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Research Article

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Optimization of Growth Performance of Micronutrient Fortified Predigested Soymilk for Complementary Feeding

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Abstract Fortified soymilk extracted from 12hrs tap water steeped and 72hrs sprouted TGX 923-2E soybean variety was optimized using central composite design (CCD). Box-Wilson (1951) experimental design matrix was used for the fortification which comprised three variable factors (K=3) namely ferric ammonium citrate (Fe), calcium carbonate (CaCO3) and vitamin C (VC). Data on each run were statistically regressed with Minitab (version 11.21) computer software while response surface plots were generated with Matlab (version R2007b) computer software. Regression models showed that the three combinations of the experimental variables had cross product effects on growth performance with maximum growth performance from fortificant combinations 5, 150, 24 for iron, calcium carbonate and vitamin C respectively.

Keywords Growth performance, micronutrient, complementary feeding, fortified soymilk, and response surface methodology

Introduction

Infancy is a period of tremendous physical growth characterized by increase in length, weight, physiological, immunological and mental development [1] which cannot be met beyond six months by human milk alone due to large compositional variations between nutrients in lactating mothers [2]. Besides, preterm infants however have growth rate approximately three times higher than that of a mature-term infant resulting in higher requirements for both proteins and minerals for skeletal development which cannot be met by mother's milk alone.

A complementary food is therefore introduced to improve both the energy and nutrient intakes since the child will no longer gain weight despite appropriate breast feeding, and will be feeling hungry always despite frequent breast feeding [3].

Complementary foods are nutritional companion to breast feeding infants and young children after six months [4] or additional foods introduced to the diet of breast-or-formula-fed infants during transition to an adult diet [4-6]. Actually, they are non-breast milk or nutritive foods which may be solid, semi solid, liquid [6-7], or specially prepared foods or modified family meals [5]. Generally, complementary foods are introduced as readily consumed and digested foods to provide additional nutrition to meet the extra needs of growing children during their vulnerable time [4, 8] most especially vitamins, minerals, proteins and carbohydrates [4] to reduce the high risk of malnutrition and illnesses [5]. This period of introduction is called complementary feeding period which usually begin at 4 to 6months till two years or beyond. Sometimes, complementary foods are also used to rehabilitate undernourished children especially those recovering from illness [6], and as an emergency intervention feeding package [9]. Good complementary foods include those foods that are affordable, rich in energy, protein, micronutrients (particularly Iron, zinc, calcium, vitamins A, C and folate), clean and safe with no pathogens (disease causing bacteria or other harmful organisms), locally available and affordable, easy to prepare and others [5].

Soymilk, also known as vegetable or imitation milk produced from whole soybean (*Glycine max*) resembles cow's milk in appearance, flavour and nutritive value. Soybean, like all other legumes are endowed with some useful human nutrients which are only available to consumers through processing into palatable foods like soymilk [10] using good manufacturing process (Clarke, 1995; Anjum *et al*., 2006). Soymilk contains 8.25% solid-not fat, not less than 3.25% fat, not more than 88.50% water and not less than 11.50% solids including 3.25% fat when properly processed [10]. These have drawn the attention of many researchers for adoption as breast milk substitute [11] and infant formulas [12]. Essential amino acids pattern of soy products, cow's milk and human milk compared to infant requirements have been highlighted [13]. However, vitamin and minerals content of soymilk are lower [14] than human milk and recommended daily intake (RDI) which calls for fortification for complementary feeding [15]. Acceptability of soymilk for infant food formulation depends not only on its nutritional and functional properties, but also on its shelf stability and nutrient bioavailability [12].

Food fortification, a deliberate addition of one or more essential nutrients to foods [16-17] whether or not it was normally contained in the food had long been adopted to complement the nutrient content and their bioavailability in foods provided the fortificants are compatible to the food matrix in question [16]. Successful fortification of varieties of foods including soymilk with vitamin B12, fat (including omega fatty acids and fish oil), ascobates and calcium to prevent development of their deficiency symptoms in infants including children and adults had been reported [15, 18]. Food fortificants like iron, calcium and vitamin C work in synergy to promote growth performance [15, 19].

Germination had long been adopted as a traditional technology to lower bulk density, increase energy and nutrient density of legumes and cereals used in formulating local complementary foods [20]. Soybean germination (sprouting) increases soymilk quality, decreases oil content, increases bioavailability of many essential nutrients [21], improves digestibility [10, 21] and stability of foods [12] both in food industries and in home preparation of local staples for complementary feeding [4,6]. Germination also eliminates soymilk anti-nutrients and endows soymilk with some desirable healthy benefits [10, 12].

Some *in vivo* studies for man, rodent animals (mainly rats and mice) and albino rats have been advanced [3, 22] along with some cost effective trails to assess growth performance after feeding the experimental diets for predetermined time. Parameters accessed include weight, height and length gains [23].

