Optimization of gamma-aminobutyric acid in three varieties of germinated brown rice using the rotatable central composite design (RCCD) of the response surface methodology (RSM)

Udofia, P.G.[1*], Eddy, N.O.[2], Udoudoh, P.J.[3]

[1,3]Department of Food Technology, AkwaIbom State Polytechnic, IkotOsurua, IkotEkpene
[2]Department of Chemistry, University of Nsukka, Nigeria

Abstract—*Gamma-aminobutyric acid (GABA) is one of the bioactive compounds required for nervous health of man especially that of the elderly. GABA is an inhibitory neurotransmitter in plants and animals. It is present in paddy rice at levels too low for an active functionality in a health food. Germination of paddy rice varieties could increase the concentration of GABA to levels required for human health. GABA is a non-protein amino acid, it is applied to relieve or prevent non-communicable diseases in man especially among the elderly. Synthetic has failed to meet the ideal requirement of a good supplement; affordability, safety and availability and renewability. The work was done to improve the content of GABA in Sesalin, Mars, and Vara rice varieties through germination process. The three rice varieties (X_1), were obtained from Ini Local Government Area of AkwaIbom State, Nigeria. Germination of the brown rice was done under the environmental conditions of germination time (X_2), steeping time (X_3), and temperature of steeping water (X_4). The rotatable central composite design (RCCD) of the response surface methodology was deployed (RSM)for the study to identify terms of the models which exhibited optimum levels of GABA concentration, sensory and market attributes of the germinated brown rice (GBR). The experiment revealed that rice variety, steeping time, germination time and their interactions synergistically influenced the germination process. The model of GABA was significant ($p<0.05$), $R^2=0.8722$ and mean value =16.03µg/100g of the samples. The models for colour, taste and general acceptability of the samples were not significant ($p>0.05$), but the response surface plots exhibited some effects on the parameters. Optimization process showed that Vara 44 rice variety, sprouting time of 91.42 hr, 0.023 hr, and steeping water temperature of 37°C produced 906.64ug/100g, 4.53, 4.19, and 7.07 of GABA, colour, taste, and general acceptability of the GBR respectively at 92.80% desirability. More studies are needed using higher values of the independent variables to obtain higher levels of the study responses and education of the consumers on the health benefits of GABA.*

Keywords— RCCD, RSM,GBR, GABA, sprouting, gamma-aminobutyric acid-enriched white rice.

I. INTRODUCTION

Gamma-aminobutyric acid is a major inhibitory neurotransmitter in plants and animals. It is a non-protein ammino acid needed for the maintenance of good nervous health in the mammalian subjects (Magali *et al*., 2016).GABA acts by sending chemical message through the brain and the nervous system, therefore communicating with the brain cells. GABA may also inhibit or reduce the activity of the neurons or nerve cells. It plays an important role behavior, cognition, and the body response to stress. According to Crawford, (1994) and Thitinunsomboon, *et al*., (2013) GABA may also inhibits the proliferation of cancer cells, blood pressure regulation. Lower-than-normal levels of GABA in the brain are linked to schizophrenia, depression anxiety and sleep disorders especially among the elderly. GABA supplements taken alone or in combination with other ingredients are marketed widely and used for the management and treatment of depression, anxiety, and insomnia. Synthetic GABA products can induce toxicity depending on the source, abuse, especially through self-medication practices and adulteration (Mie, *et al*., 2016). Unlike the synthetic counterpart, the plant source of GABA is affordable, safe, cheaper and renewable. This work is seeking plant source of GABA.

Traditionally, one major source of GABA is the brown (unpolished) rice. Its presence is the intact brown rice is at levels, which are too low to support normal human (Totayava, 2014). Germination process can improve the nutrients composition, physiochemical, and functional characteristics of raw, germinated and fermented legume, grains and seed flour (Ijarotimi *et al*., 2013). According to Totayava, (2014), graded levels of environmental factors like moisture, germination, darkness, and stimuli such as heat shock and cold shock could induce germination and increase rate of synthesis of GABA and concentration in brown rice. Mustapha *et al*., (2013) and Totayava, (2014) respectively reported that sprouting promotes phytochemical contents of cereals. Germinated rice, which was steeping in water in graded time and temperature of the steeping water, increased the content of GABA (Finney, 2020). However, according to Chaiyasut *et al*., (2017), excessive soaking rice samples led microbial enrichment, sufficient soaking retarded germination and failed to improve phytochemical content of the samples. Bown, *et al*., (2006); Chaiyasut *et al*., (2017); Fengfeng *et al*., (2013) independently reported that germination time of paddy rice played a critical role in the multiplication of the nascent bioactive chemical compounds in paddy rice. However, paddy rice cannot germinate without the presence of water at appropriate steeping water temperature Chaiyasut *et al*., (2017).

The UN Food and Agriculture Organization, Corporate Statistical Database (FAOSTAT) (2018) reported that rice s the seed of the grass species *Oryza sativa* (Asian rice) or *Oryzaglaberrima* (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia and Africa. It is the agricultural commodity with the third-highest worldwide production (rice, 741.5 million tonnes in 2014), after sugarcane (1.9 billion tonnes) and maize (1.0 billion tonnes).

Due to the high prevalence of acceptance and consumption of rice, it poses as a suitable candidate in food fortification and enrichment nutrition programme especially for the vulnerable group of the world population (Jirapa, *et al*., 2014 and Nathalie and John, 2013). The study optimized GABA levels, colour, taste, and general acceptability in the three varieties of brown rice; Sesalin, and Mars, Vara 44 (X_1), under the influence of controlled sprouting time (X_2), steeping water time (X_3) and temperature of steeping water (X_4) using the central composite design (RCCD) of the response surface methodology (RSM). The design has superior advantages over the one-factor-at-a-time (OFAT) counterpart.

