OPTIMIZATION OF OYSTER MUSHROOM YIELD BY USING SIMPLEX-CENTROID MIXTURE DESIGN

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ABSTRACT

Despite the increased recognition of the nutritional value of the Oyster mushroom, coupled with its ability to tolerate a wide range of climatic conditions, its production is still at infancy stage with low adoption rate in Kenya. The low uptake could be attributed to lack of skills for substrates preparations or cost of buying the substrates coupled with poor knowledge on its consumption benefits. The objective of this study was to optimize *Pleurotus ostreatus* (Oyster mushroom) yield by establishing the local suitable substrates mixture that maximized the yield in Machakos County, Kenya. To achieve this objective simplex-centroid mixture design was used. Based on the study findings there was a significant variability on the substrate compositions used under the study, which included sawdust, sugarcane baggase, star grass, euphoria and the cattle manure. Sawdust yielded the most under the pure blend at 1.1 kg per experimental unit while on the mixed blend sugarcane bagasse and sawdust produced the highest yield at 1.3 kg per experimental unit (1kg of dry substrate), giving 10% and 30% biological efficiency respectively. There was no pinning on the cattle manure and the euphorbia substrates hence they were eliminated at the screening stage. The mixture response was found to be more valuable than the pure blend responses then simplex- centroid mixture design to rightly proportion the substrates was recommended for improved oyster mushroom production. A further research on determining suitability of alternative locally found substrates which may be more cost effective and multiple response optimizations aimed at achieving maximal nutritional value and yield against minimal cost of spawns and substrates were recommended.

Key words: Substrates, simplex centroids mixture designs, optimization

1.0 Introduction

Mixture component is a product of two or more ingredients mixed together, such that, the components of a mixture and the response varies as the proportions vary, (Cornel, 2002).

The total amount of the mixture is normally fixed in a mixture experiment and the component settings are proportions of the total amount. Hence, the component proportions in a mixture experiment cannot vary independently as in factorial experiments since they are constrained to a constant sum of 1 or 100% for standard designs, such that for a q-component mixture;

$$0 \le x_i \le 1, \text{ for } i = 1, 2, \ldots, q \quad \text{and} \quad \sum_{i=1}^{q} x_i = 1 \qquad 1.1$$

where x_i represents the proportions of the i^{th} component in the mixture of q-components.

A lot of products are formed by putting together two or more ingredients at predetermined proportions to arrive at a desired quality product. Examples of such products include; Fruit juices, building construction concrete, paints and fertilizers, (Montgomery, 2001)

The common mixture designs include Simplex Lattice, Simplex Centroid and Axial design. The study adopted Simplex Centroid Mixture Design (SCMD). This is a method of determining a unique set of components combination at various centroids that maximize or minimize the response variable depending on the objective function. Scheffé (1963) gave the simplex centroid designs consisting of $2^q - 1$ points with q permutations of $(1, 0, 0, \ldots 0)q$ pure blends, ${}^qC_2$ permutations of $\left(\frac{1}{2}, \frac{1}{2}, 0, \ldots, 0\right)$ giving binary blends and the overall centroid $\left(\frac{1}{q}, \frac{1}{q}, \ldots, \frac{1}{q}\right)$ giving a mixture blend of every component at equal proportions.

1.1 Background Information and motivation

Different substrates on oyster mushroom cultivation have been tried and applied, in Kenya and other parts of the world. (Kimenju et al., 2009) tried bean straw, water hyacinth, rice straw and maize straw and according to his findings bean straw had the best yield. Ajonina in 2012 performed experiments using wheat straw, coffee husks and saw dust and according to the findings the wheat straw had the highest biological efficiency of over 75%, which meant 75-100 kg of fresh mushrooms were expected from 75-100 kg of a dried wheat straw. However, those substrates were not locally found within the study area hence need for alternative locally available and suitable substrates for comparative advantage gain. According to Khademi and Timmermans in 2012, the application of mixture designs cannot be overemphasized in today's decision making and optimization. Therefore this study not only tried to establish the suitable local substrates for oyster mushroom production but also sought to investigate an optimal mix among them for maximum yield.

2.0 Methodology

Simplex centroid mixture design was applied to determine the optimal substrates' mix for *Pleurotus ostreatus* maximum yield. The experiment was carried out at the Machakos University ground in Machakos County, Kenya.
A dry kilogram of the substrate in a polythene paper was treated as the experimental unit.

The study employed one-factor-at-a-time (OFAT) approach initially in order to determine the most important substrates among the star grass ,sawdust , cattle manure , euphorbia , and sugarcane bagasse substrates on which the oyster mushroom grew.

