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Effect of Thermo-extrusion Process Parameters on Selected Quality Attributes of Meat Analogue from Mucuna Bean Seed Flour

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ABSTRACT

Flour from mucuna beans (*Mucuna pruriens*) were used in producing texturized meat analogue using a single screw extruder with the intention to monitor modifications on some functional properties of the extrudate. Response surface methodology based on Box Behnken design at three levels of barrel temperature (110, 120, 130ºC), screw speed (100, 120, 140 rpm) and feed moisture content (44, 47, 50%) were used in 17 runs. Regression models describing the effect of process variables on the product quality attributes were obtained. Result obtained showed that the moisture contents of the meat analogue samples decreased from 13.23 to 6.53%. Increasing feed moisture content resulted in extruded meat analogue with a higher density (0.988), water absorption index (WAI) (2.30), oil absorption index (OAI) (2.350), swelling power (3.47) and lower lateral expansion (0.84). Lateral expansion, OAI and swelling power increased as barrel temperature increased with peak values of 1.39, 2.39 and 3.47 respectively, while bulk density and WAI decreased. The product functional responses with coefficients of determination (R^2) ranging between 0.658 and 0.894 were most affected by changes in barrel temperature and feed moisture and to lesser extent by screw speed. Optimization results based on desirability concept indicated that a barrel temperature of 120.15°C, feed moisture of 47% and screw speed of 119.19 rpm would produce meat analogue of preferable functional properties.

Keywords: Mucuna bean flour, functional properties, texturized meat analogue, optimization.

Introduction

Legumes are one of the most important sources of proteins, carbohydrates and dietary fiber for human nutrition. Generally, legumes have protein contents between 20 and 40% and a few ranges between 40 and 60% (Emenalom and Udedibie, 1998; Maneepen, 2000). One of such crops is Mucuna beans (*Mucuna spp*). The mucuna bean, commonly called velvet bean, is an annual leguminous climber, with pods that are covered with velvety hairs that irritate the skin when the fruit is mature and dry. The major use for mucuna, at present, is as a green manure/cover crop for small holder farmers in tropical regions of the world. It has nutritional potential as a rich source of protein (23 – 35%) (Bressani and Elias, 1979; Teixeira *et al.*, 2003) and metabolisable energy of about 1 kcal/g for raw seeds and 3.2 kcal/g for processed seeds (Ukachukwu *et al.*, 2002). However, the lack of knowledge of the nutritional qualities of lesser-known legumes, such as mucuna, grown in developing countries is responsible for the poor utilization of these traditional crops in different food formulations.

Lack of sufficient protein in nutrition of large percentage of people of developing countries is becoming a major setback for human development. This increasing demand by people of developing

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countries for less expensive protein and alternatives to scarce and/or high priced meat in recent years has lead to intensive research efforts aimed at finding alternative sources of protein from underutilized legume seeds in order to meet the protein demands (Lawal and Adebowale, 2004).

Meat analogues, which approximate the aesthetic qualities of certain types of meat, are blends of various protein sources such as isolates, glutens and albumin, and are also called meat substitute, mock meat, faux meat, imitation meat, or soy meat (Lucas, 1996).

One way of producing meat-like products is extrusion cooking of plant materials to texturised fibrous meat substitutes. Extrusion cooking is an important and popular food processing technique classified as a high temperature/short time (HTST) process to produce fiber-rich product (Gaossong and Vasanthan, 2000). It is a process in which moistened, expansive, starchy and/or proteinaceous food materials are plasticized and cooked in a tube by a combination of moisture, pressure, temperature and mechanical shear, resulting in molecular transformation and chemical reactions (Castells et al., 2005). The process has found numerous applications, including increasing numbers of ready-to-eat cereals; salty and sweet snacks; co-extruded snacks; indirect expanded products; croutons for soups and salads; an expanding array of dry pet foods and fish foods; textured meat-like materials from defatted high-protein flours; nutritious precooked food mixtures for infant feeding; and confectionery products (Harper, 1989; Eastman *et al.*, 2001).

