International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 981-990

Received: 21-09-2017
Accepted: 23-10-2017

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Optimization of extrusion process for development of antioxidant rich extrudates from finger-millet and horse-gram in rice matrix

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Abstract
Innovative ready-to-eat extruded snack from optimized composite flour comprising of rice, finger millet and horse gram in a ratio of 40:50:10 was produced. Statistical optimization was carried out using central composite design of response surface methodology (RSM) and second order polynomial models were fitted to responses to understand the effect of different extrusion variables (feed moisture, temperature and screw speed) on product responses. All operational variables were found to have significant ($P<$ 0.05) effect on expansion ratio, water absorption index, water solubility index, hardness, total phenolics and antioxidants. The multiple regression analysis demonstrated high significance of fitted models in predicting product response. Optimum processing condition generated from the models was: feed moisture: 11.36% wb, die head temperature: 130.58ºC and screw speed 329.03 rpm. The developed snacks had high sensory score with significantly higher total phenolic content (465.23 mg GAE/100g dw) and antioxidant activity (17.98 µmol TE/g dw) than market samples.

Keywords: Extrusion, finger millet, horse gram, response surface methodology, phenolics, antioxidants

Introduction

New product development and continuous innovation in food industry has been partially a reflection of the changing needs of consumer for health, nutrition and convenience. There have been consistent efforts to increase functionality of cereals based foods by enriching them with higher protein, phenolics, dietary fibre and mineral content for lowering glycemic index so to qualify them as functional foods. Use of millets, barley, oats, amaranth, quinoa, and kaniwa and underutilized legumes in development of extruded snacks is clearly a consequence of the recent trend to satiate the growing needs of modern consumer (Altan *et al.*, 2009; Deshpande and Poshadri, 2011; Filli *et al.*, 2013; Gumul *et al.*, 2015; Ramos Diaz *et al.*, 2015) [1-5].

Finger millet (*Eleusine coracan*) and Horse gram ((*Macrotyloma uniflorum* L.) are too candidate underutilized grains high in minerals, phenolics and known to have anti-diabetic properties thus seem promising for those suffering from diabetics. Finger millet is a unique minor cereal, excellent source of dietary fibre, calcium, iron, zinc and phenolics. Finger millet regulates glucose homeostasis by inhibiting pancreatic amylase and intestinal α-glucosidase (Shobana *et al.*, 2009) [6] and is strongly advocated for managing type-2 diabetes. Horse-gram (*Macrotyloma uniflorum* L.) commonly known as '*kulthi'* is a traditional grain legume and poor man's pulse crop in South India. It is a cheapest and excellent source of protein, dietary fiber, micronutrients (iron, molybdenum and calcium) and phytochemicals (phenolics and flavonoids) with potential health benefits (Thirukkumar and Sindumathi, 2014) [7]. The legume has been traditionally used for treatment of asthma, bronchitis, kidney stones, diabetes, obesity, hypertension and heart disease. Horse gram not only possesses anti-diabetic (anti-hyperglycemic) properties but also reduces insulin resistance by inhibiting protein-tyrosine phosphatase 1 β-enzyme. Thus combining bioactive principles from finger millet and horse-gram in rice matrix seems a healthy proposition for development of phenolics and antioxidant rich extruded snack for modern consumer.

Most of studies related to optimization of extruded snacks have largely focused on physicochemical parameters of extrudates such as expansion ratio, WAI and WSI. There is scarce information on combined use of physicochemical and antioxidant parameters in optimization studies.

Keeping this in mind, the present study attempts to develop extrudates from rice, finger millet and horse gram using statistical optimization taking into consideration both physical and functional attributes for optimization. The intention was to design snacks with high phenolics and antioxidant activity.

2. Materials and methods

2.1 Flour preparation

Rice, finger millet and horse gram grains were purchased from local commercial suppliers. The grains were grounded separately in hammer mill and sieved through 35 mesh screen to obtained particle size less than 500 μm. The proximate composition of obtained flours is presented in Table 1.

Table 1: Proximate composition of selected flours

	Moisture (wb),%	Ash,%	Protein,%	Crude fat,%	Crude fibre,%	Carbohydrate,%
Rice	5.45	1.1	5.83	2.41	1.08	84.13
Finger millet	8.99	3.51	6.88	1.57	3.43	75.62
Horse gram	10.10	3.23	20.46	1.94	5.62	58.65

2.2 Blend formulation and preconditioning

The rice flour was selected as base due to its good capacity of expansion and bland taste so that it would not cover the typical flavour of finger millet. The blend formulation was decided on the basis of preliminary trials; consisted of rice (40%), finger millet (50%) and horse gram (10%). Prior to extrusion, the composite flour was preconditioned to achieve final moisture content (as per CCD) by spraying with a calculated amount of distilled water and mixing continuously at medium speed in laboratory mixer blender (National Manufacturing Company, Lincoln, N. E.) for 5 minutes. The moistened flour was then transferred to polyethylene pouches and allowed to stay overnight to equilibrate at room temperature prior to extrusion. Preconditioning ensured uniform hydration in order to minimize variability in the state of feed material.

2.3 Extrusion

Extrusion was performed using Brabender Lab-Compounder KETSE 40/20 twin screw extruder (Germany) with length to diameter (L/D) ratio of 40:1. The steps include feeding preconditioned flour material with varying moisture (10-20% wb) to the extruder at varying screw speed (200-400 rpm) and extruding the plasticized mass through die orifice to form an extrudates without jamming extruder. The temperatures of first four zones (Z1: feed zone; Z2, Z3, Z4: barrel zones) were set to 30 ˚C/50 ˚C/90 ˚C/110 ˚C for all runs; whereas both Z5 (barrel zone) and Z6 (heating ring zone) were maintained at the same temperature (120-180 ˚C). The position and speed (20 rpm) of feeder were kept constant throughout the experiment. A die with a single circular opening (3 mm diameter), equipped with a rotary die face cutter (speed of 150 rpm) was used. The expelled hot extrudates were fed directly into a tray drier, maintained at 60 °C. The expelled hot extrudates were fed directly into a tray drier, maintained at 60 °C. The final dried samples with maximum of 5-6% (wb) moisture were packed in polythene bags and stored at -20 °C temperature until the day of analysis.