The mixture of the significant substrates $\left(x_1, x_2, ..., x_q\right)$, with $\left(x_i \geq 0\right)$ and further restriction of $\sum x_i = 1$ were investigated to establish their influence in the oyster mushroom yield through their ratios or proportions variation.
The significant substrates were randomly and repetitively tried in order to counterbalance the order of treatments effects.

2.1 Substrates preparations

Procedurally all the input materials were gathered prior to the starting of the process. The substrate materials were weighed and shred into small pieces to easy mixing, packing and soaking. The substrate materials were soaked for an overnight to absorb enough water content that could sustain the whole process of mycelia colonization and fruition. The substrates were mixed with wheat bran, lime and as designated with other substrate components. The mixed dry substrates were packed into polythene paper bags per kilogram. The Polyvinyl Chloride (PVC) plastic pipes were fixed and sealed with the cotton wool which was fastened with the rubber bands to avoid external contaminations.
The sealed bags were steam boiled for five hours to sterilize them. The bags were allowed to cool and then the spawns were placed into the substrates through the spooning chambers, in a sterilized germ free environment with the attendants' hands and mouth gloved and covered respectively. The inoculated bags were then placed on the shelves in a dark room where the temperature and humidity were controlled for at least a month for substrates' full colonization. The PVC and the cotton wool were detached to allow the pinheads to sprout out which finally transformed into the oyster fruits.

2.3 Parameter estimate in the polynomials

The constraint $\sum x_i = 1$ in the mixture models makes them differ from the usual polynomials employed in response surface methodologies.
At the points of simplex centroids design, the response variable data was fitted onto a polynomial that had the same number of parameters to be estimated as there were points in the associated design. The standard form of a mixture polynomial model is defined as

$$\eta = \sum_{i=1}^{q} \beta_i x_i + \sum_{i<j} \sum^{q} \beta_{ij} x_i x_j + \sum_{i<j<k} \sum \sum^{q} \beta_{ijk} x_i x_j x_k + \cdots + \beta_{12\cdots q} x_1 x_2 \cdots x_q \qquad 2.1$$

The parameters in equation 3.29 are expressible as linear functions of the expected responses at the points of the simplex centroid design. The parameter β_i represents the expected response to the pure blend. In case of a curvature due to a nonlinear blending between components pairs, the parameter β_{ij} represents either synergistic or antagonistic blending otherwise it is a mere additive blending.

By substituting η_i, η_{ij} and η_{ijk} into equation 2.1 for the responses $x_i = 1$, $x_j = 0, i \neq j$ to $x_i = x_j = \frac{1}{2}$ and $x_i = x_j = x_k = \frac{1}{3}$ respectively, for all *i, j* and *k* then the parameters were;

$$\beta_i = \eta_i\,,\ \beta_{ij} = 2\{2^1\eta_{ij} - 1^1(\eta_i + \eta_j)\} \text{ and}$$

$$\beta_{ijk} = 3\{3^2\eta_{ijk} - 2^2(\eta_{ij} + \eta_{ik} + \eta_{jk}) + 1^2(\eta_i + \eta_j + \eta_k)\}$$

and by extension,

$$\beta_i = \eta_i = \overline{y}_i,\ (i = 1, 2, \ldots, k),$$

$$\beta_{ij} = 4\overline{y}_{ij} - 2(\overline{y}_i + \overline{y}_j),\ (ij = \underset{i<j}{1, 2}, \ldots, n)$$

The experimental substrates complemented one another at different proportions such that;

$$x_i \geq 0,\ x_1 + x_2 + x_3 + \text{L} + x_q = 1$$

Where; x_l, for $i = 1\text{L}\ q$ represented the substrate components and each component proportion x_i took the values zero to unity and all the blends among the ingredients were tried. Since the experiment used the simplex centroid design the mixtures were located at the centroid of the $(q-1)$ dimensional simplex and at the centroids of all the lower dimensional simplexes contained within the $(q-1)$ dimensional simplex.

2.3.1 Contour Plots

The outcome produced an empirical polynomial model which gave an approximation of the true response surface over a factor region. By overlaying contour maps from the experimental responses, it was possible to find the ideal "window" of operability.

3.0 RESULTS AND DISCUSION

3.1 Simplex Centroid Mixture Design

Five different substrates were tried for oyster mushroom growth on pure blend basis but under similar conditions. The five substrates included; Sawdust (x_1), Sugarcane bagasse(x_2), Cattle manure (x_3), Euphorbia (x_4), and Star grass(x_5).

