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# Development of cowpea-based *(Vigna unguiculata)* extruded snacks with improved *in vitro* protein digestibility

Jakkanwar, S. A., Rathod, R. P. and *Annapure, U. S.

*Food Engineering and Technology Department, Institute of Chemical Technology, Matunga, Mumbai 400 019*

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**Abstract**

Cowpea contains an extensive amount of protein, carbohydrate, fibre and other nutrients but their bioavailability and utilization by humans relatively low and less explored due to the presence of antinutritional factors. The present study aimed to evaluate the physicochemical and sensory properties of extruded product by using response surface methodology, the influence of different moisture content in raw material and the temperature of extrusion processing on properties of extruded product prepared using cowpea. The study also focused on the assessment in vitro protein digestibility. The die temperature varied from 160-180°C with screw speed of 160-200 rpm at constant feed rate 16 rpm (70 g/ min) and the feed moisture of the raw material was in the range of 16-24%. From the result it was observed that increase in feed moisture content and decrease in die temperature significantly increases the density and hardness of extrudates and decreases the expansion, water absorption index, water soluble index and organoleptic scores. Furthermore, it was also found that the associated thermal treatment was the most effective in improving protein digestibility (up to 89%). The overall best quality product was obtained when using cowpea flour with 16% moisture content, under 180°C die temperature and 200 rpm screw speed.



## Introduction

Cowpea *(Vigna unguiculata)* is a member of leguminosae family and sub-family Fabaceae. It is one of the most important protein sources in the diet of the tropical region. Cowpea seeds are highly nutritious with 25% protein content and higher protein digestibility compared to other legumes in accordance with Afoakwa and Yenyi (2006). Besides nutritional components, cowpeas also contain certain amounts of anti-nutritional factors which have to be eliminated to improve their nutritional quality and organoleptic acceptability. Factors such as trypsin inhibitors, which reduce the nutritional quality of the protein, could be limited by using adequate heat treatment, as they are relatively heat-labile as studied by Doblado *et al.* (2007). Alonso *et al.* (1998) stated that a wide range of processing techniques could improve the protein and starch digestibility of legumes and therefore their utilization. However, it is already known and understood that some of the treatments could make physicochemical changes in proteins, starch and other components of legume seeds affecting their overall nutritional properties as observed by Valle *et al*. (1994) and De Pilli *et al.* (2011).

Extrusion cooking is a high-temperature short-time process commonly used for processing of starchy and proteinaceous materials (Baik *et al.*, 2004; Singh *et al.*, 2007) with low nutrient losses (Moscicki *et al*., 2003; Allan and Booth, 2004). Extrusion cooking is used worldwide for the production of expanded snack foods, as there is a huge demand of healthy and nutritious ready-to-eat products from all age groups of consumer. Response surface methodology (RSM) is an empirical statistical modeling technique employed for multiple regression analysis using quantitative data of designed experiments to solve multivariable equations simultaneously showed by Afoakwa and Yenyi (2006). In several food research studies, RSM is routinely used for the optimization of various food processes such as extraction, product development, functional food preparation etc. Afoakwa *et al.* (2006) applied RSM for optimizing the pre-processing conditions for canning of a newly developed and promising cowpea variety. Therefore the present investigations were carried out to explore the possible use of cowpea as a candidate for production of protein rich extruded product and study the effect of process parameters on the nutritional value with the aim of increasing the in vitro protein digestibility (IVPD) by using RSM.

*Corresponding author.
Email: *udayannapure@gmail.com*
Tel: +91 (022) 33612507; Fax 91-022-33611020

## Materials and Methods

*Materials*

Cowpea was purchased from a local market of Mumbai city. Cowpea was cleaned and ground to obtain flour and passed through a 0.84 mm sieve to obtain uniform particle size. The flour was packed in polyethylene bags and stored in a desiccator until further analysis. The enzyme used for the In vitro protein digestibility (IVPD) study was donated by Biocon, Bangalore (pepsin, 1200-2000 units / mg of protein). All the other chemicals used for the analysis were of analytical grade.

*Proximate composition*

Moisture content, ash content, protein content, fat content, analysis were carried out according to AOAC (1980, 2006) and carbohydrate was calculated by difference.

*Sample preparation*

Cowpea flour with different moisture levels in the range of 16-24% was used for extrusion process. To obtain the desired moisture levels, calculated amounts of distilled water were sprayed into the flour with thorough mixing. The mixture was then packed in polyethylene bags and allowed to equilibrate for 24 h prior to extrusion (Rathod and Annapure, 2015).

*Extrusion process*

Samples with different moisture contents were subjected to extrusion process using a Brabender single screw an extruder (Model No. 8 235 00, Germany), with a barrel diameter ratio of 20:1 and a screw with a compression ratio of 2:1. A die nozzle of 4 mm diameter was fitted to the extruder. The processing parameters were optimized using RSM for which the moisture content of feed was varied from 16 to 24%, die temperature from 160°C to 180°C and screw speed from 160 rpm to 200 rpm. The extrudates obtained were dried in hot air lab oven (Hally Instrument, Mumbai) at 60°C for 2 h to achieve a final moisture content of 4%. After drying, the samples were sealed in polyethylene bags and stored in a desiccator until further analysis as described by Rathod and Annapure (2015).