2.4 Experimental layout and statistical analysis

The experimental design and statistical analysis were performed using Design Expert 9.0 (State-Ease Inc., Minneapolis, USA). A five factors and three levels central composite design (CCD) was employed to predict responses based on few sets of experimental data in which all factors were varied within a chosen range. The range of each variable was established according to literature information and preliminary trials. The independent variables considered were: feed moisture content X1 (%); barrel temperature X2 (ºC) and screw speed X3 (rpm). Feed composition was kept constant. The independent variables and their variation levels are shown in Table 2.

Table 2: Independent variables and levels used for Central Composite Design (CCD)

Variables	Coded variable level					
	Symbols	-1.68	-1	0	1	1.68
Feed moisture (% wb)	X1	10	12.5	15	17.5	20
Barrel Temperature (ºC)	X2	120	135	150	165	180
Screw Speed (rpm)	X3	200	250	300	350	400

The design consisted of 17 combinations including three replicates of the centre point used to determine the experimental error (Table 3). Dependent variables considered for optimization were physicochemical properties (expansion ratio, bulk density, water absorption index, water solubility index), textural properties (hardness, crispness) and functional properties (total phenolic content and antioxidant activity) of extrudates. Based on the experimental data, an empirical second-order polynomial model was fitted for each response as shown in the following equation:

$$f(x_u) = \beta_0 + \sum_{i=1}^{v} \beta_i x_{iu} + \sum_{i=1}^{v} \beta_{ii} x_{iu}^2 + \sum_{i=1}^{v-1} \sum_{i'=i+1}^{v} \beta_{ii'} x_{iu} x_{i'u} + e_u$$

The verification experiments were carried out to confirm the adequacy of the models for predicting the optimum operating conditions.

Table 3: Response surface design for the extrusion

Execution Order	Runs	Coded values			Actual values		
		X1	X2	X3	Barrel Temp (ºC)	Screw Speed (rpm)	Feed moisture (% wb)
1	17	0	0	0	150	300	15
2	5	1	-1	-1	165	250	12.5
3	4	-1	1	1	135	350	17.5
4	1	-1	-1	-1	135	250	12.5
5	12	0	1.68	0	150	400	15
6	7	1	1	-1	165	350	12.5
7	11	0	-1.68	0	150	200	15

8	6	1	-1	1	165	250	17.5
9	3	-1	1	-1	135	350	12.5
10	14	0	0	1.68	150	300	20
11	13	0	0	-1.68	150	300	10
12	2	-1	-1	1	135	250	17.5
13	15	0	0	0	150	300	15
14	8	1	1	1	165	350	17.5
15	16	0	0	0	150	300	15
16	10	1.68	0	0	180	300	15
17	9	-1.68	0	0	120	300	15

2.5 Analysis
2.5.1 Proximate Composition
Proximate composition of extrudate flour was determined using AACC International Methods (AACC, 2000) [8].

2.5.2 Bulk density
Extrudate volume was measured by small seeds displacement method (Lopez *et al.*, 2004) [9].

2.5.3 Expansion ratio (ER)
Expansion ratio was calculated as ratio of diameter of the extrudates and the diameter of the die (Ding *et al.*, 2005) [10]. Measurements were taken on 10 randomly selected pieces of extrudates.

2.5.4 Water absorption index (WAI) and water solubility index (WSI)
WAI and WSI of extrudates were determined by the method of Anderson (1982) [11].

2.5.5 Hardness and crispness
The textural properties of extrudates in terms of hardness and crispness were measured using Texture Analyser (TA-XT2, Stable Microsystems, Surrey, UK) fitted with 49 N load cell and 36 mm diameter cylinder probe with pre and post-test speed of 1mm/s and 10 mm/s respectively. The calculations were done by 'Texture Expert' software attached to the texture analyzer.

2.5.6 Total phenolic content
Total (Free and bound) phenolic content of extrudates was estimated spectrophotometrically using Folin–Ciocalteu reagent (FCR) (Singleton *et al.*, 1999) [12]. Results were expressed as Gallic acid equivalent (mg GAE/100 g).

3. Results and discussion
3.1 Predictive model for response variables
The predictive models were obtained by fitting the second order polynomial model to responses were tested for adequacy and fitness by analyses of variance (ANOVA) and results are presented in Table 4. The results of ANOVA were confirmed using good predictive model criteria: R^2 (coefficient of determination) $\geq$ 0.80, a significance level of $P< 0.05$; lack of fit test > 0.05 and adequate precision > 4 (Myers and Montgomry, 2002) [14].

3.2 Bulk density and expansion ratio
Bulk density (BD) is a reflection of puffed structure and an intrinsic quality attribute of expanded food products. The BD values of rice-finger millet-horse gram (RFH) extrudates ranged from 0.144-0.404 g/cm^3. ANOVA of regression model fitted to experimental results of BD showed that linear coefficient terms X_2 and X_3 are significant ($P< 0.01$) model terms (Table 4). BD was significantly dependent on screw speed and feed moisture and decreased with decreasing feed moisture and increasing screw speed. However, temperature had no significant effect on BD. The model F-value of 13.03 implies the model is significant ($P< 0.05$). A non-significant lack of fit (p > 0.05) and R^2 (0.9437) indicated fair suitability of model in predicting BD of extrudates. The effect of extrusion conditions on extrudate density can also be found in the 3-D surface plot (Fig. 1).

