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# EMPIRICAL MODELS AND PROCESS OPTIMIZATION FOR PREDICTION OF NUTRITIONAL PARAMETERS OF STORED COWPEA VARIETY (IT96D-610K)

Musliu Olushola SUNMONU[1]*, Michael Mayokun ODEWOLE[1], Ibukunolowa Omotola OYELEKE[1], Grace Olufunke OTITODUN[2], Mobolaji OMOBOWALE[3], Moses Odunayo OGUNDARE[2]

[1]University of Ilorin, Kwara State, Nigeria
[2]Nigerian Stored Products Research Institute, Ilorin, Kwara State, Nigeria
[3]University of Ibadan, Oyo State, Nigeria

This paper presents a study carried out to develop empirical models and process optimization for prediction of nutritional parameters of stored cowpea variety (IT96D-610K). Twelve small scale prototype silos made of two different materials (wooden and galvanised mild steel) were constructed and used in the storage of the cowpea for a 4-month period. Seven kilograms of cowpea at 9.88% moisture content admixed with DE having two different particle sizes ($7.5 \times 10^{-5}$ m and $9 \times 10^{-5}$ m) and three different concentrations (0.0001 kg, 0.00005 kg and 0 kg) in varying combinations were loaded into each prototype silo structure. The control (zero/no concentration) was set without the use of DE in each of the wooden and galvanised mild steel structures, respectively. Temperature, relative humidity and moisture content within the storage structures were monitored. Nutritional parameters such as ash, crude protein, fat, crude fibre, and carbohydrate content were also measured alongside moisture. Significant differences ($P < 0.05$) were observed between the control sample and treated samples. Six model equations using Essential Regression Software package were further generated to determine the relationship between input and output parameters, and were checked for adequacy and validity. The model equations developed were used to get the optimum values of output parameters which are: minimum moisture content (8.87%), minimum ash content (4.07%), maximum crude protein (22.86%), maximum fat (2.04%), maximum crude fibre (2.26%) and maximum carbohydrate (60.31%) of the stored cowpea at various conditions. Study results show that all the storage conditions had significant effects at $P < 0.05$.

**Keywords:** cowpea; diatomaceous earth; storage; nutritional composition; silos

Global production of cowpeas in 2010 was 5.5 million metric tons and Africa is responsible for 94% of this value (CGIAR, 2015). Over 200 million people in Africa depend on cowpea as a source of food (Chege, 2004) mainly because the crop plays a significant role in the diet of many homes in Nigeria and other developing countries in Africa. Protein in cowpea seed is rich in amino acids, lysine and tryptophan compared to cereal grains (Davis et al., 1991). According to Jenkins (2000), cowpea grains are also a rich source of minerals. Cowpeas are low in fat and high in fibre content, respectively. Cowpea weevil is one of the major pests of stored cowpea. It thrives in a warm environment, where it requires a short time for reproduction. Due to this favourable environment, a freshly threshed store of cowpeas with only a small start-up weevil infestation can be rendered inedible and worthless in the market between two or three months (Baritbusa et al., 2010). The use of insecticides and chemicals in cowpea storage by farmers have posed serious health risks, as there is a lack of information on proper mixing amount of these substances. Development in treatment of grains with diatomaceous earth (DE), a naturally occurring organic compound with little mammalian harmfulness, has been an interesting and ongoing field of research in storage of grains. This compound has been found to replace the use of chemicals posing stark environmental and health hazards on human and animals when ingested (Faulde et al., 2006; Stadler et al., 2012). It is used as filtration media for various beverages, inorganic and organic chemicals, as well as an adsorbent for oil spills. Although the diatomite has a unique combination of physical and chemical properties, its use as an adsorbent in wastewater treatment has not been significantly investigated (Michell and Atkinson, 1991; Aytas et al., 1999).

The type of DE used in storing grains is food grade. Modelling is a low-cost means of examining the way in which a system operates and of predicting future behaviour. It can be used to: analyse the proposed changes or additions to the system; examine the system over time; test hypotheses and compare different scenarios; identify constraints on the efficient operation of the system; identify and diagnose problems and develop an understanding about the system (NIST, 2013). Hence, the aim of this paper is to develop empirical models and performance of process optimization to predict the nutritional parameters of stored cowpea variety (IT96D-610K).

**Contact address:** Musliu Olushola Sunmonu, University of Ilorin, Faculty of Engineering and Technology, Department of Food Engineering, P.M.B. 1515, Ilorin, Kwara State, Nigeria, e-mail: sholams2000@yahoo.co.uk

## Material and methods

### Sample collection of cowpea seeds

Cowpea seed (*Vigna unguiculata*) variety IT96D-610K in Fig. 1 used for the storage experiment was purchased from International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State.

### Sample collection of diatomaceous earth

Crude DE of fresh water origin as shown in Fig. 2 was obtained from Bularafa community in Yobe State, Northern Nigeria. Fig. 2 shows the crude Bularafa DE. It was oven-dried at 40 °C to 4.5% moisture content (Arnaud et al., 2005) and ground to dust by means of a laboratory mortar and pestle, sieved using US standard 200 (0.075mm openings) and 170 sieves (0.090 mm) and kept in airtight ziploc bags prior to being admixed with the cowpea seeds.

