methods on the properties of soy protein powder.

Spray drying: A laboratory-scale spray dryer with a double-fluid nozzle arrangement and a co-current flow pattern was used in this study. The spray dryer was operated at three different air inlet temperatures (170°C, 180°C, and 190°C) and three different airflow rates (0.02, 0.06, and 0.1 m³/min), while the outlet temperature was fixed at 85°C. The feed flow rate was kept constant at a low level by adjusting the control knob on the spray dryer. The aim was to optimize the spray drying conditions to maximize protein yield and achieve desired moisture content and color in the resulting powder. Optimization was conducted using Design Expert software, employing a 3-level factorial design with a total of 13 runs and 2 replications. The chosen independent variables were air inlet temperature and airflow rate,

Std	Run	Air temp (°C)	Inlet Airflow rate (m³ min-1)	Protein yield (%)	Moisture content (%)	Color (L*)
3	1	190	0.02	97.2536	5.18	75.45
9	2	190	0.1	95.1118	4.78	78.19
13	3	180	0.06	96.2994	5.82	83.18
2	4	180	0.02	97.5322	5.56	83.36
10	5	180	0.06	97.5897	6.01	82.85
8	6	180	0.1	96.1853	5.18	82.96
7	7	170	0.1	96.3914	6.61	84.22
4	8	170	0.06	97.5322	6.86	83.36
5	9	180	0.06	96.8897	5.21	81.25
12	10	180	0.06	97.2765	5.17	83.07
1	11	170	0.02	97.8843	6.34	84.31
6	12	190	0.06	97.1503	6.05	75.68
11	13	180	0.06	97.4994	5.72	82.98

Table 1. Factorial design with experimental results (spray drying)

with protein yield, moisture content, and powder color as the dependent variables.

Hot air oven drying: A laboratory-scale hot air oven was used for drying the soy protein solution obtained by ultrafiltration. The drying process was conducted using combinations of three drying temperatures (40°C, 50°C, and 60°C) and three drying times (24, 36, and 48 h) to evaluate their effects on the final product. Optimization was carried out with Design Expert software, employing a 3-level factorial design with 13 runs and 2 replications to maximize protein yield and achieve desired levels of moisture content and color in the dried protein powder. After drying, the powder was ground using a mortar and pestle and sieved through a No. 100 mesh. The independent variables of the study were drying temperature and drying time, while protein yield, moisture content, and powder color were the dependent variables.

Freeze drying: For freeze drying, protein solution was frozen for 12 h at -18°C. Frozen samples were freeze dried at -45°C compressor temperature and 0.07 mm Hg vacuum pressure. Freeze dried samples were ground using a mortar and pestle and sieved through a No. 100 mesh.

Protein yield: Protein yield of soy protein after drying was calculated using the formula below:

$$\begin{array}{l} \textit{Protein} \\ \textit{extraction} \\ \textit{yield} \end{array} = \frac{\textit{Protein content in soy protein powder} \times \\ \frac{\textit{Weight of protein powder}}{\textit{Protein content of DSF}} \times 100 \\ \end{array}$$

Moisture content: The moisture content of soy protein was determined by gravimetric method. The samples were taken in petri dishes and the initial weight (W_i) was measured by an electronic balance. Then these samples were kept in a hot air oven maintained at a temperature of 105°C for 6 h and the weight of the dried sample was taken (W_f). The moisture content was expressed as the percentage change in weight (Mansuri *et al.*, 2016).

Moisture content (%) = $((W_i - W_f)/W_f) \times 100$

Color: Color values of soy protein powder were determined according to Schuck *et al.* (2013). Before taking the measurements, HunterLab colorimeter was calibrated by placing black and white tiles to set zero and 100 values. Placed the leveled powder in the powder measurement system and noted down the L*, a*and b* values.

Results and Discussion

Spray drying

A three-level factorial design with two factors and three levels was employed to optimize the spray drying conditions using a quadratic model. The experiments were repeated 2 times. Table 1 depicts the 3 level factorial design with experimental design. 13 runs were carriedout with 2 replications.

The statistical significance of each model term was checked by regression analysis and analysis of variance (ANOVA). Table 2 depicted the regression coefficients and significance of each variable on spray drying parameters.