The Sugarcane bagasse and sawdust recorded the highest yield of o.6kg on average while cattle manure recorded the least 0.1kg for the whole fruition period. There was no single pinning on the euphorbia substrate. The last two substrates were eliminated from further trials and the study proceeded with a three component mixture experiment to establish the substrate mixture that maximized the oyster mushroom yield. The three significant substrates that is, sawdust (x_1), sugarcane bagasse (x_2), and the star grass(x_3), were tried under pure blends, binary and ternary combinations repetitively and randomly.

The substrates were mixed with wheat bran, lime and as designated with the substrate components, they were rationed to try the desired outcome. Figures 3.1 and 3.2 indicate the designated design points for the three components.

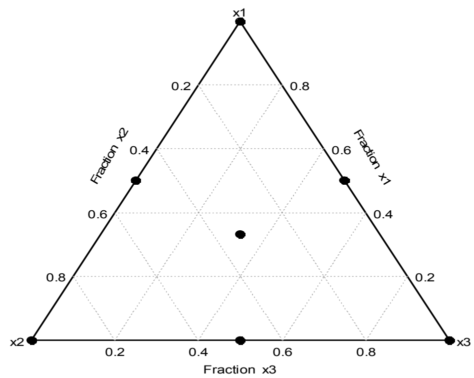

Figure 3.1 Distinct Experimental Points

Each point in the graph represented a design point for the three components; the vertices represented the pure component blends; x_1, x_2 and x_3. Binary blends, half combinations of any two substrates occurred at the midpoints of the sides on the triangle, while interior dot represent the bary centre, that is a geometric centroid of the three blends mixed at equal proportions. The measuring units were kilograms; for pure blends the spawns were inoculated in a kilogram of each substrate while for binary half of two different substrates. Then for the ternary a third of the three substrates was mixed to form a kilogram in which the spawns were inoculated. Figure 3.2 displays the response surface for the three components.

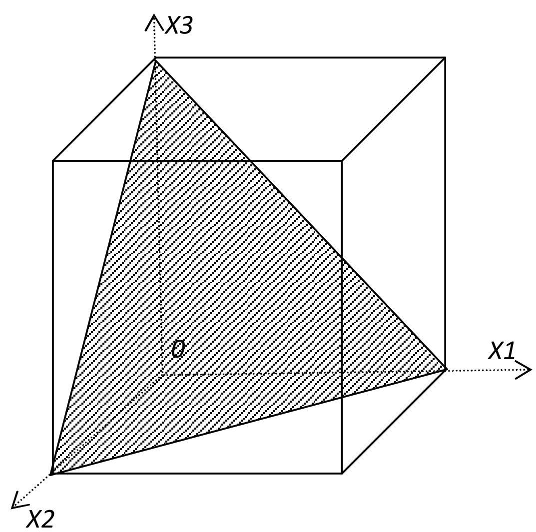

Figure 3.2 Planar Surface Spaces

Assuming each substrate increased its value from the vertex towards the centre of the planar surface space, the shaded region gives the possible responses as a function of substrates setting.
Applying the simplex centroid design, the number of distinct points were $2^3 - 1$, which corresponded to 3 permutations of (1,0, 0) on the three single component blends, $\binom{3}{2}$ permutations of $\left(\frac{1}{2},\frac{1}{2},0\right)$ i.e the binary mixtures and then the overall centroid point $\left(\frac{1}{3},\frac{1}{3},\frac{1}{3}\right)$ trinary mixture. Figure 3.3 shows the substrates combinations process; mixing, weighing, steaming and spawning.

Figure 3.3 Mixing the Substrates

The dry mixed substrates were packed into polythene paper bags per kilogram. The PVC plastic pipes were fixed and sealed with the cotton wool which was fastened with the rubber bands to reduce the chances of contamination and insect infestation.
The sealed bags were steam boiled for five hours to sterilize them. The bags were allowed to cool and then the spawns were placed into the substrates through the spooning chambers, in a sterilized germ free environment with the attendants' hands and mouth gloved and covered respectively. The growing room was cleaned and dimly lit to retain moisture in the air and simultaneously provide airflow when ventilation is needed. The room was sprayed with a solution of bleach along the walls and corners for the inoculations preparations. The inoculated bags were then placed on the shelves in a dark, temperature and humid controlled room to incubate for at least a month. During

this time the spawn ran (mycelium spreads) throughout the substrate, implying it was fully colonized. The air temperature in the spawn run room was maintained at 18–25°C. Relative humidity was maintained at 95 to 98 percent to minimize drying of the substrate surfaces. The bags were regularly checked for any mould contamination and any infected bag was immediately removed from the growing area. The PVC and the cotton wool were detached for the pinheads to sprout out which finally transformed to the oyster fruit.