Response surface methodology (RSM) had been adapted into food process optimization to predict the values of dependent response variables by determining the optimum process variable combinations that maximizes or minimizes the product response [24]. This work aims at optimizing the growth performance of the micronutrient fortificants in fortified soymilk.

Materials and Methods

TGX923-2E variety of soybean was procured from the National Cereal Research Institute (NCRI) outstation, Amakama Olokoro while National Root Crop Research Institute (NRCRI) Umudike both in Abia State, Nigeria provided analytical space.

Preparation of soymilk

A 12hrs tap water steeped previously cleaned and sorted soybeans were sprouted for 72hrs while been moistened as soon as their surfaces dried on jute sack spread on the floor [21, 25]. The sprouted beans were boiled in 0.5%$NaHCO_3$ solution for 20min, drained [26], allowed to cool and hand-dehulled. The hulls and the shoots were removed by water floatation leaving soybean cotyledons that were milled to slurry in QLink (Japan) variable speed kitchen blender with hot water (93^oC) in a ratio of 2.7 parts hot water to one part cotyledons (v/w) [14, 25]. Soymilk was obtained by screening the slurry through a double layered muslin cloth [12].

Fortification of soymilk

The oil content of soymilk (1.8%) quantified by Soxhlet method was marked up to 3.5% with soybean oil [10], and the bulk was divided into15 batches and fortified according to design matrix (Table 2). Each batch which represents an experimental run or trial was bottled separately, coded and sterilized at121^oC for 5min, cool and stored in the refrigerator for feeding trials.

Animal (*in vivo*) study

Male (n = 50) suckling albino rats purchased from the Veterinary Department of the University were used for the study [3, 22] because of their rapid growth rate than human. The rats with an initial weight range between 30 to 45g were randomly assigned into 10 groups of five rats each and housed in iron cages in the laboratory (25 to 32^0C).

The rats were fed for three days with normal feed for acclimatization before the experimental feeding. All the rats were fed *ad libitum* with the experimental samples except those in cage 0 that were fed with unfortified sample. Rats in cages 1, 2, 3, 4, 5, 6, 7, 8 and 9 were fed with the samples from experimental runs 1, 4, 5, 8, 9, 10, 11, 13, and 15 respectively. The feeding lasted for two weeks. During the feeding trial, their weights were taken at every 48hrs intervals [3, 23]. The adequacy of the formulation was ascertained (Figure 1) by plotting the weight gain against time [22].

Experimental design and statistical analysis

A central composite design (CCD) comprising 3 variables with 5 level combinations were used to generate a total of 21 experimental runs with 6 replications only at center point [27]. The runs which covered the entire range of spectrum of combinations of variables were employed to study their linear, quadratic, and cross product effects on growth performance (Table 2). Each of the variables namely ferric ammonium citrate, calcium carbonate and vitamin C was coded -0.682, -1, 0 +1, and +0.682 (Table1) and used for the fortification (Table 2) according to the experimental design matrix [28].

All the analytical determinations were conducted in triplicate to obtain data that were statistically regressed and analyzed for variance using Minitab (version 11.21). Plots of the fitted significant responses were made using Matlab (version R2012) software to visualize the effects clearly. Coefficient of determination (R^2) and correlation coefficient (R) were used to determine the precision while analysis of variance (ANOVA) was used for adequacy or fitness of the model to predict the responses. Statistical significance was accepted at 5% probability level ($p \leq 0.05$).

Table 1: Experimental variables (k = 3) with their 5 coded combinations used for the central composite rotatable design.

Independent variables	**Code**	**K = 3**	**Variable levels**				
(mg/100ml)			-1.682	-1	0	+1	+1.682
Ferric ammonium citrate	Fe	X_1	1	2	3	4	5
Calcium carbonate	Ca	X_2	50	100	150	200	250
Vitamin C	VC	X_3	8	16	24	32	40

Table 2: Experimental design matrix for coded, real independent process variables and responses

