

Optimizing the extrusion conditions for the production of expanded intermediate wheatgrass (*Thinopyrum intermedium*) products

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Abstract: In this study, the effects of extrusion conditions such as feed moisture content (20%, 24%, and 28%), screw speed (200, 300, and 400 rpm), and extrusion temperature (130, 150, and 170°C) on the physical and functional properties (moisture content, expansion ratio, bulk density, hardness, water absorption index [WAI], water solubility index [WSI]) of intermediate wheatgrass (IWG) were investigated for the first time. Response surface methodology was used to model and optimize the extrusion conditions to produce expanded IWG. The model coefficient of determination (R^2) was high for all the responses (0.87–0.98). All the models were found to be significant ($p < 0.05$) and were validated with independent experiments. Generally, all the extrusion conditions were found to have significant effects on the IWG properties measured. Increasing the screw speed and decreasing the extrusion temperature resulted in IWG extrudates with a high expansion ratio. This also resulted in IWG extrudates with generally low hardness and bulk density. Screw speed was found to have the most significant effect on the WAI and WSI, with increasing screw speed resulting in a significant ($p < 0.05$) decrease in WAI and a significant ($p < 0.05$) increase in WSI. The optimum conditions for obtaining an IWG extrudate with a high expansion ratio and WAI were found to be 20% feed moisture, 200–356 rpm screw speed, and 130–154°C extrusion temperature.

KEYWORDS

extrusion, intermediate wheatgrass (IWG), optimization, physical and functional properties, response surface methodology (RSM)

Practical Application: Extrusion cooking was employed in the production of expanded IWG. This research could provide a foundation to produce expanded IWG, which can potentially be used as breakfast cereals and snacks. This is critical in the efforts to commercialize IWG for mainstream food applications.

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1 | INTRODUCTION

Intermediate wheatgrass (*Thinopyrum intermedium*, IWG) is the world's first commercially viable perennial grain crop currently being developed and explored for mainstream food applications in the United States. Its trade name is Kernza[®], and it belongs to the *Triticeae* tribe of the *Poaceae* family. IWG originated from Eurasia and was introduced to the United States in 1932. It was originally used as a forage crop until the Rodale Research Centre in Pennsylvania, United States, initiated its domestication in the 1980s (Bajgain et al., 2020). One of the main reasons why IWG has received much attention for its development and use is the environmental benefits it provides, attributed to its extensive root system. This root system effectively sequesters carbon, thus helping to improve soil health while reducing greenhouse gas emissions (Culman et al., 2013; Glover et al., 2010). IWG was shown to have 15 times more root growth and almost double the amount of aboveground biomass than annual wheat (Sprunger et al., 2018), leading to a 13% increase in carbon sequestration and 86% less nitrate leaching when compared to wheat (Culman et al., 2013). Moreover, nitrate leaching from the IWG was found to be one to two orders of magnitude lower than that of annual maize (Jungers et al., 2019).

Recent efforts have focused on breeding programs aimed at improving the domestication and agronomic traits of the IWG. The first breeding cycles of IWG relied on traditional breeding approaches and resulted in increases in grain yield (144%), free-threshing ability (129%), and seed size (52%) (Tyl et al., 2020). The University of Minnesota, through the Forever Green Initiative, started its IWG breeding program in 2011 using germplasms from the Land Institute (Zhang et al., 2016). This breeding program led to the development of MN Clearwater, which was officially released as the first IWG for commercial use in the fall of 2019 and has been met by interest from consumers and the food industry (Bajgain et al., 2020).

The remarkable progress made toward IWG development and improvement has resulted in efforts to utilize IWG for mainstream food applications. Previous studies have focused on the compositional and functional properties of IWG as impacted by varietal differences, bran content, storage conditions, and dough conditioners to enhance baking quality (Banjade et al., 2019; Marti et al., 2016; Rahardjo et al., 2018; Tyl & Ismail, 2019; Zhong et al., 2019a), determining the starch hydrolysis kinetics of IWG (Zhong et al., 2019b), establishing tempering conditions for the production of refined IWG flour (Tyl et al., 2019), and pretreating IWG bran with xylanase to enhance the properties of IWG bread (Dai et al., 2021). In one such study (Tyl & Ismail, 2019), it was shown that IWG had higher protein, dietary fiber, and antioxidant contents than wheat. Accord-

ingly, IWG is a promising cereal grain that can potentially be utilized in food products to contribute to protein, fiber, and antioxidant intake. It is, therefore, critical to progress research focused on the effects of processing on the functionality, chemistry, storage, and safety of IWG in efforts to commercialize IWG for mainstream food applications.

In our current research, extrusion cooking was explored as a processing option for IWG for potential food applications such as breakfast cereals and snacks for the first time. This is critical in our current efforts to diversify IWG's use in food applications, as this could potentially increase its demand and drive its production and development. Extrusion cooking is an efficient, versatile, high temperature short time process in which food raw materials are cooked using a combination of moisture, pressure, temperature, and mechanical shear (Oliveira et al., 2015). It is widely used in the food industry for making ready-to-eat breakfast cereals, snacks, pasta, baby foods, and meat analogs (Oliveira et al., 2015). Extrusion technology has been successfully used to process cereal grains such as rice (Ding et al., 2005; Rani et al., 2021), wheat (Ding et al., 2006), corn (L. Liu et al., 2017; Y. Liu et al., 2000; Singha, Singh, et al., 2018), and oats (Y. Liu et al., 2000). These studies showed that extrusion could be used to produce breakfast cereals and snacks from the grains. Furthermore, extrusion conditions such as feed moisture content, extrusion temperature, screw speed, and the raw materials greatly influenced the physicochemical and functional characteristics of extruded products (Ding et al., 2005, 2006; Y. Liu et al., 2000; L. Liu et al., 2017; Rani et al., 2021).

To produce extruded products with targeted physical and functional properties, response surface methodology (RSM) has been used for process optimization to obtain such targets (Charunuch et al., 2014). For instance, Charunuch et al. (2014) used RSM to optimize extrusion conditions such as feed moisture and extrusion temperature, as well as defatted rice bran content to produce a rice-based breakfast cereal with high expansion and a low water solubility index. RSM is a statistical method used to study the relationships between several independent variables or factors and one or more response variables, with the aim of obtaining optimal responses. Compared to a full factorial design, the RSM's experimental design reduces the number of experiments required to evaluate independent variables and their interactions (Kalitsis et al., 2021). RSM has been extensively used for process optimization in the production of extruded cereal grains (Charunuch et al., 2014; Román-Gutiérrez et al., 2021; Singha, Singh, et al., 2018).

The development of extruded IWG is important for improving the commercial viability of grains. However, there is no information on how extrusion conditions affect the characteristics of IWG. Therefore, the goal of this study

TABLE 1 Variable combinations as determined using a three-factored face centered central composite design (FC-CCD) with six cube points

Run	Coded variables			Actual variables		
	X_1	X_2	X_3	Feed moisture (%)	Screw speed (rpm)	Extrusion temperature (°C)
1	-1	-1	-1	20	200	130
2	-1	0	0	20	300	150
3	-1	1	-1	20	400	130
4	-1	1	1	20	400	170
5	-1	-1	1	20	200	170
6	0	0	1	24	300	170
7	0	-1	0	24	200	150
8	0	0	-1	24	300	130
9	0	0	0	24	300	150
10	0	0	0	24	300	150
11	0	0	0	24	300	150
12	0	1	0	24	400	150
13	1	0	0	28	300	150
14	1	-1	1	28	200	170
15	1	-1	-1	28	200	130
16	1	1	-1	28	400	130
17	1	1	1	28	400	170

was to investigate the effect of some extrusion conditions on the physical and functional characteristics of expanded IWG extrudates and further optimize the extrusion conditions using RSM to obtain extrudates with high expansion properties and water absorption index.

2 | MATERIALS AND METHODS

2.1 | Materials

IWG grains (variety: “MN Clearwater”) were harvested in Stearns County, Minnesota, in August 2020. They were provided by the Agricultural Utilization Research Institute (Saint Cloud, MN, USA). The grains were cleaned, dehulled, and stored at 4°C prior to use. The cleaned, dehulled grains were milled to flour using a Break mill SM 3 (Brabender®, South Hackensack, NJ, USA) prior to extrusion.