Source	Protein	yield	Moisture content		Color	
	Coefficient	p value	Coefficient	p value	Coefficient	p value
Intercept	97.17	0.021	5.66	0.021	82.59	< 0.0001
Temp.	-0.38	0.077	-0.63	0.004	-3.76	< 0.0001
Airflow	-0.83	0.003	-0.085	NS	0.37	NS
Interaction	-0.16	NS	-0.17	NS	0.71	0.095
Temp ²	0.023	NS	0.62	0.026	-2.89	0.0003
Airflow ²	-0.46	NS	-0.46	0.074	0.75	NS
\mathbb{R}^2	0.80		0.80		0.97	
Adj R²	0.66		0.66		0.94	
F value	5.67		5.67		41.33	
CV (%)	0.47		6.38		0.47	
LOF	0.37	0.78	0.87	0.53	0.63	0.64

All the quadratic models were observed to be significant at p <0.0209 and lack of fit was non-significant. Coefficient of variance (CV) for all the responses was less than 10% and hence the experiments were carried out with adequate precision.

Effect of spray drying parameters on protein yield: The Model F-value of 5.67 implies the model is significant at 2.09%. In this case, inlet air temperature is significant at 7.68% and airflow rate is significant at 0.28%. Interaction effect and square terms are observed to be non-significant. The "Pred R-Squared" of 0.802 is in

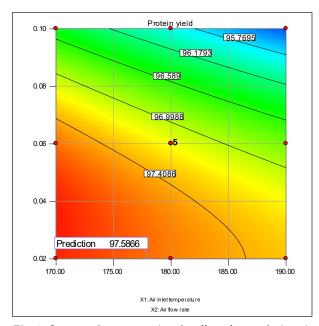


Fig. 1. Contour plot representing the effect of spray drying air temperature and airflow rate on protein yield.

reasonable agreement with the "Adj R-Squared" of 0.6606. lack of fit value is non-significant.

Protein yield showed a decreasing trend with airflow rate and air inlet temperature (Fig. 1). Maximum protein yield was obtained with inlet air temperature in the range of 170-175°C and airflow rate 0.02-0.04 m³/min with a predicted protein yield 97.57%.

Regression equation to find out protein yield in terms of actual factors is given:

Protein yield = +107.43244 -0.097797*Air inlet temperature +86.68106*Airflow rate -0.40554*Air inlet temperature*Airflow rate +2.33127E-004*Air inlet temperature² -286.99880* Airflow rate²

Effect of spray drying parameters on moisture content: The Model F-value of 5.67 implies the model is significant at p <0.0500. In this case, air inlet temperature is significant at 0.38% whereas airflow rate is non-significant. Interaction effect is observed to be non-significant and both the squares of main effects are significant at 10% significance level. R² value 0.8021 indicated that variability in moisture content can be well explained by the independent parameters.

Moisture content has an inverse relation with air inlet temperature in general. As airflow rate increases, moisture content shows an initial increase and thereafter a slight decrease (Fig. 2). Lowest moisture content was obtained with the inlet air temperature in the range 180-190°C and airflow rate either between 0.02- 0.04 m³ min-1 above 0.08-1 m³ min-1.

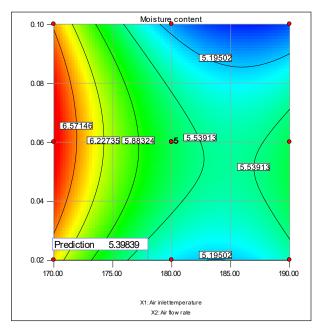


Fig. 2. Contour plot representing the effect of spray drying air temperature and airflow rate on moisture content.

Regression equation to find out moisture content in terms of actual factors is given below:

Moisture content = +213.17030 -2.27766* Air inlet temperature +107.96983*Airflow rate -0.41875* Air inlet temperature*Airflow rate +6.22069E-003*Air inlet temperature² -289.33190* Airflow rate²

Effect of spray drying parameters on color: The Model F-value of 41.33 implies the model is significant at p <0.0001. In this case air inlet temperature is significat at 0.01% and airflow rate is non-significant. Interaction effect is significant at 9.51%. square term of inlet air temperature also observed to be significant at 0.03%. lack of fit is non-significant. R square value of 0.9672 indicates the good fit of model. Also, the "Pred R-Squared" of 0.8666 is in reasonable agreement with the "Adj R-Squared" of 0.9438.Experiments were carried out with adequate precision since the CV value was less that 10% (0.90%).Inlet air temperature

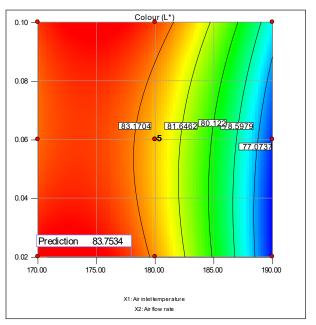


Fig. 3. Response surface (a) and contour plot (b) representing the effect of spray drying air temperature and airflow rate on color.

