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### **Optimization of Roba1 extrusion conditions and bean extrudate properties using response surface methodology and multi-response desirability function**

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#### **Citation:**

Natabirwa, Hedwig, Nakimbugwe, Dorothy, Lung'aho, Mercy, Muyonga, John H. (2018). Optimization of Roba1 extrusion conditions and bean extrudate properties using response surface methodology and multi-response desirability function. *LWT - Food Science and Technology*, 96, 411–418.

#### **Publisher's DOI:**

<https://doi.org/10.1016/j.lwt.2018.05.040>

#### **Access through CIAT Research Online:**

<http://hdl.handle.net/10568/93204>

#### **Terms:**

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1 Optimization of Roba1 bean extrusion conditions and extrudate properties using  
2 Response surface methodology and multi-response desirability function

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12  
13 **Abstract**

14 Effects of extruder die temperature, screw speed and ingredient feed moisture on  
15 Roba1 bean extrudate nutritional and physicochemical properties were evaluated by  
16 response surface methodology (RSM) and extrusion processing conditions  
17 optimized for optimal extrudate attributes by multi-response desirability function.  
18 Responses taken were protein content, protein digestibility, polyphenols, phytates,  
19 extrudate expansion, bulk density, water absorption and water solubility index, as  
20 well as texture.. Feed moisture, die temperature and screw speed significantly ( $p <$   
21  $0.05$ ) influenced the physicochemical properties of Roba1 extrudates ( $R^2 \geq 0.500$ ).  
22 Increase in feed moisture at low die temperatures resulted in decrease in extrudate

23 expansion ratio (~3.96%) and water solubility (~10%). Increases in expansion, and  
24 reduction in bulk density and water absorption index due to increase in screw speed  
25 and die temperature were also observed. Predictive desirability optimization  
26 generated optimal attributes (expansion ratio, 2.59; bulk density, 1.32; protein  
27 digestibility, 81.58%; and hardness, 24.4 N) for snack with desirability index of 0.75.  
28 Information from this study can be useful for optimization of bean snack extrusion  
29 process and product in the food industry.

30 **Key words:** Extrusion, common beans, optimization, desirability function,  
31 physicochemical properties, RSM

## 32 1. INTRODUCTION

33 Common beans (*Phaseolus vulgaris L.*) are a nutritious food consumed as a staple  
34 by a large population throughout the world, especially in developing countries  
35 (Anderson *et al.*, 2016). Beans not only provide proteins, but are also rich in  
36 vitamins, minerals and fibre (Nyombaire, Siddiq, & Dolan, 2011), and average daily  
37 per capita consumption ranges between 0.01 and 0.18 kg/day (Blair, 2013).  
38 Recently, new common bean varieties enriched with iron and zinc through  
39 conventional breeding were developed with the aim of reducing micronutrient  
40 malnutrition among vulnerable populations. Among these is Roba1 **containing**  
41 **about 66.7 and 27.6 µg/g of Fe and Zn, respectively (Natabirwa, Muyonga,**  
42 **Nakimbugwe, & Lungaho, 2018). Roba1 is a high-yielding and multiple disease-**  
43 **resistant haricot bean variety currently produced in Sub-Saharan African countries**  
44 **(Ethiopia, Tanzania and Uganda), with average yield of about 1.8 tons/ha**  
45 **(Mukankusi, Nkalubo, Katungi, et al, 2015).**Biofortified and iron-rich beans in  
46 general obtain higher levels of iron (~70 - 96 µg/g) and zinc and (~26 - 35µg/g) than  
47 the conventional beans (Bouis & Welch, 2010). The use of biofortified beans as

48 major ingredients in processed foods would help to improve the nutritional quality.  
49 Bean consumption however has generally remained relatively low due to the lengthy  
50 cooking time which translates into high fuel costs (Rocha-Guzman *et al.*, 2008), and  
51 monotonous preparation techniques involved. Moreover, common beans have  
52 hardly been explored as raw material in the food industry, except canning and to  
53 some extent, flour production (Nyombaire *et al.*, 2011; Pedrosa *et al.*, 2015). Even  
54 though sprouting, soaking, fermentation and roasting have been explored for  
55 nutritional improvement, the processes are cumbersome and can only be used on  
56 small-scale (Nkundabombi, Nakimbugwe, & Muyonga, 2015; Rehman, Salariya, &  
57 Zafar, 2001; Rocha-Guzman *et al.*, 2008).

58 Extrusion cooking, a high temperature-short time industrial processing technique,  
59 has been singled out as the most promising method which can transform food raw  
60 material into highly nutritious, palatable and quality food (Ghumman, Kaur, Singh,  
61 & Singh, 2016; Siddiq, Kelkar, Harte, Dolan, & Nyombaire, 2013). Extrusion  
62 produces a range of food products from cereals and legumes (including flours,  
63 snacks, breakfast cereal, etcetera) with distinctive characteristics (Anton, Fulcher, &  
64 Arntfield, 2009; Meng, Threinen, Hansen, & Driedger, 2010; Nyombaire *et al.*,  
65 2011). The technology offers high efficiency in terms of fuel cost and output, but it  
66 is affected by a range of factors including ingredient type and extrusion conditions  
67 which determine the properties of extrudates (Ghumman *et al.*, 2016; Steel *et al.*,  
68 2012). Notably, any variations in parameters such as extruder barrel temperatures,  
69 ingredient moisture, specific mechanical energy and screw speed, affect process  
70 variables as well as product quality.

71 Reports have shown that mild extrusion conditions (high moisture content, low  
72 residence time, low temperature) improve the nutritional quality of beans, while high  
73 extrusion temperatures (>200 °C), low moisture contents (< 15%) and/or improper

74 formulation (such as presence of high-reactive sugars) can adversely impair the  
75 nutritional quality (Berrios, Ascheri, & Losso, 2012; Siddiq et al., 2013). However,  
76 studies on effects of extrusion processing conditions on extrudate properties have  
77 majorly been undertaken on conventional beans, other legumes and cereals  
78 (Ghumman *et al.*, 2016; Korus, Gumul, & Czechowska, 2007; Nyombaire *et al.*,  
79 2011; Rathod & Annapure, 2017; Siddiq *et al.*, 2013).