### Experimental design and arrangement

A 2 × 3 × 2 experiment in Completely Randomised Design was used in this research work. The factors taken into consideration were storage structure (M); particle size of DE (*P*); concentration of DE (C). The storage structures investigated were wooden (M1) and galvanised mild steel (M2). Particle size of DE were (P1) – if passed through $7.5 \times 10^{-5}$ m openings – and (P2) – if passed through $9 \times 10^{-5}$ m sieve openings – for US sieve No 200 and 170, respectively, after being oven-dried and ground to powder. Concentrations of DE used were (C1) as 0.0001 kg; (C2) as 0.00005 kg and (C3) as 0 kg, zero/no concentration. Each treatment combination was replicated five times, leading to the total number of 60 runs of the test trials.

Experiment took place between May 31, 2016 and September 19, 2016 for a period of four months (16 weeks). The experimental design and layout is presented in Table 1.

**Fig. 1** Clean cowpea seeds (IT96D-610K) diatomaceous variety

**Table 1** Experimental design and layout

| Concentration (C) (kg) | Storage materials (M) / Particle size (P) | | | |
|---|---|---|---|---|
| | M1 | | M2 | |
| | P1 (m) | P2 (m) | P1 (m) | P2 (m) |
| C1 | M1P1C1 | M1P2C1 | M2P1C1 | M2P2C1 |
| C2 | M1P1C2 | M1P2C2 | M2P1C2 | M2P2C2 |
| C3 | M1P1C3 | M1P2C3 | M2P1C3 | M2P2C3 |

Storage materials (M1 – wooden, M2 – galvanised mild steel)

### Storage procedure

Twelve prototype small scale storage structures were fabricated and used for storage. Six were made of wood and another six were made of galvanized mild steel (GMS). The total height of the structure was 0.48 m with the conical frustum measuring of 0.15 m total height. The internal silo diameter was 0.254 m with 0.6908 $m^2$ area of storage floor. The storage structure components included loading cover, storage chamber, offloading door and base stand. Admixed portion of the cowpea with DE with different concentrations and particle size was stored in each of the silos. DE concentration admixed with the cowpea seeds was expressed in the ratio 0.0001 kg/0.1 kg of cowpea seeds. Eighty four kilograms of clean cowpea seeds were measured with a weighing scale (Hana manufactured by Precision Hana Scales Pvt, India) and divided into twelve equal portions of 7 kg each. Nwabauni et al. (2014) reported that 0.0001 kg/0.1 kg of Bularafa DE was admixed with grains such as cowpea and maize to prevent insect infestation. Samples of 0.0001 kg and 0.00005 kg with DE concentration

**Fig. 2** Crude bularafa earth

were used in cowpea treatment. Crude DE was ground into fine powder and sieved using $7.5 \times 10^{-5}$ m and $9.0 \times 10^{-5}$ m sieves. The 0.021 kg of the ground DE was each obtained from the $7.5 \times 10^{-5}$ m and $9.0 \times 10^{-5}$ m sieves. The 0.007 kg of DE with particle size of $7.5 \times 10^{-5}$ m was admixed with each of two equal portions of the cowpea and stored in two different wooden and GMS prototype storage structures, respectively. The 0.0035 kg of DE with particle size opening of $7.5 \times 10^{-5}$ m was also admixed with two equal portions of the cowpea and stored in two different wooden and GMS structures, respectively. Another 0.007 kg of DE with particle size of $9.0 \times 10^{-5}$ m was admixed with each of two equal portions of the cowpea and stored in the storage structures made of wood and GMS, respectively. The 0.0035 kg of DE with particle size opening of $9.0 \times 10^{-5}$ m was admixed with each of two equal portions of the cowpea and stored in the storage structure made of wood and GMS, respectively. No DE was admixed with two equal portions of cowpea stored in two wooden structures respectively and no concentration of DE was also admixed with two equal portions of cowpea stored in two galvanized mild steel structures, respectively. Cowpea with no DE added served as a control for the experiment. Capacity of each storage structure was 12 kg.

### Moisture content

Moisture content of cowpea seeds was determined in the laboratory using the oven method. A clean crucible was first dried in oven for about 30 min and then cooled in a desiccator. Cooled crucible was weighed as (W1) using an analytical balance. Weighed quantity of 2 g of the cowpea seed variety was then introduced into the previously dried and weighed crucible and weighed as (W2) before drying. This was placed in an electric oven (Model 10GC Lab Oven) which was thermostically set at 105 °C for an interval of 24 h. After 24 h, the crucible with dried cowpea seeds was removed from the oven and immediately cooled in a desiccator and then weighed as (W3). Crucible with dried cowpea seeds was again placed in the oven to heat, cooled in desiccator and re-weighed. This procedure was repeated until constant weight was obtained (AOAC, 2002). Subsequently, moisture content represented by the losses in weight was calculated according to following formula and samples were analysed weekly:

$$\%\ moisture\ content = \frac{W2 - W3}{W2 - W1} \times 100 \qquad (1)$$

where:
W1 – weight of clean crucible, g
W2 – weight of clean crucible plus sample, g
W3 – weight of clean crucible plus dried sample, g
W2 – W3 – total loss in weight, g
W2 – W1 – weight of sample, g