Feed moisture was found to have most pronounced effect on extrudate BD. Increased feed moisture lead to a significant ($P< 0.05$) increase of BD value at all temperature levels. This could be attributed to changes in amylopectin molecular structure and plasticization of melt which was reflected in reduced elasticity and bubble growth of starch-based material, thus resulting in higher BD (Ding *et al*, 2006) [16]. Our results are in conformity with the earlier findings of Bhise *et al.* (2012), Camacho-Hernandez *et al.* (2014) [15] and Hagenimana *et al.* (2006) [18]

Increased extrusion temperature caused a slight decrease in BD of extrudate; however observed effect was not significant (p > 0.01). Several authors have reported decreased BD with increasing temperature, which is due to degree of gelatinization and starch degradation as an effect of thermal process (Camacho-Hernandez *et al.*, 2014) [15]. In addition, high temperature at die end flashed-off super-heated water from extrudates and made them lighter in weight (Koksel *et al.*, 2004) [19]. The non-significance of extrusion temperature for BD in our study; might be because of raw material composition and range of processing parameters.

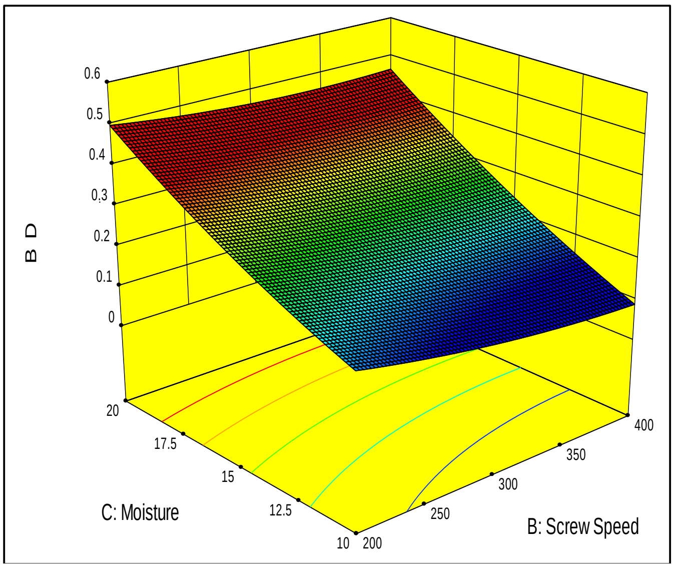


Fig 1: The bulk density (BD) expansion ratio (ER) of extrudates as a function of screw speed and feed moisture at optimized temperature (130.52 ºC)

Linear effect of screw speed, significantly affected the BD ($P< 0.05$), being lower for samples extruded at high screw speed. This might be because of dual effect of the structural breakdown of amylopectin networks and low residence time; influencing greater elastic effect and greater expansion (Chakraborty *et al.*, 2011) [20]. The intensified shearing effect at higher screw speeds could be reflected in lighter and puffer product; as a result of stretching and weakening of starch molecules and increased gelatinization (Yagci and Gogus, 2008) [21]. Our results are in accordance with the reports in literature for the extruded products (Ding *et al.*, 2005; Keawpeng *et al.*, 2014) [10, 22].

Table 4: Analysis of variance (ANOVA) for second order polynomial regression model for response variables

Coefficients	**Responses**						
	Bulk density	**Expansion ratio**	**WAI**	**WSI**	**Hardness**	**Crispness**	**TPC**
Intercept							
β_0	0.248	2.927	5.949	10.835	84.415	33.182	401.235
Linear							
β_1	-0.001	0.189***	-0.368*	1.233*	-8.069**	-5.047**	-72.082***
β_2	-0.027***	0.007	-0.830***	1.732**	-12.857***	-9.825***	41.032***
β_3	0.073***	-0.323***	0.742***	-1.910***	12.209***	8.566***	33.891***
Quadratic							
β_{11}	0.011	0.011	-0.085	0.691	-1.053	-0.448	-12.916
β_{22}	0.004	0.022	-0.285	1.080*	-1.377	-0.282	-2.015
β_{33}	0.006	0.016	0.107	0.026	-1.099	-0.780	8.010
Interaction							
β_{12}	-0.009	-0.073	-0.051	0.1562	3.741	0.838	-15.067
B_{13}	-0.010	-0.091	0.118	-0.265	-9.134**	-6.093*	-11.375
β_{23}	0.004	0.040	-0.279	0.301	-1.609	-0.224	-13.603
Model (F- value)	13.03	12.19	6.17	3.82	7.97	6.17	15.50
R^2	0.9437	0.9400	0.8880	0.8310	0.9111	0.8881	0.9522
Lack of fit (p-value)^a	0.0583	0.1036	0.6928	0.071	0.1723	0.1283	0.0711
Adequate precision	12.976	11.891	9.068	6.912	10.076	8.388	14.356

1: Temperature, 2: Screw speed, 3: Feed moisture
* Significant at $P < 0.1$.
** Significant at $P < 0.05$.
*** Significant at $P < 0.01$; ns: non-significant.
$\beta_0, \beta_1, \ldots, \beta n$ = Regression coefficients
a: want the selected model to have non-significant lack of fit ($p > 0.05$).

3.3 Expansion ratio

Expansion ratio (ER) represents the extent of degree of puffing of extrudate and higher value is desirable in the production of extruded snacks. The values of ER observed in our study ranged from 2.294 and 3.819. The coefficients of the model and other statistics of polynomial model for ER are given in Table 4. The Model F-value (12.19) indicated high significance ($P < 0.05$) of the obtained model. In addition, non-significant lack of fit ($p = 0.1036$), R^2 (0.94) and adequate precision (11.89) depicted that the model designed using the CCD is fairly appropriate and can be used for prediction of ER. ANOVA (Table 4) revealed a significant ($P < 0.05$) effect of feed moisture and temperature on expansion of extrudates as main effect whereas there was a non-significant ($p > 0.05$) effect of screw speed on expansion. All quadratic and interaction term coefficients were found to be non-significant ($p > 0.05$). Feed moisture had a significant negative linear effect, while temperature has a significant positive linear effect ($P < 0.05$) on the ER. It is evident from response surface plot (Fig. 2) that extrudates showed high ER at low feed moisture and high temperature.