80 Response Surface methodology (RSM) and multi-response desirability function can  
81 be used for optimization of processing conditions through exploration of relationship  
82 between several processes and responses (Altan, McCarthy, & Maskan, 2008;  
83 Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008; Jain, Monika; Singh, Chetna;  
84 Gupta, Kushboo; Jain, 2014). The RSM approach is important in design,  
85 development and formulation of new products, as well as improvement of existing  
86 product design (Bezerra *et al.*, 2008). With the desirability function approach,  
87 operating conditions that meet the criteria set for optimization and provide the best  
88 value of compromise for combined responses, are established (Vera Candiotti, De  
89 Zan, Cámara, & Goicoechea, 2014). Various product quality characteristics can be  
90 optimized together. For the extrusion of biofortified beans to be successful, critical  
91 control of processing conditions and a precise study of the variations that occur in  
92 product properties are necessary. The objective of this study was to investigate the  
93 effects of feed moisture, screw speed and die temperature on physicochemical and  
94 nutritional properties of Robal bean extrudate and; to determine the best  
95 combination of extrusion processing parameters for a desirable snack extrudate.

96

## 97 **2. MATERIALS AND METHODS**

### 98 **2.1 Experimental design**

99 Roba1 bean extrudates were developed following a Box Behnken design with three  
 100 independent variables including ingredient feed moisture, extruder die temperature,  
 101 and screw speed. Levels of each variable were established basing on preliminary  
 102 trials and works from previous authors (Anton *et al.*, 2009; Berrios *et al.*, 2012). The  
 103 three levels of process variables were coded as -1, 0 and 1, making the total number  
 104 of experiments equal to 15 by Box Behnken design. Coded and actual values for  
 105 process variables are given in Table 1.

106 Table 1 Coded and actual values used in developing experimental data

Factors	Factor codes	Level codes and actual values		
		-1	0	1
Die temperature (°C)	$X_1$	120	135	150
Feed moisture (%)	$X_2$	15	17.5	20
Screw speed (Hz)	$X_3$	35	40	45

107

## 108 2.2 Material preparation

109 Newly harvested dry beans of variety Roba1, a plain cream coloured bean enriched  
 110 with iron and zinc through biofortification, were purchased from farmers in Rakai  
 111 district, Uganda. The beans were sorted, washed with clean tap water and solar dried  
 112 at temperatures 30 – 55°C for approximately 20 hours. Dried bean grains were  
 113 milled using a commercial mill (Model YZMF, Yize, Shuliy Henan, China), to pass  
 114 through a 1.5 mm sieve.

## 115 2.3 Extrusion

116 Beans were extruded in a Twin Screw Extruder (Model DP 70-III, Jinan, China) at  
 117 barrel temperatures 60/100/120°C, 60/110/135 °C and 60/110/150 °C, and feed  
 118 moisture 15 to 20 % and screw speeds (35, 40 and 45 Hz), as described in Table 1  
 119 above. The die diameter, screw diameter and length to diameter ratio of extruder  
 120 were 5 mm, 27 mm and 18:1, respectively. Resultant extrudates were cooled to room

121 temperature, and milled using a Stainless Steel mill (Model 30B-C, Changzhou,  
122 China) to pass through a 1.5 mm pore size sieve.

## 123 **2.4 Extrudate analysis**

### 124 2.4.1 Physicochemical properties

125 Extrudate expansion ratio (*ER*) and bulk density (*BD*) were determined according to  
126 methods described (Natabirwa, Muyonga, Nakimbugwe, & Lungaho, 2017). Water  
127 absorption index (*WAI*) and water solubility index (*WSI*) were determined using  
128 methods described (Natabirwa *et al.*, 2017; Nyombaire *et al.*, 2011). The final  
129 pasting viscosity of extruded flour was determined using extrusion profile on a Rapid  
130 Viscoanalyzer, Model RVA 4500, (Perten Instruments, Australia) using methods  
131 described (Natabirwa *et al.*, 2017).

### 132 2.4.2 Extrudate texture

133 The texture of cylindrical extrudates (approximately 3 – 4 cm long pieces) was  
134 measured using a Stable Microsystems Texture Analyzer (Model TA.XT-Plus  
135 42095, UK) by compression with a cylindrical probe of 6 mm diameter (SMS P/6)  
136 following methods described (Altan *et al.*, 2008) with modifications. Hardness in  
137 newtons (N) was determined by measuring the maximum force required to break the  
138 extruded samples (~ 40 mm long), while crunchiness was determined as the average  
139 area under the force-deformation curve. The test speed was 2mm/s and the  
140 penetration distance was 5mm, with a trigger force 0.049 N. Post-test speed was  
141 10.00 mm/sec. The return distance of the probe was kept at 20 mm. A force time  
142 curve was recorded and analyzed by Texture Exponent 32 Software programme. Ten  
143 (10) measurements were performed on each sample and averaged.

### 144 2.4.3 Protein digestibility

145 A multi-enzyme in vitro technique (Hsu, Vavak, Satterlee, & Miller, 1977; Krupa-  
146 Kozak & Soral-Šmietana, 2010) was applied for determination of protein  
147 digestibility since it could avoid under-predicting the digestibility of proteins. The  
148 digestibility was calculated using the regression equation (eq. 1) (Hsu *et al.*, 1977).

$$149 \text{ Protein digestibility (\%)} = 201.464 - 18.103 \times H \quad (1)$$

150 where, *H* is the pH value of the sample suspension after 10 minutes digestion with  
151 the multi-enzyme solution.

#### 152 2.4.4 Total polyphenol content

153 Total polyphenol content was determined using the Folin-Ciocalteau reagent, as  
154 described (Makkar, 2000; Natukunda, Muyonga, & Mukisa, 2016), with  
155 modifications. Briefly, 0.20 g of finely ground bean flour was measured into a 50 ml  
156 polypropylene tube and extracted twice using 5 ml of methanol:water (50:50, v/v)  
157 with ultrasonication (20 min). The extraction solution was centrifuged at 3000 x g,  
158 for 10 min.), and the supernatant (extract) was stored at 4°C in a refrigerator until  
159 time for use. Total polyphenols in the extract were then determined as described  
160 (Natukunda *et al.*, 2016). A standard curve was prepared with gallic acid at  
161 concentrations 0.00, 0.02, 0.04, 0.06, 0.08 and 0.10 mg/ml. Final results were  
162 expressed as gallic acid equivalents (mg GAE/100 g of bean flour).