These were determined in the laboratory using nutritional guidelines (AOAC, 2002). Nutritional values determined were crude protein, carbohydrate, fat, ash and crude fibre. Crude protein content was determined using the Kjedahl method to determine the total nitrogen content of the cowpea and subsequently multiplied by a constant factor to obtain the protein content. Ash was determined in the laboratory using the dry ashing method in a muffle furnace set at 550 °C. Fat was determined using the continuous solvent extraction method with petroleum ether as the extracting solvent. Crude fibre content was also determined according to aforementioned nutritional guidelines. Total amount of carbohydrates in the cowpea samples was determined by the difference method.

### Temperature and relative humidity

Temperature and relative humidity were checked and monitored by using Hobo data logger sensors placed inside the prototypes structures and was set throughout the period of the experiment. A moisture meter (EMC grain probe IL 1-800-284-5779) recording moisture content, temperature and relative humidity was also used for measuring whenever sampling of the cowpea seeds was done for proximate analysis. Indoor temperature of the storage room was also recorded by using a Pro V2 Weather temperature data logger (Onset Hobo – U23-003). Hobo Pro V2 External Temperature/ Relative Humidity Data Logger is a weatherproof data logger with an external temperature and relative humidity sensor. It has accuracy of ±0.21 °C from 0° to 50 °C and is produced by ONSET Company in the United States.

### Statistical analysis

Data obtained from the experiments were subjected to a statistical analysis of variance (ANOVA) using SPSS 16.0. (SPSS for Windows, version 16.0. produced in Chicago, United States by SPSS Inc.) Essential Regression Software Package in Math-lab format was used to generate model equations. Furthermore, the means were compared using Duncan's New Multiple Range Test (DNMRT). *T*-test was also carried out to compare the observed and predicted values, as well as numerical and graphical approaches were used to check the adequacy and validity of all model equations.

### Modelling and Optimization Techniques and Model Verification and Validation

The data obtained for moisture content, crude protein, carbohydrate, ash, crude fibre and fat were modelled using the STATA computer software (ver. 11, produced by Statacorp LP: Stata Statistical software, college station, Texas, USA) and from the regression analysis carried out, the best performing functional models were selected – one for each measured parameter.

All the models generated were verified and validated using graphical method. The graphs of Expected Normal values (rankit) versus Residual; and Residual versus predicted values for all output (dependent) parameters were plotted and compared in order to achieve this purpose.

## Results and discussion

From the regression analysis carried out, the best performing functional models (Eq. (2) to Eq. (7)) were selected, one for each measured parameter.

$$MC = 20.22 + 0.356M\tau - 0.259MT - 0.01338\tau^2 - 0.01469T^2 - 10.28M + 0.865T, \ (R^2_{adj} = 96.6\%) \qquad (2)$$

$$Ash = 4.023 + 0.08794C + 0.00609T - 0.000251MT - 0.01383C^2 - 0 - 0.00256TC + 0.000175\tau T, (R^2_{adj} = 90.7\%) \quad (3)$$

$$CP = 22.94 - 0.000402T^2 + 0.00392Tc - 0.000504\tau T - 0.143PC + 0.01854C^2 - 0.07398C, (R^2_{adj} = 88.1\%) \quad (4)$$

$$F = 2.067 - 0.000569T^2 + 0.00383Tc + 0.00383\tau T - 0.00136C - 0.220\tau + 0.01105T + 0.02900\tau C, (R^2_{adj} = 79.8\%) \quad (5)$$

$$CF = 2.126 + 0.07667C + 0.00577T - 0.380MP + 0.00109M\tau + 5.403P^2 - 0.00963C - 0.00129\tau C - 0.00169TC, (R^2_{adj} = 75.6\%) \quad (6)$$

$$CAB = 49.62 - 0.362M\tau + 0.00741\tau T + 0.261MT + 0.01205\tau^2 + 0.01645T^2 + 10.42M - 1.078T, (R^2_{adj} = 96.3\%) \quad (7)$$

where:

*T* – storage period
*M* – storage structure
τ – temperature, °C
*C* – concentration of DE
*P* – particle size of DE
*MC* – moisture content
*CP* – crude protein
*F* – fat
*CF* – crude fibre
*CAB* – carbohydrate

## Model adequacy checking

In this section, the adequacy of models 2 through 7 was checked using regression statistics and graphical method. This ensured that these models are valid for predicting the nutritional qualities of the stored cowpea when used in similar experiments. Gosukunda et al. (2017) also predicted thermal properties of sorghum bagasse using regression models.

From Table 2, it can be inferred that the adjusted coefficient of multiple determination which defined the percentage of total variability explained by the model was 96.6%, 90.7%, 88.1%, 79.8%, 75.6% and 96.3% for moisture content, ash, crude protein, fat, crude fibre and carbohydrate, respectively. These high percentages of total variability explained by the models imply good fits. Adjusted coefficient of multiple determinations is preferred over the coefficient of determination because it takes into account the number of process parameters (degrees of freedom) in the model.