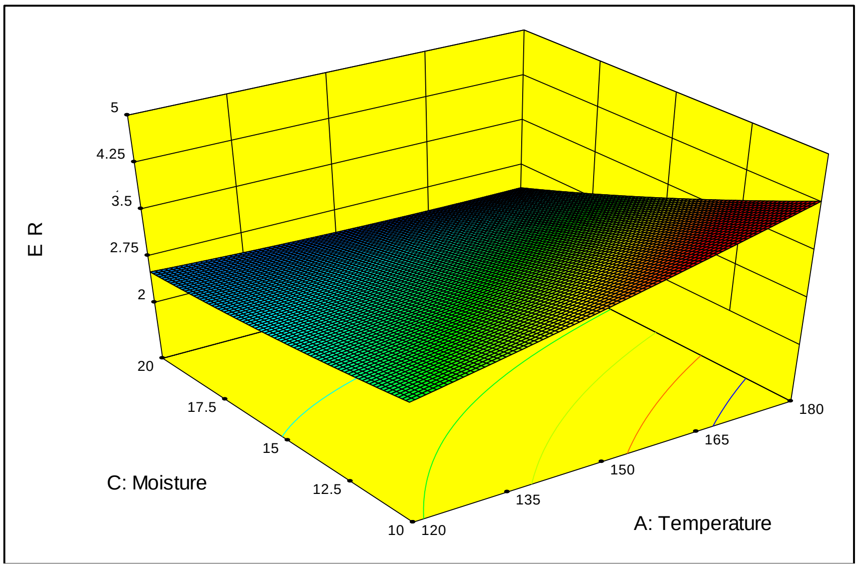


Fig 2: The expansion ratio (ER) of extrudates as a function of temperature and feed moisture at optimized screw speed (329.08ºC)

Increased feed moisture (10 to 20%) led to marked decrease (about 15.33%) in extrudate ER (Fig. 2). The highest ER value of 3.819 was observed at low moisture and high temperature. The commonly observed opposite trend between feed moisture and ER can be explained on the basis of lubricating effect of moisture. Reduced viscosity of melt at high moisture tend reduce the pressure between the differential interior of the growing bubble and atmospheric pressure thus leading to a less expanded product (Fill *et al.*, 2013) [3]. Omwamba and Mahungu (2014) [23] assigned the effect high moisture to reduced shear strength and energy input to the material, resulting in decreased moisture evaporation at the die end, consequently decreased ER.

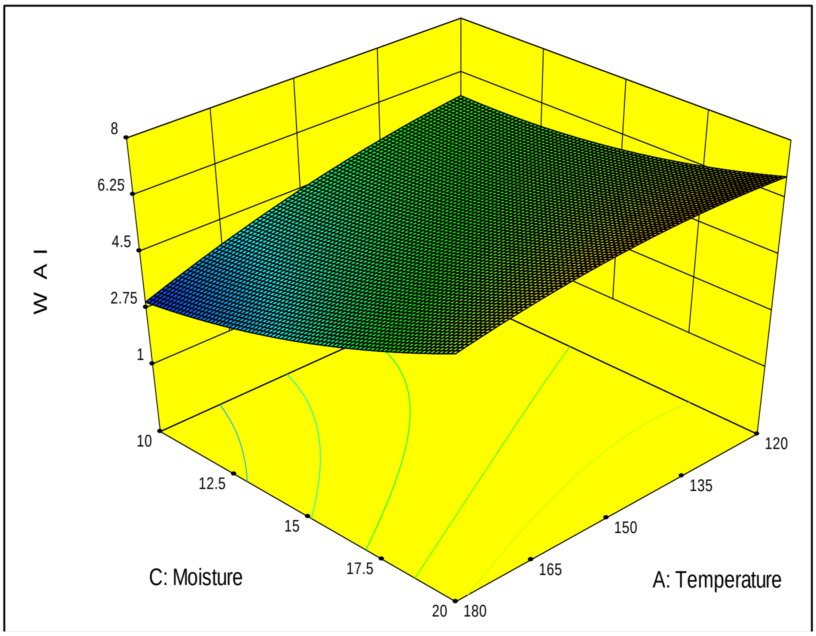
The temperature also had a significant effect on ER (Table 4). At optimized screw speed (329.08 rpm), increase in temperature from 120 °C to 180 °C caused significant increase (30.65%) in ER especially at high moisture content (Fig. 2). Expansion is mainly governed by two factors; dough viscosity and elastic force. High temperature induced higher level of starch granules breakdown, superheating of water, leading to a reduction in dough viscosity which favors bubble formation and increased expansion (Ding *et al.*, 2006; Omwamba & Mahungu**,** 2014) [16, 23]. Camacho-Hernandez *et al.* (2015) [15] mentioned that the expansion usually occurs at high temperature and low moisture as a result of several phenomenons such as structural transformations of biopolymers, phase transitions and nucleation, swelling, growth, and collapse of air bubbles, all of them contributing to the expansion.

The non-significant ($p > 0.05$) effect of speed screw on ER of RFH extrudates is in agreement with reports Omwamba & Mahungu (2014) [23] in extruded snack developed from composite blend of rice, sorghum and soybean flour. Thymi *et al.* (2008) [24] also showed that ER of extrudates from corn grits was dependent more upon the feed moisture and temperature, but screw speed had no effects. Screw speed is generally believed to have little effect on extrudate expansion in twin-screw extrusion (Ding *et al.*, 2005) [10].