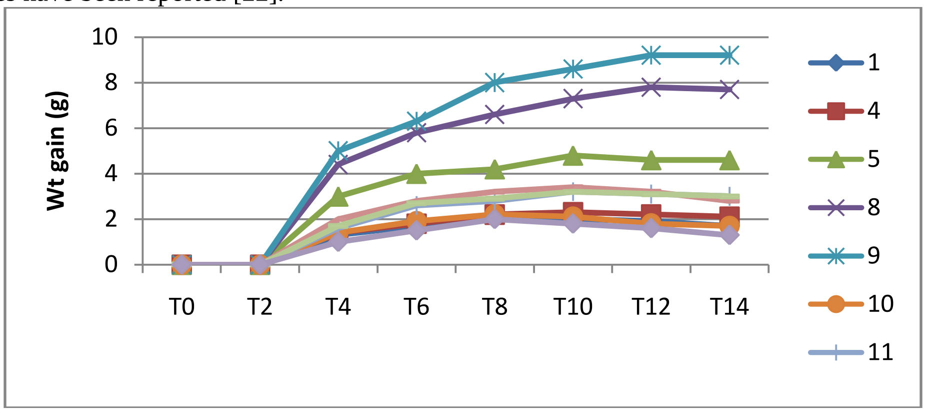
Experimental Runs	**Coded Variables**	**Independent**	**Processes**	**Real Independent**	**Process Variables**		**Responses**
	X_1	X_2	X_3	Fe (X_1)	Ca(X_2)	C (X_3)	Y
1	-1	-1	-1	2	100	16	1.7
2	-1	- 1	1	2	100	32	1.8
3	-1	1	-1	2	200	16	1.7
4	-1	1	1	2	200	32	2.1
5	1	-1	-1	4	100	16	4.6
6	1	-1	1	4	100	32	6.4
7	1	1	-1	4	200	16	4.8
8	1	1	1	4	200	32	7.7
9	1.682	0	0	5	150	24	9.2
10	-1.682	0	0	1	150	24	1.7
11	0	1.682	0	3	250	24	3.0
12	0	-1.682	0	3	50	24	2.8
13	0	0	1.682	3	150	40	2.8
14	0	0	-1.682	3	150	8	2.8
15	0	0	0	3	150	24	3.0
16	0	0	0	3	150	24	2.8
17	0	0	0	3	150	24	2.9
18	0	0	0	3	150	24	3.0
19	0	0	0	3	150	24	3.1
20	0	0	0	3	150	24	2.9
21	0	0	0	3	150	24	2.7
0	0	0	0	-	-	-	1.3

X_1(Fe), X_2(Ca) and X_3(Vit C) represent respective concentrations (mg/100ml) of ferric ammonium citrate, calcium carbonate and vitamin C fortificants used in the fortification trials. Each row represents a fortification trial run or adjustment levels of the process variable combination of one run. Y represents responses of growth performance for the runs.

Results and Discussion

Growth Performance of Fortified Soymilk on Albino Rats

Weight gains and growth rates in each group of albino rats obtained during a 14 days *Ad libitum* feeding trials are shown in Table 2 and Figure 1 respectively. Rats fed with fortified samples had more weight gains and higher growth rates than those fed with unfortified sample depending on the types and levels of fortificants in the experimental runs. Mortality rate of three rats were recorded only in group fed with unfortified soymilk. Maximum growth was recorded from rats fed with fortificant combinations 5,150, 24 mg/100ml for X_1, X_2 and X_3 respectively on the 12th day. After the peak growths, all the rats started exhibiting weight decline (Figure 1). Similar weight gains and growth rates have been reported [22].



Nos 1, 4, 5, 8, 9, 11, 13, 15 and 0 each represents an experimental run fed to each group of albino rats.

Figure 1: Plots demonstrating growth performance of 8 groups of albino rats fed Ad libitum with some selected fortified soymilk samples for 14 days

Table 3: Regression Co-efficient and ANOVA of Fortified Soymilk on Growth Performance

Predictor	**Constant**	**X_1**	**X_2**	**X_3**	**X_1X_2**	**X_1X_3**	**X_2X_3**	**X_1^2**	**X_2^2**	**X_3^2**
Co-efficient	0.23434	0.02344	0.01914	0.01958	-0.10270	-0.10295	-0.12278	-0.05114	-0.00236	-0.00306

X_1, X_2 and X_3 are the linear coded terms of respective fortificants. X_1X_2, X_1X_3 and X_2X_3 are the interaction coded terms of the respective fortificants. X_1^2, X_2^2, and X_3^2 are the quadratic coded terms of the respective fortificants.
R=9.39, R^2=88.3% and ANOVA=0.064

(a) (b)

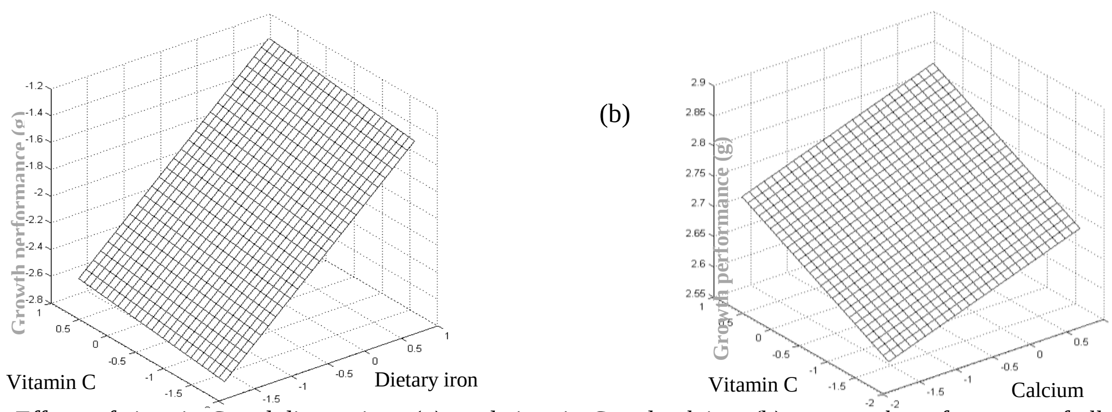


Figure 2: Effects of vitamin C and dietary iron (a), and vitamin C and calcium (b) on growth performance of albino rats fed with fortified soymilk.