Despite the increased use of extrusion process, it is still a complex process that has to be optimized for specific applications based on the nature of raw materials and desired final product. Even within a given extrusion process, small variations in processing conditions affect process variables as well as product quality (Desrumaux *et al.*, 1999). Nutritional concern about extrusion cooking is reached at its highest level when extrusion is used specifically to produce nutritionally balanced or enriched foods, like weaning foods, dietetic foods and meat replacers (Cheftel, 1986; Plahar *et al.*, 2003).

Despite the vast literature on texturization of vegetable proteins, soya in particular, very scarce information is available on optimization of the proximate composition and functional properties during extrusion of mucuna bean flour (MBF) into meat analogues. Therefore, the objectives of this study were to study the effect of extrusion process variables on the proximate composition and functional properties of extruded meat analogue from mucuna bean flour (MBF) and to determine the optimized extrusion parameters.

Material and Methods

Sample preparation protocol

Seeds of *Mucuna pruriens* were obtained from the International Institute of Tropical Agriculture/International Livestock Research Institute (IITA/ILRI), Ibadan, Nigeria. The seeds were cleaned, dehulled manually using pestle and mortal, ground in Christy Laboratory Mill (Cheff Food Processor, Japan), sieved through a screen of 20 mesh sizes and packaged in air-tight polyethylene bag at 30 ± 2ºC prior to use. Mucuna paste was prepared ensuring three different levels of water content, 44, 47 and 50% (w.b.) based on the process design. The amount of water added depended on the initial water content of the dry mucuna flour and was adjusted to ensure that all the paste contained the specified amount. To do this, the exact water content of the flour was determined experimentally by drying in a forced air oven at 105ºC for 24 h (to constant mass). To form the paste, distilled water was added to the specific dry flour until it reached 44, 47 or 50% water content (w.b.). Half of the water was added at 28ºC while mixing for 2 min using a mixer. After mixing for 3 min, the rest of the water was added. This fraction of water was heated to 100ºC and was poured while mixing for 2 min. The paste was them allowed to stay for about 1 hour before being fed into the extruder.

Extrusion cooking

A laboratory scale single screw extruder with screw length per diameter (L/D), screw diameter and length of 16.43:1, 18.5 mm and 304 mm as earlier described by Sobukola *et al.* (2012) was used for this work. The extruder has a power of 0.25 hp and composed of two sections, transmission and die zone. The barrel section was heated with band heater. It was operated at full speed in all runs under the following conditions: barrel and die temperature (110, 120 and 130°C), screw speed (100, 120 and 140 rev/min) and feed moisture content (44, 47 and 50 %). During extrusion, the barrel temperature and screw speed were recorded when stable. A rod die (12 mm diameter) and 5 mm nozzle was used to extrude the mucuna paste samples fed manually through a standard bin feed hopper. Extruded samples were cut immediately as they exit the die, allowed to cool and then packaged in polyethene bags prior to analysis.

Sample analysis

Proximate composition

Protein was determined by the Kjeldhal digestion and distillation methods of AOAC (1995), fat by Soxhlet distillation method of AOAC (1995), moisture content, crude fibre and ash by AOAC (1995) while carbohydrate was by difference. The pH determination of the flour was done using Kent pH meter (Model 7020, Kent Ind. Measurement Ltd, Surreyl) with a glass electrode as described by Oyewole and Odunfa (1988).

Functional properties

Lateral expansion determination

Sectional expansion, the ratio of diameter of extrudate and the diameter of die, was used to express the expansion of extrudate (Fan *et al.*, 1996). Six replicates of extrudate were selected at random and the average taken.

$$\text{Lateral expansion} = \text{Diameter of extrudate}/\text{Diameter of hole} \quad (1)$$

Bulk density

Each of the samples was weighed using a laboratory balance. The length and diameter of the sample was measured using a digital vernier caliper. The bulk density of the extrudate was calculated as shown below (Ali *et al.*, 1996).

$$\text{Bulk density} = 4m/\pi d^2L \quad (2)$$

where m is mass (g), d is diameter (cm) and L = length (cm) of the extrudate.