One argument in support of model adequacy was the closeness between *R*-square adjusted, *R*-square for prediction and *R*-square itself (David et al., 1998). Table 2 shows all these regression statistics to be close in range and this is what is expected from good models. David et al. (1998) also argued that models with minimum prediction error sums of squares (PRESS) and coefficient of variations imply good fits. Values of these two statistics were minimal compared to other models generated during the regression analysis.

Reliability of the model was also examined using the analysis of variance (ANOVA). Analysis of variance tests for all models were significant at 5% level.

## Model validations

Developed models were verified and validated to ensure that they meet intended requirements in terms of the methods employed and the results obtained (Macal, 2005). Therefore, obtained models were validated using the graphical method. In this method, plots of residuals from fitted model provide information on the validity of the model developed (David et al., 1998).

Fig. 3 shows a typical normal probability plot of the expected normal values versus the residuals. The plots in Fig. 3 examine the error structure to ensure that the residuals behave as expected, i.e., if the errors are distributed normally. The graphs clearly showed that the residual plots are not heavy tailed, i.e. spread about the straight line. For all data observed, residuals rather fell on straight line. This implies that assumption of normality of the residuals was not violated. Assumption of constant error variance was also checked using the plots of residuals vs predicted responses. Plots in Fig. 4 show bands around 0 with constant width, implying that the error variance was stable with respect to predicted responses for all observed parameters. This conclusion applies to Figs. 3–14. Therefore, it can be concluded that the models developed are valid and can be used for the purpose of predicting the nutritional qualities of stored cowpea under same condition as demonstrated below.

**Table 2** Multiple regression statistics on the nutritional parameters of stored cowpea

| Regression statistics | Moisture content (%) | Ash content (%) | Crude protein (%) | Fats & oil (%) | Crude fat (%) | Carb. (%) |
|---|---|---|---|---|---|---|
| **\|R\|** | 0.983 | 0.954 | 0.940 | 0.897 | 0.875 | 0.982 |
| **$R^2$** | 0.967 | 0.910 | 0.884 | 0.805 | 0.766 | 0.964 |
| **$R^2$ adjusted** | 0.966 | 0.907 | 0.881 | 0.798 | 0.756 | 0.963 |
| **Standard error** | 0.403 | 0.010 | 0.023 | 0.022 | 0.009 | 0.417 |
| **PRESS** | 35.068 | 0.020 | 0.113 | 0.111 | 0.018 | 38.038 |
| **$R^2$ for prediction** | 0.964 | 0.902 | 0.875 | 0.777 | 0.740 | 0.960 |
| **Durbin-Watson d** | 1.402 | 2.178 | 1.559 | 1.560 | 1.814 | 1.389 |
| **Coefficient of variation** | 3.314 | 0.236 | 0.101 | 1.133 | 0.407 | 0.735 |

$R^2$ – coefficient of determination; $R^2$ adjusted – coefficient of multiple determination; PRESS – prediction error sums of squares