2.2 | Experimental design

RSM was used to optimize the conditions for the extrusion of IWG. A three-factored face centered central composite design (FC-CCD) with six cube points was developed using Design-Expert® software (version 13, Stat-Ease, Inc., Minneapolis, MN, USA). The factors included feed moisture (%) as X_1 , screw speed (rpm) as X_2 , and extrusion

temperature (°C) as X_3 . The lowest and the highest values of the variables were coded as -1 and 1, respectively, with the mid-value coded as 0 (Table 1). These ranges were established based on preliminary experiments. The ranges for feed moisture were chosen because feed moisture content below 20% resulted in pressures close to the extruder's physical limits, while values above 28% did not result in expansion. The ranges for screw speed were chosen because values below 200 rpm resulted in torque close to the extruder's physical limits, whereas values above 400 rpm approached the maximum screw speed of the extruder. The ranges for extrusion temperature were selected because values below 130°C resulted in pressures close to the extruder's physical limits, whereas values above 170°C caused burning of the material. Furthermore, at 28% feed moisture, 400 rpm screw speed, and 170°C extrusion temperature, expansion was not observed (expansion ratio = 0.97 ± 0.01). Seventeen variable combinations with three center points were generated from the FC-CCD design (Table 1).

2.3 | Extrusion process

Extrusion of IWG flour was performed using a corotating twin-screw extruder (Brabender® TwinLab-F 20/40). A long screw with a length (L) of 800 mm and a diameter (D) of 20 mm was used, resulting in an L/D ratio of 40:1. The extruder was equipped with six heating zones. Heating

zones 1, 2, and 3 were kept at fixed temperatures of 50, 80, and 120°C, respectively. Heating zones 4–6 were set at the same temperature, and the temperature in these zones was varied according to the experimental design (Table 1). The screw speed and the feed moisture were also varied as presented in Table 1. The feed moisture content was adjusted to the desired levels by injecting a corresponding amount of water into the extruder using a peristaltic pump. The feed rate was kept constant at 2 kg/h. A circular die with a diameter of 3 mm was used at the end of the extruder. The extrudates were collected and dried at 50°C for 18 h. The dried samples were then stored under refrigeration conditions (~4°C) until analyzed.

2.4 | Moisture content

A loss-on-drying moisture analyzer (model: MB45 Basic AM; Ohaus®, Switzerland) was used to determine the moisture content of the samples. The extrudates were milled to flour prior to moisture determination. The conditions used were as follows: temperature, 180°C; time, 4 min; and sample weight, 1 g. Moisture determination was performed in triplicate.

2.5 | Expansion ratio

The expansion ratio was determined by dividing the cross-sectional diameter of the extrudates by the diameter of the circular die (Ding et al., 2006). The diameter of the extrudates was measured using a digital Vernier caliper (Toolshop®, Menard Inc., Eau Claire, WI, USA). Ten different extrudate pieces of each experimental run were randomly selected for the diameter measurements.

2.6 | Bulk density

The bulk density was determined in triplicate using the method reported by Charunuch et al. (2008), with some modifications. Briefly, the extrudates were poured into a 100 ml measuring cylinder and tapped 20 times on a flat platform. The volume and mass of the samples were recorded, and the bulk density was calculated by dividing the mass by volume.

2.7 | Hardness

Hardness was determined using a TA.XT.plus Texture Analyzer (Texture Technologies Corp, Scarsdale, NY, USA) as described by L. Liu et al. (2017), with some modifications. A cylindrical probe of 13 mm diameter was used for the double-compression test cycle with the following conditions: pretest speed, 2 mm/s; test speed and posttest speed, 0.4 mm/s; distance, 50% strain; hold time, 5 s; and trigger force, 5 g. The test was conducted at ambi-

ent temperature (~22°C). The hardness of the samples was calculated using Texture Analyzer software and recorded. Six randomly selected samples of each extrudate were analyzed.

2.8 | Water absorption index and water solubility index

The water absorption index (WAI) and water solubility index (WSI) were determined in triplicate using a technique developed for cereals (Ding et al., 2006), with some modifications. Specifically, 2 g of powdered sample was suspended and mixed with 20 ml distilled water in a 50 ml centrifuge tube. This was followed by stirring for 30 min at room temperature. Then, the suspension was centrifuged at 3000 g for 10 min at ambient temperature (~22°C). The supernatant was decanted into a tarred aluminum pan of known weight and dried at 135°C for 2 h to evaporate the moisture. The aluminum pan was then cooled to room temperature and weighed to obtain the weight of dry solids in the supernatant. The WAI was calculated as the weight of the sediment obtained after removal of the supernatant per unit weight of original dry solids. The WSI was calculated as the weight of dry solids in the supernatant expressed as a percentage of the original weight of the sample.

2.9 | Statistical analysis

The experimental runs (Table 1) were analyzed to investigate the effect of the independent variables on the physical and functional characteristics of IWG extrudates. The responses measured were moisture, expansion ratio, bulk density, hardness, water absorption index, and water solubility index. The obtained experimental data were fitted into quadratic equations, as shown in Equation (1) below:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (1)$$

where, Y is the response variable and is predicted using the factors represented as X_1 , X_2 , and X_3 . β_0 is the constant coefficient, and β_1 , β_2 , and β_3 are the linear regression coefficients. β_{11} , β_{22} , and β_{33} correspond to the coefficients of the squared independent variables, and β_{12} , β_{13} , and β_{23} correspond to the interaction coefficients. Then, a stepwise backward regression procedure was used to find the best-fit model. Akaike's information criterion (AIC) was used

as the model selection criterion for the stepwise backward regression procedure. AIC is a mathematical method used to evaluate how well a model fits the data. Lower AIC values are preferred, and this can help to choose the best-fit model to avoid underfitting or overfitting of the data.

Analysis of variance (ANOVA) was performed using Design-Expert[®] (version 13, Stat-Ease, Inc.) to determine the goodness of the fit and the significance of the coefficients on the responses at the 95% confidence level. Correlations among response variables were performed using Microsoft[®] Excel 2019 (Microsoft Corporation, Redmond, WA, USA). Correlations were deemed significant at $p < 0.05$.

2.10 | Optimization and model validation

Multiresponse optimization was carried out graphically using Design-Expert[®]. Once the individual responses had been numerically optimized, the criteria for each response and independent variables were set for the multiresponse optimization process.

To validate the RSM models, two independent experiments were conducted using the methods and number of replicates as described in Section 2. One of the conditions was selected in the optimum region, whereas the other set of conditions was selected outside the optimum conditions. Moreover, the two sets of conditions used for the validation study were selected such that they did not replicate any experimental run used in the RSM. The measured responses were compared to the predicted values from the response surface design by conducting a two-tailed, one-sample t -test using Microsoft[®] Excel 2019.

3 | RESULTS AND DISCUSSION

3.1 | RSM models

The results obtained for the responses (moisture, expansion ratio, bulk density, hardness, WAI, and WSI) are presented in Table 2. As shown in Table 3, for all responses, the models were significant ($p < 0.05$), and the lack-of-fit tests were not statistically significant ($p > 0.05$), implying goodness of fit. ANOVA was used to determine the significance ($p < 0.05$) of each term in the models (Table 4). The coefficient of determination (R^2) was also used to determine the appropriateness of the models. A relatively higher R^2 indicated that the model was a good fit. As shown in Table 4, R^2 was high for all responses (0.87–0.98). Moreover, the predicted R^2 and adjusted R^2 were within 0.2 of each other for all responses, suggesting good agreement between these two values. The closeness of adjusted R^2 and predicted R^2 is an indication that the model did not overfit the data.

3.2 | Moisture

The moisture content of the extrudates ranged from 5.14% to 6.13%. Moisture is critical for the shelf life and stability of ingredients and products. Low moisture of expanded products is desired for shelf stability and safety. The low moisture contents (<0.1) of the samples suggest that they would be shelf stable, and enzyme and microbial reactions that could affect their quality and stability would be minimal.

As shown in Table 4, all the independent variables had significant negative effects on moisture content. In addition, the interaction term of screw speed and extrusion temperature, as well as the quadratic term for screw speed, had significant positive effects on the moisture content. The best-fit equation is given below:

$$\text{Moisture} = 5.44 - 0.12X_1 - 0.12X_2 - 0.28X_3 + 0.21X_2X_3 + 0.32X_2^2. \quad (2)$$

The surface plots for moisture content are presented in Figure 1. The minimum moisture content was obtained when the feed moisture was 28%, the screw speed was 300 rpm, and the extrusion temperature was 150°C (Figure 1c), whereas the maximum moisture content was obtained when the feed moisture was 20%, the screw speed was 200 rpm, and the extrusion temperature was 130°C (Figure 1a). It is also evident from Figure 1 that at higher extrusion temperatures, the moisture content was low for the extrudates. This is due to the enhanced evaporation of moisture at higher temperatures. Increasing the screw speed reduced the moisture content of the extrudates. A high screw speed causes frictional heating of the screw, which can also aid in moisture evaporation. Similar observations on the effect of screw speed and extrusion temperature on the moisture content of extrudates were reported by Rani et al. (2021) for rice-black gram flour. Moisture evaporation is important for the expansion of extrudates. When an extrudate is melted because of high temperature and screw speed, the sudden drop in pressure at the end of the extruder results in a rapid evaporation of moisture as the product exits the die of the extruder. This causes a rapid expansion of the extrudate (Ye et al., 2018).