Figure 3.4 Packing and Spraying

A successful cultivation of mushroom requires proper sterilization of the substrates prior to inoculation with the quality spawn (Musieba, Okoth, Mibey, Wanjiku, &Moraa, 2012). Figure 3.4a shows parked and sealed substrates, figure 3.4b shows an infected bag, figure 3.4c shows unsealed but parked substrates and figure 3.4d shows spraying of the polythene bags for sanitation taking place. The labeled polythene bags were arranged in a completely randomized design on shelves in the mushroom growing room and incubated at ambient temperature and relative humidity controlled by manually spraying water on the walls and placing open containers filled with water in the corners of the room.The fruition continued reproducing for a period of 3-4 months, and the harvesting was done daily by plucking the whole fruit with sterilized hands. The fruit was sold fresh and dry. Table 3.1 gives the summary of the fruition and harvested yield per experimental unit.

Where; x_1, x_2 and x_3 represented the sawdust, sugarcane baggase and star grass substrates respectively

Table 3.1: Experimental output

Design Points	Substrates Proportions							Observed weight (kg) values	Average
	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3	$x_1x_2x_3$	**y**	$\bar{y}$
η_i	1	0	0	0	0	0	0	1.0,1.2,1.3,1.1,1.1,0.9	1.1
η_j	0	1	0	0	0	0	0	1.1,0.7,0.9,0.9,0.5	0.8
η_k	0	0	1	0	0	0	0	0.4,0.2	0.3
η_{ij}	0.5	0.5	0	0.25	0	0	0	1.1,1.4,1.5,1.4,1.6,1.2,0.8	1.3
η_{ik}	0.5	0	0.5	0	0.25	0	0	0.4,0.6,0.5,0.7,1.1,0.3	0.6
η_{jk}	0	0.5	0.5	0	0	0.25	0	1.0,0.8,0.4,0.6,0.1,0.2	0.5
η_{ijk}	0.33	0.33	0.33	0.1089	0.1089	0.1089	0.0360	0.6,0.5,0.9,1.0,0.7,0.4	0.7

The design points in Table 3.1 refers to one polythene paper with one setting for each of the substrate/s of the experiment but replicated in different times and for which a single value for the response was observed, that is the yield in kilogram. The results indicated that there was a synergism in the binary mixture between the sugarcane bagasse and the sawdust in excess of 0.3 kg, while the binary mixture between the star grass and the sawdust as well as between the Sugarcane bagasse and the Star grass registered antagonism of the mixtures. Among the pure blends, sawdust was the best at 1.1kg on average followed by the sugarcane baggase and lastly star grass at 0.8kg and 0.3kg respectively.

Figure 3.5 Harvesting and Packing

Figure 3.5a shows the plucked out flesh mushroom fruits and figure 3.5b shows the packed flesh oyster mushroom. The harvested fruit were packed into 200g, 1kg or 2kg units. The average price was ksh 600 per kg when fresh while about ksh 4000 when dry. Implying one kilogram of dry oyster was approximately equal to seven kilograms when fresh in terms of both the quantity and value.

3.1.1 ANOVA Test Statistics for Substrates

The one way between groups Analysis of Variance (ANOVA) was conducted to explore the impact of substrates mixture variation on the mushrooms yield. The subjects were categorized into seven groups based on the mixture blend, which are pure blends, binary blends and the triad blend.

To ensure ANOVA test statistics assumptions were not violated during the experimentation period, complete randomization of the polythene bags was done and the polythene bag labelled but randomly and independently placed. The levene's test for the assumption of homogeneity of variance was conducted whose significance value was 0.370 (≥ 0.05) implying the assumption of homogeneity of variance was not violated.

Therefore precautionary actions were taken prior to the data analysis to ensure the data conformed to the parametric test statistics assumptions.

3.1.1.1 The ANOVA Table

The results for one way ANOVA conducted to explore the impact of mixing different proportions of substrates on Oyster mushroom yield are summarized in table 3.2.

Table 3.2: ANOVA Table

Source of variance	Sum of Square	df	Mean Square	F	Sig.
Between Groups	3.484	6	0.581	8.767	0.000
Within Groups	2.053	31	0.066		
Total	5.537	37			

There was a statistical significant difference among the mean yield for the seven mixture groups at the $p < 0.05$ level in the expected yield.

The computed $F = 8.767 > F_{0.05,\ 6,\ 31} = 2.42$, therefore the null hypothesis (H_0) was rejected with a conclusion that the seven substrates mixture groups differed significantly in their yielding amount as measured by the average size of their yield. This meant that the yield difference per mixture blend could not be attributed to chance but the proportions of the substrates included in the mixture.