#### 163 2.4.5 Phytate determination

164 Phytate content was determined according to the method described (Gao *et al.*, 2007)  
165 with modifications. Briefly, 0.5 g of raw and extruded bean flour (ground to pass  
166 through a 1.0 mm screen) was weighed into clean 15 mL polypropylene tubes, and  
167 10 mL of 2.4% HCl extraction solution was added. The tubes were shaken at 220  
168 rpm for 16 h in an Orbital Incubator/Shaker (Model, Stuart S1600C, Wagtech) and  
169 centrifuged at 1000 x g for 10 min. at 10 °C. The extract was collected into a new



170 set of falcon tubes containing 1 g NaCl each. The contents were vortexed for approx.  
171 1 min. to dissolve the salt and allowed to settle at 4 °C for 60 min. The mixtures were  
172 centrifuged again at 1000 x g for 10 minutes, and clear supernatants were collected  
173 for colour development. An aliquot of clear supernatant (1 mL) was diluted 25 times  
174 in a 50-mL polypropylene tube with distilled deionized water. A portion (3 mL) of  
175 the diluted sample was combined with 1 mL of modified Wade reagent (0.03%  
176 FeCl<sub>3</sub>.6H<sub>2</sub>O + 0.3% sulfosalicyclic acid) in a 15-mL falcon tube, thoroughly mixed  
177 on a vortex and absorbance of colour reaction determined at 500 nm using a UV  
178 spectrophotometer (UVLine 9400, Schott Instruments, France).

179 A standard curve was prepared using series of calibration standards containing 0,  
180 1.12, 2.24, 3.36, 5.6, 7.84, or 11.2 mg L<sup>-1</sup> PA-P from phytic acid sodium salt  
181 hydrate (Sigma, *P8810*) and phytic acid content determined as above.

## 182 **2.5 Statistical analysis and process optimization**

183 Means and standard deviations for experimental data were computed using Statistica  
184 7.0 (Tulsa, OK, USA). Response surface methodology (RSM) was used to relate  
185 product characteristics to extrusion variables. Response surface plots were generated  
186 as a function of two variables, while keeping the third variable constant at its  
187 intermediate value. Regression coefficients were generated by a second order  
188 polynomial:

$$189 \quad Y_i = B_0 + B_i \sum_{i=1}^3 X_i + B_{ii} \sum_{i=1}^3 X_i^2 + B_{ij} \sum_{i,j=1}^3 X_i X_j + \varepsilon \quad (2)$$

190 where,  $Y_i$  is a response variable;  $B_0$  is a constant;  $B_i$ ,  $B_{ij}$  are gradients;  $X_{i,j}$  are  
191 factors; and  $\varepsilon$  is error term.

192 The simultaneous optimization of the process conditions and product responses  
193 (protein digestibility, WAI, WSI, extrudate expansion ratio (ER), bulk density and  
194 hardness) for Robal bean snack was accomplished using a multi-response

195 desirability method (Derringer & Suich, 1980). Individual desirability functions for  
196 each response variable were manipulated to achieve optimum values (Granato,  
197 Ribeiro, Castro, & Masson, 2010). In this study, WAI, bulk density and extrudate  
198 hardness demanded minimization, while protein digestibility, WSI and ER were  
199 maximized.

200

201

## 202 3. RESULTS AND DISCUSSION

### 203 3.1 Extrudate physicochemical properties

204

205 Robal bean extrudate properties differed significantly with variation in die  
206 temperature, feed moisture and screw speed (Tables 2, 3 and 4). The coefficients of  
207 determinations ( $R^2$ ) for regression equations varied between 0.484 and 0.842 with  
208 significant probability values ( $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ ) (Table 4).  
209 Significant negative quadratic effects ( $p < 0.01$ ) of die temperature and positive  
210 interaction effects ( $p < 0.05$ ) of die temperature with feed moisture on protein  
211 digestibility were observed (Table 4). Total polyphenols were significantly  
212 decreased with increase in feed moisture (linear terms,  $p < 0.05$ ) and interactions of  
213 die temperature (linear) with feed moisture (quadratic). Increases in screw speed  
214 (linear terms) and feed moisture (quadratic terms) resulted in increases in total  
215 polyphenols. The increases in screw speed (quadratic) and interaction effects of die  
216 temperature (quadratic) with screw speed (linear) resulted in decrease in total  
217 polyphenols. Possibly high screw speed limits the time of exposure of polyphenols  
218 to heat destruction (Anton *et al*, 2008).

219 Phytates are considered undesirable in foods since they form complexes with major  
220 divalent and trivalent cations (Ca, Fe, Zn, Mg and Cu) bearing an effect on mineral  
221 uptake, and also bind with proteins affecting their nutritional quality and absorption  
222 (Greiner, R., Konietzny, U., Jany, 2006). In this study, reduction in phytate content  
223 (Table 3) by at least 12 % as a result of extrusion was observed. Raw Robal flour  
224 was initially analyzed with average phytate content of 38.3 mg/g. No significant  
225 effect of change in feed moisture, die temperature and/or screw speed (linear,  
226 quadratic or interaction terms) on phytates were observed ( $p > 0.05$ ) for Robal  
227 extrudate as seen from regression models.

228 Table 2 Effect of extrusion conditions on Roba1 extrudate nutritional properties

229 Values represent means of three replicates  $\pm$  standard deviations.

<b>Exp. run</b>	<b>Feed moisture (%)</b>	<b>Die Temp. (°C)</b>	<b>Screw speed (Hz)</b>	<b>Protein content (g/100g)</b>	<b>Protein digestibility (%)</b>	<b>Polyphenol content (mg/100g)</b>	<b>Phytate content (mg/g)</b>
<b>1</b>	15.0	120	40	23.49 $\pm$ 0.01	82.33 $\pm$ 0.48	27.86 $\pm$ 3.12	28.89 $\pm$ 1.39
<b>2</b>	17.5	120	35	24.04 $\pm$ 0.34	82.89 $\pm$ 0.52	23.72 $\pm$ 4.92	28.52 $\pm$ 1.34
<b>3</b>	17.5	120	45	24.04 $\pm$ 0.07	82.16 $\pm$ 0.90	24.15 $\pm$ 1.65	30.41 $\pm$ 1.70
<b>4</b>	20.0	120	40	24.88 $\pm$ 0.78	80.59 $\pm$ 1.80	23.15 $\pm$ 0.53	25.89 $\pm$ 2.65
<b>5</b>	15.0	135	35	22.26 $\pm$ 1.37	83.40 $\pm$ 0.10	22.72 $\pm$ 0.87	27.99 $\pm$ 2.11
<b>6</b>	15.0	135	45	24.29 $\pm$ 0.41	81.44 $\pm$ 0.55	31.22 $\pm$ 1.11	31.02 $\pm$ 3.48
<b>7</b>	17.5	135	40	24.05 $\pm$ 0.05	83.64 $\pm$ 0.43	24.34 $\pm$ 4.20	30.49 $\pm$ 1.83
<b>8</b>	20.0	135	35	24.00 $\pm$ 0.13	82.64 $\pm$ 0.76	17.91 $\pm$ 2.66	29.15 $\pm$ 0.41
<b>9</b>	20.0	135	45	24.10 $\pm$ 0.04	82.34 $\pm$ 0.77	20.20 $\pm$ 1.64	32.12 $\pm$ 3.46
<b>19</b>	15.0	150	40	24.16 $\pm$ 0.49	82.41 $\pm$ 1.26	30.73 $\pm$ 0.55	26.38 $\pm$ 4.47
<b>11</b>	17.5	150	35	23.59 $\pm$ 0.08	81.08 $\pm$ 0.30	24.53 $\pm$ 4.42	33.58 $\pm$ 3.24
<b>12</b>	17.5	150	45	24.72 $\pm$ 0.40	81.59 $\pm$ 0.71	18.58 $\pm$ 2.12	29.48 $\pm$ 0.16
<b>13</b>	20.0	150	40	24.22 $\pm$ 0.31	82.40 $\pm$ 0.14	35.71 $\pm$ 1.73	33.01 $\pm$ 1.87