*Experimental design*

The response surface methodology was applied using a central composite design (CCD) for three independent variables explained by Barros-Neto *et al.* (2010), namely: the moisture content of the raw material, the extrusion temperature (die temperature) and the screw speed. The dependent variables used in the study were the overall expansion, bulk density, water soluble index, water absorption index and hardness. Twenty tests were performed: eight tests of factorial points ($2^3$) (three levels for each factor), six axial points (two for each variable) and six repetitions of the central point.

The results from the dependent variables were subjected to multiple regression analysis using Design expert software 7.0.0 full version (Stat-Ease, Minneapolis, USA) and coefficients with p values below 0.05 were considered significant. Linear and quadratic models were tested to explain the influence of independent variables on the response variables, because in Response Surface Methodology, the relationship between these variables is unknown and, therefore, it is necessary to find an adequate approximation to the true relationship between the response and the independent variables.

*Extrudate characteristics*

*Expansion ratio*

The diameters of 10 extruded products were measured using Vernier calipers (Absolute Digimatic Caliper, Series-500, Innox, Japan). Expansion index of the samples was determined by dividing the average diameter of the products by the diameter of the die nozzle as explained by Ding *et al*. (2005) and Rathod and Annapure (2015).

*Bulk density*

The bulk density was determined from 10 random measurements on the diameter (D, cm) and length (L, cm) of the extrudates using digital calipers, and the weight (m, g) was determined on an analytical balance. The bulk density was obtained from following formula as showed by Ding *et al*. (2005),

$$\text{Bulk Density} = 4^{*}m / (\pi^{*} D^{2}\,{}^{*}L)$$

*Porosity*

The porosity of extrudates was determined from the bulk and apparent volumes. Porosity was calculated using the equation (Rathod and Annapure, 2015),

$$\text{Porosity} = (\text{Bulk volume} - \text{Apparent volume}) / \text{Bulk volume}$$

Where, Bulk volume= $(1/\rho_b)$ and Apparent volume= $(1/\rho_{s)}$

As $\rho_b$ -bulk density, $\rho_s$ -apparent density

*Water solubility index (WSI) and water absorption index (WAI)*

Extrudates were ground to powder using a laboratory Cyclotec mill (IKA India Pvt. Ltd., Bengaluru, India) and passed through a 60 mesh sieve for uniform size distribution. 2.5 g ground powder was suspended in 25 ml water at room temperature for 30 min, with intermediate stirring and then centrifuged at 3000 x g for 15 min. The supernatant was decanted into the preweighed evaporating dish and water was evaporated untill constant weight to get dry solids. The WSI is the weight of dry solids in the supernatant expressed as a percentage of the original weight of sample whereas WAI is the weight of residue obtained after removal of the supernatant per unit weight of original dry solids as per accordance with Ding *et al.* (2005) and Rathod and Annapure (2015).

*Color*

A Hunter Lab Colorimeter (Model DP-9000 D25A), (Hunter associates laboratory, Reston, VA, USA) was used to measure the color of extrudates in terms of L value (lightness, ranging 0-100 indicating black to white). The samples were ground and passed through a 0.42 mm sieve and reflectance was measured using a Xenon flash lamp as the source of light.

*Hardness*

The hardness of the extrudates was determined using a TAXT2i texture analyzer (Serial No.4650, TEE version no.2.64 UK) with a 2 mm cylindrical probe. The force required for the cylindrical probe to penetrate the sample was recorded as grams (g). Ten randomly collected samples of each extrudate were subjected to the hardness test and a mean value of the readings was taken as the final value explained by Rathod and Annapure (2015).

In vitro *protein digestibility*

IVPD of cowpea extrudates was determined by a reported method (Metrz *et al*., 1984; Rathod and Annapure, 2015). 200 mg of ground extrudate sample were weighed and dispersed in 35 ml of pepsin solution (1.5 mg / 1ml) in 0.1 M HCl-KCl buffer (pH 2). The mixture was incubated at 37°C for 2 h with continuous stirring. 2 ml of 2 M NaOH solution was added to stop the action of the pepsin enzyme. The suspension was centrifuged at 4800 rpm, for 20 min, at 4°C. The supernatant was discarded and the residue was washed with 15 ml of 0.1 M phosphate buffer (pH 7) and again centrifuged at 4800 rpm, 20 min at 4°C. The residue was transferred to Whatman No.4 filter paper and the filter paper containing the undigested residue was vacuum dried for 2 h at 80°C.