II. MATERIALS AND METHODOLOGY

2.1 Sample collection

The paddy of Sesalin, Mars and Vara 44 (Oriza sativa L.) varities were cultivated at the rice belt of Ini Local Government Area, AkwaIbom State, Nigeria. The freshly harvested paddy rice samples were sundried to about 15% moisture content and stored for germination.

2.2 Reagents and equipment

Standard GABA and other reagents were of analytical grade. All equipment were available from Eurofins Global Control GmbH, Germany.

2.3 Preparation and germination of paddy rice

The germination of paddy rice was carried out according to the method adopted by Panatda et al., (2013) with slight modification to meet the local condition. Three varieties of the paddy rice were steeped in graded temperature of distilled water (pH 6.6) at room temperature and relative humidity of 90-95% and placed in straw baskets which bottom were covered with thick moistened paper and covered with a muslin cloth. The set up was allowed to germinate according to the conditions in (Montgomery, 1998) Table 1. The germinated grains from each experimental run were dried at 50^{o}C to approximately 10% moisture content. The hull, root and shoot were separated from the germinated paddy rice using a laboratory de-husker. All rice samples originated from each experimental run. Germinated rice samples were stored at room temperature for analysis.

2.4 Experimental design

Response surface methodology was carried out according to the method of Montgomery, (1998). The design optimized the variables; variety (X_1), sprouting time (X_2), steeping time (X_3), temperature of steeping temperature (X_4) that might affect the content of GABA during germination of the paddy rice varieties. In this design a two interaction factor models, (Equation 1), were used to describe the relationship between the independent and the responses (concentration of GABA).

$$Y_i = \beta_o + \sum\beta_i X_i + \sum\beta_{ij} X_i X_j + e \quad (1)$$

Where Y_i = a predicted response and β_o'β_i,β_{ij} represent coefficient of the linear and interactive effects respectively; X_i, X_iX_j represent the linear, quadratic, and interactive effects of the independent variables respectively. Three-dimensional response surface plots were drawn to illustrate the interactive effects of the 4factors on concentration of GABA, colour, taste and acceptability taking two factors at a time. The analysis of variance (ANOVA) was used to determine effects of variety (X_1), sprouting time (X_2), steeping time, (X_3) steeping water temperature (X_4) on the temperature. To find significant differences, *F* and p-values at probability levels of 0.05 were studied. Design-Expert 12. Version Software Package (State-Ease Corp., Minneapolis, Minn., U.S.A) was used to generate designs to fit the response surface models to the experimental data and drawn response surface plots.

Response surface methodology is an effective statistical technique which is deployed to for the optimization of complex experimental processes. The main advantages of the design is the reduced number of experimental runs needed to adequately assess multiple variables on responses singly, their interactions and quadratic and cubic form with less labour, time and cost (Montgomery, 2002).

2.5 Extraction of gamma-aminobutyric acid

GABA was determined according to the method of (Woraharn *et al.*, 2016; Chaiyasut, *et al*., 2017) with slight modifications. The rice samples were steeped in 4% acetic acid and then in 50% ethanol solution separately and agitated at 150 rpm for one (1) hour and centrifuged at 6000 rpm for 20 minutes. The supernatant was collected and evaporated at 50 °C using the vacuum evaporator (Rotavapor R-210, BUCHI) to obtain the GABA extract.

2.6 Determination of GABA

Determination of gamma-aminobutlyric acid in the germinated varieties of paddy rice was determined according to the HPLC method adopted by Chaiyasut et al., (2017). A gradient elution was used for the separation and quantification of GABA after pre-colum derivatization with phenylisothiocyanate (PITC). The column was Pico x Tag for free amino acids. GABA was determined with UV detector at 254 nm. Good linearity was observed within the ranges from 0.125 to 6.25 micromo/L. The average recoveries were 95.4%. The intra-and intre-precision values were within 3.56% and 7.47%. The concentration of GABA in each sample was recorded in Table 2 (a).

2.7 Preparation of germinated brown rice

Germinated brown rice was produced according to the method of (Bown, *et al*., 2006) with some modifications to suit the local conditions. The 21 samples of the germinated paddy rice samples were dried to about 15% to 30% moisture content. The shoot and roots were removes from the brown rice after drying. Some bran of the dried paddy rice was not removed which tented the samples hence, the name 'germinated brown rice'. The brown germinated brown rice meal was prepared according to the local method. Product of each experimental run was measured into separate 250 ml beakers and boiled to softness on Bunsen flame (Okada, *et al*., 2000). The meal was presented for sensory evaluation.

2.8 Sensory properties of the germinated brown rice

Sensory attributes of the GBR was determined according to the method of Lin, (2000). The taste panel was constituted from the database of the village in which the work was carried out. The panel 64untrained panelists, aged between 40 to 60 years.

The panelists were guided to complete a questionnaire, specifying their attitudes on the acceptance and consumption of rice in respect to their health status. Conditions for participation in the study included familiarity and consumption of various forms and varieties of rice for a long time, lack of allergy to any ingredients used in the preparation of the meals and availability for the taste sessions (Okada, *et al*., 2000). Each participant was to received honorarium of five hundred Naira (N500) each at the end of the sessions. Nine 9-point hedonic scale (9 = like extremely, 5 = neither like nor dislike, 1 = dislike extremely) was used by the panelists to evaluate color, taste and overall acceptability of the 21 samples. GBR meal samples were served warm with all panelists of a given session receiving the same coded sample at the same time. Left over samples was warmed to 95 to 102^{o}C before served again to the panelists at new and repeated sessions. The samples were randomized among the sessions, panelists were asked to actually eat the sample, expectorate and rinse their mouths with water between samples.