3.1.1.2 Post Hoc Tests

The post hoc tests were carried out among the component means with a significant difference from each other. The differences were revealed by Tukey's Highest Significant Difference (HSD) analysis

Table 3.3: Significant Mean Difference

Source of Difference	Mean Difference	Sig.	Std. error
x_1 € x_3	0.8000	0.010	0.21
x_{13}	0.5000	0.030	0.15
x_{23}	0.5833	0.007	0.15
x_{12} € x_3	0.9857	0.001	0.21
x_{13}	0.6857	0.001	0.14
x_{23}	0.7690	0.000	0.14
x_{123}	0.6024	0.003	0.14

The highest mean yield difference was between the sawdust and sugarcane bagasse binary blend and the star grass at 0.9857. The second highest mean yield difference was between the saw dust and the star grass from pure blends at 0.8000.

3.2 Parameter estimate in the polynomials

The coefficients of the simplex centroid mixture model were obtained through the R statistical computer package. The output summary of the oyster mushroom yield as influenced by varying the substrate's mixture component is summarized in table 3.4.

Table 3.4: Polynomial Parameter Estimate Output

Mix	N	Est	Std. Dev	Std. Error	95% CI for Mean Lower Bound	95% CI for Mean Upper Bound	Min	Max
x_1	6	1.100	0.1414	0.0577	0.952	1.248	0.9	1.3
x_2	5	0.820	0.2280	0.1020	0.537	1.103	0.5	1.1
x_3	2	0.300	0.1414	0.1000	-0.971	1.571	0.2	0.4
x_{12}	7	1.386	0.2734	0.1033	1.033	1.539	0.8	1.6
x_{13}	6	-0.400	0.2828	0.1155	0.303	0.897	0.3	1.1
x_{23}	6	-0.200	0.3488	0.1424	0.151	0.883	0.1	1.0
x_{123}	6	-3.248	0.2317	0.0946	0.440	0.926	0.4	1.0

The highest onetime yield recorded was 1.6 kg from the sawdust and sugarcane bagasse binary blend set, from which the best average mean was also realized of 1.286 kg with a 95% confidence interval of 1.033 to 1.539 mean values. The best pure blend was the sawdust with a mean yield of 1.1kgs and a 95% confidence interval mean value of 0.952 to 1.248. The sawdust pure blend also registered the smallest standard error of 0.0577, an indication that the sample mean was a more accurate reflection of the actual population mean.

The minimum average yield was 0.3 kg, obtained from Star grass pure blend with a 95% confidence interval of -0.971 to 1.571 mean values. The minimum one set single yield was 0.1kg from the sugarcane bagasse and Star grass binary blend. Therefore from the output in table 3.1, the yield could be predicted using the model.

$$\hat{y}(x) = 1.1x_1 + 0.8x_2 + 0.3x_3 + 1.4x_1x_2 - 0.4x_1x_3 - 0.2x_2x_3 - 3.1x_1x_2x_3 \qquad 3.1$$

3.2.1 Manually Computed Parameters Estimate in the Polynomials

The polynomial parameters could also be calculated by using the formulas for the parameter estimate and the values in table 3.1 as shown in the following section.

$$\beta_i = \eta_i = 1.1$$

$$\beta_j = \eta_j = 0.8$$

$$\beta_k = \eta_k = 0.3$$

$$\beta_{ij} = 2\left\{2^1(1.3) - 1^1(1.1+0.8)\right\} = 1.4$$

$$\beta_{ik} = 2\left\{2^1(0.6) - 1^1(1.1+0.3)\right\} = -0.4$$

$$\beta_{jk} = 2\left\{2^1(0.5) - 1^1(0.8+0.3)\right\} = -0.2$$

$$\beta_{ijk} = 3\left\{3^2\eta_{ijk} - 2^2(\eta_{ij} + \eta_{ik} + \eta_{jk}) + 1^2(\eta_i + \eta_j + \eta_k)\right\}$$

$$\beta_{ijk} = 3\left\{3^2(0.7) - 2^2(1.3+0.6+0.5) + 1^2(1.1+0.8+0.3)\right\} = -3.3$$

hence the fitted model in the three components is;

$$\hat{y}(x) = 1.1x_1 + 0.8x_2 + 0.3x_3 + 1.4x_1x_2 - 0.4x_1x_3 - 0.2x_2x_3 - 3.3x_1x_2x_3 \qquad 3.2$$

Which is the same as equation 3.1 to a great extend.