Water absorption index (WAI)

This was determined using the method of Anderson *et al.* (1969). A suspension of 2.5 g of ground extrudate sample (100 mesh) was prepared in 30 ml distilled water at room temperature for 30 min by gently stirring during this period, and then centrifuged at 3,000 rpm for 15 min. The supernatant was decanted carefully into an evaporating dish of known weight. The evaporating dish containing the supernatant was placed on the plate until all the water has evaporated. WAI was calculated as gel weight of the original dried solids.

Oil absorption index (OAI)

Same method of Anderson *et al.* (1969) was used with the replacement of distilled water with groundnut oil.

Swelling power

One gram of sample was weighed into a 50 ml plastic centrifuge tube. Then 50 ml of distilled water was added into the meat analogue granule and mixed gently. The slurry was heated in a water bath at 60, 70, 80, 90 and 100°C, respectively for 10 min. The solution was shaken gently during heating to prevent clumping of the starch and the solution was centrifuged at 3,000 rpm for 10 min using SPECTRA, UK (Merlin 503) centrifuge. The supernatant was decanted and dried to determine the amount of soluble solid, and was used to calculate solubility index. The weight of the sediment was recorded and moisture content of the sediment gel was determined (Takashi and Sieb, 1988).

$$\text{Swelling power} = \frac{\text{Weight of the wet mass of sediment}}{\text{Weight of dry matter in the gel}} \quad (3)$$

Experimental design and statistical analysis

A three factor experimental set up was used with barrel temperature (X_1), screw speed (X_2) and moisture levels (X_3) as the independent factors at three levels each. The data obtained were analyzed by response surface methodology (RSM) based on Box Behnken design (Table 1) to optimize process variables. Seventeen combinations including five replicates of the centre point was performed in random order according to the design. A second order polynomial model for the dependent variables as shown in Eq. (4) was established to fit the experimental data. An ANOVA test was carried out using design expert version 8.0.4 to determine level of significance at 5%.

Table 1: Coded and Uncoded Levels for the Response Surface Design

	Levels		
Variables	-1	0	+1
Screw speed (rpm, X_1)	100	120	140
Temperature (°C, X_2)	110	120	130
Moisture level (%, X_3)	44	47	50

X_1 – Screw speed, X_2 – Barrel temperature, X_3 – Feed moisture

$$Y = \beta_o + \Sigma^3_{i=1} \beta_i X_i + \Sigma^3_{i=1} \beta_{ii} X_i^2 + \Sigma\Sigma^3_{i<j=1} \beta_{ij} X_i X_j + \varepsilon \quad (4)$$

where Y is the response, β_o is a constant while β_i, β_{ii} and β_{ij} are linear, quadratic and interaction coefficients; and β_i is error.

Optimization procedures

Numerical and graphical optimization procedures were applied to determine the optimum level of the three independent variables (barrel temperature, screw speed and feed moisture levels) investigated in this work using design expert version 8.0.4. Based on desirability concept whose value must be close to 1, process conditions were varied to obtain meat analogue with the maximum lateral expansion, WAI, OAI, swelling power and minimum bulk density. However, the lowest possible level of moisture content is expected for increased shelf stability. A meat analogue is expected to supply almost similar level of nutrient obtainable from traditional animal meat sources.