Nitrogen content of the ground sample prior to pepsin digestion (Total sample N) and the undigested residue (Residual N) was determined by a semi-automatic Kjeldhal method (KELPLUS). Percent pepsin digestibility was calculated as follows

Percent *in vitro* pepsin digestibility
= [(Total sample N – Residual N)/ Total sample N] $^{*}$100

*Sensory analysis*

Ten trained students from Food Engineering Department evaluated the extruded snacks for appearance, flavor, texture, taste and overall acceptability in triplicate. Panelists were instructed to eat and swallow each sample and rate the intensity of each attribute using a nine-point scale (1 = dislike extremely and 9 = like extremely) (ISO 11136:2014). The sessions were performed on the same day (with a minimum 2 h break between the sessions) at the sensory laboratory of the Food Engineering and Technology Department (Mumbai, India) designed in accordance with ISO guidelines (2007) Assessors were asked not to smoke, eat or drink anything, except water, at least 1 h before the tasting sessions. For each sample, panelists received a sample served in plate coded with a digit number. Participants were provided with mineral water to clean their mouth between tastings. Presentation orders were systematically varied over assessors and replicate in order to balance the effects of serving order and carryover (MacFie *et al*., 1989).

## Results and Discussion

*Proximate composition*

Cowpea flour contains 1.35% fat, 24.68% protein, 59.73% carbohydrate, 11.03% moisture and 3.21% ash content and is fairly rich in starch with 54.78%, as well as in vitro protein digestibility of cowpea flour is 56.0%. All proximate analyses are as mean values (± standard deviation) of at least three replicates and the mean values are expressed as g per 100 g of sample (dry and wet weight basis).

*Expansion ratio*

The Expansion Ratio of the extrudates varied from 1.11 to 1.49 as shown in Figure 1A. Expansion ratio of extrudate was significantly affected by extrusion conditions namely feed moisture, die temperature and screw speed. Increase in feed moisture content and decrease in die temperature decreased the expansion ratio of extrudates from 1.49

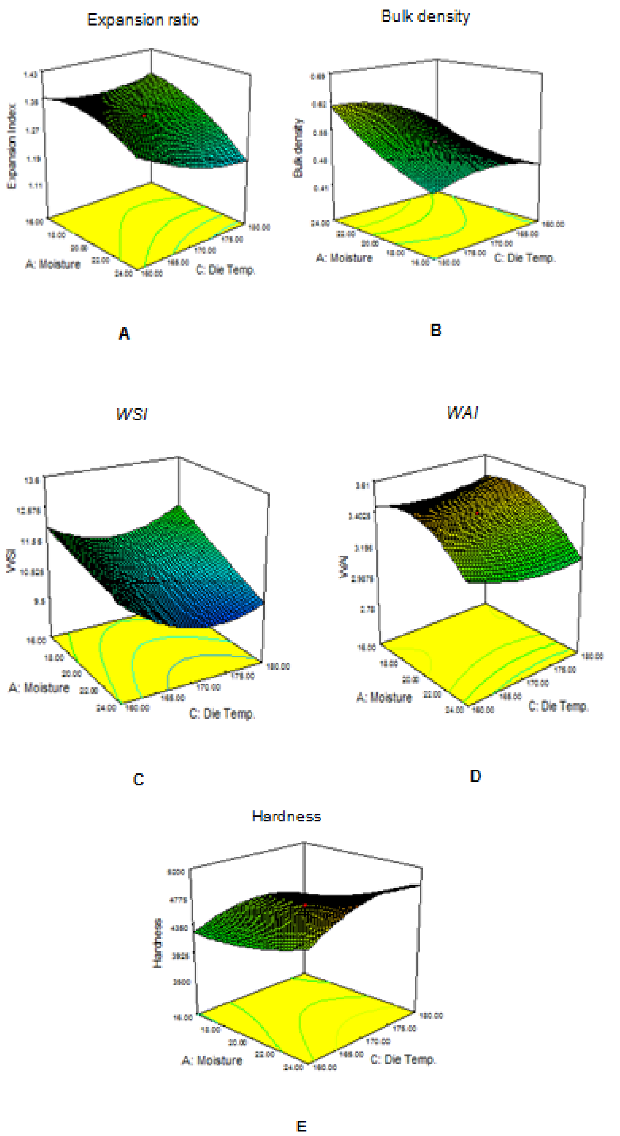

Figure 1. 3D surface plots for effect of feed moisture and screw speed on extrudate properties as expansion ratio, bulk density, WSI, WAI, hardness.

to 1.11 (Table 1). This could be due to the pressure and temperature difference within the barrel and the outside atmosphere leads to the sudden flash off of the internal moisture in terms of vapours, which resulted in bubble formation as explained by Moraru and Kokini (2003). Die temperature is one of the most important factors that contribute to starch modification during extrusion. As the temperature of extrusion cooking increased, starch becomes more fully cooked and thus better able to expand and Low moisture content in feed may restrict the flow of the material and increase shearing rate and residence time, which might increase the degree of gelatinization and expansion. Similar results were found by Singh *et al.* (2007). The expansion ratio was found to be highest at screw speed of 213 rpm but the sensory value for this is very low as compare to optimized batch. Optimized value of expansion ratio is 1.45 which is obtained at 16 % moisture content, 200 rpm screw speed and 180°C die temperature. Screw speed has generally a positive effect on extrudate expansion due to the increase in shear, thus decrease in melt viscosity induced by high screw speeds (Kokini *et al.*, 1992).