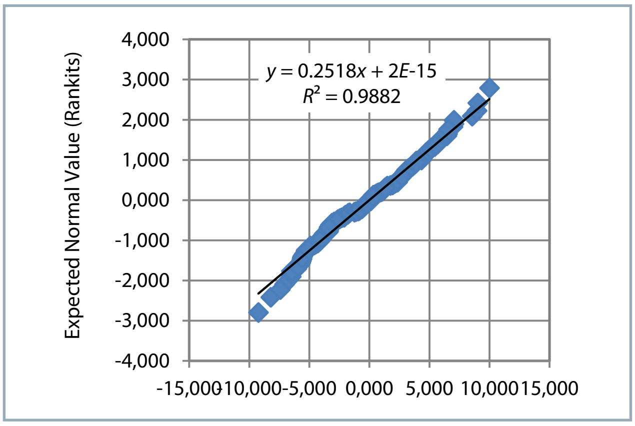


**Fig. 3** Residuals for moisture content

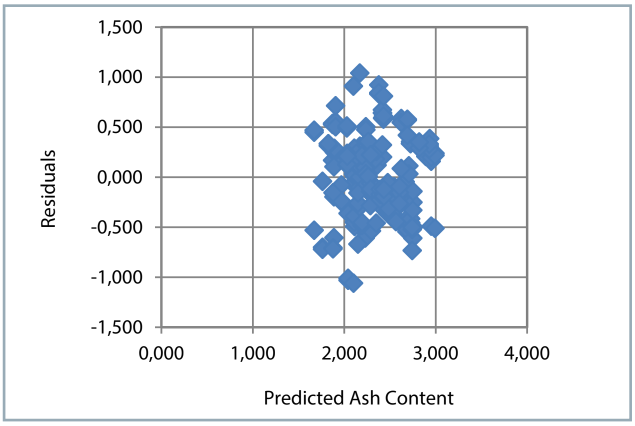


**Fig. 6** Expected normal value for ash

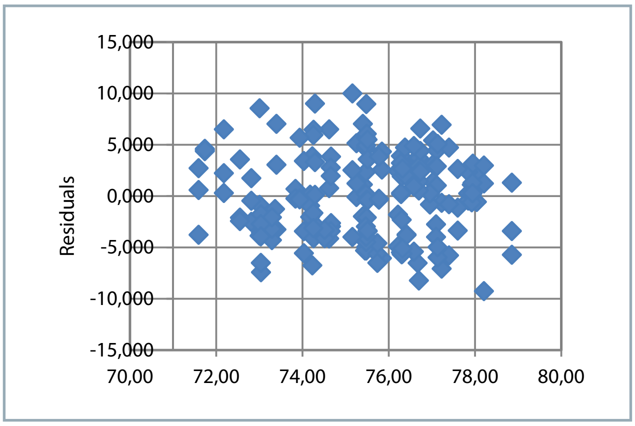


**Fig. 4** Expected normal value for moisture content

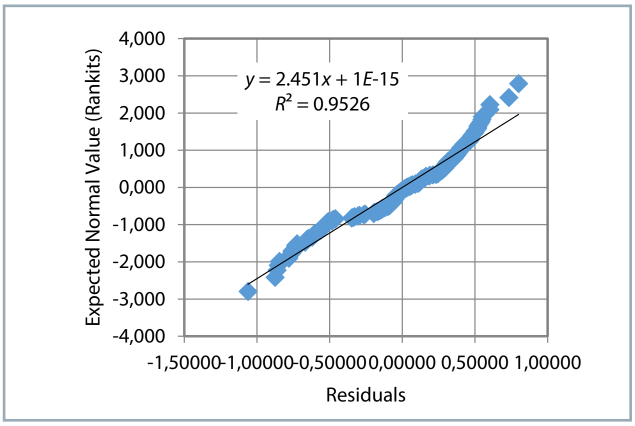


**Fig. 7** Residuals for crude protein

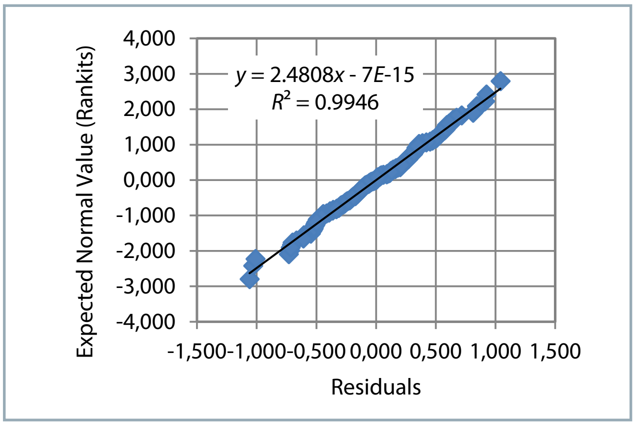


**Fig. 5** Residuals for ash

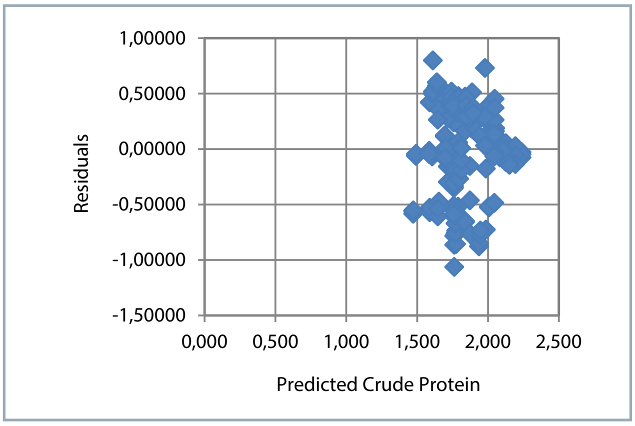


**Fig. 8** Expected normal value for crude protein

### Optimized value of process conditions and output

Optimization means finding the optimum (maximum or minimum) value of process parameters in mathematical model that would most adequately give the desired output or response value. Optimization was done for all models developed in order to find the optimum values of process conditions, namely: storage period, storage structure, particle size, and concentration and minimum values possible as presented in Table 3; while Figs. 15–20 illustrate the results obtained from Table 3. Optimized values of process conditions and outputs are shown in Table 3. Desirable optimum conditions for this study are: minimum moisture (%), minimum ash (%), maximum crude protein (%), maximum fat (%), maximum crude fibre (%) and maximum carbohydrate (%) content. The results in Table 3 show that the optimum (minimum) value of moisture content was achieved by storing cowpea in galvanized mild steel storage structure at the first week of storage at an ambient temperature of 22.3 °C. The concentration and particle size of DE did not have any significant impact, as shown by the * sign. This combination gave a maximum value of 8.87%.