Even though screw speed and extrusion temperature had significant negative effects on the moisture content of the extrudates, their interaction, however, had a significant positive effect. At a lower extrusion temperature (130°C), increasing the screw speed resulted in a decrease in the moisture content of the extrudates, possibly due to the increased frictional heating and higher rate of water evaporation with the mixing effect of the screw speed. However, at an extrusion temperature of 170°C, a higher screw speed

TABLE 2 Experimental and predicted values for the response variables from the central composite design

Feed moisture (%)	Screw speed (rpm)	ET (°C)	Moisture (%)	Expansion ratio	Bulk density (kg/m ³)	Hardness (N)	WAI (g/g dry solids)	WSI (%)
20	400	170	6.22 ± 0.02 (5.68)	1.59 ± 0.11 (1.53)	100.45 ± 0.95 (111.10)	20.39 ± 1.36 (19.21)	3.75 ± 0.00 (3.80)	30.98 ± 0.62 (30.34)
20	300	150	5.47 ± 0.04 (5.56)	2.21 ± 0.01 (2.11)	144.70 ± 0.40 (158.17)	27.55 ± 0.56 (23.89)	4.14 ± 0.01 (4.20)	23.47 ± 0.17 (23.04)
20	200	130	6.53 ± 0.26 (6.48)	1.82 ± 0.03 (1.96)	354.20 ± 0.40 (329.60)	33.66 ± 0.44 (38.39)	4.38 ± 0.00 (4.41)	18.58 ± 0.53 (18.68)
20	400	130	5.62 ± 0.06 (5.83)	2.34 ± 0.08 (2.32)	174.05 ± 0.25 (164.30)	26.49 ± 1.46 (25.37)	3.83 ± 0.01 (3.80)	27.43 ± 0.43 (28.29)
20	200	170	5.65 ± 0.21 (5.51)	1.77 ± 0.02 (1.62)	144.55 ± 0.35 (157.94)	29.29 ± 0.60 (32.23)	4.35 ± 0.00 (4.41)	20.62 ± 0.05 (20.72)
24	400	150	5.88 ± 0.02 (5.64)	1.97 ± 0.08 (1.92)	156.25 ± 0.15 (137.69)	28.59 ± 1.31 (31.43)	3.92 ± 0.03 (3.80)	26.57 ± 0.15 (27.19)
24	300	170	5.22 ± 0.16 (5.16)	1.25 ± 0.00 (1.32)	173.40 ± 0.40 (167.08)	29.53 ± 0.30 (29.95)	4.33 ± 0.00 (4.20)	22.06 ± 0.09 (21.94)
24	300	130	5.81 ± 0.07 (5.72)	1.90 ± 0.04 (1.88)	250.40 ± 0.20 (279.51)	38.21 ± 0.08 (36.12)	4.23 ± 0.00 (4.20)	20.38 ± 0.18 (19.90)
24	300	150	5.46 ± 0.04 (5.44)	1.66 ± 0.02 (1.85)	204.30 ± 1.05 (190.73)	36.38 ± 1.49 (33.04)	4.14 ± 0.04 (4.20)	21.01 ± 0.52 (20.92)
24	200	150	5.69 ± 0.11 (5.87)	1.78 ± 0.02 (1.79)	229.25 ± 0.25 (243.76)	45.35 ± 1.71 (44.45)	4.34 ± 0.00 (4.41)	18.47 ± 0.04 (17.58)
28	200	130	6.28 ± 0.17 (6.24)	1.59 ± 0.08 (1.44)	392.95 ± 1.75 (394.72)	57.12 ± 0.18 (56.68)	4.50 ± 0.00 (4.41)	15.91 ± 1.85 (16.16)
28	300	150	5.14 ± 0.11 (5.32)	1.82 ± 0.02 (1.59)	207.65 ± 1.45 (223.29)	33.13 ± 0.89 (42.18)	4.15 ± 0.01 (4.20)	20.37 ± 0.52 (20.53)
28	400	170	5.38 ± 0.16 (5.44)	0.97 ± 0.01 (1.01)	175.45 ± 0.35 (176.22)	35.15 ± 3.07 (37.49)	3.78 ± 0.01 (3.80)	27.76 ± 0.42 (27.82)
28	200	170	5.22 ± 0.08 (5.27)	1.01 ± 0.04 (1.11)	241.55 ± 0.35 (223.06)	56.85 ± 2.89 (50.51)	4.46 ± 0.01 (4.41)	17.77 ± 0.03 (18.20)
28	400	130	5.68 ± 0.14 (5.60)	1.76 ± 0.07 (1.80)	225.95 ± 1.15 (229.42)	46.55 ± 1.37 (43.66)	3.70 ± 0.01 (3.80)	26.68 ± 0.28 (25.78)

Note: Data are expressed as the mean ± standard deviation followed by the predicted values in parentheses. Abbreviations: ET, extrusion temperature; WAI, water absorption index; WSI, water solubility index.

TABLE 3 Analysis of variance (ANOVA) for the best-fit models for the responses

Source	Sum of squares	Degrees of freedom	Mean square	F value	p Value
Moisture					
Model	1.80	5	0.36	14.94	0.0002
Residual	0.24	10	0.02		
Lack of fit	0.24	9	0.03	10.79	0.2323
Pure error	0.0025	1	0.0025		
Total	2.04	15			
Expansion ratio					
Model	1.85	5	0.37	16.80	0.0001
Residual	0.22	10	0.02		
Lack of fit	0.22	9	0.02	19.42	0.1744
Pure error	0.0013	1	0.0013		
Total		15			
Bulk density					
Model	81326.09	5	16265.22	46.02	<0.0001
Residual	3534.65	10	353.46		
Lack of fit	3532.44	9	392.49	178.00	0.0581
Pure error	2.21	1	2.21		
Total	84860.74	15			
Hardness					
Model	1444.92	4	361.23	19.11	<0.0001
Residual	207.89	11	18.90		
Lack of fit	203.48	10	20.35	4.61	0.3485
Pure error	4.41	1	4.41		
Total	1652.81	15			
WAI					
Model	0.97	2	0.4848	82.51	<0.0001
Residual	0.08	13	0.0059		
Lack of fit	0.07	12	0.0060	1.49	0.5717
Pure error	0.0041	1	0.0041		
Total	1.05	15			
WSI					
Model	272.61	5	54.52	113.61	<0.0001
Residual	4.80	10	0.48		
Lack of fit	4.26	9	0.47	0.88	0.6871
Pure error	0.54	1	0.54		
Total	277.41	15			

Abbreviations: WAI, water absorption index; WSI, water solubility index.

resulted in a slight increase in the moisture content of the extrudates. This explains the significant quadratic effect of screw speed had on the moisture content of the extrudates.

Interestingly, lower moisture contents were obtained at higher feed moisture levels. This confirms our observation that not only feed moisture but also screw speed and extrusion temperature influence the final moisture content of the extrudates. From Equation (2), it is apparent that screw speed and extrusion temperature had the most significant effect on extrudate moisture.