The corresponding second order response model equation for the expansion with R2 (0.9189):

$$\text{Expansion ratio} = 1.31 - (0.056A) + (0.062B) - (0.027C) - (0.033A^2) + (0.022\,B^2) + (0.024\,C^2) + (0.015\,AB) - (0.028\,AC) + (0.020\,BC)$$

Where A, B and C represents moisture content, screw speed and die temperature respectively.

From ANOVA (Table 2) it was found that, moisture content ($p<0.0002$), screw speed ($p<0.0001$) and temperature ($p<0.0201$) has the most significant effect on expansion ratio of cowpea extrudates, as they have largest coefficients. The negative sign of coefficient (A) suggest that an increased value of feed moisture resulted in decreased expansion ratio, whereas positive value of coefficient (B) suggests that, expansion ratio increases with increased screw speed.

*Bulk density*

Density reflects extrudate expansion and open cell area. In general the extrudates having lower expansion shows higher density and vice versa. The increase in density of cowpea extrudate from 0.37-0.69 g/ml (Figure 1B) was observed as the expansion ratio decreased from 1.49 to 1.11. Screw speed showed a highly significant effect on the density of extrudates. Increase in die temperature can increase the temperature of the moisture above the boiling point so that when the extrudate exits from the die, a part of the moisture would quickly flash-off as steam and result in an expanded structure with large alveoli and low density and decrease in temperature was not helps to enough flash-off the moisture (because of low die temperature and high feed moisture), less expansion occurs resulting in a high BD product with collapsed cells. The lowest density of 0.37g/ml was found at highest screw speed of 213 rpm but the sensory value for this is very low as compare to optimized batch. Optimized value of bulk density which is 0.39 g/ml obtained at 16 % moisture content, 200 rpm screw speed and 180°C die temperature. Higher screw speed may be expected to lower the melt viscosity of the mixture increasing the elasticity of the dough, resulting in increases in the density of the extrudates as also observed by Ding *et al.* (2006) and Rathod and Annapure (2015).

Table 1. Central composite design matrix of independent variables and their experimental responses

| Moisture (g/100g) | Screw speed (RPM) | Die Temp. (°C) | *Expansion ratio | *Density (g/ml) | **WSI* (%) | **WAI* (%) | *Porosity | *Color (L*) | (a*) | (b*) | *Hardness (g) | **In vitro* protein digestibility (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | 160 | 160 | 1.36±0.03 | 0.44±0.01 | 12.21±0.11 | 3.42±0.07 | 0.36±0.02 | 68.62 | 3.23 | 17.87 | 4436.11±0.12 | 79.51±0.15 |
| 24 | 160 | 160 | 1.29±0.03 | 0.52±0.01 | 11.17±0.02 | 3.17±0.07 | 0.28±0.01 | 65.85 | 3.57 | 18.24 | 4601.99±0.15 | 76.09±0.06 |
| 16 | 200 | 160 | 1.41±0.01 | 0.42±0.03 | 13.12±0.11 | 3.55±0.01 | 0.39±0.01 | 70.15 | 2.74 | 17.56 | 3791.95±0.24 | 78.22±0.11 |
| 24 | 200 | 160 | 1.35±0.02 | 0.45±0.03 | 12.16±0.05 | 3.37±0.11 | 0.35±0.02 | 67.88 | 3.42 | 18.32 | 4594.22±0.35 | 77.08±0.09 |
| 16 | 160 | 180 | 1.37±0.04 | 0.43±0.01 | 12.69±0.24 | 3.52±0.09 | 0.36±0.04 | 68.99 | 3.06 | 17.72 | 4503.94±0.52 | 81.14±0.13 |
| 24 | 160 | 180 | 1.14±0.02 | 0.64±0.02 | 10.51±0.11 | 3.11±0.05 | 0.21±0.02 | 60.91 | 4.19 | 19.46 | 4930.32±0.22 | 78.53±0.05 |
| 16 | 200 | 180 | 1.45±0.03 | 0.39±0.01 | 13.73±0.22 | 3.63±0.02 | 0.44±0.01 | 70.78 | 2.68 | 17.39 | 3512,78±0.64 | 78.05±0.02 |
| 24 | 200 | 180 | 1.33±0.04 | 0.46±0.02 | 11.86±0.21 | 3.36±0.14 | 0.32±0.01 | 66.32 | 3.51 | 18.03 | 4591.41±0.32 | 77.13±0.04 |
| 13 | 180 | 170 | 1.28±0.01 | 0.51±0.01 | 11.22±0.24 | 3.14±0.04 | 0.27±0.02 | 65.56 | 3.61 | 18.31 | 4719.82±0.31 | 82.47±0.05 |
| 27 | 180 | 170 | 1.11±0.02 | 0.69±0.01 | 9.52±0.32 | 2.78±0.18 | 0.11±0.01 | 60.11 | 4.27 | 19.62 | 5118.75±0.31 | 78.06±0.01 |
| 20 | 147 | 170 | 1.21±0.05 | 0.55±0.03 | 11.12±0.31 | 3.12±0.08 | 0.27±0.03 | 65.08 | 3.83 | 18.59 | 4878.79±0.46 | 83.91±0.03 |
| 20 | 213 | 170 | 1.49±0.03 | 0.37±0.01 | 13.86±0.31 | 3.64±0.06 | 0.47±0.04 | 71.81 | 2.39 | 17.09 | 3469.43±0.55 | 79.12±0.06 |
| 20 | 180 | 153 | 1.43±0.04 | 0.41±0.03 | 13.57±0.07 | 3.61±0.11 | 0.43±0.04 | 71.22 | 2.43 | 17.22 | 3579.91±0.42 | 76.68±0.04 |
| 20 | 180 | 186 | 1.28±0.01 | 0.54±0.04 | 11.28±0.09 | 3.35±0.01 | 0.27±0.02 | 65.47 | 3.97 | 18.41 | 4509.61±0.11 | 84.95±0.06 |
| 0 20 | 180 | 170 | 1.31±0.01 | 0.52±0.04 | 10.57±0.21 | 3.42±0.04 | 0.31±0.02 | 66.26 | 3.53 | 18.59 | 4643.79±0.36 | 80.73±0.03 |