Therefore the response value can be predicted at any point, for instance $\hat{y}\left(\frac{1}{2},\frac{1}{2},0\right)$ would be

$$\hat{y}\left(\frac{1}{2},\frac{1}{2},0\right) = 1.1\left(\frac{1}{2}\right) + 0.8\left(\frac{1}{2}\right) + 0.3(0) + 1.4\left(\frac{1}{2}\right)\left(\frac{1}{2}\right) - 0.4\left(\frac{1}{2}\right)(0) - 0.2\left(\frac{1}{2}\right)(0) - 3.3\left(\frac{1}{2}\right)\left(\frac{1}{2}\right)(0)$$

; 1.3

3.2.2 The Variance and the Standard errors

The standard errors were determined by using the sample standard deviations $\left(S^2\right)$ since the population standard deviation $\left(\sigma^2\right)$ could not be obtained

$$S^2 = \sum_{i=j=k}^{2 \text{ or } 5 \text{ or } 6} \sum_{ij=ik=jk}^{6 \text{ or } 7} \sum_{ijk}^{6} \frac{\left(y_{iu} - \bar{y}_i\right)^2}{\sum_{i=1}^{7}\left(r_i - 1\right)} \qquad 3.3$$

$$S^2 = \frac{(1.0-1.1)^2 + (1.2-1.1)^2 + \cdots + (0.4-0.7)^2}{5+4+1+6+5+5+5} = 0.07$$

and the estimates of the variance of the parameter were obtained by;

$\widehat{\text{var}}(b_i) = \frac{s^2}{r_i}$ for the pure blends, $\widehat{\text{var}}(b_{ij}) = s^2\left\{\frac{16}{r_{ij}} + \frac{4}{r_i} + \frac{4}{r_j}\right\}$

and

$$\widehat{\text{var}}(b_i) = \frac{s^2}{r_i} = \frac{0.07}{6} = 0.012$$

$$\widehat{\text{var}}(b_j) = \frac{s^2}{r_j} = \frac{0.07}{5} = 0.014$$

$$\widehat{\text{var}}(b_k) = \frac{s^2}{r_k} = \frac{0.07}{2} = 0.035$$

$$\widehat{\operatorname{var}}(b_{ij}) = s^2\left\{\frac{16}{r_{ij}} + \frac{4}{r_i} + \frac{4}{r_j}\right\} = 0.07\left\{\frac{16}{7} + \frac{4}{6} + \frac{4}{5}\right\} = 0.263$$

$$\widehat{\operatorname{var}}(b_{ik}) = s^2\left\{\frac{16}{r_{ik}} + \frac{4}{r_i} + \frac{4}{r_k}\right\} = 0.07\left\{\frac{16}{6} + \frac{4}{6} + \frac{4}{2}\right\} = 0.373$$

$$\widehat{\operatorname{var}}(b_{jk}) = s^2\left\{\frac{16}{r_{jk}} + \frac{4}{r_j} + \frac{4}{r_k}\right\} = 0.07\left\{\frac{16}{6} + \frac{4}{5} + \frac{4}{2}\right\} = 0.383$$

hence the fitted second- degree model to the observed data was

$$\hat{y}(x) = \underset{(0.110)}{1.1x_1} + \underset{(0.118)}{0.8x_2} + \underset{(0.187)}{0.3x_3} + \underset{(0.513)}{1.4x_1x_2} - \underset{(0.612)}{0.4x_1x_3} - \underset{(0.619)}{0.2x_2x_3} \qquad 3.4$$

3.2.3 Adequacy of the Fitted Model

The adequacy of each design point in the fitted model could be tested through the null hypothesis that the estimate of the response at the designated check point is not significantly different. To estimate the variance of a fitted point, the following formula was used.

$$\widehat{\operatorname{var}}\left[\hat{y}(x)\right] = s^2\left\{\sum_{i=1}^{3}\frac{a_i^2}{r_i} + \sum\sum_{i<j}^{3}\frac{a_{ij}^2}{r_{ij}}\right\}$$

For $a_i = x_i(2x_i - 1)$ and $a_{ij} = 4x_ix_j$

For instance to estimate the variance of $\hat{y}(x)$ at the centroid point $\hat{y}\left(\frac{1}{3},\frac{1}{3},\frac{1}{3}\right)$ in the model, and using the formula;

$$\widehat{\operatorname{var}}\left[\hat{y}(x)\right] = s^2\left\{\sum_{i=1}^{3}\frac{a_i^2}{r_i} + \sum\sum_{i<j}^{3}\frac{a_{ij}^2}{r_{ij}}\right\}$$