The optimum (minimum) value of ash content was achieved by storing cowpea in galvanized mild steel structures treated with 0 kg/0.1 kg of DE (no

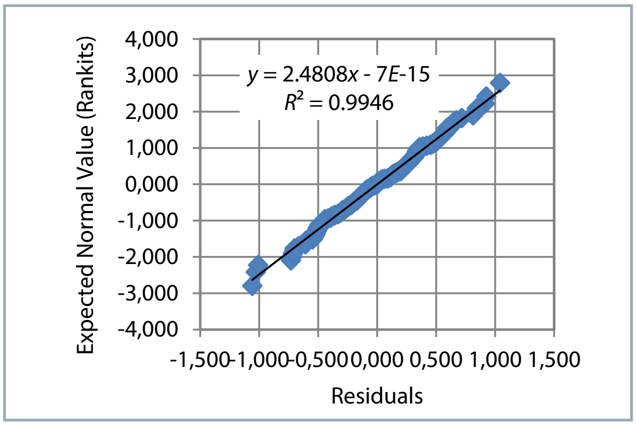


**Fig. 9** Residuals for fat

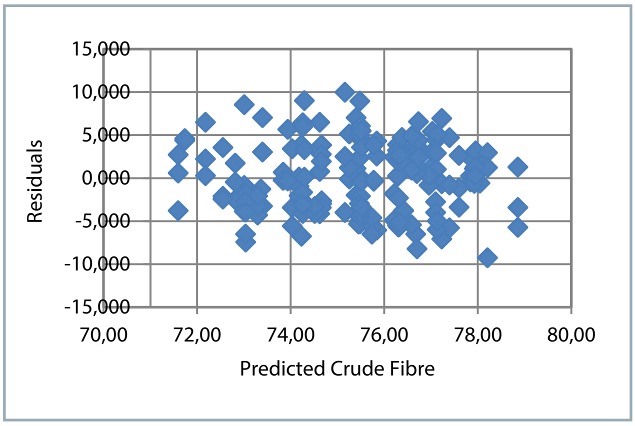


**Fig. 12** Expected normal value for crude fibre

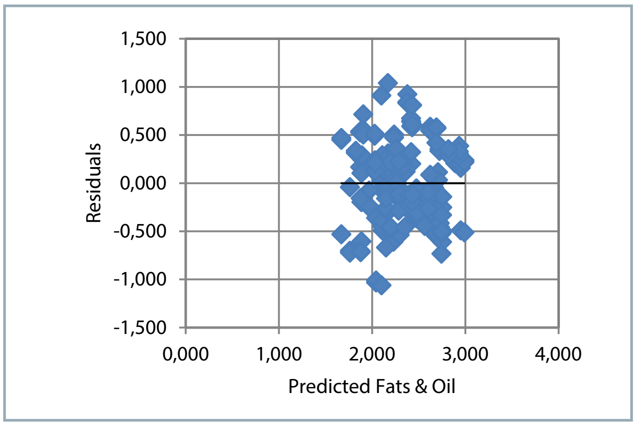


**Fig. 10** Expected normal value for fat

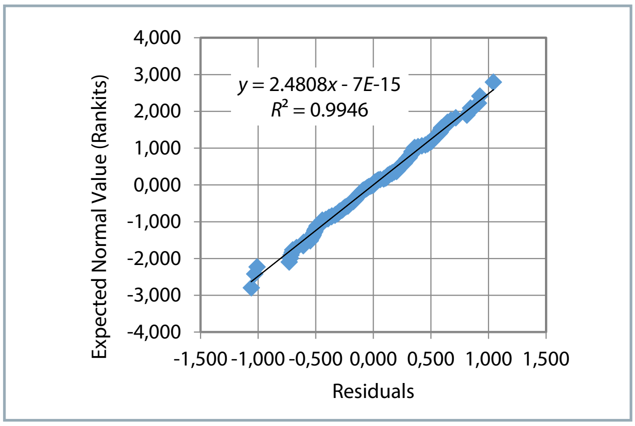


**Fig. 13** Residuals for carbohydrates

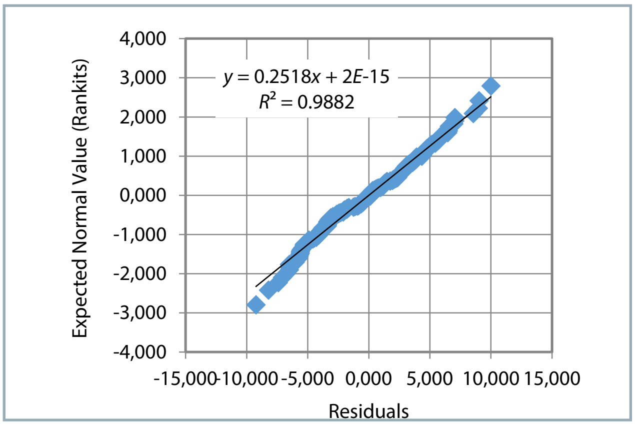


**Fig. 11** Residuals for crude fibre

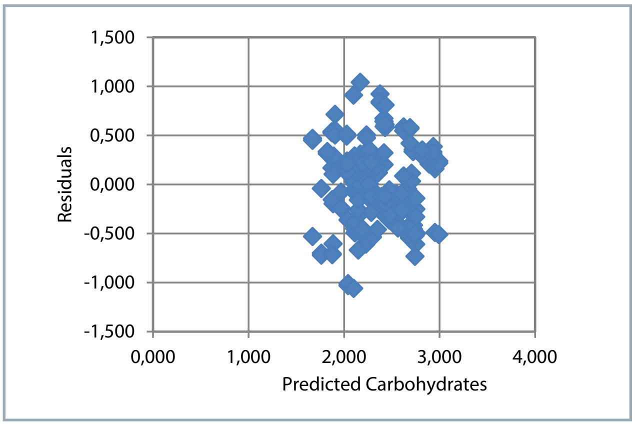


**Fig. 14** Expected normal value for carbohydrates

concentration) and stored for only one week at a stable ambient temperature of 28.8 °C. This combination yielded a maximum of 4.07%.