Result and Discussion

Proximate composition result of the mucuna bean flour is as shown in Table 2. This result was observed to be similar with the findings of Tuleum *et al.* (2008) and Ezeagu *et al.* (2003). The crude protein content, with the mean value of 31.29 %, makes this variety of mucuna beans superior to cowpea (23.7 %), groundnut (24.7 %) and pigeon pea (26.3 %) but inferior to soybeans (38.7 %) (FAO, 1994). MBF therefore has potential as a protein supplement for low-protein foods and feeds such as cereal grains, a view also held by Ezeagu et al. (2003). The moisture content of the flour (9.68 %) is low enough to prolong its shelf-life. In dry food system, moisture content of between 6-10 % has been established to prolong the shelf-life of foods, beyond which the storability of the system could be impeded by chemical and microbiological agents (Harper and Jansen, 1985). The low fat content observed in this legume allows it to be suitable for use in low cholesterol food formulations while the fairly low crude fibre content of the flour is an advantage in terms of digestibility.

Table 2: Proximate Composition of Mucuna Bean Flour

Parameters	Flour
Moisture (%)	9.68 ± 0.10
Crude Protein (%)	31.29 ± 0.39
Available Carbohydrate (%)*	50.22 ± 0.07
Ash (%)	3.25 ± 0.00
Crude Fibre (%)	1.98 ± 0.00
Fat	3.58 ± 0.40
pH	6.25 ± 0.71

Values are means of duplicate ± standard deviation

* Obtained by difference

Moisture content of samples is presumed as one of the most important determination of shelf stability. Values between 6.53 – 11.93 % were observed for the extruded meat analogue samples. This is within a range that could prevent microbial activity that enhances spoilage and also ensure shelf stability of the extrudates. High moisture products usually have shorter shelf-life stability compared to lower moisture products (Ashworth and Draper, 1992). From Fig. 1, the feed moisture content decreases as barrel temperature and screw speed increase but increases expectedly as feed moisture increases. Extrusion cooking aided by high temperature and screw speed induces structural changes in food proteins in the form of shrinkage that causes a pressure-driven flow of water out of the extrudates which resulted in a reduction in water holding capacity. Feed moisture content was observed to significantly ($p < 0.05$) affect the final moisture content of the extrudates. This is similar to the findings of Asare *et al.* (2010), despite the fortification with legumes; and also to the findings of Sobukola *et al.* (2012).

Functional properties of extrudates

The lateral expansion (Y_1), bulk density (Y_2), water absorption index (Y_3), oil absorption index (Y_4) and swelling power (Y_5) of the extruded meat analogue ranged between 0.084 and 0.139; 0.832 and 0.988; 1.677 and 2.320; 1.761 and 2.389; and 0.740 and 3.470, respectively (Table not shown). The statistical results of the response data obtained using multiple linear regression equation (Equation 1) are displayed in Table 4 and varied between 0.658 and 0.894, with Y_3 having the highest value and Y_2 with the lowest.

Table 3: The Coefficients of Regression of Equation (1) with Respect to Process Variables for the Functional Properties of the Meat Analogue

Coefficients	LE	BD	W.A.I	O.A.I	SP
X_o	0.11	0.91	2.07	1.92	0.97
X_1	-0.003	0.031	-0.19*	-0.14*	-0.25
X_2	0.013*	0.004	-0.078*	0.022	0.099
X_3	-0.008	0.013	0.031	0.07	0.32
X_1X_2	-0.004	0.021	0.008	0.093	0.22
X_1X_3	-0.01	0.023	-0.062	-0.038	0.17
X_2X_3	-0.002	-0.016	0.011	-0.19	0.52
X_{12}	0.012	-0.02	-0.086	-0.04	-0.21
X_{22}	-0.012	-0.022	-0.11*	0.13	0.68*
X_{32}	0.001	0.042	0.047	0.12	0.23
R_2	0.766	0.658	0.894	0.846	0.7
F-value	2.54	1.5	6.57	4.29	1.81
P-value	0.116	0.304	0.011	0.034	0.222

*Significant values at 5 % level

X_1- Screw speed, X_2- Barrel temperature, X_3- Feed moisture, LE- Lateral expansion, BD- Bulk density, W.A.I- Water absorption index, S.P- Swelling power, O.A.I- Oil absorption index