was negatively correlated with color whereas airflow rate has a positive correlation with color (Fig. 3). The optimum color value was obtained with a combination of inlet air temperature 170-180°C and airflow rate 0.8-1 m³min⁻¹with a predicted L value 83.75

Regression equation to find out color (L*)in terms of actual factors is given as:

Color (L*) = -766.11065 +9.92481*Air inlet temperature -365.18534*Airflow rate +1.76875* Air inlet temperature*Airflow rate -0.028909*Air inlet temperature² + 468.21121*Airflow rate²

Optimization of spray drying conditions: The optimum conditions for spray drying to get maximum protein yield were obtained from the desirability function approach, having a desirability of 0.838, inlet air temperature of 177.98°C, and flow rate of 0.02 m³ min-¹ and the optimized protein yield was 97.59%, moisture content was 5.4% and color (L*) was 83.75. For

Table 3. Verification of optimized conditions of spray drying

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Independent parameters	Optimized Value	Experimental value		
Inlet air temperature (°C)	177.98	180		
Airflow rate (m³ min-1)	0.02	0.02		
Dependent variables	Predicted value	Experimental value	RMSE	
Protein yield (%)	97.59 ^a	96.76ª	0.989	
Moisture content (%)	5.4ª	5.8ª	0.457	
Color (L*)	83.75 ^a	82.64 ^a	1.35	

Table 4. Factorial design with experimental results to optimize oven drying

Std	Run	Drying temp (°C)	Drying time (h)	Protein yield (%)	Moisture content (%)	Color (L*)
13	1	50	36	97.89	4.64	73.23
3	2	60	24	96.80	3.4	60.65
5	3	50	36	98.09	4.34	72.43
10	4	50	36	98.18	4.83	74.78
4	5	40	36	99.09	5.89	77.94
12	6	50	36	97.57	4.11	72.92
11	7	50	36	98.06	4.67	75.06
7	8	40	48	98.72	5.6	78.16
8	9	50	48	98.12	3.52	75.16
6	10	60	36	97.55	3.36	64.62
9	11	60	48	98.80	3.4	50.65
2	12	50	24	97.05	4.9	74.39
_ 1	13	40	24	99.07	6.44	76.71

optimization, independent parameteres were kept in range and protein yield as maximum and moisture content and color as minimum.

The validation of the optimum solution was done by experimenting with the optimum conditions. It was observed that result obtained was similar to optimum solution which is presented in Table 4.

Oven drying: Table 4 depicts the 3 level factorial design with experimental design. 13 runs were conducted with 2 replications.

The statistical significance of each model term was checked by regression analysis and ANOVA. Table 5 depicts the regression coefficients and significance of each variable on oven drying parameters. All the quadratic models were observed to be significant at p <0.001 and lack of fit was non-significant. CV for all the responses were less that 10% and hence the experiments were carried out with adequate precision. Lack of fit was observed to be non-significant for all variables than color (L*).

Effect of oven drying conditions on protein yield

The Model F-value of 18.96 implies the model is significant at p<0.0006. In this case, drying temperature, drying time, its interaction and square term of temperature were significant model terms. These main effects and their interaction were significant at 1% significant level. The "Lack of Fit F-value" of 1.12 implies the Lack of Fit is not significant relative to the pure error.

Table 5. Regression coefficients and significance of each variable in quadratic model

Source	Protein yield		Moisture content		Color (L*)	
	Coefficient	p value	Coefficient	p value	Coefficient	p value
Intercept	97.90	0.0006	4.46	0.0004	74.49	0.0010
Temp	-0.62	0.0005	-1.30	< 0.0001	-9.48	0.0001
Time	0.45	0.0028	-0.37	0.028	-1.30	NS
interaction	0.59	0.0021	0.21	NS	-2.86	0.094
Temp ²	0.56	0.0073	0.30	NS	-5.22	0.022
Time ²	-0.18	NS	-0.12	NS	-1.72	NS
\mathbb{R}^2	0.93		0.94	0.91		
Adj R²	0.88		0.89	0.86		
F value	18.96		21.1	16.04		
CV (%)	0.25		7.20		4.15	
LOF	1.12	0.44 (NS)	1.66	0.31 (NS)	13.63	0.014 (S

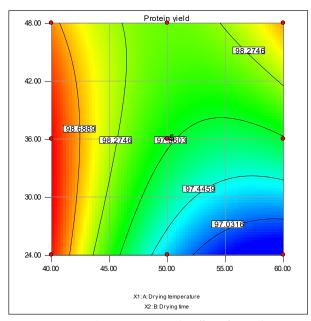


Fig. 4. Contour plot representing the effect of oven drying air temperature and drying time on protein yield.