*All the values are mean ± SD of three values

The corresponding second order response model equation for the bulk density with $R^2$ (0.9153) shown in Table 2.

Bulk density = 0.52 + (0.051A) – (0.045B) + (0.023C) + (0.019A$^2$) – (0.030B$^2$) – (0.025C$^2$) – (0.024AB) + (0.021AC) – (0.016BC)

From ANOVA it was observed that the model F-value of 12.01 (Table 3) implies the significance of the model. Value of “Prob>F” is 0.003 shown in table 3 which is less than 0.05 indicates model terms are significant. Moisture content ($p<0.0002$), screw speed ($p<0.0001$) and temperature ($p<0.0249$) were found to have largest coefficients, showing the most significant effect on density of cowpea extrudates.

*Porosity*

Porosity is the volume fraction of air or the void fraction in the sample. It was greatly affected by feed moisture and die temperature. From Table 1, it was observed that an increase of feed moisture content from 16 to 24 g/100g during extrusion decreased the porosity values from 0.47 to 0.11, whereas increase of die temperature (160 to 180°C) had the opposite effect, resulting in increased porosity (0.11-0.47). Also increase in screw speed resulted in increased porosity. The positive correlation of porosity with die temperature is attributed to the puffing of product at higher temperature which favors the bubble growth and resulted in more porous structure due to air cells, which leads to increased porosity of the product (Thymi *et al.*, 2005). Increased porosity with increased screw speed may be due to the greater dissipation of mechanical energy to the melt as similarly found by Moraru and Kokini (2003).

The corresponding second order response model equation for the porosity with $R^2$ (0.9066):

Porosity=0.31–(0.044A)+(0.016B)+(1.731E-007C) + (0.016A$^2$) + (0.018B$^2$) –(5.165E-003C$^2$) – (0.014AB) – (0.019AC) – (8.750E-003BC)

From ANOVA (Table 2) it was observed that, moisture content ($p<0.0004$) screw speed ($p<0.0005$) and temperature ($p<0.249$) were found to have largest coefficients, showing the most significant effect on porosity of cowpea extrudates. Interaction of AB (feed moisture and screw speed) and AC (feed moisture and die temperature) was found most significant.

*Water solubility index (WSI) and water absorption index (WAI)*

The extrudates exhibited WSI and WAI in the range of 9.52 to 13.86% and 2.78 to 3.64 as shown in Figure 1C and Figure 1D, respectively. WSI is used as an indicator of degradation of molecular components, measures the amount of soluble components released from the starch after extrusion. WAI measures the amount of water absorbed by starch and is used as an index of starch gelatinization. WSI of extrudates increased with decrease in feed moisture and increase in screw speed. Increased WSI, with decreased moisture content, may be attributed to higher degradation of starch (Singh *et al*., 2007). Increased in screw speed can increases pressure and shear rate barrel and because of high pressure, shear and temperature, higher degradation was occurred hence increased WSI. Also some researcher (Onyango *et al*., 2004; Ding *et al*., 2005) showed that increasing WSI is caused by greater shear degradation of starch during extrusion at low moisture conditions. Similar effects of moisture and screw speed on WSI during