Product expansion ratio, an index of degree of puffing, is one of the important physical characteristics of extrudate. Barrel temperature had a significant ($p < 0.05$) effect on the lateral expansion of the meat analogue (Table 4). From the response surface graph (Fig. 2) there was an appreciable increase in lateral expansion as barrel temperature increases while increase in screw speed did not give a remarkable increase at constant moisture content (47%). From the response surface plot, the highest and lowest values of Y_1 were observed to be 0.122 and 0.092, respectively. According to Bhattacharya (1997), high input of thermal energy due to high residence time leads to the creation of enhanced level of superheated steam; hence the product will have good expansion which creates flashy and porous structures due to formation of air cells. When extrusion cooked melt exits the die, they suddenly go from high pressure to atmospheric pressure. This pressure drop causes a flash-off of internal moisture and the water vapour pressure, which is nucleated to form bubbles in the molten extrudate and allows the expansion of the melt (Arhaliass *et al.*, 2003). The goodness of fit of the mathematical model was checked by the determination coefficient (R^2). In this case, the value of the R^2 (76.6 %) for Eq. (1) indicated that the sample variation of 76.6 % for lateral expansion was attributed to the independent variables and that 23.4 % of the total variation could not be explained by the model.

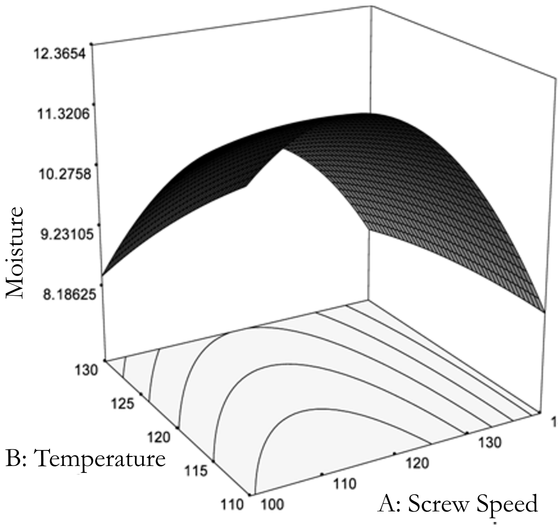

Fig. 1: **Response surface plot of moisture content (%) of the extruded meat analogue from mucuna bean flour as a function of screw speed and temperature at constant moisture content (47 %)**

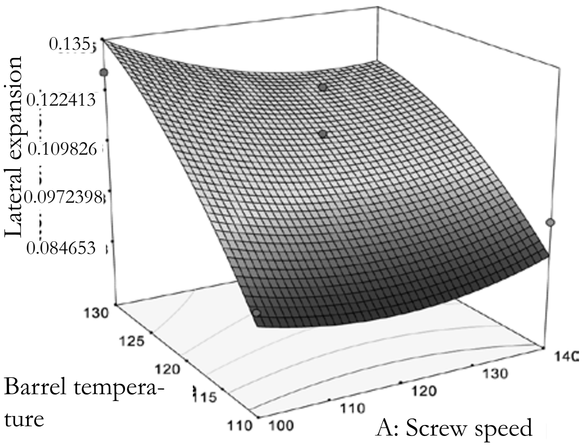

Fig. 2: **Response surface plot of lateral expansion of the extruded meat analogue from mucuna bean flour as a function of screw speed and temperature at constant moisture content (47 %)**

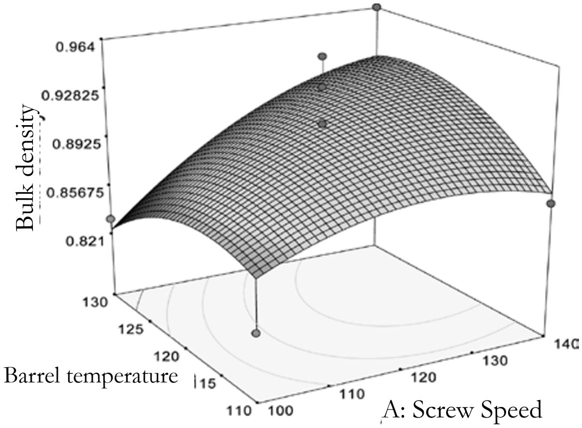

Fig. 3: **Response surface plot of bulk density of the extruded meat analogue from mucuna bean flour as a function of screw speed and temperature at constant moisture content (47 %)**