Drying temperature had a negative correlation with protein yield whereas protein yield showed an initial increasing trend and thereafter a decreasing trend with drying time (Fig. 4).

It can be inferred from the Fig. 4 that protein yield was highest at drying temperature of around 40°C and drying time 35 to 40 h with a predicted value of 98.69%.

Regression equation to find out protein yield in terms of actual factors is given below:

Protein yield = + 120.73120-0.79360*Drying temperature -0.11754*Drying time +4.89098E-003* Drying temperature*Drying time +5.55397E -003*Drying temperature² -1.24008E-003* Drying time²

Effect of oven drying conditions on moisture: The Model F-value of 21.10 implies the model is significant at 1% significance level. And both the main effects drying temperature and drying time are significant model terms. Drying temperature was affected more significantly (at 1% significance level) than drying time (at 5% significance level). The "Lack of Fit F-value" of 1.66 implies the Lack of Fit is not significant. CV was 7.20% and hence the experiments were carried out with adequate precision.

Moisture content showed a decreasing trend with the increase in drying temperature and drying time (Fig. 5). Lowest moisture content

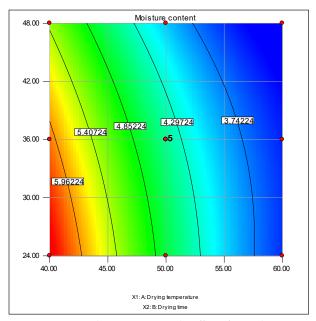


Fig. 5. Contour plot representing the effect of oven drying air temperature and drying time on moisture content.

was obtained with a drying temperature aof 55-60°C and drying time above 36 h with a predicted moisture content of 3.74%.

Regression equation to find out moisture content in terms of actual factors:

Moisture content = 21.55241 -0.48922* Drying temperature -0.059195* Drying time +1.75000E-003*Drying temperature*Drying time +2.96724E-003*Drying temperature² -8.21360E-004* Drying time².

Effect of oven drying conditions on color: The Model F-value of 16.04 implies the model is significant at 1% significance level. Here, drying temperature (p <0.0001) and its square term (p<0.0220) were significant model terms. Interaction effect of drying temperature and drying time had significant effect on color (L*) value at 10% significance level. Drying time is not affecting significantly on color (L*) value of protein powder.

Drying temperature had an inverse correlation with color value (Fig. 6) and highest color value was achieved with dryer temperature in the range of 40-50°C with a predicted color (L*) value of 74.91.

Regression equation to obtain color (L*) in terms of actual factors as given below:

Color (L^*) = -63.10003 +5.12834* Drying temperature +1.94603* Drying time -0.023854*

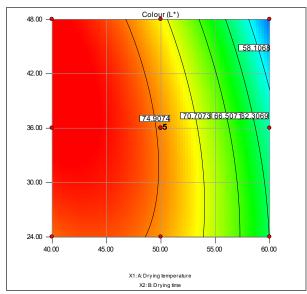


Fig. 6. Contour plot representing the effect of oven drying air temperature and drying time on color

Drying temperature * Drying time -0.052178* Drying temperature² -0.011964* Drying time²

Optimization of oven drying conditions: The optimum conditions for oven drying to get maximum proteinyield were obtained from the desirability function approach, having a desirability of 0.722, dryer temperature of 46.2°C, and drying time 48 h, and the optimized protein yield was 98.27%, moisture content was 4.42% and color (L*) was 75.37. For optimization, independent parameters were kept in range and protein yield as maximum and moisture content and color in minimum.

The validation of the optimum solution was done by conducting the experiment at the optimum conditions generated numerically. It was observed that result obtained were similar to optimum solution which is presented in Table 6.

Comparison of spray drying and oven drying of soy protein isolates

Protein yield, moisture content, and color (L*) of soy protein powders produced by spray

drying and oven drying were compared, and the results were analyzed using a t-test (α = 0.05). Oven drying produced a slightly higher protein yield (98.35%) compared to spray drying (96.76%), though this difference was not statistically significant. In terms of moisture content, oven-dried powder had significantly lower moisture (4.65%) than spray-dried powder (5.8%), indicating better drying efficiency. For color (L*), spray-dried powder appeared lighter (82.64) compared to oven-dried powder (74.27), and this difference was statistically significant. These findings highlight the distinct effects of drying methods on the quality attributes of soy protein powders.