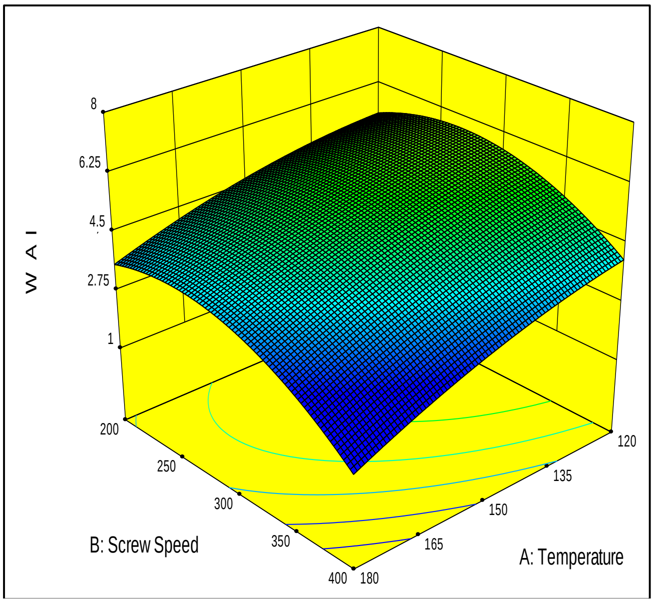
3.4 Water absorption index (WAI)

WAI is considered as an indicator of the degree of starch gelatinization, representing amount of water immobilized by the extrudate. WAI of extrudates observed in present study varied from 2.79 to 7.90 g/g. The values are in good agreement with range reported by several authors in snacks prepared from different type of composite blends (Camacho-Hernandez *et al.*, 2014; Filli *et al.*, 2013) [15, 3].

The selected regression model is well fitted to the obtained data of WAI (Table 4). The Model F-value (6.17), R^2 (0.888), adequate precision (9.068) and non-significant lack of fit ($p > 0.05$) implies that the model can be used for prediction purposes. Feed moisture and screw speed had significant ($P < 0.05$) impact on WAI of extrudates. The effect of temperature however was found to be significant at 10% level of significance ($P < 0.1$). The linear coefficients of X_1 and X_2 were negative, whereas that of X_3 was positive (Table 4); signifying increased WAI with increasing feed moisture and decreasing temperature and screw speed. The maximum WAI at design point (–1, –1, –1) was about 3 times more than the minimum WAI at the design point (1.68, 0, 0).



(A)



(B)

Fig 3: The water absorption index (WAI) of extrudates as a function of extrusion variables (temperature, screw speed and feed moisture) at optimized conditions

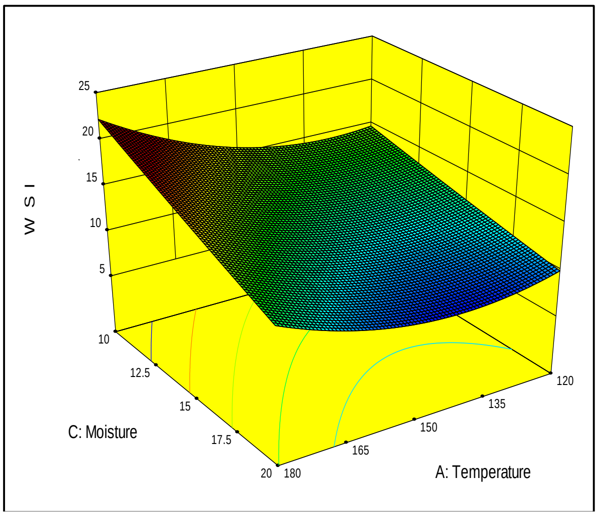
Increasing feed moisture significantly ($P< 0.05$) increased the WAI (Fig. 3a). The high feed moisture tends to reduce the viscosity of the starch, allowing for extensive internal mixing and uniform heating; accounting for enhanced starch gelatinization which may lead to increased WAI (Yagci and Gogus, 2008) [21]. Bhise *et al.* (2013) [17] postulated that high moisture may act as a plasticizer during extrusion cooking, reduces the degradation of starch granules and result in an increased capacity for water absorption. Similar effects have been reported earlier for extrudates made from pearl millet-groundnut (Filli *et al.*, 2013) [3] and barnyard millet- pigeon pea (Chakraborty *et al.*, 2011) [20].

The response surface plot (Fig. 3b) clearly demonstrated that WAI was higher for lower screw speed and lower temperature. As expected, under low shear conditions at low screw speed, had more undamaged polymer chains and hydrophilic groups; binding more water, thus resulting in high WAI (Bhise *et al.*, 2013) [17]. The decrease in WAI with increasing temperature can be assigned to high dextrinization or starch melting that prevailed over the gelatinization phenomenon (Rodriguez-Miranda *et al.*, 2011) [25].

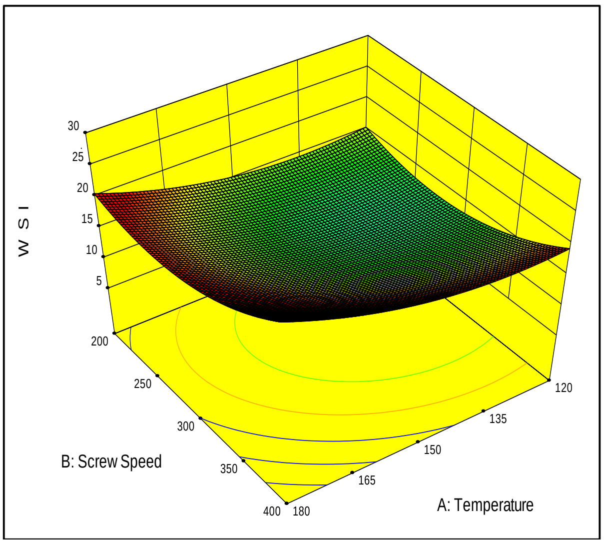
3.5 Water solubility index (WSI)

Water solubility indicates the amount of small molecules solubilized in water, hence can be used as the degree of starch conversion during extrusion (Rweyemamu *et al.*, 2015) [26]. High WSI encourages stickiness of extruded products which is not desirable by consumers. In our study, WSI of RFH extrudates varied in the range 8.510 to 19.127%. The reported WSI values of rice-based extrudates developed from various composite blends ranged from 3.3-9.5% (Awolu *et al.*, 2015; Filli *et al.*, 2013; Kumar *et al.*, 2010) [27, 3, 28]. The wide variation in WSI values might be attributed to composition and proportion of raw material formulations being used.