Table 2. Estimated coefficients and sum of square values of the fitted model representing the relationship between the responses and the process variables

| Factor | Expansion ratio | | Density | | *WSI* | | *WAI* | | Porosity | | Color | | Hardness | | *In vitro* protein digestibility | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CE | SS | CE | SS | CE | SS | CS | CS | CS | SS | CS | SS | CS | SS | CS | SS |
| Model | 1.31 | 0.9051 | 0.52 | 0.11 | 10.56 | 27.95 | 3.42 | 4643.56 | 3.815E+006 | 0.74 | 0.31 | 0.12 | 66.26 | 159.66 | 80.52 | 75.47 |
| A | -0.056 | 0.3462 | 0.051 | 0.035 | -0.66 | 5.87 | -0.13 | 230.22 | 7.238E+005 | 0.22 | -0.049 | 0.033 | -1.96 | 52.38 | -1.21 | 19.95 |
| B | 0.062 | 0.1555 | -0.045 | 0.027 | 0.65 | 5.81 | 0.11 | -318.69 | 1.387E+006 | 0.18 | 0.046 | 0.029 | 1.62 | 35.69 | -1.21 | 19.94 |
| C | -0.027 | 0.0189 | 0.023 | 6.975E-003 | -0.27 | 1.02 | -0.024 | 122.85 | 2.061E+005 | 7.842E-003 | -0.023 | 7.455E-003 | -1.11 | 16.85 | 1.43 | 27.83 |
| $A^2$ | -0.033 | 0.2392 | 0.019 | 5.387E-003 | -4.760E-004 | 3.265E-006 | -0.14 | 98.88 | 1.409E+005 | 0.27 | -0.036 | 0.019 | -1.04 | 15.49 | -0.31 | 1.35 |
| $B^2$ | 0.022 | 0.0116 | -0.030 | 0.013 | 0.75 | 8.16 | 0.013 | -164.58 | 3.904E+005 | 2.295E-003 | 0.029 | 0.012 | 0.95 | 12.91 | 0.28 | 1.11 |
| $C^2$ | 0.024 | 0.0716 | -0.025 | 8.906E-003 | 0.73 | 7.67 | 0.048 | -210.31 | 6.374E+005 | 0.033 | 0.022 | 7.022E-003 | 0.91 | 11.97 | -0.47 | 3.12 |
| AB | 0.015 | 0.0010 | -0.024 | 4.513E-003 | 0.050 | 0.020 | 0.026 | 161.08 | 2.076E+005 | 5.512E-003 | 8.750E-003 | 6.125E-004 | 0.52 | 2.12 | -0.13 | 0.13 |
| AC | -0.028 | 0.0078 | 0.021 | 3.613E-003 | -0.26 | 0.53 | -0.031 | 67.11 | 36027.33 | 7.813E-003 | -0.019 | 2.812E-003 | -0.94 | 7.03 | 0.25 | 0.49 |
| BC | 0.020 | 0.0561 | -0.016 | 2.112E-003 | 0.063 | 0.031 | 3.750E-003 | -84.77 | 57484.23 | 1.125E-004 | 0.011 | 1.012E-003 | 0.46 | 1.66 | 0.40 | 1.27 |
| $R^2$ | 0.9189 | | 0.9153 | | 0.9192 | | 0.8832 | | 0.9066 | | 0.9426 | | 0.8955 | | 0.8684 | |

Where: CE-Coefficient estimate, SS- Sum of squares

extrusion cooking have been reported earlier by Gujral *et al.* (2001).

WAI is a gelatinization index and it is generally agreed that barrel temperature and feed moisture exert greatest effect on the extrudate by promoting gelatinization (Ding *et al*., 2005). WAI generally increases along with the increase in temperature, after which it decreases, probably due to increased dextrinization. WAI was also found to increase with decreased in screw speed. High input of thermal energy due to high residence time (at low screw speeds) may lead to an enhanced level of starch gelatinization and increased WAI.

The corresponding second order response model equations for the WSI and WAI with $R^2$ (0.9192 and 0.8832) respectively:

$$WSI = 8.52 + (0.30A) + (0.43B) + (0.45C) + (0.76A^2) + (0.85B^2) + (0.23C^2) - (0.67AB) - (0.66AC) - (0.32BC)$$

$$WAI = 6.18 - (0.19A) + (0.17B) + (1.00C) + (0.087A^2) + (0.12B^2) + (0.12C^2) + (0.020AB) + (0.055AC) + (0.090BC)$$

From ANOVA it was observed that, moisture content (0.0007) and screw speed (0.0007)) were found to have the most significant effect on WSI. AC (feed moisture and die temperature) was found to have most significant effect on WSI of extrudate. As well as Feed moisture (< 0.0009) and screw speed (0.0016) were found to have significant effect on WAI. Interaction of AC (moisture and screw speed) was found to have significant effect WAI of extrudate.

*Color*

Color is the characteristic of food product as far as the consumer appeal is concerned. Color changes can give information about the extent of browning reactions such as caramelization, Maillard reaction, degree of cooking and pigment degradation during the extrusion process (Ilo and Berghofer, 1999). The maximum value for lightness (L value) is 100 indicating white color. L value less than 100 indicates a decrease in whiteness. The redness and yellowness are denoted by the 'a' and 'b' values, respectively. The parameter $L^*$ was negatively correlated with $a^*$ and b* values. On the other hand, $a^*$ and $b^*$ values were positively correlated with each other. Color 'L' decreased as die temperature increased. The low value of 'L' was obtained at high temperatures with high moisture content. The reduction in lightness with increasing temperature may be resulted from occurrence of browning reaction such as Maillard and caramelization reaction during extrusion process. Negative correlation was observed between die temperature and lightness (Altan *et al*., 2008; Shoar *et al*., 2010). 'a' and 'b' value increased with increased in die temperature that positive correlation was observed between die temperature and redness as well as between die temperature and yellowness. Table 1 summarizes the color characteristics of the