TABLE 1
ASSIGNMENT OF INDEPENDENT VARIABLES IN RCCD EXPERIMENTAL DESIGN

Name	Unit	Low level	Middle level	-alpha level	+alpha level
Variety (X_1)	Cat.	1	3	0.38	3.68
Sprouting time (X_2)	Hr	0	94	32.04	113.47
Steeping time (X_3)	Hr	0	12	4.09	16.09
Steeping water temperature (X_3)	DegC	37	60	29.16	67.84

Cat. = categorical variable, DegC = degree centigrade.
Source: Montgomery, (1998)

TABLE 2
EXPERIMENTAL RUNS AND RESULTS MATRIX

Std	X_1	X_2	X_3	X_4	GABA(a)	Colour(b)	Taste(c)	Gen.Accept(d)
13	2	47	4.1	49	201.8	6	5	5
11	2	32	6	49	204.6	6	5	5
20	2	47	6	49	201.9	6	5	5
21	2	47	6	49	8.1	4	3	3
10	4	47	6	49	321.1	6	5	5
18	2	47	6	49	215.7	8	7	7
4	1	94	0	60	81.2	5	4	4
19	2	47	6	49	199.2	4	3	3
15	2	47	6	29	211.7	5	3	3
1	3	94	12	37	98.6	7	6	5
3	3	0	12	60	7.7	9	7	8
12	2	126	6	49	98.7	4	4	4
2	3	94	0	37	7.8	5	5	8
17	2	47	6	49	1.7	7	6	5
6	1	0	12	37	9.8	8	4	4
16	2	47	6	68	125.1	5	4	4
5	3	0	0	60	7.9	7	7	7
8	1	0	0	37	2.6	8	4	4
7	1	94	12	60	98.7	6	6	6
9	0	47	6	49	202.8	6	5	6
14	2	47	16.1	49	215.9	8	7	7

Small of the rotable central composite design (RCCD) with alpha =1.681
Std = standard run, Run=natural order of run, NA=Response not available
Variety = (X_1), Sprouting time = (X_2), Steeping time = (X_3), Steeping water temperature = (X_3)

III. RESULTS AND DISCUSSION

Table 2 presents the influence of variety, sprouting time, steeping time and temperature of steeping water on the increase of GABA content in three varieties of germinated paddy rice. The table also shows the responses of (2b), (2c) and (2d) for colour, taste and general acceptability respectively.

TABLE 3
MODELS, INTERCEPT, AND COEFFICIENTS OF ESTIMATE FOR GABA AND SENSORY ATTRIBUTES OF GERMINATED BROWN RICE

Source	DF	GABA	Colour	Taste	General acceptability
Model (p-value)		0.0027	NS	NS	NS
Intercept		179.90	174.90	5.00	5.17
X_1	1	34.95	34.95	0.00	-0.2973
X_2	1	-47	-47.00	-0.5078	-0.6516
X_3	1	-74.01	-74.01	0.5824	0.2871
X_4	1	-25.76	-25.76	0.298	0.2988
Interaction					
X_1X_2	1	87.78	87.78	-0.3263	-0.2012
X_1X_3	1	-114.89	-114.89	-0.1250	-0.5000
X_1X_4	1	-202.41	-202.41	-0.4489	-0.6516
X_2X_3	1	-110.46	-110.48	0.3750	-0.2500
X_2X_4	1	-79.39	-79.39	-0.8750	-1.55
X_3X_4	1	113.04	113.04	0.1250	0.7000
Quadratic					
R^2		0.8722	0.5149	0.5073	0.50873
Mean		167.03	6.19	5.14	5.56
SD		107.07	1.45	1.55	1.45

Table 3 shows models, intercept, and coefficients of estimate for GABA and sensory attributes of the germinated brown rice. The values show positive (synergistic), negative (antagonistic) effect of the variables in the process. Values of the coefficient of estimates the weight of contribution of the variables singly and in interaction in either direction.

The RCCD of the RSM was utilized to detect optimum conditions of the environmental variables on the optimum concentration of GABA. ANOVA of the data, Table 2, showed that the model of concentration of GABA was significant ($p< 0.05$), $R^2 = 0.8722$.The mean concentration of GABA was 167.03mg/100g of GBR. The table further revealed that steeping time (X_3) alone, interaction of variety (X_1) and temperature of steeping water (X_4), sprouting time (X_2) and steeping time were significant on the values of the concentration of GABA. X_1, X_2, and X_3 were not significant ($p>0.05$). Fig. 1, 2 and 3 shows the variation of concentration of GABA against the independent variables. Equation 2 shows the numerical weight of influence of the variables on the models. The observation was in agreement with the observation of Verma and Verma, (2002) and Thitinunsomboon, *et al*., (2013) who reported that germination increase nutrients content in seeds, legumes and grains. Panatda *et al*., (2013) reported an increase of GABA concentrations in some varieties of rice follows: 1.45-3.14mg/g, 1.36-2.85 mg/g, 2.39-2.52 mg/g, 0.82-2.09mg/g and 1.33-1.50 mg/g in normal rice PL2, CN1, KDML 105, SP1 and PT1, respectively.

The increment in the concentration of GABA in the rice samples could be attributed to the biochemical mechanisms in the samples which take place during germination and growth (Panatda *et al*., 2013). Seed, cereal and legume germinations increases hormone production and enzyme activity (Verma and Verma, 2002). The slight variations between the works and the current work could be attributed to the experimental design adopted, soil where the rice samples were planted, and variety including climate of origin. These results support effective uses of germinated rice grains for consumption and rice leaves for pharmaceutical application.