For $a_i = x_i(2x_i - 1)$ and $a_{ij} = 4x_ix_j$

$$\widehat{\operatorname{var}}\left[\hat{y}(x)\right] = 0.07\left\{\frac{\left(-\frac{1}{9}\right)^2 + \left(-\frac{1}{9}\right)^2 + \left(-\frac{1}{9}\right)^2}{6+5+2} + \frac{\left(\frac{4}{9}\right)^2 + \left(\frac{4}{9}\right)^2 + \left(\frac{4}{9}\right)^2}{7+6+6}\right\};\ 0.034$$

To test the satisfaction and fitness to the fitted model, the t-test statistics formula in the following equation was used

$$t = \frac{\bar{y}_{obs} - \hat{y}_{est}}{\sqrt{\text{var}\left(\bar{y}_{obs}\right) + \text{var}\left(\hat{y}_{est}\right)}}$$

$$= \frac{0.7 - 0.9}{\sqrt{0.07 + 0.034}} = 0.62\,4.21$$

but tabulated $t_{0.025,\ 6} = 2.447 > computed$ t=0.62

therefore, the H_0 is not rejected at $\frac{\alpha}{2} = 0.025$ and the response at this point $\hat{y}\left(\frac{1}{3}, \frac{1}{3}, \frac{1}{3}\right)$ is not significantly different from the mean.

Equally the estimate of the variance of $\hat{y}(x)$ at the point $\hat{y}\left(\frac{1}{2}, \frac{1}{2}, 0\right)$, $\hat{y}\left(\frac{1}{2}, 0, \frac{1}{2}\right)$ and $\hat{y}\left(0, \frac{1}{2}, \frac{1}{2}\right)$ can be made.

3.2.4 Graphical Yield Representation

The yield for each type of substrate was recorded and represented in a multiple linear graph, figure 3.6. The cumulative yield was noted and summarized in figures 3.7, in the form of linear graph, bar graph, pie char and the box plots. Figure 3.8 displayed the optimal mixture proportions in the form of contours.

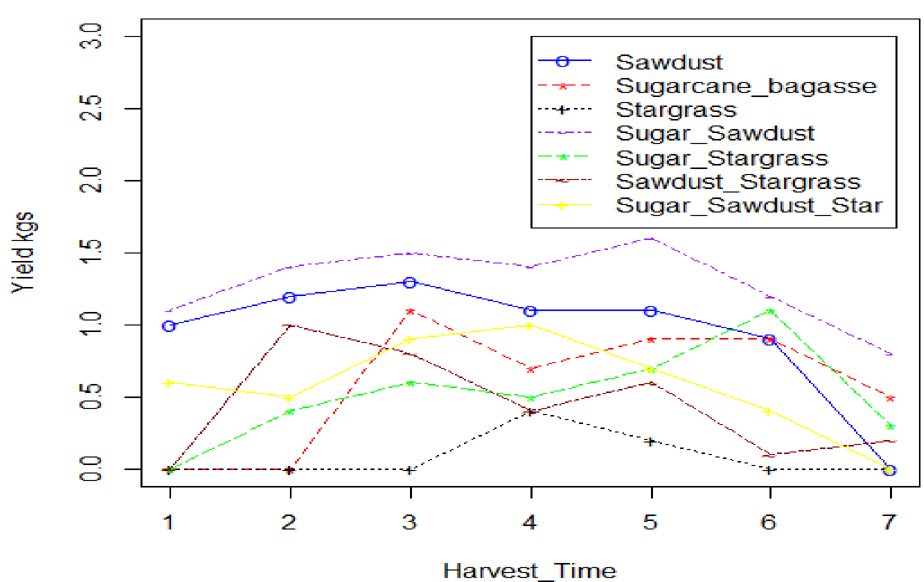

Figure 3.6: Substrates Performance

The yield from the sugarcane bagasse and sawdust binary blend was the highest all along the harvest times. Among the pure blend sawdust gave the best yield while star grass performed most dismally.

Cumulatively the mixture of sugarcane bagasse and sawdust was the best as summarized in figures 3.7a, 3.7b, 3.7c and 3.7d.