3.3 | Expansion ratio

Expansion properties are the most important physical properties of expanded products, especially for snack-type products (Patil et al., 2007). Expansion is critical to the acceptability of puffed products. The best-fit equation obtained for the expansion ratio is given below:

$$\text{Expansionratio} = 1.85 - 0.26X_1 + 0.07X_2 - 0.28X_3 - 0.11X_2X_3 - 0.25X_3^2 \quad (3)$$

TABLE 4 Regression coefficients and coefficients of determination for the models

Variables	Bulk density					
	Moisture (%)	Expansion ratio	(kg/m ³)	Hardness (N)	WAI (g/g dry solids)	WSI (%)
Intercept	5.45*	1.85*	190.72*	33.03*	4.20*	20.92*
X ₁	-0.11*	-0.26*	32.56*	9.14*	0.01	-1.26*
X ₂	-0.11*	0.07*	-53.04*	-6.51*	-0.31*	4.81*
X ₃	-0.28*	-0.28*	-56.21*	-3.08*	0.003	1.02*
X ₁ X ₂	0.03	-0.03	-1.11	-2.03	-0.04	0.19
X ₁ X ₃	-0.18	-0.07	10.17	-0.15	0.02	-0.33
X ₂ X ₃	0.21*	-0.11*	29.62*	-1.61	0.01	0.09
X ₁ ²	-0.12	0.13	-5.63	-1.74	-0.08	0.87*
X ₂ ²	0.32*	-0.01	10.94	4.89	-0.10*	1.46*
X ₃ ²	0.09	-0.25*	32.57*	1.79	0.06	0.12
R ²	0.89	0.89	0.96	0.87	0.93	0.98
Adjusted R ²	0.82	0.84	0.94	0.83	0.92	0.97
Predicted R ²	0.70	0.72	0.89	0.72	0.89	0.95

Note: X₁—feed moisture, X₂—screw speed, X₃—extrusion temperature. R²—Coefficient of determination.

Abbreviations: WAI, water absorption index; WSI, water solubility index.

*Statistical significance at $p < 0.05$.

The expansion ratio ranged from 0.97 to 2.34. The minimum expansion ratio was achieved when the feed moisture was 28%, the screw speed was 400 rpm, and the extrusion temperature was 170°C, whereas the maximum expansion ratio was achieved at low feed moisture (20%), 400 rpm screw speed, and 130°C extrusion temperature. The screw speed was found to have a positive effect on the expansion ratio. A similar observation of a positive effect of screw speed on the expansion ratio was reported for corn-based extrudates (Singha, Singh, et al., 2018) and water yam starches (Oke et al., 2013). The positive effect of screw speed on the expansion ratio has been attributed to the increase in shear and decrease in melt viscosity that is induced by high screw speeds (Oke et al., 2013). However, other studies have reported that screw speed had little to no effect on the expansion ratio: for wheat-based extrudates see Ding et al. (2006), and for extruded oat-corn puff see Y. Liu et al. (2000). As shown in Figure 2 and Table 4, increasing the extrusion temperature resulted in a significant decrease in the expansion ratio of the extrudates. This was in contrast with observations made in other studies. Temperature did not affect the expansion ratio of a wheat-based extrudate (Ding et al., 2006), whereas temperature had a positive effect on rice-black gram flour extrudate (Rani et al., 2021). Such differences in observations could be due to the differences in extrusion conditions used as well as the different feeds used. IWG has a lower starch content and higher protein and fiber content than wheat (Tyl & Ismail, 2019) and rice (Rani et al., 2021), and these variations in composition could influence how extrusion

conditions affect their expansion properties. It must be noted in the study by Rani et al. (2021) that black gram flour was also added to rice at a ratio of 1:3 and fermented prior to extrusion.

The quadratic term for extrusion temperature as well as its interaction term with screw speed had a significant negative effect on expansion. At a lower temperature (130°C), increasing the screw speed led to an increase in the expansion ratio, as expected. Conversely, increasing the screw speed at a higher temperature (170°C) led to a slight decrease in the expansion ratio. Rani et al. (2021) observed that at higher temperatures, the effect of screw speed diminished, and changes in the expansion ratio were driven by the extrusion temperature.

Feed moisture was found to have a significant negative effect on the expansion ratio (Figure 2, Table 4). Similar observations have been reported for wheat-based extrudates (Ding et al., 2006), yam starch (Oke et al., 2013), cassava starch (Leonel et al., 2009), and corn-based extrudates (Singha, Singh, et al., 2018). This is attributed to the fact that low moisture feeds tend to increase the pressure at the die due to high drag force, resulting in greater expansion of extrudates compared to high moisture feeds at the exit of the die (Oke et al., 2013). Ding et al. (2006) also reported that high moisture feeds can reduce dough elasticity and hence reduce gelatinization, thereby decreasing expansion of the extrudate. It is evident from Figure 2a that to attain a high expansion ratio, a lower temperature, lower feed moisture, and higher screw speed were needed.

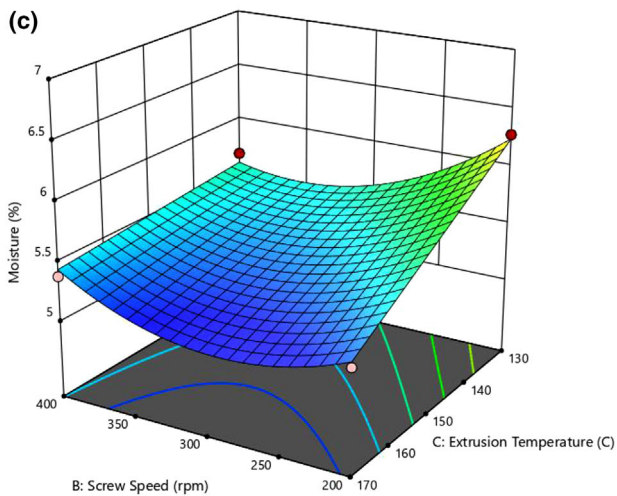
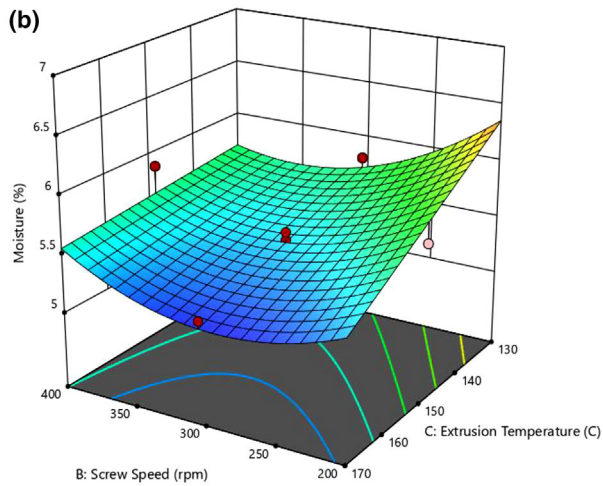
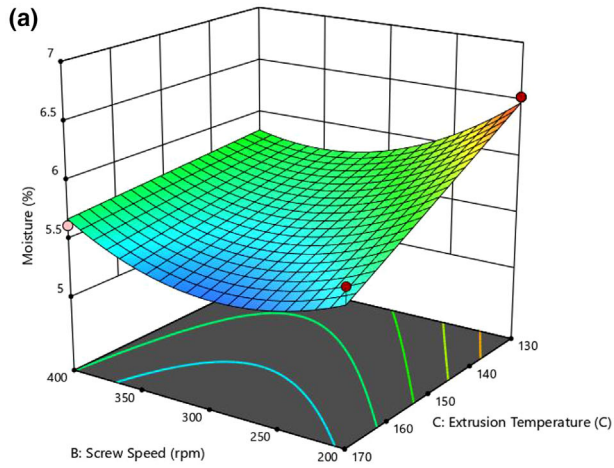


FIGURE 1 Three-dimensional (3D) surface plot showing the effects of screw speed and extrusion temperature on the moisture content of intermediate wheatgrass (IWG) extrudates at (a) 20% feed moisture, (b) 24% feed moisture, and (c) 28% feed moisture