The model F-value (3.82) revealed significance ($P< 0.05$) of predictive model fitted to experimental results of WSI. Furthermore, R^2 (0.8310), adequate precision (6.912) and non-significant ($p > 0.05$) lack of fit indicated that the experimental model was adequate and reproducible. It is noted from Table 4 that all independent variables (temperature, screw speed and feed moisture) significantly ($P< 0.05$, $P< 0.1$) influenced WSI values in a linear fashion. Feed moisture was the variable that most influenced the WSI having a negative effect, followed by screw speed and temperature having positive effect ($P< 0.05$). Screw speed also had significant ($P< 0.05$) positive quadratic effect on WSI. The maximum WSI at design point (0, 0, 1.68) was about 2.25 times more than the minimum WAI at the design point (1, –1, –1).



(A)



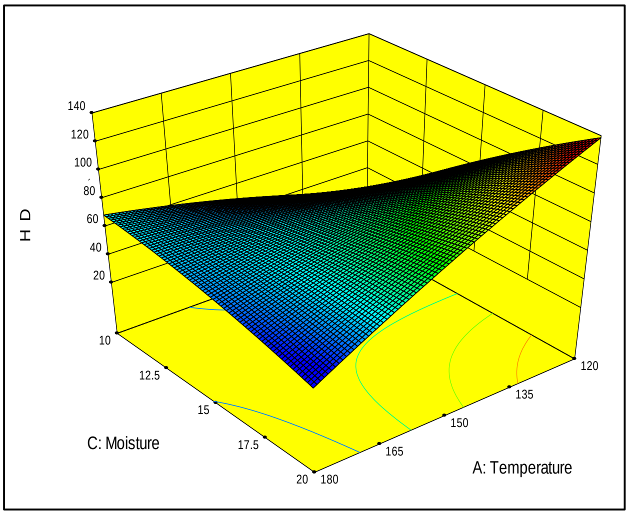
(B)

Fig 4: The water solubility index (WSI) of extrudates as a function as a function of extrusion variables (temperature, screw speed and feed moisture) at optimized conditions

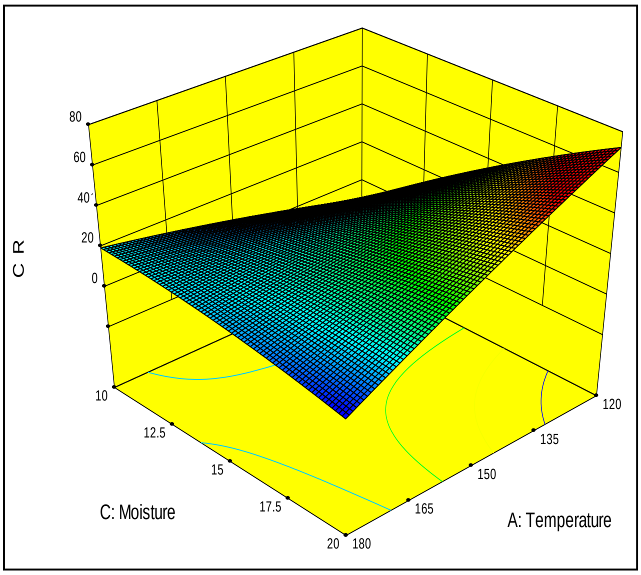
Results indicated that higher feed moisture in extrusion process can diminish starch degradation and protein denaturation, hence decreases WSI. At optimized screw speed (329.08 rpm) and high temperature (180 ^{0}C), increase in feed moisture from 10 to 20%, caused about 40.66% reduction in WSI (Fig. 4a). Similar trend was observed for all levels of temperature. The negative effect of feed moisture on WSI may be due to the fact that an increase in moisture reduces friction of the dough in the extruder; limiting material fragmentation and dextrinization. Furthermore, the lubricating effect imparted by the water causes the material to pass faster through the extruder; thus reduces the shearing effect of temperature and screw speed, causing less degradation and lower WSI (Camacho-Hernandez *et al.*, 2014) [15]. Similar effects have been reported in literature for blue corn snacks

(Camacho-Hernandez *et al.*, 2014) [15] and maize-soybean-moringa leaf powder snacks (Rweyemamu *et al.*, 2015) [26].
Examination of response surface plot (Fig. 4b) for the result of WSI of extrudates showed that increasing temperature and screw speed significantly ($P< 0.05$) increased the WSI. At optimized feed moisture (11.36%), increased screw speed from 200-400 rpm, promoted about 23.38% rise in WSI. Whereas, increased temperature from 120–180 ºC, caused 27.94% rise in WSI. Results demonstrated greater effect of screw speed on WSI than that of temperature. This behaviour can be explained on the basis of combined effect of heat and mechanical shear. High temperature and high screw speed caused severe thermal–mechanical damage; resulting in de-polymerization and degradation of macromolecules to small molecules with higher solubility (Bhise *et al.*, 2013) [17]. WSI is related to the amount of soluble solids in a dry sample, allowing for the verification of the severity of the extrusion process with increasing temperature and screw speed. Our findings are consistent with those reported by several authors who found the similar positive trend between WSI, screw speed and temperature (Bhise *et al.*, 2013; Camacho-Hernandez *et al.*, 2014; Ding *et al.*, 2006; Hagenimana *et al.*, 2006; Kumar *et al.*, 2010) [17, 15, 16, 18, 28].