Table 3. F values and P values (Probe>F) of the fitted model representing the relationship between the responses and the process variables

| Factor | Expansion ratio | | Density | | *WSI* | | *WAI* | | Porosity | | Color | | Hardness | | *In vitro* protein digestibility | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F value | Prob > F | F Value | Prob > F | F value | Prob > F | F value | Prob > F | F value | Prob > F | F value | Prob > F | F value | Prob > F | F value | Prob > F |
| Model | 12.59556 | 0.0002 | 12.01337 | 0.0003 | 12.53599 | 0.0002 | 8.404712 | 0.0013 | 10.79988 | 0.0005 | 18.36569 | 0.0001 | 9.516565 | 0.0008 | 5.314118 | 0.0098 |
| A | 32.24652 | 0.0002 | 34.98926 | 0.0001 | 23.55601 | 0.0007 | 21.93009 | 0.0009 | 27.52144 | 0.0004 | 54.1481 | <0.0001 | 16.25222 | 0.0024 | 5.29195 | 0.0352 |
| B | 39.80085 | 0.0001 | 27.37435 | 0.0004 | 23.49822 | 0.0007 | 18.2413 | 0.0016 | 24.85603 | 0.0005 | 36.89867 | 0.0001 | 31.14254 | 0.0002 | 3.631601 | 0.0748 |
| C. | 7.618104 | 0.0201 | 6.945457 | 0.0249 | 4.109887 | 0.0701 | 0.798158 | 0.3926 | 6.450652 | 0.0294 | 17.42055 | 0.0019 | 4.627824 | 0.0569 | 7.018802 | 0.0175 |
| AB | 1.351315 | 0.2720 | 4.49349 | 0.0600 | 0.07706 | 0.7870 | 0.56103 | 0.4711 | 0.529959 | 0.4833 | 2.193452 | 0.1694 | 4.660688 | 0.0562 | - | - |
| AC | 4.541919 | 0.0589 | 3.597282 | 0.0871 | 2.129157 | 0.1752 | 0.79511 | 0.3935 | 2.433487 | 0.1498 | 7.268691 | 0.0225 | 0.808926 | 0.3896 | - | - |
| BC | 2.402337 | 0.1522 | 2.103601 | 0.1776 | 0.121644 | 0.7345 | 0.01145 | 0.9169 | 0.876055 | 0.3713 | 1.712129 | 0.2200 | 1.290701 | 0.2824 | - | - |
| A^2 | 11.51015 | 0.0069 | 5.364573 | 0.0431 | 0.000388 | 0.9847 | 27.07772 | 0.0004 | 15.15919 | 0.0030 | 15.66693 | 0.0027 | 3.163585 | 0.1057 | - | - |
| B^2 | 5.324132 | 0.0437 | 13.05623 | 0.0047 | 33.04122 | 0.0002 | 0.233539 | 0.6393 | 10.32259 | 0.0093 | 13.6714 | 0.0041 | 8.764749 | 0.0143 | - | - |
| C^2 | 6.206482 | 0.0319 | 8.868711 | 0.0139 | 31.0529 | 0.0002 | 3.375595 | 0.0960 | 5.872304 | 0.0359 | 12.68088 | 0.0052 | 14.31241 | 0.0036 | - | - |

Where, A-Moisture, B-Screw speed, C- Die temperature

cowpea extrudates (L). From Table 1, it was observed that, measured values of the color parameters of extruded varied in the range from 71.78 to 60.11 for lightness (L value), 2.39 to 4.27 for redness (a) and 17.09 to 19.62 for yellowness (b).

The corresponding second order response model equation for the color with $R^2$ (0.9426):

$$\text{Color} = 51.40 - (1.17A) + (0.64B) + (1.02C) + (1.13A^2) + 0\ (.97B^2) + (0.58C^2) + (0.51AB) - (0.53AC) + (0.17BC)$$

From ANOVA it was showed that, feed moisture ($p< 0.0001$), screw speed ($p<0.0001$) and die temperature (<0.0019) were found to have significant effect on color of extrudate. Also Interaction of AC (feed moisture and die temperature) ($p<0.0225$) was found most significant.

*Hardness*

Hardness is the average force required for a probe to penetrate the extrudate (Mercier *et al.,* 1989).The quality of extruded snack food is judged from its crispness, which in turn was determined by expanded volume. Feed moisture, screw speed and die temperature were found to have the significant effect on extrudate hardness. From Table 1, it was observed that increase in feed moisture content significantly increases the hardness of extrudate, whereas an increase in die temperature and screw speed resulted in decreased hardness. Same observations were found by Ding *et al*. (2006) and Rathod and Annapure (2015). Increasing temperature would decrease melt viscosity, but it also increases the vapor pressure of water. This favors the bubble growth which is the driving force for expansion that produces low density products and thus decreasing hardness of extrudate, Ding *et al.* (2005) agreed with this result. Increase in hardness of extrudates with increase in the feed moisture content might be due to the reduced expansion by the increase in moisture content which results in a dense and hard structure of extrudates (Liu *et al.*, 2012). Hardness of the extrudates varied from 3469.43 to 5118.75 as shown in Figure 1E.