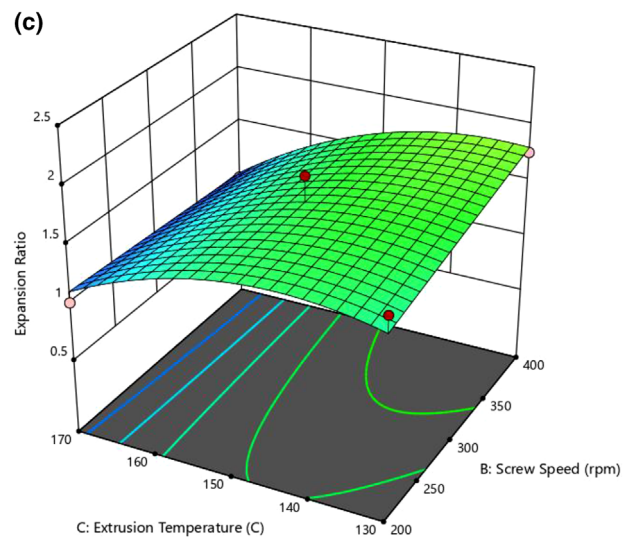
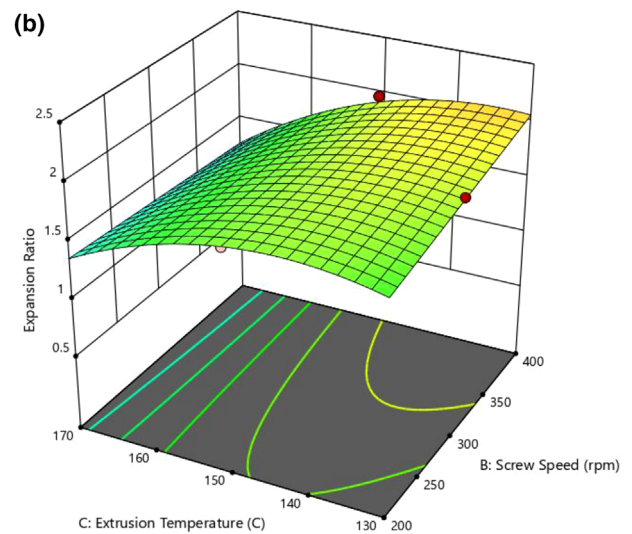
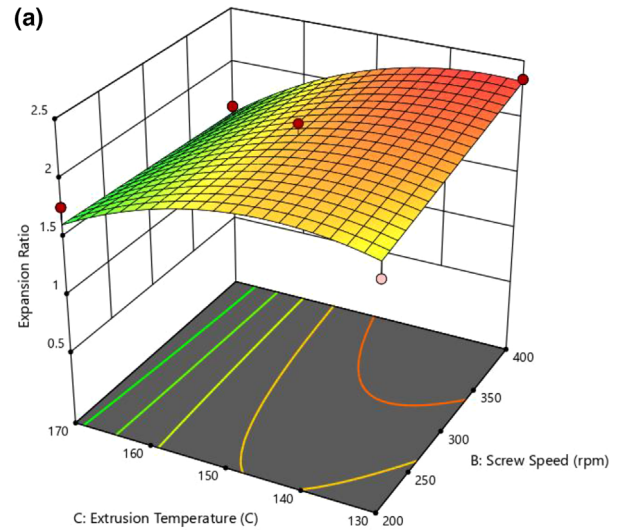


FIGURE 2 Three-dimensional (3D) surface plot showing the effects of screw speed and extrusion temperature on the expansion ratio of intermediate wheatgrass (IWG) extrudates at (a) 20% feed moisture, (b) 24% feed moisture, and (c) 28% feed moisture

3.4 | Bulk density

Bulk density is another important quality characteristic of expanded ready-to-eat products (Delgado-Nieblas et al., 2019; Rani et al., 2021), as it has been found to be negatively correlated with the expansion ratio in cereal-based extrudates (Ding et al., 2006; Singha, Singh, et al., 2018). The bulk density ranged from 100.45 to 392.95 kg/m³, with the lowest value obtained at low feed moisture (20%), higher screw speed (400 rpm), and higher extrusion temperature (170°C), whereas the highest value was obtained at high feed moisture (28%), low screw speed (200 rpm), and low extrusion temperature (130°C). From Table 4, the best-fit equation for bulk density is given below:

$$\begin{aligned} \text{Bulk density} = & 190.72 + 32.56X_1 - 53.04X_2 - 56.21X_3 \\ & + 29.62X_2X_3 + 32.57X_3^2. \end{aligned} \quad (4)$$

The response surface plots are presented in Figure 3. Feed moisture, screw speed, and extrusion temperature were found to have significant effects on bulk density. An increase in feed moisture content resulted in an increase in the bulk density of the extrudates. A similar trend was reported by other researchers: for wheat-based expanded snacks see Ding et al. (2006), for chickpea flour-based extrudate see Meng et al. (2010), and for corn-based extrudate see Singha, Singh, et al. (2018). Ding et al. (2006) reported that high feed moisture negatively influenced dough elasticity and gelatinization, decreasing the density of extrudates.

Increasing temperature resulted in a decrease in bulk density, which was in agreement with previous studies on chickpea flour-based extrudate (Meng et al., 2010) and corn-based extrudate (Singha, Singh, et al., 2018). This observation is due to an increase in the superheating of water at high temperatures, favoring the formation of bubbles and increasing the volume of the extrudates (Singha, Muthukumarappan, et al., 2018). Consequently, there is a decrease in the density of the extrudates. Singha, Singh, et al. (2018) also reported that higher screw speeds result in high shear, which can lower melt viscosity and increase dough elasticity, thereby reducing density. It is, therefore, not surprising that screw speed had a negative significant effect on bulk density in this study.

Interestingly, the extrusion temperature and screw speed interaction had a significant positive effect on bulk density, even though their individual terms had a negative effect on bulk density. It is apparent in Figure 3 that at

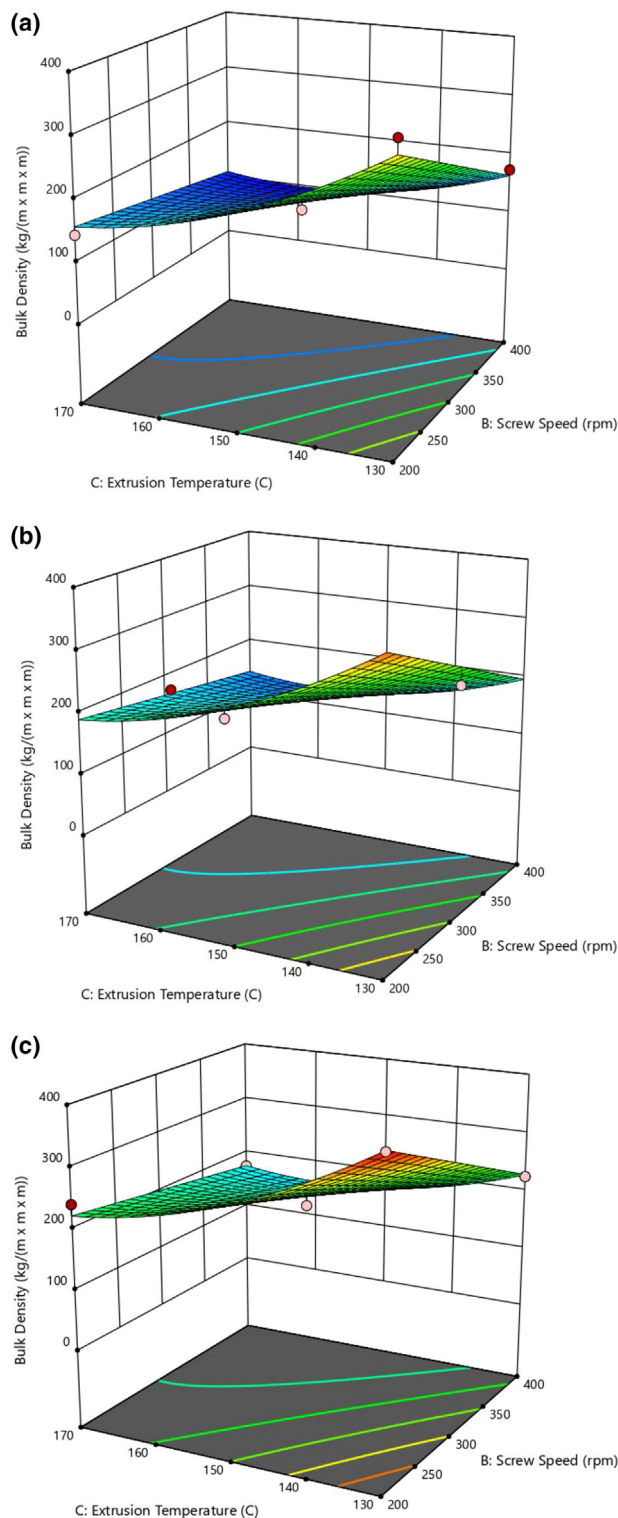


FIGURE 3 Three-dimensional (3D) surface plot showing the effects of screw speed and extrusion temperature on the bulk density of intermediate wheatgrass (IWG) extrudates at (a) 20% feed moisture, (b) 24% feed moisture, and (c) 28% feed moisture

low screw speed, increasing the temperature had a greater negative effect on bulk density than at high screw speed. Another interesting observation was the positive effect of the quadratic term of extrusion temperature on bulk density. At higher extrusion temperatures, the negative effect of extrusion temperature diminished. At a screw speed of 400 rpm, increasing the extrusion temperature above 160°C caused a slight increase in bulk density, confirming the interaction effect observed. These findings were not observed for corn-based extrudates (Singha, Singh, et al., 2018). It is possible that this might be due to other conditions or parameters not included in this study, differences in extrusion conditions, and the different feeds used. In the study by Singha, Singh, et al. (2018), for instance, garbanzo flour and food grade distiller's dried grains, which are very high in dietary fiber and protein, were added to corn grits. The presence of dietary fiber and protein could interfere with starch gelatinization and affect the expansion and density of the extrudates (Singha, Singh, et al., 2018).