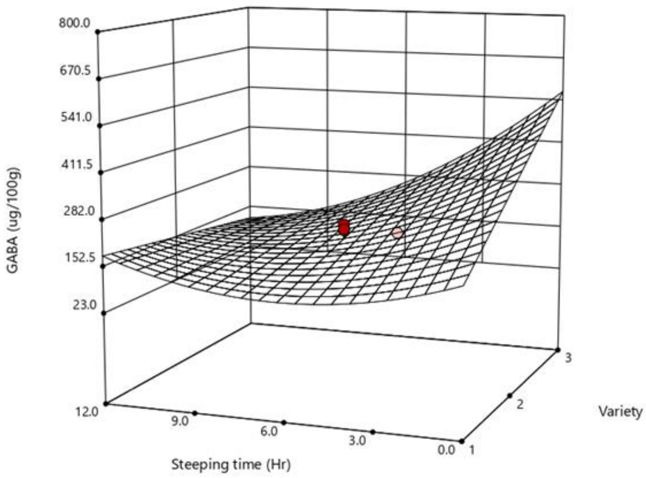

FIGURE 1: Response surface plot of GABA against steeping time and rice variety

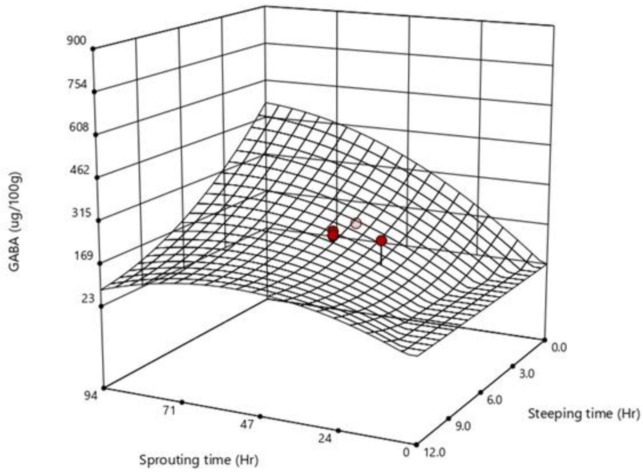

FIGURE 2: Response surface plot of GABA against sprouting time and rice variety

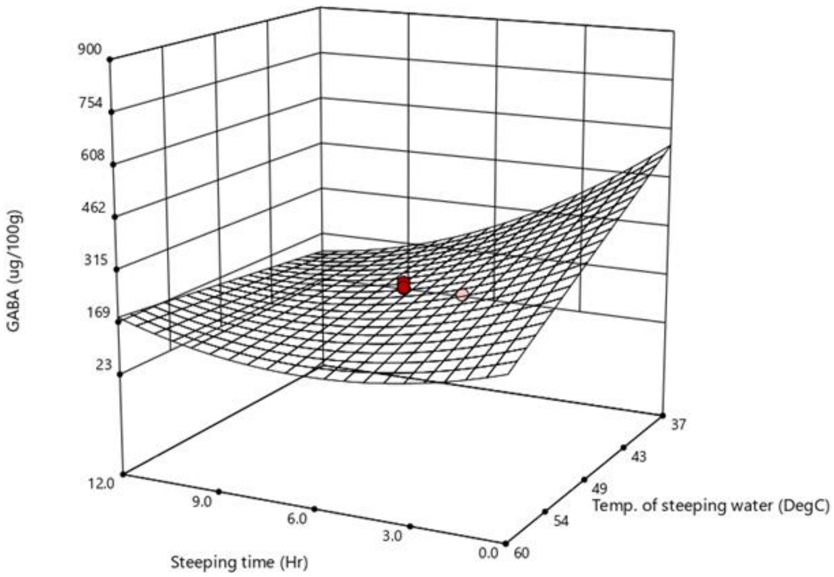

FIGURE 3: Response surface plot of GABA against sprouting time and rice variety

3.1 Predictive model of gamma-amino butyric acid

GABA = $174.94 + 34.95 X_1 - 47X_2 - 74.01X_3 - 25X_4 - 87.78X_1X_2 - 114.89X_1X_3 - 202.41X_2X_3 - 110.46X_2X_4 + 113.04X_3X_4$ (2)

Colour

Table 3 presents effect of variety, sprouting time, steeping time and steeping water temperature on colour of GBR. The model of the parameter was not significant ($p>0.05$), the linearity coefficient of the model appeared to be reliable (R^2 = 0.5149) with mean score of 6.19 and standard deviation of 1.45.Colour plays an important role in food choice. The attribute influences taste thresholds, sweetness perception, food preference, pleasantness, and acceptability (Varanyabind *et al.*, 2005). Colour is elusive and difficult to quantify, but is really a psychological attribute in food choice. The nature of colour places the attribute in a secondary role to the other sensory characteristics, a position not entirely consistent with the facts. According to Totayava *et al*., (2015) color in a quantitative sensehas been shown to be able to replace sugar and still maintain sweetness perception in flavored foods. Colour interferes with judgments of flavor intensity and identification and in so doing has been shown to dramatically influence the pleasantness and acceptability of foods (Totayava*et al*., 2015).Due to the complexity of colour attributes, it was not possible to relate effect of any of the variables to colour and acceptance of the rice samples. Fig. 4, 5, 6 and 7 show the influence of variety (X_1), sprouting time (X_2), steeping time (X_3) and steeping water temperature (X_4) of colour. Model 3 corroborates the observations that sprouting time and variety of colour in the products.

Predictive model for the colour of germinated brown rice

$$\textbf{Colour} = 6.22+0.0004X_1 - 1.25X_2 + 0.737X_3 + 0.0009X_4 + 0.125X_1X_2 + 0.375X_1X_3 - 0.126X_1X_4 + 0.125X_2X_3 - 0.125X_2X_4 + 0.125X_3X_4 \quad (3)$$

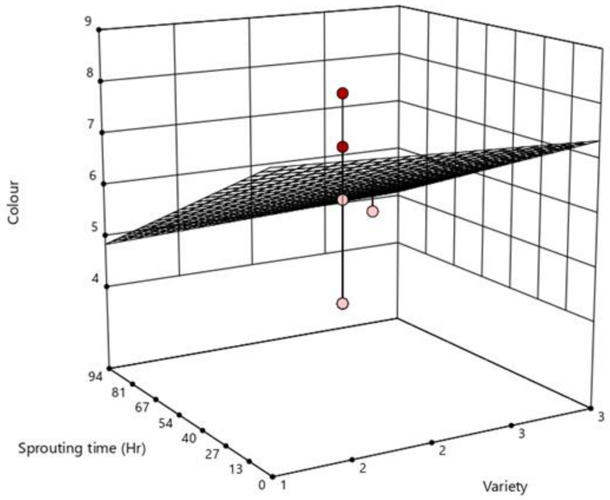

FIGURE 4: Response surface plot of colour of GBR against sprouting time and rice variety