Furthermore, the optimum (maximum) value crude protein of stored cowpea was achieved in wooden storage structure at a short storage period of approximately one week. A 0 kg/0.1 kg (no concentration) of DE is required and a minimal temperature of 22.3 °C is adequate in storage. This combination yielded a maximum value of 22.86%.

Similarly, the maximum value of fat was achieved at first week of storage using the 0 kg/0.1 kg (no concentration) of DE (control) at an ambient temperature of about 28.8 °C. The storage structure and particle size of DE did not matter much as shown by the * sign. This yielded a maximum value of 2.04%.

For higher crude fibre however, the optimum (maximum) value was achieved by storing cowpea in wooden structures treated with 0. 0001 kg/0.1 kg of DE having particle size of $9.0 \times 10^{-5}$ m stored for sixteen weeks. A minimal ambient temperature of about 22.3 °C will yield an optimum value of crude fibre of about 2.26%.

Finally, the optimum (maximum) carbohydrates value of 60.31% was achieved for cowpea stored in wooden structures for a period of one week and with an ambient

**Table 3** Optimized values of process parameters and output

| Parameters | C | M | P | T | Tc | Optimized value | Nature of solution |
|---|---|---|---|---|---|---|---|
| **Moisture content** | * | 1 | * | 16 | 22.3 | 17.979 | maximized |
| | * | 2 | * | 0 | 22.3 | 8.875 | minimized |
| **Ash** | 1 | 1 | * | 16 | 28.8 | 4.20 | maximized |
| | 3 | 2 | * | 0 | 28.8 | 4.073 | minimized |
| **Crude protein** | 3 | 1 | 1 | 1 | 22.3 | 22.86 | maximized |
| | 1 | * | 2 | 16 | 28.8 | 22.60 | minimized |
| **Fat** | 3 | * | * | 1 | 28.8 | 2.04 | maximized |
| | 1 | * | * | 16 | 28.8 | 1.789 | minimized |
| **Crude fibre** | 1 | 1 | 2 | 16 | 22.3 | 2.256 | maximized |
| | 3 | 1 | 1 | 0 | 28.8 | 2.187 | minimized |
| **Carbohydrates** | * | 2 | * | 0 | 22.3 | 60.306 | maximized |
| | * | 1 | * | 16 | 22.3 | 51.734 | minimized |

C – concentration (1–0.0001 kg/0.1 kg; 2–0.00005 kg/0.1 kg; 3–0 kg/0.1 kg (no DE added)); M – storage structure (1 – wooden; 2 – GMS); P – particle size (1–7 × $10^{-5}$ m; 2–9 × $10^{-5}$ m); T – storage period (0 – initial; 1, 2, 3…16 weeks); Tc – temperature (random variable); * – it did not significantly influences the level of process parameter used as long as it is within the experimental level used for this study