3.5 | Hardness

Hardness is the maximum force needed for the probe to break the extrudate. Hardness, like expansion properties, is an important quality characteristic for the acceptance of expanded products. The hardness of expanded extrudates is mainly a perception of the consumer and has been associated with extrudates' expansion and cell structure (Charunuch et al., 2014; Ding et al., 2005).

The hardness of the extrudates ranged from 20.39 to 57.12 N. The minimum hardness was achieved at 20% feed moisture, 400 rpm screw speed, and 170°C extrusion temperature, whereas the maximum value was achieved at 28% feed moisture, 200 rpm screw speed, and 130°C extrusion temperature. The effects of extrusion conditions on the hardness of the extrudates are shown in Figure 4 and Table 4. All the independent variables were found to have significant effects on the hardness of the extrudates. The best-fit equation is given below:

$$\text{Hardness} = 33.03 + 9.14X_1 - 6.51X_2 - 3.08X_3 \quad (5)$$

Increasing the feed moisture resulted in an increase in the hardness of the extrudates. A similar trend has been reported for rice-based expanded snacks (Ding et al., 2005), wheat-based expanded snacks (Ding et al., 2006), and chickpea flour-based expanded snacks (Meng et al., 2010). Ding et al. (2005) reported that with high feed moisture, water acted as a plasticizer to the starch-based material and reduced its viscosity. This reduced bubble growth in the

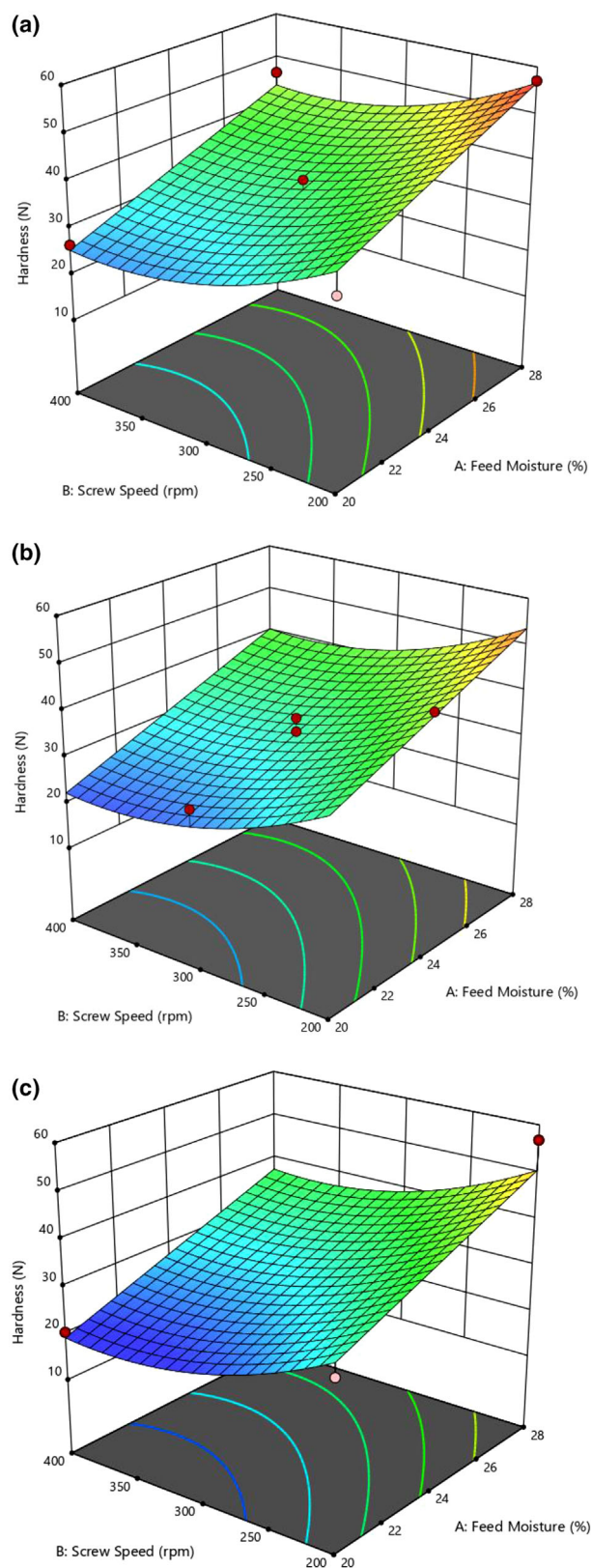


FIGURE 4 Three-dimensional (3D) surface plot showing the effects of feed moisture and screw speed on the hardness of intermediate wheatgrass (IWG) extrudates at (a) 130°C extrusion temperature, (b) 150°C extrusion temperature, and (c) 170°C extrusion temperature

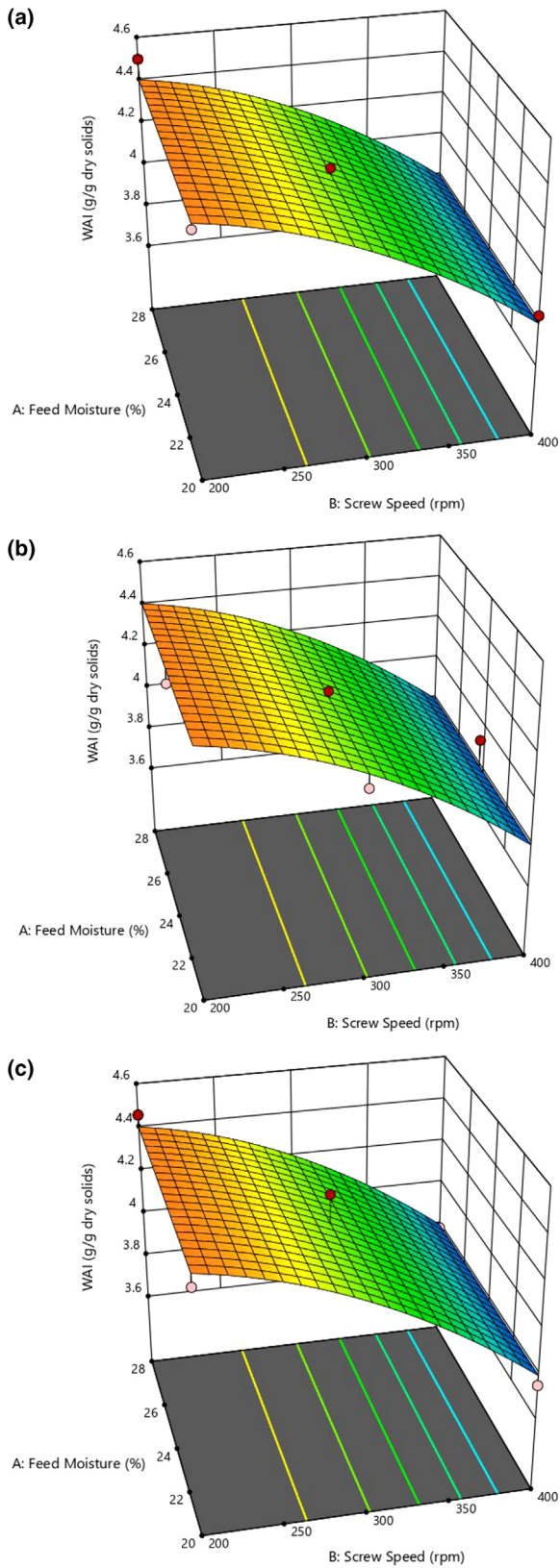


FIGURE 5 Three-dimensional (3D) surface plot showing the effects of feed moisture and screw speed on the water absorption index (WAI) of intermediate wheatgrass (IWG) extrudates at (a) 130°C extrusion temperature, (b) 150°C extrusion temperature, and (c) 170°C extrusion temperature