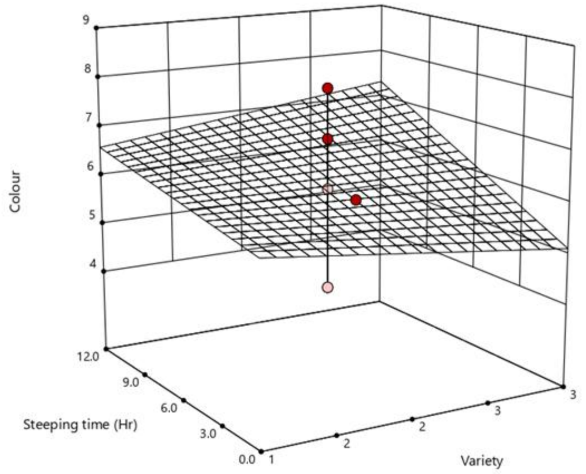

FIGURE 5: Response surface plot of colour of GBR against steeping time and sprouting time

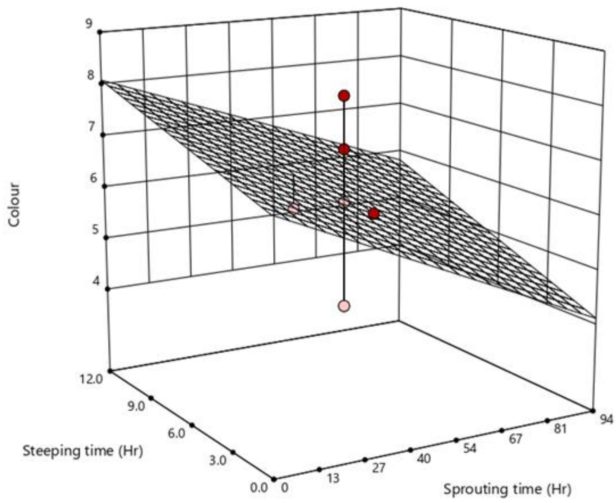

FIGURE 6: Response surface plot of colour of GBR against temperature of steeping time and steeping time

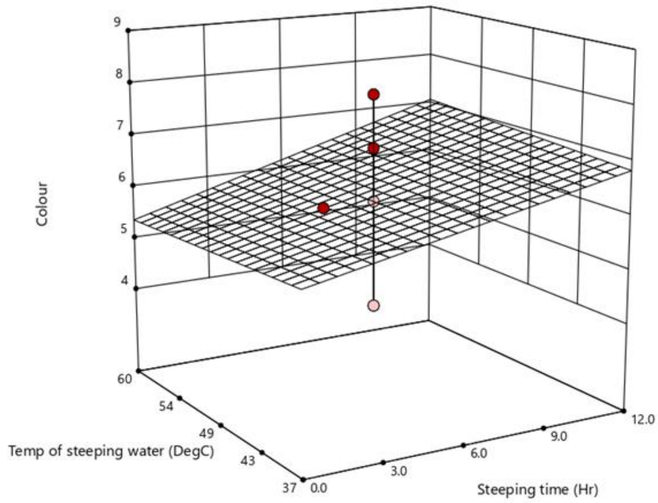

FIGURE 7: Response surface plot of colour against temperature of steeping water and steeping time
Taste

Taste

Table 3(c) presents the coefficient of estimates of taste model of GBR. According to the table, the model was not significant ($p>0.05$), R^2 of 0.5073 appeared to be significant, but the mean score on the parameter was 5.15 of the 9-point herdonic scale. No single or interactive terms seemed to be significant. However, Figs. 8, 9, 10 showed some level of influence of the variables in the terms of the model of colour. Model 4 shows the mathematical contribution of the variables to the parameter. Taste is a primary determinant of food choice and acceptability hence both go often. Genetic differences in the ability to perceive bitter taste are believed to play a role in the willingness to eat bitter-tasting vegetables and in the preferences for sweet taste and fat content of foods (Rossella *et al.*, 2011). Taste of a new product can increase acceptability if health claims is attached.

Predictive model for taste of germinated brown rice

$$\textbf{Taste} = 5.0 - 0.001X_1 - 0.5739X_2 + 0.582X_3 + 0.299X_4 - 0.326X_1X_2 - 125X_1X_3 - 0.449X_1X_4 + 0.375X_2X_3 - 0.875X_2X_4 + 0.125X_3X_4 \quad (4)$$

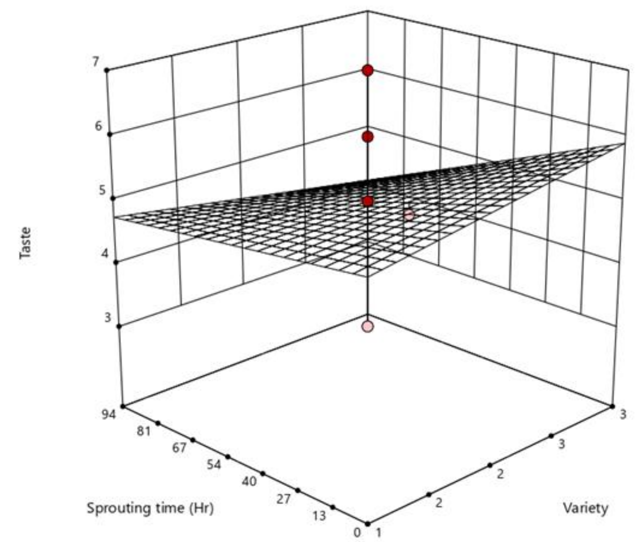

FIGURE 8: Response surface plot of sprouting time and variety on taste of GBR samples