3.6 Hardness and crispness
The hardness (HD) and crispness (CR) is associated with the expansion and cell structure of the extruded product (Ding *et al.*, 2005) [10]. HD is the maximum force required for a probe to penetrate the extrudate. The higher the value of peak force, the more is the hardness. CR denotes jaggedness of the representing fragility of snack foods. The CR was estimated by the number of peaks of the compressive curve for extrudate. The lower the number of peaks, thinner the cell walls of the extruded snacks due to the expansion and consequently higher the crispness of extrudates (Lazou & Krokida, 2010) [29]. HD of extrudates ranged from 49.84-129.74 N. However, HD values have been reported for rice extrudates as 10.58-77.29 N, wheat extrudates as 10-100 N and barley-grape pomace extrudate as 4.62-27.23 N (Ding *et al.*, 2005 & 2006; Altan *et al.*, 2009) [10, 16, 1]. The values are not comparable due to differences in type of probe used in measurement of hardness. CR (number of peaks) was varied from 7-31. Chakraborty *et al.* (2011) [20] observed CR of barnyard millet-pigeon pea extrudate as 22-50.
Regression analysis of models fitted to experimental data (Table 4) elucidates high significance of model ($P< 0.05$) and non-significant lack of fit ($p > 0.05$) for both HD and CR. The predicted model displayed high R^2 values for HD (0.9111) and CR (0.8881). Further, adequate precision of predicted model was found to be 10.076 and 8.388 for HD and CR respectively. In this context, the selected model represented the data adequately for both HD and CR. ANOVA (Table 4) clearly explained that HD and CR of extrudates were highly dependent on all processing variables (temperature, screw speed and feed moisture) and significantly ($P< 0.05$) affected by their linear effects. Feed moisture had significant positive effect ($P< 0.05$) on HD, while temperature and screw speed had negative effect on HD of extrudates. The interaction of temperature and feed was also found to have significant ($P< 0.05$) negative effect on HD. The inverse trend was noticed between HD and CR as evidenced by high negative correlation (r = – 0.983) between them.



(A)



(B)

Fig 5: The hardness (HD) and crispness (CR) of extrudates as a function as a function of temperature and feed moisture at optimized screw speed (329.08 ºC)

Feed moisture had pronounced effect on HD of RFH extrudates. Increased moisture significantly increased the hardness and reduced crispness (i.e. more number of peaks) (Fig. 5a and Fig. 5b). It might due to the reduced expansion with increasing feed moisture. Reduction in ER hindered the air bubble formation and starch conversion; consequently resulted in denser and less crispy extrudate. This commensurate with our previous findings on high BD, low ER coupled with low WSI in extrudates processed at high feed moisture. Our results are in conformity with previous work in rice snacks (Ding *et al.*, 2005 & Keawpeng *et al.*, 2014) [10, 22] and corn snacks (Ding *et al.*, 2006; Da costa *et al.*, 2010) [16, 30].
The temperature rise produced more expanded, crispier and consequently softer products. The increase of temperature triggers the starch dextrinization, superheating of water and bubble formation, which simultaneously produced more expanded product with low wall thickness. The thinner shell wall is probably responsible for the low hardness and high crispness of snacks (Omwamba & Mahungu, 2014; Lazou & Krokida, 2010) [23, 29]. Several studies reported the high negative correlation between ER/crispness and hardness

(Altan *et al.*, 2009; Ding *et al.*, 2006; Keawpeng *et al.*, 2014; Kumar *et al.*, 2010) [1, 16, 22, 28].

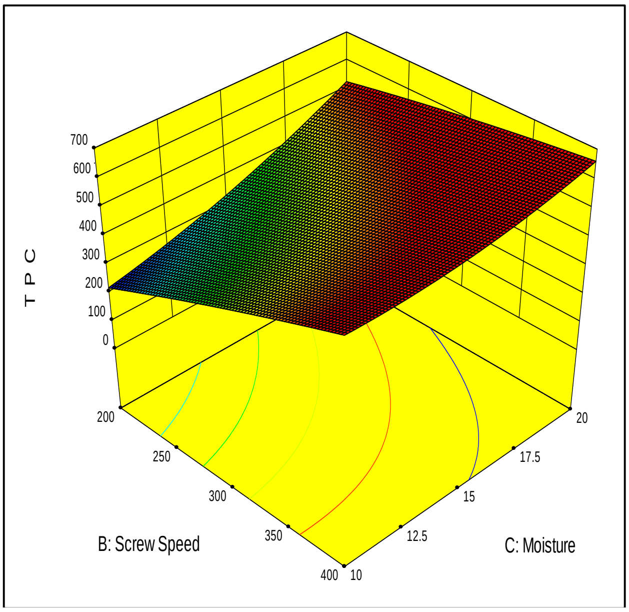
The effect of screw speed on hardness was related to extrudate BD. Increased screw speed decreased BD of extrudate and caused more expanded product with soft texture which in turn decreased HD. Similar findings were observed in wheat extrudates (Ding *et al.*, 2006) [16], barley–grape pomace extrudates (Altan *et al.*, 2009) [1] and rice-carrot pomace-pigeon pea extrudates (Kumar *et al.*, 2010) [28].

3.7 Total phenolic content (free and bound)

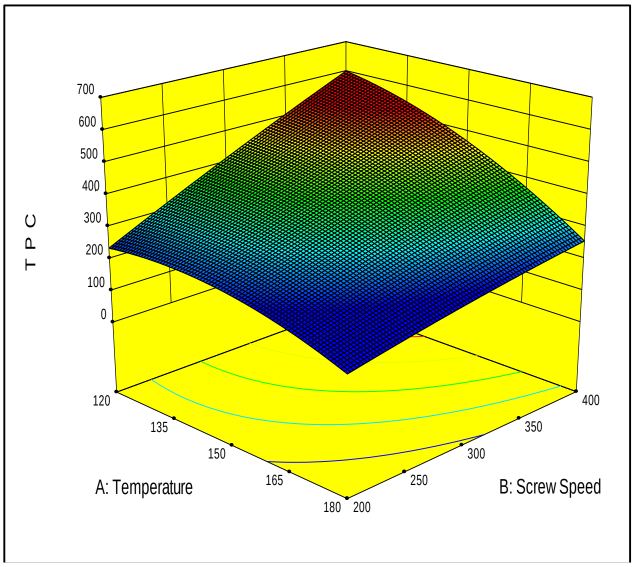
The Phenolic compounds are potent antioxidants; known to work synergistically to promote human health. Therefore, snacks with high phenolics are of great interest now-a-days. Total phenolic content (TPC) as a sum of free and bound phenolics of RFH extrudates are presented in Figure 6. Control (unextruded) RFH composite flour exhibited 619.10 mg GAE/100g dw TPC.