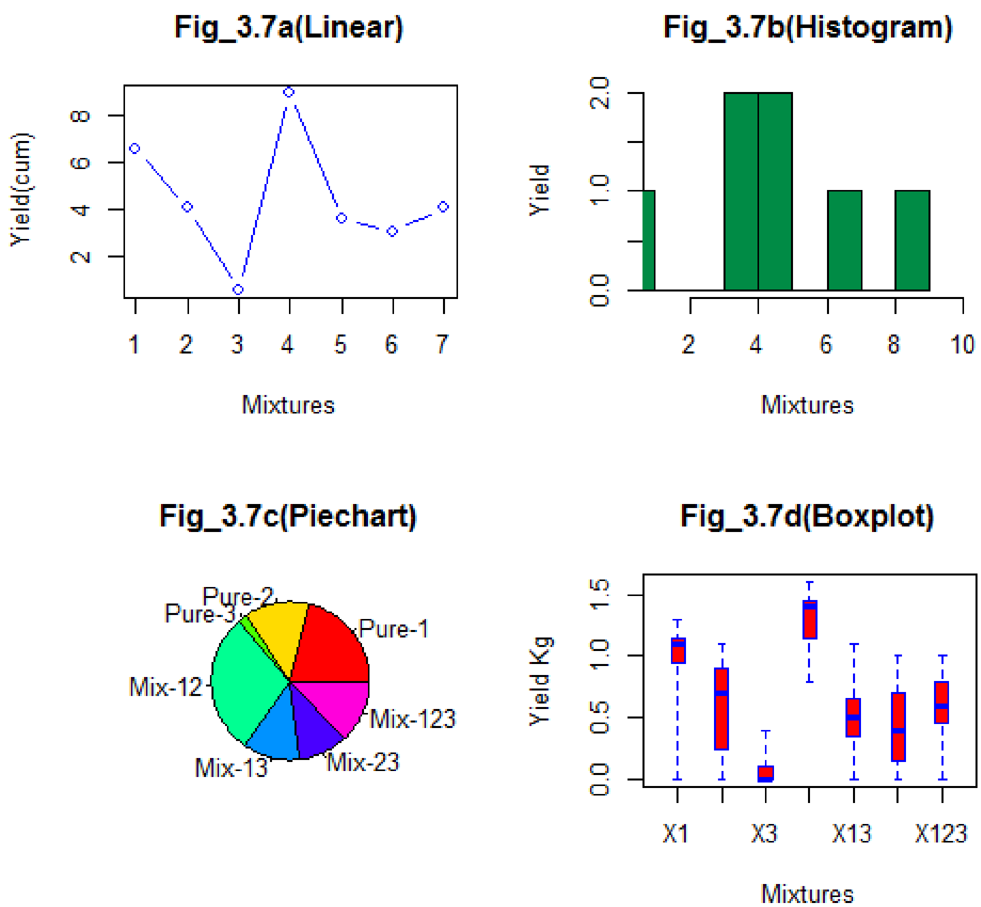

Figure 3.7: Cumulative Yield

By inspection from figures 3.7 the sugarcane bagasse and saw dust mixture recorded the highest yield while with pure blend the star grass labeled as pure-3 in figure 3.7c recorded the lowest yield with the sawdust substrate yielding the best. The box plots displayed the middle and the quartiles distribution of the yield per substrate composition. Based on the box plots displays star grass was the most compact while the sawdust pure blend, star grass and sawdust binary blend were among the most dispersed.

3.2.5 Surface Contour Plot for Mixtures

Generally contour lines for a function of variables connect the points where the function has the same value. Contour plot for the yield as a function of the three mixture substrate combination is shown in figure 3.8

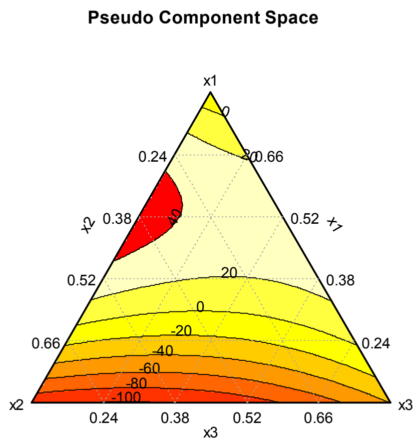

Figure 3.8 Mixture Contour Plots

The grey and yellow colours are the highest in the design space with little of maroon and red colours. The optimal mixture yield could be spotted at the around seventy percentage of sawdust , about twenty percent of sugarcane bagasse and ten percent of the star grass.

Conclusions and Recommendations

Truly the mixture experiments are widely used today in formulation experiments, blending experiments, and marketing choice experiments, where the goal is to determine the most preferred attribute composition of a product at a given price.

The simplex centroid mixture design was found to be very efficient and effective in determining and discovering the optimal substrate combination for the best oyster mushroom yield. The best yields were realized at; 100% saw dust, or 50% to 50% of saw dust and sugarcane bagasse or 70% to 20% to 10% of sawdust, sugarcane bagasse and star grass respectively depending on what is available.

Venturing into oyster mushroom farming not only provides a protein- rich food but reduces the environmental pollution and requires a very small land to operate. It is a transformative link for inedible wastes into edible biomass of high monetary value. Therefore it is recommended that most of the farmers should be exposed to the activity and be trained to understand the factors which individually or interactively affect oyster mushroom production.

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