230

231 Table 3 Mean values of extrudate physical and functional properties

Exp. run	Feed moisture (%)	Die Temp. (°C)	Screw speed (rpm)	WAI (g / g)	WSI (mL/g)	Radial expansion ratio (ER)	Bulk density (g/cm <sup>3</sup> )	Peak force (N)	[Area F-D (cm) <sup>2</sup> *	Mean force (N)	Final viscosity <sup>1</sup> (cP)
1	15.0	120	40	3.98±0.61	0.39±0.06	2.52±0.07	1.50±0.06	30.22±1.48	123.72±4.93	16.50±0.68	259.00±22.00
2	17.5	120	35	4.67±0.10	0.33±0.02	2.45±0.07	2.06±0.05	46.17±5.71	158.58±10.62	21.82±0.65	323.67±7.38
3	17.5	120	45	4.35±0.35	0.36±0.02	2.50±0.01	1.81±0.08	34.08±1.63	132.64±5.79	17.49±1.07	307.20±16.80
4	20.0	120	40	4.20±0.18	0.35±0.05	2.40±0.03	2.17±0.33	48.20±1.28	172.82±8.74	23.29±1.21	333.50±24.10
5	15.0	135	35	3.69±0.29	0.41±0.03	2.52±0.05	1.49±0.25	32.66±1.84	130.85±5.35	17.47±0.74	302.70±10.90
6	15.0	135	45	3.47±0.08	0.46±0.00	2.59±0.07	1.33±0.05	25.62±1.15	110.14±3.72	14.71±0.50	298.80±23.70
7	17.5	135	40	3.98±0.30	0.38±0.02	2.45±0.06	1.84±0.04	35.65±3.67	140.31±9.36	18.72±1.25	286.94±9.27
8	20.0	135	35	4.43±0.17	0.36±0.02	2.36±0.04	1.85±0.58	35.17±4.85	137.35±13.76	18.33±1.84	335.80±14.70
9	20.0	135	45	3.86±0.49	0.38±0.01	2.47±0.05	1.78±0.01	29.50±3.43	120.66±8.90	16.34±1.31	292.00±9.02
19	15.0	150	40	3.63±0.27	0.36±0.10	2.54±0.04	1.58±0.31	26.38±4.79	112.90±13.79	15.08±1.84	298.00±33.40
11	17.5	150	35	4.47±0.13	0.36±0.01	2.42±0.01	2.15±0.05	43.10±3.33	159.03±8.39	21.23±1.14	320.50±45.90
12	17.5	150	45	3.97±0.08	0.42±0.01	2.48±0.03	1.63±0.04	29.78±0.58	122.17±2.70	16.31±0.38	278.70±28.40
13	20.0	150	40	3.71±0.30	0.42±0.03	2.53±0.01	1.72±0.07	31.86±2.86	131.40±20.09	17.54±2.70	308.80±19.50

232 Values represent means of three replicates ± standard deviations

233 <sup>1</sup>Final pasting viscosity determined at 95°C using RVA

234 \* Area F-D, Area of force deformation curve, (cm)<sup>2</sup>

235 Table 4 Regression coefficients of the linear, quadratic and interaction effect of feed moisture die temperature and screw speed on Robal  
 236 extrudate properties

237

Variable	Protein digestibility	Total polyphenols	WAI	WSI	Radial expansion ratio	Bulk density	Hardness (Peak Force)	Area F-D	Mean force
Constant	<b>83.640***</b>	<b>24.823***</b>	<b>3.983***</b>	<b>0.384***</b>	<b>-2.451***</b>	<b>1.837***</b>	<b>35.647***</b>	<b>140.310***</b>	<b>18.723***</b>
Die temperature (X <sub>1</sub> )	-0.597	-1.171	-0.060	<b>0.022*</b>	-0.011	-0.024	-1.844	-2.505	0.442
Feed moisture (X <sub>2</sub> )	0.038	<b>-3.623**</b>	<b>0.283**</b>	<b>-0.031**</b>	<b>-0.069***</b>	<b>0.204**</b>	1.600	4.256	-0.623
Screw speed (X <sub>3</sub> )	-0.566	<b>3.032**</b>	<b>-0.198*</b>	0.003	<b>0.042**</b>	-0.120	<b>-3.179*</b>	<b>-9.348**</b>	<b>-1.186*</b>
X <sub>1</sub> *X <sub>1</sub>	<b>-1.117**</b>	1.979	0.147	-0.019	-0.001	0.054	<b>3.033*</b>	<b>6.628*</b>	0.381
X <sub>2</sub> *X <sub>2</sub>	-0.589	<b>2.561*</b>	<b>-0.257**</b>	0.014	<b>0.035*</b>	<b>-0.197**</b>	<b>-4.512**</b>	<b>-11.728***</b>	<b>-0.939*</b>
X <sub>3</sub> *X <sub>3</sub>	-0.597	<b>-4.038***</b>	0.136	0.003	0.013	-0.029	-0.398	-3.834	<b>-1.559***</b>
X <sub>1</sub> *X <sub>2</sub>	0.432	2.425*	<b>-0.195*</b>	<b>0.023*</b>	0.029	<b>-0.131*</b>	<b>-3.125**</b>	<b>-7.651*</b>	<b>-1.083**</b>
X <sub>1</sub> *(X <sub>2</sub> <sup>2</sup> )	<b>1.070*</b>	<b>-5.028**</b>	-0.142	-0.013	<b>0.049*</b>	-0.070	<b>-3.201*</b>	<b>-10.552*</b>	<b>-1.350*</b>
(X <sub>1</sub> <sup>2</sup> )*X <sub>2</sub>	-0.474	<b>3.691*</b>	-0.048	<b>0.035*</b>	0.009	-0.001	<b>4.264**</b>	<b>12.644**</b>	<b>1.689**</b>
X <sub>1</sub> X <sub>3</sub>	0.308	-1.578	0.004	0.005	0.004	-0.069	-0.306	-2.731	-0.147
(X <sub>1</sub> <sup>2</sup> )*X <sub>3</sub>	0.513	<b>-4.396*</b>	-0.072	0.003	0.012	-0.138	<b>-3.172*</b>	-6.352	<b>-1.127*</b>
X <sub>2</sub> *X <sub>3</sub>	0.415	-1.219	-0.085	-0.001	-0.013	0.024	0.342	1.005	0.190
R <sup>2</sup>	0.484	0.653	0.651	0.499	0.645	0.659	0.842	0.803	0.817