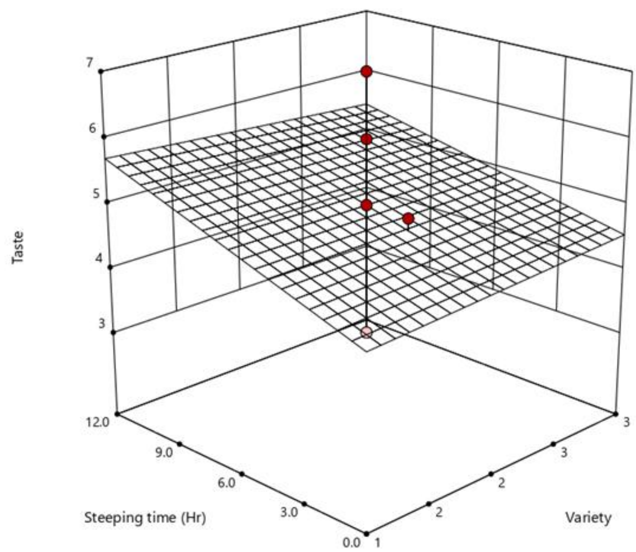

FIGURE 9: Response surface plot of steeping time and variety on taste

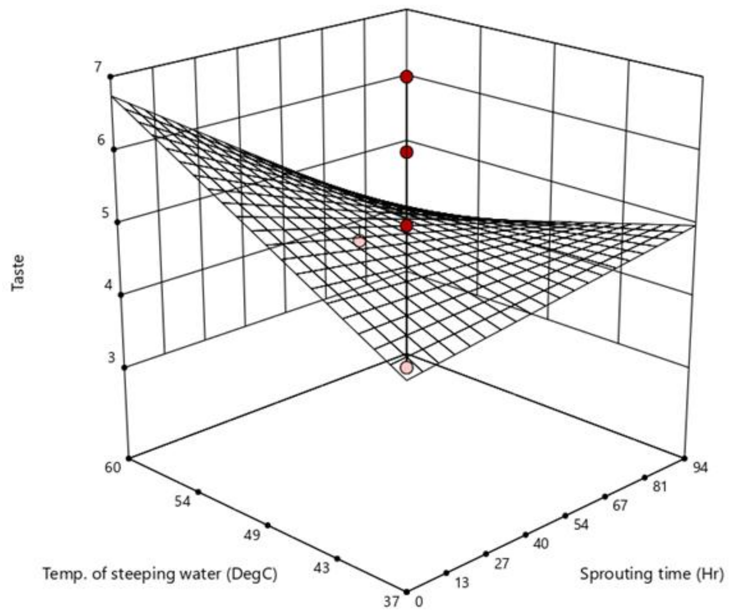

FIGURE 10: Plot of influence of temperature of steeping water and sprouting time on samples on the taste of GBR meal

Table 3(d) presents the contribution of the processing variables to the general acceptability of the germinated brown rice meal. The table shows that the model was not significant ($p>0.05$), the linearity coefficient ($R^2=0.5087$) appeared to be significant, with a mean herdonic score of 5.56, and a standard deviation of 1.45. In this study, the observation was expected since acceptability is influenced by colour and taste (Charles, 2015). According to (Rogers, *et al.*, 1993), a defect in any of the parameters could have a very serious effect on acceptability. Simply put, food acceptability directly relates to the interaction it might exercise on the consumer at a given moment in time. The key factors that determine food acceptability are the sensory characteristics of food especially since consumers seek foods with specific sensory properties. Figs. 10, 11 and model 5 illustrate the observation.

3.2 General acceptability of the germinated brown rice meal

The model of general acceptability of the germinated brown rice samples was not significant (p>0.05, the $R^2$of 0.5073 appeared to be significant, but not reliable for the prediction of the parameter under different values of the same variables. The low standard deviation suggested unanimity of low acceptance score of the samples of hedonic mean score of 5.14. Germinated brown rice was an unfamiliar version of rice product; like any new product, high acceptability was not expected. The observation was in agreement with the observation of Murray and Baxter, (2003) in Sensory Evaluation who reported that food acceptability is affected by many factors, which are mostly specific to the individual consumers, the food, or the environment in which the food is consumed.

Acceptability is a subjective measure based on hedonics (pleasure), which in turn is influenced by the sensory properties of the food, previous exposure to it and subsequent expectations, contextual factors, an individual's culture, physiological status (i.e., hunger, thirst, and presence/absence of illness), and many other variables. The measurement of food acceptance is highly complex and relies on psychometrics (scales) and/or behavioral models (food-choice models) (Rogers, *et al*., 1993). Figure 11, 12, 13, and 14 show the relationship between the parameter and the process variables. The response of the parameters to the independent variables did not seem to be consistent. Equation 5 supports the observation.

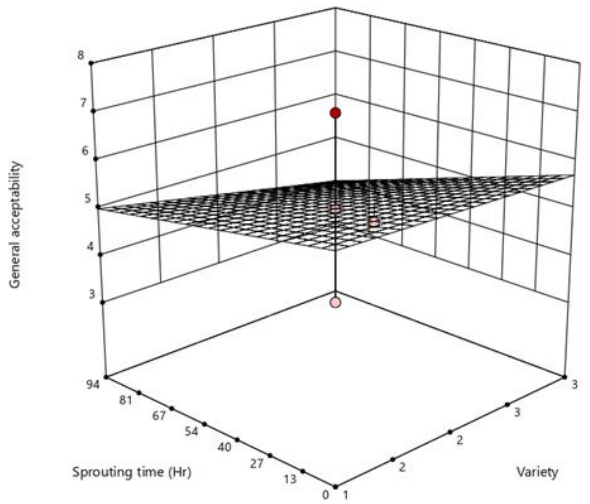

FIGURE 11: Plot of influence of steeping time and variety on general acceptability of samples of GBR meal