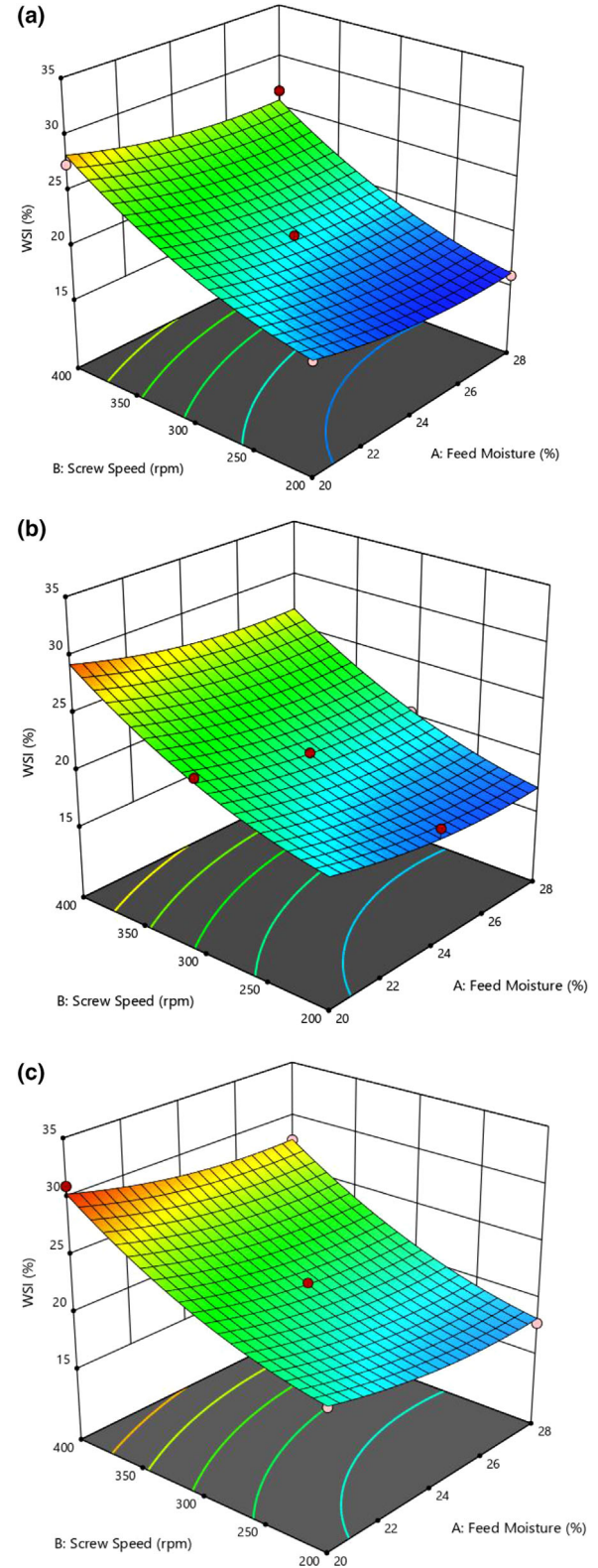


FIGURE 6 Three-dimensional (3D) surface plot showing the effects of feed moisture and screw speed on the water solubility index (WSI) of intermediate wheatgrass (IWG) extrudates at (a) 130°C extrusion temperature, (b) 150°C extrusion temperature, and (c) 170°C extrusion temperature

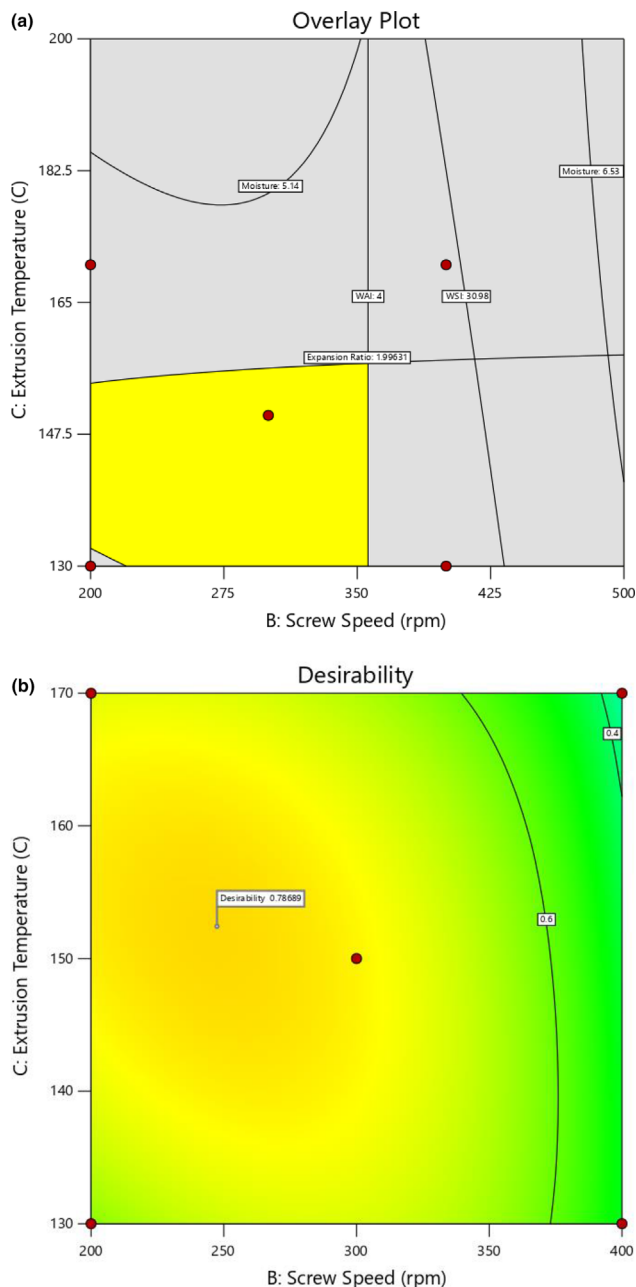


FIGURE 7 Multiresponse optimization (a) overlay plot and (b) desirability plot at 20% feed moisture for minimum water solubility index (WSI), minimum hardness, minimum bulk density, expansion ratio (>2), and WAI (>4 g/g dry solids) shown by the shaded yellow regions

material, resulting in denser and, consequently, relatively harder products. This was also observed in our study, with product hardness having a strong positive correlation with bulk density. The correlation results are explained later in this study.

Extrusion temperature and screw speed were found to have a significant negative effect on extrudate hardness. Contrary to high feed moisture, a higher screw speed and

extrusion temperature lower the melt viscosity and favor bubble growth, resulting in less dense extrudates with a higher degree of expansion. As such, one would expect that to improve the expansion ratio of IWG extrudates, an extrudate with relatively lower density and hardness would be desired. Other studies have also reported a negative effect of screw speed and extrusion temperature on extrudate hardness: for wheat-based expanded snacks see Ding et al. (2006), and for chickpea flour-based expanded snacks see Meng et al. (2010).

3.6 | Hydration properties

3.6.1 | Water absorption index

WAI measures the amount of water absorbed by the ground extrudate, mainly by the starch component. As such, WAI is often used as an index of gelatinization and dextrinization during extrusion (Anderson et al., 1970). Gelatinization of starch causes a disruption in the crystalline structure due to the breaking of intra- and intermolecular hydrogen bonds between the crystalline and amorphous regions. This results in the starch granules forming hydrogen bonds with water, thereby increasing WAI after extrusion. Moreover, water molecules can easily diffuse into the amorphous regions of starch after extrusion.

A high WAI is an *in vitro* indicator of good starch digestibility (Pardhi et al., 2019), which is important in the production of breakfast cereals and snacks. It is important to note that this might not be desirable for weight management. As starch granules are disrupted during extrusion, more water is able to bind to the starch molecule. However, this can cause the extrudate to be more susceptible to microbial and enzymatic reactions that can negatively affect the safety and stability of the extrudate during storage. Therefore, packaging as well as the addition of preservatives should be considered during the production of such breakfast cereals and snacks.

The minimum WAI (3.7 g/g dry solids) was achieved at 28% feed moisture, 400 rpm screw speed, and 130°C extrusion temperature, whereas the maximum WAI (4.5 g/g dry solids) was achieved at 28% feed moisture, 200 rpm screw speed, and 130°C extrusion temperature. The best-fit equation for WAI is given below:

$$\text{WAI} = 4.20 - 0.31X_2 - 0.10X_2^2 \quad (6)$$

Of all the independent variables, only screw speed was found to have a significant effect on WAI, with both its linear and quadratic terms having a significant negative effect on WAI (Table 4). As shown in Figure 5, increasing

the screw speed resulted in a decrease in WAI, irrespective of feed moisture content and extrusion temperature. A decrease in WAI with an increase in screw speed has been previously reported for soy white flake-based aquafeed (S. K. Singh & Muthukumarappan, 2016) and corn-based extrudates (Singha, Singh, et al., 2018). A high screw speed has been associated with high mechanical shear, which leads to the breakdown of starch polymers into smaller molecules with higher solubility (Altan et al., 2009). This could also explain the significant quadratic effect of screw speed on WAI, as the increase in screw speed above 250 rpm had a greater negative effect on WAI than the initial increase from 200 to 250 rpm.