238 \* Significant at  $p < 0.05$   
 239 \*\* Significant at  $p < 0.01$   
 240 \*\*\* Significant at  $p < 0.001$

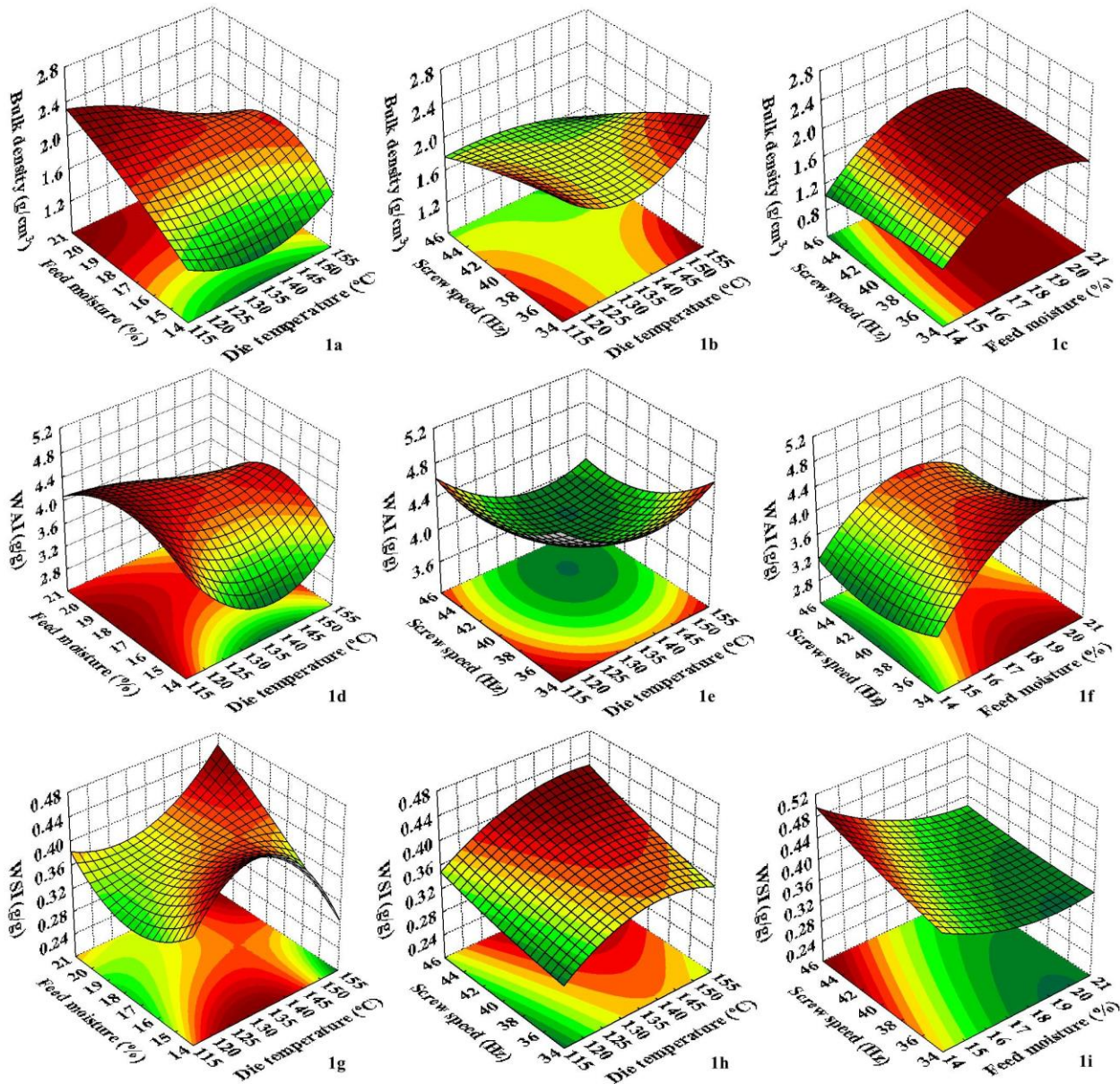
241

242 *Extrudate expansion and bulk density*

243 Increases in feed moisture by linear terms resulted in reduced expansion of Robal  
244 extrudate, while increases in screw speed (linear), feed moisture (quadratic) and  
245 interaction terms of die temperature with feed moisture resulted in increased  
246 extrudate expansion (Table 4). Decreases in expansion at high feed moisture might  
247 be due to reduced elasticity of dough through plasticization of melt in the extruder  
248 (Ding, Ainsworth, Plunkett, Tucker, & Marson, 2006; Hagenimana, Ding, & Fang,  
249 2006). The significant increases in expansion ( $p < 0.01$ ) due to increased screw  
250 speed may be attributed to bubble growth resulting from increased water vapour  
251 pressure at the die nozzle (Hagenimana *et al.*, 2006). The results suggest that low  
252 feed moisture and high screw speed would be important for attainment of high  
253 expansion of extrudates.

254 Bulk density is related to the extent of extrudate expansion (Hagenimana *et al.*, 2006)  
255 and is very important in the production of expanded and formed food products.  
256 Increases in feed moisture at low die temperatures ( $<130\text{ }^{\circ}\text{C}$ ) and high feed moisture  
257 at high die temperatures ( $>145^{\circ}\text{C}$ ) resulted in increased bulk density (Table 3; Fig.  
258 1). High bulk density at high feed moisture was probably due to the lubricating and  
259 plasticizing effect of water, which lowers the mechanical shear effects and disruption  
260 of starch in the extruder (Altan & Maskan, 2011). High bulk density could also be  
261 due to rupture of the starch cell walls caused by fibre particles before the gas bubbles  
262 in the starch attained full expansion (Altan & Maskan, 2011; Chiu, Peng, Tsai, Tsay,  
263 & Lui, 2013). Chiu *et al* (2013) reported that high bulk density may result from  
264 binding of water to non-starch polysaccharides (fibre) which inhibits water loss at  
265 the die thus reducing expansion. Low bulk density at high screw speed (Fig. 1b and  
266 1c) for all temperatures was probably due to starch gelatinization and increased

267 expansion of extrudate caused by increased water vapour pressure at the die  
268 (Hagenimana *et al.*, 2006).