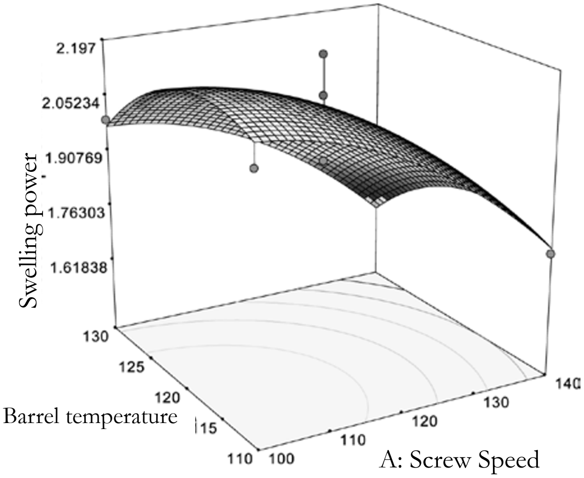

Fig. 4: **Response surface plot of water absorption index of the extruded meat analogue from mucuna bean flour as a function of screw speed and temperature at constant moisture content (47 %)**

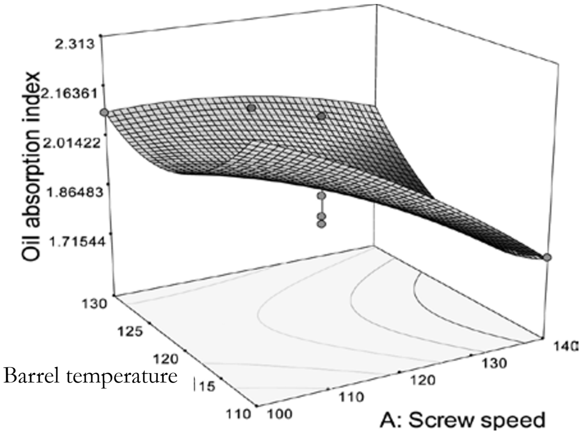

Fig. 5: **Response surface plot of oil absorption index of the extruded meat analogue from mucuna bean flour as a function of screw speed and temperature at constant moisture content (47 %)**

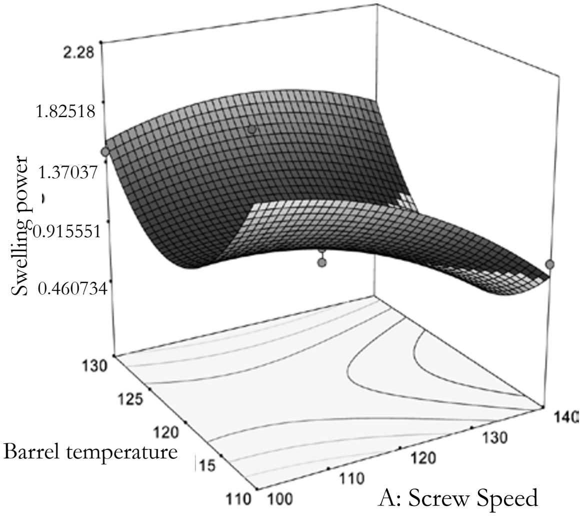

Fig. 6: **Response surface plot of swelling power of the extruded meat analogue from mucuna bean flour as a function of screw speed and temperature at constant moisture content (47 %)**