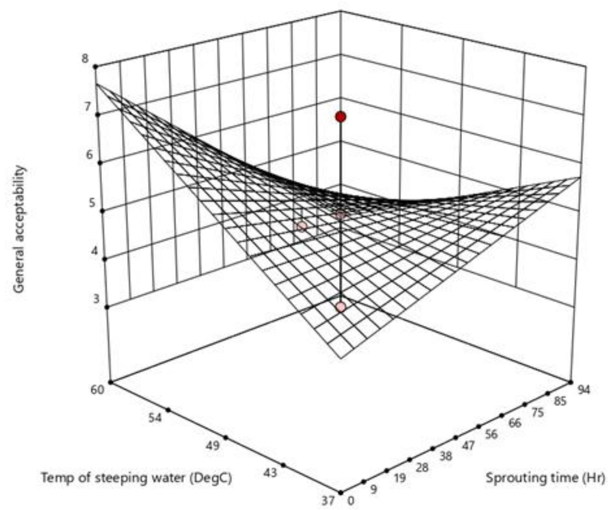

FIGURE 12: Plot of influence of temperature of steeping water and sprouting time on general acceptability of samples of GBR meal

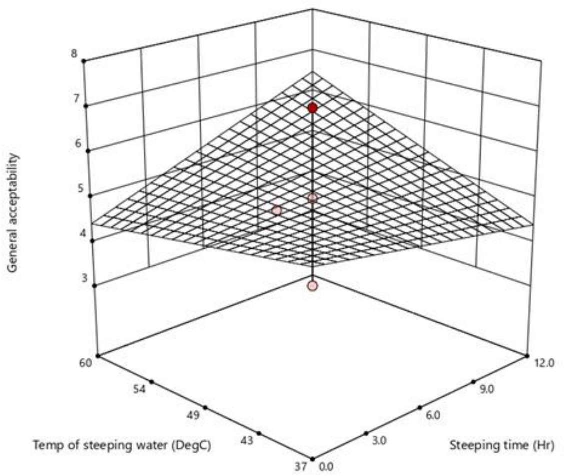

FIGURE 13: Plot of influence of temperature of steeping water and steeping time on general acceptability of samples of GBR meal

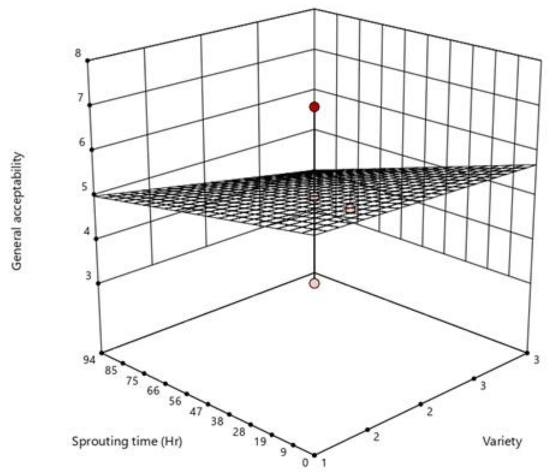

FIGURE 14: Plot of influence of sprouting and variety on general acceptability of samples of GBR meal

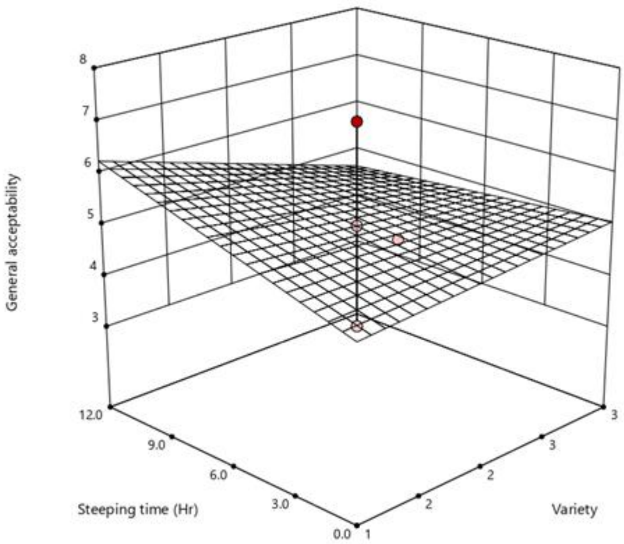

FIGURE 15: Effect of variety and sprouting time on general acceptability of GBR samples

3.3 Prediction model of acceptability

Acceptability = $5.17 – 0.297X_1 - 0.652X_2 + 0.287X_3 + 0.299X_4 – 0.201X_1X_2 - 0.50X_1X_3 – 0.652X_1X_4 - 0.250X_2X_3 – 0.1.55X_2X_4 + 0.750X_3X_4$ (5)

3.4 Optimization

Optimization procedure showed that Vara 44 rice variety, sprouting time of 91.42 hours, 0.023 hour and steeping water temperature of 37^oC produced 906.64 µg/100g of GBR, 4.53, 4.19 and 7.073 GABA, colour, taste and acceptability on the GBR respectively at 92.8% desirability.

IV. CONCLUSION

The present study confirmed that sprouting and sprouting time could increase the concentration of gamma-amino butyric acid in paddy rice irrespective of variety. The absence of moisture in adequate levels cannot induce germination. The brown rice that originated from the experiment showed lower acceptability due to strangeness of the product, hence organoleptic parameters. Education of the target population on the function of GABA and the need to accept the product may lead to higher acceptability irrespective of poor hedonic levels. It is recommended that important of brown rice and related health products should be restricted to encourage local production.

ACKNOWLEDGEMENTS

The authors acknowledge the sponsorship of Tertiary Education Trust Fund (TET Fund) on the Institution-Based Research Grant, Batch 5, (2017) AkwaIbom State Polytechnic, IkotOsurua, to complete the work. The Department of Science Laboratory Technology, AkwaIbom State Polytechnic and the Department of Chemistry, University of Uyo, AkwaIbom State are appreciated for providing facilities for chemical analysis of the plant samples. The rice farmers of Ini Local Government Area are appreciated for providing advice on rice cultivation.