269

270

271 Figure 1a to 1h      Response surfaces curves of bulk density, WAI and WSI as affected by die  
272 temperature, feed moisture and screw speed

273 In this study it is notable that high bulk density was a function of increase in feed  
274 moisture (linear terms) and the interactions of feed moisture (linear terms) with die



275 temperature (Table 4). Low feed moisture and high screw speed therefore would be  
276 necessary for obtaining an extrudate with low bulk density.

### 277 *Water absorption and solubility index*

278 Water absorption index (WAI) was significantly influenced by feed moisture in both  
279 linear and quadratic terms, screw speed (linear terms) and the interaction of die  
280 temperature and feed moisture (Table 4, Fig. 1d to 1f). The negative coefficients of  
281 the linear terms of screw speed, quadratic terms of feed moisture and interaction  
282 terms of die temperature and feed moisture indicate that WAI decreases with  
283 increase of those variables. Positive coefficients of the linear terms of feed moisture  
284 indicated that WAI increased with increases in feed moisture. The increase in WAI  
285 with increasing feed moisture could be attributed to the dispersion of starch in excess  
286 water, the increased degree of starch damage by gelatinization and the extrusion  
287 induced fragmentation of starch granules (Chiu *et al.*, 2013; Ding, Ainsworth,  
288 Tucker, & Marson, 2005; Hagenimana *et al.*, 2006; Yagcı & Gögüs, 2011). High  
289 moisture content builds low viscosity, thus allowing for internal mixing and uniform  
290 heating of the dough which would account for enhanced gelatinization (Yagcı &  
291 Gögüs, 2011). Additionally, protein denaturation, starch gelatinization and swelling  
292 of fibre at high feed moisture could be responsible for increase in WAI (Altan &  
293 Maskan, 2011).

294 Increases in die temperature (linear) and interactions of die temperature and feed  
295 moisture (linear and quadratic terms) caused increase in WSI, while increases in feed  
296 moisture (linear) resulted in reduction of WSI (Table 3). Similarly increases in  
297 screw speed caused significant increase in WSI at low feed moisture and at high die  
298 temperature (Fig. 1h and 1i). Increase in WSI at high die temperatures was probably  
299 associated with the disintegration of starch granules and low molecular compounds  
300 from extrudate melt during the extrusion process, thus increasing the soluble

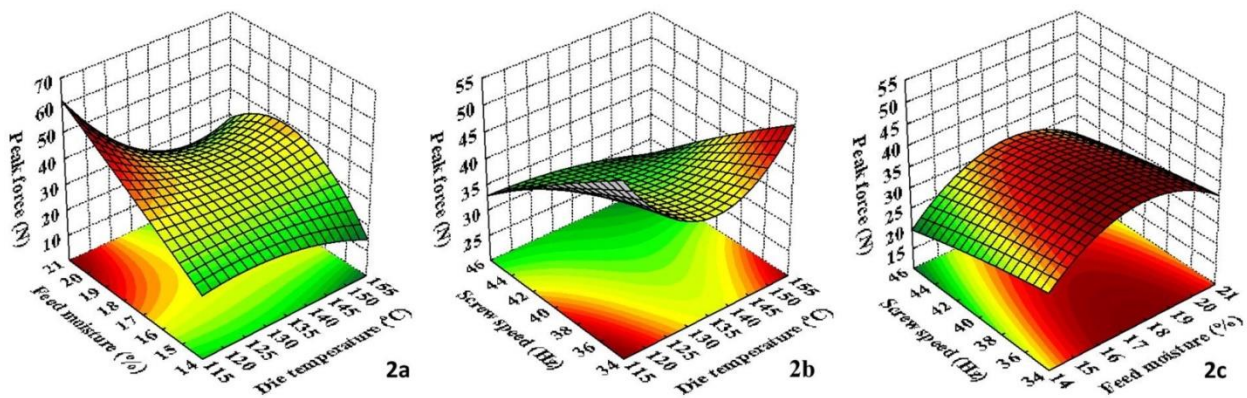
301 material.(Yağcı & Göğüş, 2008) The increase in WSI with increase in screw speed  
302 was in agreement with previous works (Altan & Maskan, 2011; Altan *et al.*, 2008)  
303 and may be due to increased specific mechanical energy, mechanical shear and  
304 degradation of macromolecules. Increased WSI at high temperature and high screw  
305 speed (Fig. 1h) was possibly associated with the starch degradation at high  
306 temperature and greater shear action at high screw speed (Altan & Maskan, 2011;  
307 Seth, Badwaik, & Ganapathy, 2015)

308 High WSI at high feed moisture could be explained by the complete changes of food  
309 components from native forms, that is starch gelatinization and protein denaturation,  
310 respectively (Yu, Liang; Ramaswamy, 2012). The low WSI at high feed moisture  
311 and low die temperature, as well as at low screw speed and low die temperature  
312 could be explained by the low degree of starch transformation, the reduced shear  
313 degradation of starch and the low tendency to dextrinization (Hernandez-Diaz,  
314 Quintero-Ramos, Barnard, & Balandran-Quintana, 2007; Liu *et al.*, 2011). High  
315 moisture content in extrusion processes may reduce protein denaturation and starch  
316 degradation (Hernandez-Diaz *et al.*, 2007). Alternatively, high feed moisture acts as  
317 a plasticizer thus hindering full expansion and rupture of starch in the extruder (Liu  
318 *et al.*, 2011). WSI is an indication of the ease of solubilization and extent of water  
319 absorption of the cooked product (Altan & Maskan, 2011). While WAI measures the  
320 volume occupied by starch after swelling in excess water, thus its integrity in  
321 aqueous dispersions (Ding *et al.*, 2005). Both WAI and WSI can be useful indicators  
322 of the suitability for use of extruded starchy products in suspensions or solutions  
323 (Yagcı, & Göğüş, 2011). Results from this study were in agreement with Ding *et*  
324 *al.*, (2006) who reported increases in WSI as extrusion temperature increased at feed  
325 moisture of 18.2%. High WAI obtained at moderate feed moisture and low-to-high  
326 die temperatures possibly reflected the ability of Roba1 extrudate to absorb moisture  
327 and the stability of starch polymer composites upon exposure to water treatment;

328 which would be a good attribute in flour applications for aqueous dispersions  
329 (Sarifudin & Assiry, 2014) such as soups and gruels. This study therefore reveals  
330 that both extrusion conditions and material composition appear to influence the  
331 functional properties of bean extrudates (WAI, WSI, bulk density and expansion).