Bulk density considers expansion of extrudate in all directions. In this study, none of the process independent variables had any significant ($p < 0.05$) effect on the bulk density. It was observed that an increase in screw speed resulted in an extrudate with higher density while an increase in barrel temperature gave an extrudate of lower density (Fig. 3). According to Fletcher *et al.* (1985), an increase in the barrel temperature will increase the degree of superheating of water in the extruder and encourages bubble formation and also a decrease in melt viscosity, leading to reduced density, as observed in this work. Temperature and feed moisture have been found to be the main factors affecting extrudate density and lateral expansion (Ilo *et al.*, 1999). The effects of temperature and screw speed were found to be dependent on each other. The increase in bulk density as screw speed increases might be due to intensified effect of temperature on extrudate melt under increased shear environment (high screw speed) which may increase the extent of gelatinization process and so gave higher extrudate, which is also in agreement with the report of Li *et al.* (2005). The model showed that it had the lowest coefficient of determination ($R^2 = 0.658$), which means that a high proportion of the variability was not explained by the model with a non-significant lack of fit value of 2.54.

WAI has been generally attributed to the dispersion of starch in excess water, and the dispersion is increased by the degree of starch damage due to gelatinization and extrusion induced fragmentation, it measures the degree of volume occupied by the starch granule after swelling in excess water (Altan *et al.*, 2008). Gelatinization, the conversion of raw starch to a cooked and digestible material by the application of water and heat, is one of the important effects that extrusion has on the starch components of foods (Ding *et al.*, 2006). WAI of extrudate, which relate to product juiciness or moistness upon hydration, decreases with increase in temperature and screw speed at constant moisture content (Fig. 4). These factors exerted a significant negative effect on water absorption index (Table 4). So many studies have shown that WAI increases at a higher extrusion temperature whereas it decreases with increasing screw speed. This statement correlate with the findings of Anderson *et al.* (1969) on cereals, but it is however contrary to the findings of this study. It is possible however in this study that WAI decreased with increase in temperature if dextrinization or starch melting prevails over the gelatinization phenomenon (Ding *et al.*, 2006). A decrease in WAI with increasing temperature was probably due to decomposition or degradation of starch (Pelembe *et al.*, 2002). In this study, the effect of processing variables on water absorption index presented in Table 4 shows that the coefficient of determination for the model equation was calculated to be 89.4 %, which means that the R^2 value was considered sufficiently accurate for prediction purposes.

Oil absorption index is the ability of a product to entrap oil and this is known to improve flavor and increase mouth feel of a food material (Eke and Akobundu, 1993). In this study, oil absorption index of the extrudates increases with increase in barrel temperature and decreases with increase in screw speed (Fig. 5). The increase in oil absorption might be attributed to high level of starch degradation in the extrudate as a result of high input of thermal energy. Increase in the oil absorption of the extrudates could be of advantage if the product is to be used in the preparation of soup or sauce. Meat analogue is expected to imitate meat from animal protein source in its functional characteristics. The quadratic model developed for oil absorption index can explain 84.6 % of the variation in the data with a non-significant lack of fit of 4.29.

Swelling power is the ability of the starch in the meat analogue to absorb water such that the starch granules increase in size, which indicates the degree of exposure of the internal structure/ matrix of the granules to action of water (Raules *et al.*, 1993). From the response surface plot (Fig. 6), swelling power capacity of the extrudates increases with increase in barrel temperature and decreases with increase in screw speed. From Table 4, the coefficient of determination R^2 for the model equation was calculated to be 70%, which means that the R^2 value was considered sufficiently accurate for prediction purposes.

Conclusion

Flour from mucuna bean seeds was successfully used to develop extruded meat analogue at different barrel temperature, screw speed and feed moisture content. Proximate composition of the mucuna bean flour (MBF) revealed that the flour is high in protein content and low in fat. The moisture content and the functional properties of the developed product were mostly affected by barrel temperature and moisture level and to a lesser extent by screw speed. However, the optimization results based on desirability concept indicated that a barrel temperature of 120.15°C, feed moisture of 47% and screw speed of 119.19 rpm would produce meat analogue of preferable functional properties. This work has shown the potential of Mucuna pruriens in food formulation as well as the development of acceptable products from neglected agricultural crops.

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