REFERENCES

[1] Bown, A. W., Macgregor, K, B., &Shelp, B. J. (2006). Gamm-aminobutyrate: Defense against invertebrate pests? *Trends in plant Science*, 11(9), 424-427. PMid: 16890474. http.//dx.doi.org/10.1016/j.tplants.2006.07.002.

[2] Charles,S. (2015). On the psychological impact of food colour. *Flavour*.4:21.DOI 10.1186/s13411-015-0031-3.

[3] Chaiyasut, C., Bhagavathi, S.S., Noppawat, P., Manee, S.P.K., Sasithorn, S., Pranom, F., Korawee, J., Suchanat, K. and Sartjin P. (2017). Optimization of conditions to achieve high content of gamma amino butyric acid in germinated black rice, and changes in bioactivities. *Food Science and Technology*, 37(1), 318 – 326. https://doi.org/10.1590/1678-457x.33416

[4] Fengfeng, W., Na, Y., Alhassane, T., Zhengyu, J. and Xueming, X, (2013). Germinated riceand its role in human health: critical reviews in food science and nutrition, 53(5): 451-463.

[5] Finney, P. L. (2002). Potential for the Use of Germinated Wheat and Soybeans to Enhance Human Nutrition,Advances in Experimental Medicine and Biology, 105 Nutr. Improv. Food Feed Proteins, 681-701, CODEN: AEMBAP ISSN: 0065-2598, 1978.

[6] Hagiwara, H., Seiki, T., Ariga, T. (2004). Effect of pre-germinated brown rice intake on blood glucose and PAI-1 levels in Streptozotocin-induced diabetic rats. *BiosciBiotechnolBiochem,* 68:444-447.

[7] Jirapa, K., Jarae, Y., Phanee, R., &Jirasak, K. (2016). Changes of bioactive components in germinated paddy rice *(Oryza sativa L.) International Food Research Journal,* 23(1), 229-236.

[8] Lin, E. O. (2000). Handbook of sensory Methods and Analysis. PROJOINT Communications Services Ltd. Enugu. Pp 70-72.

[9] Magali, L., Arnaud, L., Sabuktagin,R., Tahmeed,A., Ahmed, S. R., Nurul,A., Santhia,I.,and Dora, P. (2016). An assessment of the potential impact of fortification of staples and condiments on micronutrient intake of young children and women of reproductive age in Bangladesh. *Nutrients*. pp 1-12.S.

[10] Mie, Nishimura,Shin-ichi Yoshida, MasafumiHaramoto, Hidenori Mizuno, Tomohiko Fukuda, HiroyoKagami-Katsuyama, Aiko Tanaka, Tatsuya Ohkawara (2016).J Tradit Complement Med. 6(1): 66–71. doi: 10.1016/j.jtcme.2014.11.022.

[11] Montgomery, D. C. (1998). Experimental design for product process design and development. The Statistician, 48, Part 2, pp 159-177.

[12] Murray, J. M. and Baxter, I. A. (2003). Sensory Evaluation. Food Acceptability and Sensory EvaluationinEncyclopedia of Food Sciences and Nutrition (Second Edition),

[13] Mustapha, U. I., Mazna, I., Hairuszah, I. and Abdul, R. O. (2013). Effect of germinated brown rice and its bioactivecompounds on the expression of the peroxisome proliferation activated receptor gamma gene. Nutrients, (5): 468-447.

[14] Nathalie, M. M. and John, S. M. (2013). The role of rice in changing fod consumption patterns in West Africa Foundation for sustainable agriculture. 3rd Africa Rice Congress. 21st to 24th October, 2013. Nairobi, Camaron.

[15] Okada, T., Sugishita, T., Muria, H., Saikusa, T. (2000). Effect of the defated rice germ enriched with GABA for sleeplessness, depression, autonomic disorder by oral administration. Nippon ShokihimKaishi 47: 596-603.

[16] Rogers, E. J., rice, S. M., Nicolos, R. J. Carpenter, D. R. McClelland, C. A. and Romanxzyk, L. J. (1993). Identification and quantification of –orizanol components and simultaneous assessment of tocoals in rice bran. Joirnal Oil Chemist' Society, 70:301-307.

[17] RossellaNegri, _Mariarosaria Di Feola, _Simone Di Domenico, _M. GiuseppaScala,GinevraArtesi, _Serena Valente, _Andrea Smarrazzo, _Francesca Turco, yGabriellaMorini, and _Luigi Greco (2011). Taste Perception and Food Choices. Journal of pediatric gastroenterology and nutrition 54(5):624-9.

[18] ThithimaKaosa-ard and SirichalSongsermpong (2012). The influence of germination time on the GABA content and physical properties of germinated brown rice. As. J. Food Ag-Ind. 5(4): 270-283.

[19] Totayava, D. D., Hettiarachey, N., Horex, Ronny, Eswaranandam, S., Jha, A. (2014). Effect of germination on nutrient composition of long grain rice and its protein physicochemical and functional properties, J of Food Nutr 1: 1-9.

[20] UN Food and Agriculture Organization, Corporate Statistical Database (FAOSTAT). (2018). Archived from the original on May 11, 2017. Retrieved October 11, 2019.

[21] Varanyabind, W., Tungrtkul, P., Suronjanaametakul, V., Watanasiritham, L. and Luxiang, W. (2005). Effects of water soaking on gamma-aminobutyric acid (GABA) in germ different Thai rice varieties. Kasetsart Journal (Natural Science) 39:-411-415.

[22] Verma, S. K. and Verma, M. (2002). A Textbook of Plant Physiology, Biotechnology and Biotechnology. Chand & Company Ltd. New Delhi. pp 111.

[23] Woraharn, S., Lailerd, N., Sivamaruthi, B. S., Wangcharoen, W., Sirisattha, S., Peerajan, S., &Chaiyasut, C. (2016). Evaluation of factors that influence the L-glutamic and γ-aminobutyric acid production during Hericiumerinaceus fermentation bylatic acid bacteria. *CyTA-Journal of Food*, 14(1), 47-54. http://dx.doi.org/10.1080/19476337.2015.1042525.