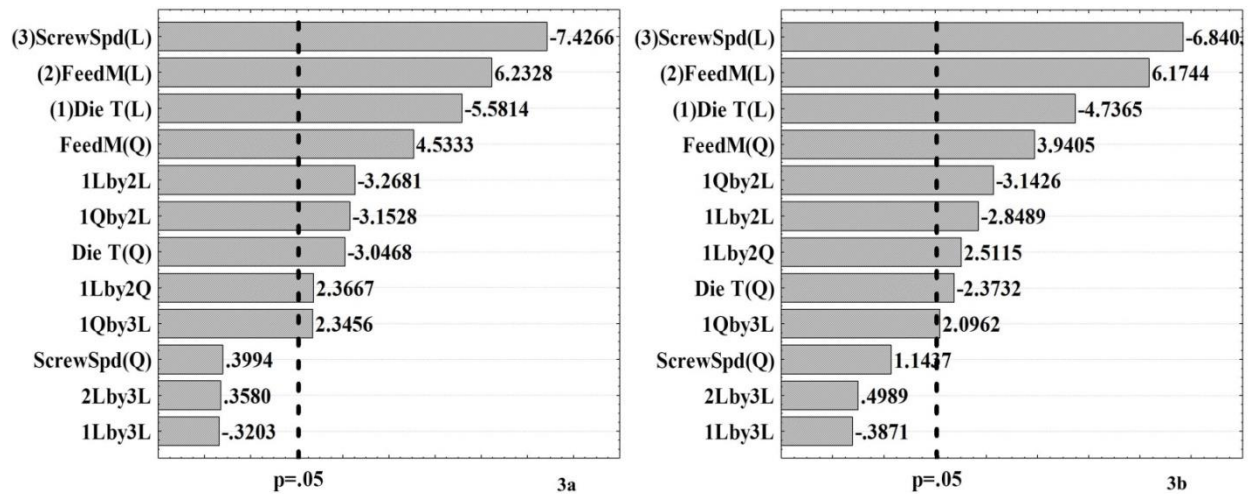
### 332 *Extrudate texture*

333 The hardness, crunchiness and crispiness of Roba1 extrudates measured in terms of  
334 peak force and area under force deformation curve were determined. Peak force  
335 represents the resistance of extrudate to initial penetration and is believed to be the  
336 hardness of the extrudate (Anton & Luciano, 2007; Ding *et al.*, 2006). The regression  
337 model of extrudate hardness was significant as a function of screw speed (linear  
338 terms), feed moisture (quadratic terms), and the interactions of feed moisture with  
339 die temperature (Table 4). High screw speed and low feed moisture resulted in soft  
340 extrudates. Increase in feed moisture at low die temperatures resulted in increased  
341 extrudate hardness (Fig. 2 and 3).



342  
343 Figure 4 Response surfaces showing effect of die temperature, feed moisture and screw  
344 speed on peak force (hardness) of Roba1 extrudate

345



346

347 Figure 5 - 6 Pareto charts showing significance of effects of extrusion conditions on Robal  
 348 extrudate hardness and crispiness.

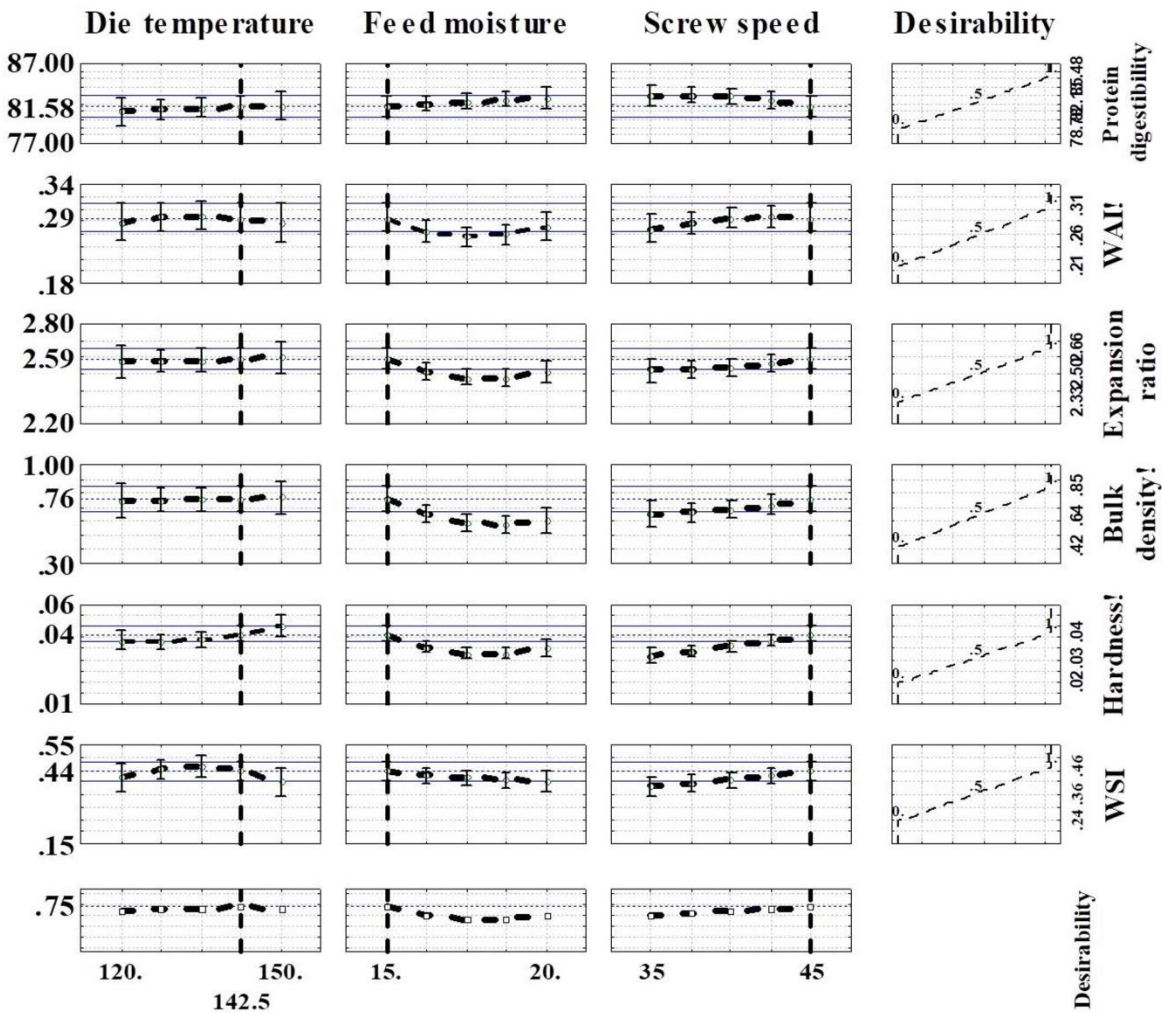
349 Positive coefficients of feed moisture (LT and QT) and interactions of die  
 350 temperature (QT) with screw speed resulted in increased extrudate hardness (Table  
 351 4, Fig. 3). This could partly be due to the reduced dough friction in the extruder,  
 352 permitting rapid extrusion and full expansion of extrudate at the die exit at high  
 353 temperatures (Hagenimana et al., 2006). Increase in screw speed (LT) significantly  
 354 ( $p < 0.05$ ) decreased Robal extrudate hardness, particularly at low feed moisture  
 355 and high die temperature (Figures 2b, 2c, and 3). Low hardness, a favoured property  
 356 of extrudates (Meng et al., 2010), was observed at low feed moisture and high screw  
 357 speed. Results from this study are similar to reports from previous works (Altan *et*  
 358 *al.*, 2008; Ding *et al.*, 2005), which showed that extrudate hardness increased with  
 359 increase in feed moisture at low die temperatures. This may be due to the reduced  
 360 pressure resulting from lubrication of extruder walls at high feed moisture, thus  
 361 lowering expansion. The hardness of extrudates was highly related to the bulk  
 362 density (Fig. 1 and 2). Low screw speed resulted in harder extrudates, which  
 363 probably was due to increase in melt viscosity of the mix (Ding *et al.*, 2006; Maskus  
 364 & Arntfield, 2015).