Freeze drying of soy protein isolate

The soy protein solution was freeze-dried for 36 hours to achieve constant moisture content (5.73±0.99%) of dried protein powder. Freeze drying is widely recognized as a superior drying technique for preserving the quality of food products, including maintaining their natural color, texture, and functionality. Previous studies (e.g., Schuck et al., 2013; Ghribi et al., 2015) have consistently demonstrated the ability of freeze drying to yield high-quality products with minimal changes in color. Hence, the focus of this research was to compare spray drying and oven drying, which are more commonly employed at commercial scales, to evaluate their effects on soy protein powder quality under varying conditions. The sov protein powders obtained by freeze drying, oven drying and spray drying are shown in Fig. 7.

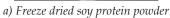
Overall protein yield from different drying methods.

The base material used for extracting the soy protein was defatted soy flour. The ultrafiltered soy protein solution was dried using different drying methods. Some losses in protein quantity may occur due to the difference in drying methods. Drying food involves factors like temperature, humidity, change in shape,

Table 6. Verification of optimized conditions of oven drying

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Independent parameters	Optimized value	Experimental value					
Dryer temperature (°C)	46.24	47					
Drying time (h)	48	48					
Dependent parameters	Predicted value	Experimental value	RMSE				
Protein yield (%)	98.27ª	98.35ª	0.7747				
Moisture content (%)	4.42a	4.65ª	0.4549				
Color (L*)	75.37a	74.27a	2.1794				











c) Spray dried soy protein powder

Fig. 7. The soy protein powders obtained by freeze drying, oven drying and spray drying.

crystallization and different chemical reactions. Maintaining these conditions in different drying methods is challenging which affects drying outcomes due to variations in drying parameters like pH, temperature and diffusivity. Hence there will be a difference in the total protein yield of dried protein from different drying methods even though the base material used was same. Therefore, the overall protein yield was calculated with reference to the base material 50 g of defatted soy flour with 53.92% moisture content and is shown in Table 7.

In the spray dryer, 100 ml produced 11.38 g of protein powder (78.34%). The oven dryer yielded 16.14 g protein powder (80.56%), and the freeze dryer produced 17.25 g protein powder (83.94%). These results highlight the differences in protein extraction efficiency among the three drying techniques. Maximum protein yield was obtained for freeze dried soy protein powder (53.69±3.39%) and yield was least for spray dried protein powder (32.935±1.335%).

Conclusion

The meticulous optimization of drying conditions for soy protein solutions using *Table 7. Overall protein yield from different drying methods*

both spray drying and oven drying methods has provided valuable insights into enhancing protein yield and product quality. The established optimal parameters for the spray dryer, including an inlet air temperature of 178°C and airflow rate of 0.02 m³ min⁻¹, resulted in a commendable 97% protein yield, 5.4% moisture content, and color (L*) value of 83. Similarly, the oven dryer, under optimized conditions of 46°C dryer temperature and 48 h drying time, exhibited impressive outcomes with a 98% protein yield, 4.5% moisture content, and color (L*) value of 75. Comparatively analyzing the obtained results against the freeze-dried counterpart, it is evident that while the spray dryer showed the least overall protein yield, the freeze dryer emerged as the most effective method, yielding the maximum protein content. This study underscores the significance of choosing the appropriate drying method based on the desired outcome and intended application. As we navigate the delicate balance between yield and quality, these findings contribute valuable knowledge to the field, paving the way for further advancements in protein drying technology.

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Amount of defatted soy flour (DSF) (g)	50.00
Protein content in DSF (%)	53.92
Protein in 50 g DSF (g)	26.96
Amount of protein extract obtained (ml)	335.00
Soluble protein content in extract (%)	61.73
Protein in 23.66 g dry weight of extract (g)	14.61

	Spray dryer	Oven dryer	Freeze dryer
Amount of feed (ml)	100.00	100.00	100.00
Amount of protein powder (g)	11.38±0.50	16.14±1.02	17.25±1.09
Protein content (%)	78.34	80.56	83.94
Amount of protein (g)	8.88±0.36	13.01±0.83	14.475±0.915
Protein yield (%)	32.935±1.335	48.26±3.08	53.69±3.39

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