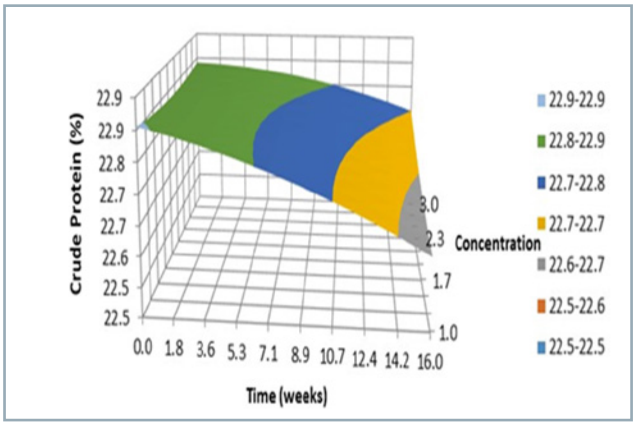


**Fig. 15** Optimized value of crude protein content

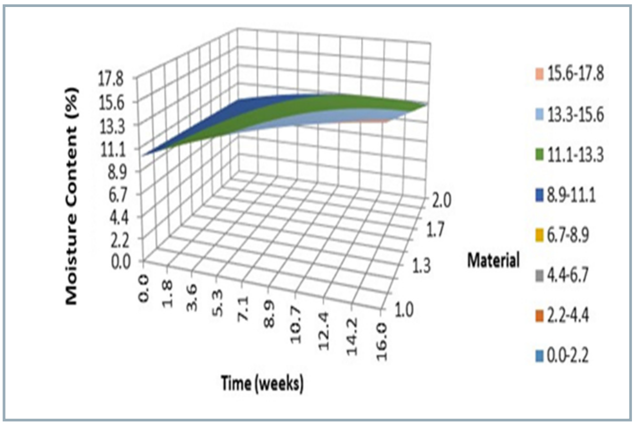


**Fig. 17** Optimized value of moisture content

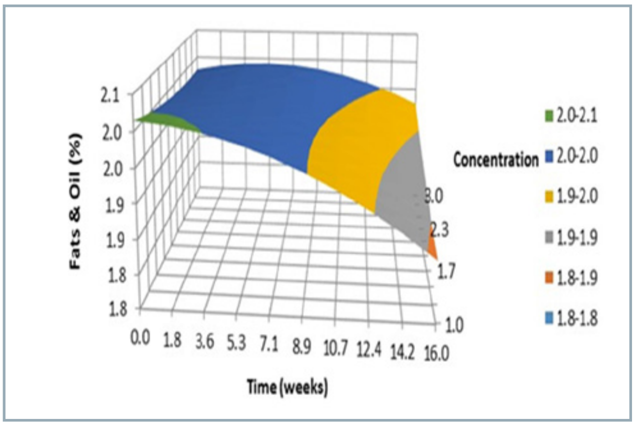


**Fig. 16** Optimized value of fat content

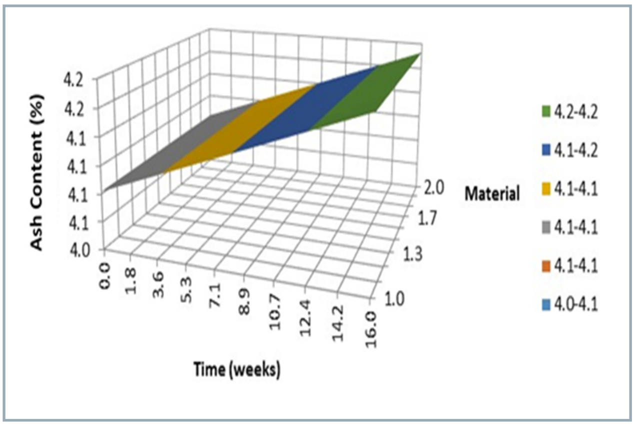


**Fig. 18** Optimized value of ash content

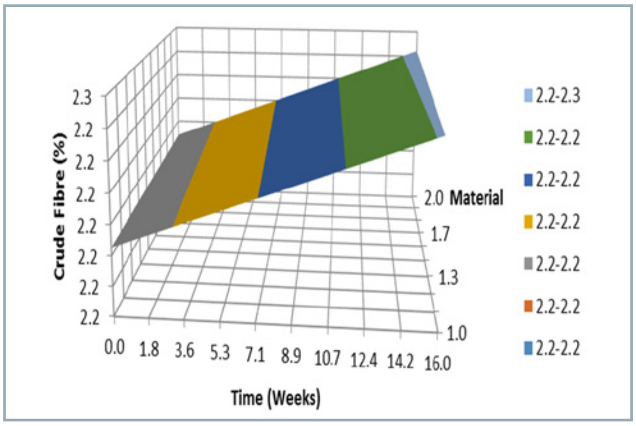


**Fig. 19** Optimized value of crude fibre content

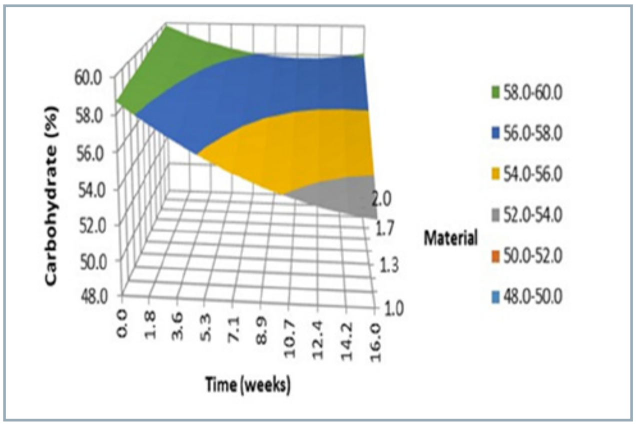


**Fig. 20** Optimized value of carbohydrates content

temperature of 22.3 °C. The concentration and particle size of DE did not matter much as shown by the * sign.

### Optimized values of process parameters

The graphs in Figs. 15–20 corroborate the optimized value for each process parameter. Colours have a legend of the number range at the graph right-hand corner. The legend number range in the table of optimized values shows the colour level to which the process output is optimized and at what level of process parameters. An example is in Fig. 15, in which the optimized value of crude protein was 22.86%, this was displayed in the green area of the graph and the legend for this shows the range 22.8–22.9.

## Conclusion

Model equations developed in the study showed functional relationship between the process conditions and output parameters and were adequately developed. The equations were checked for adequacy and validity and hence are suitable for prediction, estimation and optimization of cowpea storage. Optimum values of output parameters were determined with respect to process conditions using Eq. (2) to Eq. (7). The minimum moisture content, minimum ash content, maximum crude protein, maximum fats and oil, maximum crude fibre and maximum carbohydrate of 8.88%, 4.07%, 22.86%, 2.04%, 2.26% and 60.31% were respectively achieved at specific levels of process conditions for the stored cowpea.

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