365 The area under the force determination curve (Table 2) represents the energy  
366 required for a given displacement and can measure the crispiness and brittleness in  
367 texture of a product (Anton & Luciano, 2007). Roba1 extrudate brittleness tended to  
368 increase with increase in screw speed (LT,  $p < 0.01$ ) and decrease in feed moisture  
369 (QT,  $p < 0.001$ )(Table 4; Figure 3b). Increase in die temperature (QT) increased the  
370 crispiness of Roba1 extrudate. Interactions between die temperature (quadratic) and  
371 feed moisture (linear) significantly ( $p < 0.05$ ) decreased the area under the force-  
372 deformation curve (Table 4; Fig 3b), thus increased the brittleness/crispiness of  
373 extrudate. Die temperature (QT) and screw speed (LT) interaction significantly ( $p <$   
374  $0.05$ ) increased the extrudate crispiness. The results suggested that low feed moisture  
375 (QT), high screw speed (LT) and die temperature-feed moisture interactions during  
376 bean extrusion result into soft crispy extrudates, which is in agreement with Altan  
377 et al., (2008) and Ding et al., (2006) who reported similar findings.

378

### 379 **3.2 Multi-response desirability optimization of Roba1 extrusion conditions** 380 **and extrudate properties**

381 Optimal extrusion conditions that simultaneously satisfied all selected extrudate  
382 responses and could improve the efficiency of the process for Roba1 snack extrudate  
383 were identified (Fig. 4). Notable, individual optimal desirability values ( $d$ ) (Fig. 4)  
384 differed from those obtained with a global optimal desirability ( $D$ ). This was due to  
385 the conversions that occurred statistically to reach a simultaneously optimal values  
386 (Tumwesigye et al., 2016; Vera Candiotti et al., 2014).



4

387

388 Figure 7 Desirability index and predicted response variables in multi-response optimization  
 389 of Robal extrusion conditions and product properties

390 Results revealed that die temperature 142.5 °C, feed moisture 15 % and screw speed  
 391 45 Hz, were sufficient for producing best characteristics for Robal extrudate with  
 392 bulk density 1.32 g/cm<sup>3</sup>, expansion ratio 2.60 and hardness 24.4 N at optimal  
 393 desirability of 0.75. High expansion, low bulk density and hardness were identified  
 394 as important attributes in extruded beans snack processing, properties also  
 395 established by previous workers (Altan & Maskan, 2011; Jyothi, Sheriff, & Sajeev,  
 396 2009; Maskus & Arntfield, 2015). These findings show that low feed moisture and

397 high screw speed are essential for obtaining bean snack extrudate with desirable  
398 expansion, hardness, bulk density and protein digestibility.

399

## 400 CONCLUSION

401 The results of this study revealed that die temperature, feed moisture and screw  
402 significantly influenced Roba1 bean extrudate properties. Regression coefficient  
403 ( $R^2$ ) values showed significant effect of individual factors and their interactions in  
404 influencing bean extrudate properties. Increases in WAI, bulk density and extrudate  
405 hardness and reductions in expansion and WSI were a function of the linear,  
406 quadratic and interaction relationships of feed moisture, die temperature and screw  
407 speed. Low feed moisture and high screw speed were found essential to produce a  
408 soft crispy snack extrudate with low bulk density and high water solubility index.  
409 Die temperature 142.5 °C, feed moisture (15%) and screw speed 270 rpm were found  
410 as optimal process conditions for producing an expanded snack extrudate from  
411 Roba1 bean with optimal desirability >0.7. Results of this study therefore can be  
412 very important for application in the food industry, providing extrusion conditions  
413 that for generating acceptable and nutritious products. This in turn will contribute to  
414 increased diversity of bean products on the market, reduce monotony and overcome  
415 the challenge of hard-to-cook defects. Subsequent inclusion of Roba1 beans in  
416 extruded snacks shall increase protein, fibre and mineral intake by the consumers.

417

418

419

## 420 **Acknowledgement**

421 The authors acknowledge the special funding by the African Development Bank  
422 (ADB) to Government of Uganda through the Centre for International Tropical  
423 Agriculture (CIAT). We gratefully acknowledge Peak Value Industries Ltd (U) for  
424 providing the extrusion facility.

425

## 426 **Highlights**

- 427 • Optimal extrusion conditions for Robal bean snack extrudate were  
428 determined
- 429 • Extrusion conditions linear, quadratic and interaction terms were  
430 significant
- 431 • High extrudate bulk density and hardness were associated with high feed  
432 moisture
- 433
- 434 • High screw speed and die temperature resulted in increased extrudate  
435 expansion

436

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