3.6.2 | Water solubility index

WSI is often used as an index of degradation caused in macromolecules such as starch granules and fiber during extrusion. It is generally used as a measure of the amount of soluble components released from the starch component of the material after extrusion (Ding et al., 2006). Low WSI is desired for breakfast cereals since these products are mostly consumed in liquid milk or water and being excessively soft or mostly soluble in the liquid medium is not desirable (Charunuch et al., 2014; Delgado-Nieblas et al., 2019). The WSI for the extrudates ranged from 15.91% to 30.98%. The minimum WSI was recorded at a feed moisture content of 28%, a screw speed of 200 rpm, and an extrusion temperature of 130°C, whereas the maximum WSI was recorded at 20% feed moisture, 400 rpm screw speed, and 170°C extrusion temperature. The response surface plots for the WSI are presented in Figure 6. From Table 4, the best-fit equation obtained for the WSI is given below:

$$\text{WSI} = 20.92 - 1.26X_1 + 4.81X_2 + 1.02X_3 + 0.86X_1^2 + 1.46X_2^2. \quad (7)$$

All the independent variables had significant effects on WSI, with screw speed having the most significant effect. Feed moisture was found to have a significant negative effect on WSI. However, its quadratic term had a significant positive effect on the WSI of the extrudates. Higher moisture content can reduce the level of shearing and starch degradation (Hernández-Díaz et al., 2007), which could explain the negative effect of feed moisture content on WSI. However, at increasingly high feed moisture levels (quadratic term of feed moisture), it is possible that the positive effect of feed moisture on WSI is driven by the amount of water present rather than starch degradation.

Screw speed and extrusion temperature had significant positive effects on the WSI of the extrudates. With high

screw speed, high mechanical shear damaged the macromolecules, which resulted in lower molecular weight starch granules and increased water solubility (Altan et al., 2009; Mezreb et al., 2003). Moreover, elevated temperatures cause starch degradation into smaller molecular weight fractions with increased solubility, resulting in increased WSI (Ding et al., 2006). Furthermore, high temperature and screw speed have been shown to increase the soluble dietary fiber fraction and decrease the insoluble fraction (Singha, Singh, et al., 2018), resulting in increased water solubility. With the high fiber content of IWG (Tyl & Ismail, 2019), this could have also contributed to the increased WSI.

3.7 | Response correlations

Correlations were performed to ascertain the relationships among the response variables. The expansion ratio was found to have a negative correlation with hardness ($r = -0.40$, $p = 0.1320$) and bulk density ($r = -0.10$, $p = 0.7560$). Such correlations have been reported for wheat-based extrudates (Ding et al., 2006) and corn-based extrudates (Singha, Singh, et al., 2018), albeit not significant in our study. The difference in significance might be due to the raw materials used in these studies as well as the extruder operating conditions. Extrudates with high expansion have lower bulk density due to the high volume created from bubble growth, resulting in low hardness as well. It was, therefore, not surprising that bulk density had a strong positive correlation with hardness ($r = 0.71$, $p = 0.0023$). WAI was found to have a strong negative correlation with WSI ($r = -0.94$, $p < 0.0001$), consistent with observations in other studies for wheat-based extrudates (Ding et al., 2006) and corn-based extrudates (Singha, Singh, et al., 2018). This can be explained by the high amount of starch in these cereal-based extrudates and how the transformation of starch during extrusion is linked to WAI and WSI. As already explained, a high WSI indicates that there is significant degradation of starch into relatively smaller molecules with higher solubility. Conversely, WAI is decreased since there is less high molecular weight starch that can bind water.

3.8 | Optimization and model validation

The optimum conditions for each individual response are presented in Table 5. Since a high expansion ratio and low WSI are desired for breakfast cereals and snacks (Charunuch et al., 2014; R. K. R. Singh & Majumdar, 2014), they were maximized and minimized, respectively, for their optimization. Additionally, bulk density and hardness were minimized, and WAI was minimized due to

TABLE 5 Optimization of individual response models

Response constraint	Optimum conditions			Predicted value	Desirability
	Feed moisture (%)	Screw speed (rpm)	Extrusion temperature (°C)		
Maximum expansion ratio	20.00	400.00	130.00	2.33	0.99
Minimum bulk density (kg/m ³)	20.00	399.56	158.15	99.91	1.00
Minimum hardness (N)	20.00	400.00	170.00	19.21	1.00
Maximum WAI (g/g dry solids)	24.00	200.00	150.00	4.41	0.88
Minimum WSI (%)	26.92	200.00	130.00	16.10	0.99

Abbreviations: WAI, water absorption index; WSI, water solubility index.

TABLE 6 Validation of optimized models

Name	Predicted values ^a	Experimental values ^a	Predicted values ^b	Experimental values ^b
Feed moisture (%)	20.00	20.00	24.00	24.00
Screw speed (rpm)	200.00	200.00	400.00	400.00
Extrusion temperature (°C)	150.00	150.00	130.00	130.00
Moisture (%)	5.99	5.81 ± 0.09	5.70	5.79 ± 0.12
Expansion ratio	2.14	2.20 ± 0.08	2.04	1.79 ± 0.07
Bulk density (kg/m ³)	211.20	200.85 ± 4.63	199.95	206.80 ± 1.70
Hardness (N)	35.31	35.78 ± 2.60	35.38	36.64 ± 2.02
WAI (g/g dry solids)	4.41	4.39 ± 0.06	3.80	3.81 ± 0.02
WSI (%)	19.70	18.12 ± 1.04	26.06	27.12 ± 0.68

^aPredicted and experimental values for conditions in the optimum range.

^bPredicted and experimental values for conditions outside the optimum range.

their desired levels (Charunuch et al., 2014; R. K. R. Singh & Majumdar, 2014), as well as their correlations with expansion ratio and WSI. The desirability of the optimized models ranged from 0.88 to 1. Multiresponse optimization was also carried out graphically using Design-Expert[®], using the constraints set for each response as shown in Table 5, in addition to targets for expansion ratio (>2) and WAI (>4). A high expansion ratio and WAI would result in low bulk density, hardness, and WSI. The overlay and desirability charts generated by the constraints are shown in Figure 7. The conditions were best achieved only at 20% feed moisture content. No optimum regions were observed when the feed moisture content was 24% or 28%. At 20% feed moisture content, a range of screw speeds and extrusion temperatures were possible, as shown by the shaded yellow region in Figure 7a. Extrusion at 20% feed moisture content, a screw speed range of 200–356 rpm, and an extrusion temperature range of 130–154°C would result in IWG extrudates with a relatively higher expansion ratio and lower WSI.

The results of the validation study are presented in Table 6. Validation is performed to ensure the accuracy of the models. A model's ability to accurately predict a response from unseen data is critical for ensuring scala-

bility and quality and minimizing overfitting and underfitting. The validation study showed that there were no significant differences between the predicted values and the experimental values of the responses, indicating good agreement between the two sets of values.

4 | CONCLUSIONS

This study highlighted the variability in the physical and functional properties of expanded IWG as impacted by extrusion conditions. This study demonstrated that RSM could be used to optimize extrusion conditions for the production of expanded IWG. Furthermore, to produce an IWG extrudate with desirable expansion and functional properties, low moisture was critical. This information is useful for designing extrusion conditions for the extrusion of IWG for products such as breakfast cereals and snacks. This is critical in the efforts to commercialize IWG and increase its use for food applications. Future studies with a focus on the physicochemical and nutritional characterization of IWG extrudates would be useful for further considerations in expanded snack and breakfast cereal applications. Additionally, studies focused on

sensory analysis would be useful for assessing consumer preference and acceptability of expanded products made from IWG.

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AUTHOR CONTRIBUTIONS

Prince G. Boakye: Conceptualization; Data curation; Formal analysis; Investigation; Writing-original draft; Writing-review and editing. **Akua Y. Okyere:** Data curation; Formal analysis; Investigation; Methodology; Writing-review and editing. **Ibilola Koungblenou:** Data curation; Investigation; Methodology. **Ryan Kowalski:** Data curation; Investigation; Methodology. **Baraem P. Ismail:** Conceptualization; Funding acquisition; Writing-review and editing. **George A. Annor:** Conceptualization; Funding acquisition; Supervision; Writing-review and editing.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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