The corresponding second order response model equation for the hardness with $R^2$ (0.8955):

$$\text{Hardness} = 3447.83 + (674.78A) - (528.02B) - (119.13C) + (226.51A^2) - (99.81B^2) + (95.07C^2) - (14.59AB) + (418.60AC) - (49.53BC)$$

From ANOVA, feed moisture (0.0024) and screw speed (0.0002) were found most significant. Interaction of AB (feed moisture and screw speed) was found to present a significant effect on hardness of extrudate.

In vitro *protein digestibility*

*In vitro* protein digestibility of extruded cowpea (84.95%) was found significantly higher than that of whole raw cowpea (56%). Die temperature was found to have the most significant effect on extrudate protein digestibility. Protein digestibility of extruded cowpea was increased from 76.09 to 84.95% with increased die temperature from 160 to 186oC agreed with Wang (Wang *et al.*, 2008). This may be due to the higher destruction of antinutritional factors at higher temperature. A thermal treatment causes the denaturation of the trypsin inhibitors and also partial denaturation of proteins that makes them more digestible than native proteins which in turn results

Table 4. Sensory results for cowpea extrudates

| Trial No. | Appearance | Taste | Flavor | Texture | Overall Acceptability |
|---|---|---|---|---|---|
| 1 | 6.84±0.09 | 6.30±0.1 | 7.24±0.1 | 6.79±0.11 | 6.8±0.12 |
| 4 | 6.8±0.04 | 6.34±0.11 | 7.20±0.08 | 6.71±0.14 | 6.5±0.09 |
| 7 | 7.4±0.11 | 7.46±0.13 | 7.82±0.11 | 7.62±0.1 | 7.8±0.11 |
| 12 | 6.8±0.07 | 5.7±0.06 | 7.12±0.09 | 6.43±0.12 | 6.12±0.10 |
| 15 | 7.32±0.12 | 6.86±0.16 | 7.58±0.13 | 7.24±0.16 | 7.2±0.07 |

All the values are mean ± SD of ten individual determinations.

in increased pepsin digestibility (Rivas Vega *et al.*, 2006).

The corresponding second order response model equation for the IVPD with $R^2$ (0.8684, Table 2)

$$IVPD = 80.53 - (1.21A) - (1.21B) + (1.67C) - (0.38A^2) + (0.20B^2) - (0.19C^2) - (0.13AB) + (0.25AC) - (0.40BC)$$

From ANOVA it was observed that, Feed moisture (0.035) and die temperature (0.0175) were found most significant.

*Sensory analysis*

Sensory analyses for all cowpea based extrudates samples were done and given only five randomly batches with optimized batch as per standard. Sensory analysis was showed in Table 4. The sensory scores for overall acceptance obtained for cowpea-based extrudates were 6.8, 6.5, 7.8, 6.12 and 7.2 for runs (16%, 160 rpm, 160°C), (24%, 200 rpm, 160°C), (16%, 200 rpm, 180°C,), (20%, 213 rpm, 170°C) and (20%, 180 rpm, 170°C) respectively. From these results, it was very clear that extrudates can be produced by using cowpea with good sensory acceptability. Extrudates with moisture content of 16% produced under a die temperature of 180oC at 200 rpm were found to be most acceptable using both sensory evaluation and statistical analysis.

*Validation of model*

Table 5 shows the predicted and experimental values of the responses at optimum conditions which explain the suitability of the model developed for the experiment.

## Conclusion

The present study revealed that cowpea can be used to produce a highly acceptable extruded snack. RSM was successfully employed to determine the optimal variables to obtain high quality extruded product and was used to establish the correlation between these process variables and physical properties of extruded product. It was also proved that extrusion process increased the nutritional value of the extruded product by improved IVPD. Further, it is revealed that cowpea has a great potential for extrusion to produce ready-to-eat snacks with good acceptance. The overall best quality product was optimized and obtained at 16% moisture, 180oC die temperature and 200 rpm screw speed. Thus, results indicate that cowpea may be a good candidate to be used as an industrial raw material for the production of extruded snacks with great nutritional value.

Table 5. Predicted and experimental values of the responses at optimum conditions: moisture content (16%), die temperature (180oC) and screw speed (200 rpm)

| Responses | Predicted value | *Experimental value |
|---|---|---|
| Expansion | 1.446 | 1.49±0.03 |
| B.Density(g/ml) | 0.398 | 0.37±0.01 |
| *WSI* (%) | 13.34 | 13.86±0.31 |
| *WAI* (%) | 3.566 | 3.64±0.06 |
| Porosity | 0.417 | 0.47±0.04 |
| Color (L value) | 70.421 | 71.81±0.02 |
| Hardness (g) | 3528.96 | 3469.43±0.55 |

*All the values are mean ± SD of three values

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