Regression analysis for experimental values of TPC revealed high significance of model ($P< 0.05$). The other statistics (R^2, adequate precision, lack of fit) of predictive model demonstrated suitability of model in predicting TPC of extrudates (Table 4). ANOVA showed that extrusion under selected range of processing variables significantly ($P< 0.05$) affected TPC of RFH extrudates. The linear coefficients of X_2 and X_3 were positive, whereas that of X_1 was negative (Table 3); implying high TPC at low temperature, high feed moisture and high screw speed.

The response surface plots (Fig. 6a, 6b) revealed stabilizing effect of high feed moisture on TPC, protecting phenolics from degradation due to mild or gentle processing in the extruder barrel. This positive effect of moisture on TPC was observed at all levels of temperature and screw speed (Fig. 6a). At optimum temperature (130.52 °C) and higher screw speed (400 rpm), retention of TPC ranged from 48.45 to 68% at 10 to 17.5% moisture respectively. However, further increase in moisture to 20% resulted in increased TPC (6.56%) over the raw RFH composite flour. A similar beneficial effect of feed moisture on the degree of retention of TPC during extrusion of bean has been observed by Korus *et al.* (2007) [34]. However, a group of researchers have reported contrasting results in green banana flour (Sarawong *et al.*, 2014) [36] and barley flour (Sharma *et al.*, 2012) [37].



(A)



(B)

Fig 6: Total phenolic content (TPC) of extrudates as a function as a function of extrusion variables

Increasing extrusion temperature caused significant reduction in TPC, at all moisture and screw speed levels (Fig. 6b). At optimized feed moisture (11.36%) and higher screw speed (400 rpm), rise in temperature from 120 ºC to 180 ºC brought about 57.78% loss in TPC of extrudates over selected range of screw speed. This loss in the TPC is expected to occur; as phenolic compounds are heat-liable and tend to degrade upon exposure to high temperatures. The reduction in TPC may be attributed to either reduced chemical reactivity of phenols because of their altered molecular structure or reduced extractability due to occurrence of polymerization promoted by high temperature during extrusion (Sharma *et al.*, 2012) [37]. Our findings are in conformity with the findings reported by Gat & Ananthanarayan (2015) [38] in rice-horse gram extrudates. However in certain cases, a concomitant increase in extrusion temperature, accounted for increase in TPC as reported in for extruded oats (Zielinski *et al.*, 2001) [39], purple potato-dry pea extrudates (Nayak *et al.*, 2011) [40]. This was accredited to formation of high molecular weight Maillard reaction products or release of bound phenolics.

Interestingly, screw speed followed positive linear trend with TPC of extrudates. Increased screw speed induced increased retention of TPC at all levels of temperature and moisture; the highest (97.1%) being observed at low temperature (Fig. b). Higher retention of TPC at high screw speed accredited mainly to the twin effect of high shear and low residence time. Most of the phenolics (>80%) in cereals and millets are bound or attached primarily to hemicelluloses in cell walls of the pericarp, aleurone layer and germ. High shearing and friction at higher screw speeds effectuated degradation and depolymerization of condensed tannins and bound phenols to low molecular weight oligomers that are more extractable. The liberated compounds may contribute to high TPC of extrudates (Awika *et al.*, 2003) [41]. Additionally, high screw speed reduced the residence time and consequently prevented thermal degradation of flour, thus resulting in high retention of phenolics (Mora-Rochin *et al.*, 2010) [42].

3.9 Optimization of extrusion process

Numerical optimization technique was applied to determine the optimum processing conditions (feed moisture, die head temperature and screw speed) using Design Expert software. The main criteria for optimization were desirable characteristics of ready-to-eat snacks– good physicochemical attributes (low bulk density, high expansion, high WAI and low WSI), good texture (low hardness, high crispness) and better functional properties (high total phenolic content and antioxidant activity). To achieve optimum conditions satisfying the imposed criteria, the goals are combined into an overall composite function called the desirability function (Myers and Montgomery, 2002) [14]. The optimum processing condition obtained for the development of desirable or best extrudates was: Optimum condition generated from the models was: feed moisture: 11.36% wb, die head temperature: 130.58ºC and Screw speed: 329.03 rpm. The predicted responses in terms of bulk density, expansion ratio, water absorption index, water solubility index, hardness, crispness, total phenolic content and antioxidant activity were 0.145 g/cm^3, 3.064, 5.350, 13.734%, 46.347 N, 7.088, 465.234 mg GAE/100g dw and17.983µmol TE/g dw respectively at maximum desirability of 0.7.

Verification was performed to confirm the suitability of the second order polynomial models for predicting optimum response values. The predicted values registered non-significant ($p > 0.05$) difference from experimental values at selected optimum conditions (ANOVA not shown). The results demonstrated the validation of the RSM model, indicating that the model was adequate for predicting optimum conditions in RFH extrudates development with better physicochemical, textural and functional properties.

4. Conclusions

The study demonstrated that response surface methodology can be a practical and efficient tool to optimize extrusion conditions for development of ready-to-eat snacks. Rice-finger millet-horse gram (40:50:10) snack with better physicochemical, textural and functional properties were developed employing extrusion parameters as 11.36% wb feed moisture, 130.52 ºC die head temperature and 329.08 rpm Screw speed. The developed snacks had significantly higher TPC (465.26 mg GAE/100g dw) and AOX (17.98 µmol TE/g dw). Such information revealed potential of underutilized legumes and millets in developing acceptable snacks for functional food market.

5. Acknowledgments

The authors are highly thankful to Division of Food Science and Postharvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi, India, for providing laboratory facilities.

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