Regression analysis (Table 3) showed that all the three variables had significant ($P \leq 0.05$) effects only at interactive level on growth performance with that between calcium and vitamin C having the strongest with positive coefficient to increase growth performance. All the three interactions between any two of the three variables accounted for 88.3% of the total variations in the growth performance. Correlation coefficient (R) of 0.94 indicated a very high correlation between the experimental and predicted values of the model (Table 3). Second order model will best fit into this work. The predictive equation on growth performance (GP) after removing the non-significant terms becomes:

$$GP = 0.234 + 0.124\, X_2X_3 - 0.103\, X_1 X_2 - 0.103X_1X_3 \quad (2)$$

This equation indicated that GP is dependent on the interactive effects of the fortificants which implies that the fortificants work in synergy to effect significant growth as no single fortificant had significant growth effect. All the three fortificants are therefore major contributors to GP.

ANOVA (Table 3) indicated significant ($p > 0.05$) interactive effects of the three variables on growth performance (GP). Effects of any two of the three variables on GP can be visualized (Figure 2a and b). General increase in consumption of the samples by all groups of albino rats after the adjustment periods of 0 to 2days (Figure 3) points to adequacy of the processing methods which translated into palatability, tolerance and acceptability. This adjustment period may be attributed to tolerance period during which the rats were trying to adjust to the drinkers that are different from the teats of udder they were used to. This may have resulted in little or no feed intake without apparent weight change. Reduction in appetite and suckling stimulus at the nipple in infants with early supplementation of breast milk with artificial foodstuffs had been acknowledged [29].

Poor growth rate of rats fed with unfortified samples could be attributed to inability of the sample nutrients to meet the demand of the growing rats or inadequate nutrient intake, hence the lower peak growth (<8 days) compared to those from fortified samples (>8 to 12days). General decline in weight gains of the rats after their peak days possibly may be due to inability of the samples to continue meeting their nutrient needs due to their rapid growth rate which increases with time. This may mean that the rats at this point may need introduction of semi solid and solid foods to meet their nutrient and energy needs for proper growth and development [3].

Synergetic effect may be attributed to the individual contributions of the fortificants and soymilk components on GP. Vitamin C and soymilk oligosaccharides (prebiotics) enhance iron absorption [30]. Absorbed iron metabolizes protein for absorption and increased protein efficiency ratio [15] which supports growth performance [31]. Protein provides energy and enhances calcium absorption with the aid of vitamins C and D. Further more, calcium enhances vitamin B_{12} absorption and metabolizes vitamin D [15]. Proteins, minerals and water are needed for growth, repairs, energy and regulations of body processes. Therefore, soymilk which is predominantly water and protein [10] may have supported growth. Multiple fortification of soymilk in this study is therefore appropriate, adequate and validated the report of [32] that diet related micronutrient deficiencies rarely occur in isolation. Besides, dietary iron is essential to most life forms and normal human physiology. Iron is an integral part of many proteins that maintain good health, and regulates cell growth and differentiation, DNA synthesis, energy metabolism and forms myoglobin [15]. Calcium is important during phases of growth, helps nutrient flow across the cell walls, helps in healthy development and growth of baby's bones, teeth, [33] muscle and heart [34]. Further more, $CaCO_3$ is highly bioavailable form of calcium [35] which is well absorbed when taken in small amounts [19]. However, the levels of calcium recorded in this study, possibly may have contributed to growth. Similarly, vitamin C is required for formation and maintenance of bones, blood vessels and skin [36]. Vitamin C helps to maintain healthy diets, which in turn maintains good health necessary for human survival and aids iron absorption (Rahman Khan *et al*., 2006). Vitamin C also is very important for the break down of carbohydrates, proteins and lipids which are energy components of the diet as well as building blocks in the body [37].

Other factors that may have contributed to GP are bean germination which had been reported to reduce anti-nutritional factors like phytic acid and phytates [38], degrades proteins and carbohydrates macromolecules into smaller units to increase their surface area for a facilitated digestion and subsequent absorption [31] which validated the aim of introducing CF_S [8]. Germination also reduces nutrient-nutrient interactions and may have supported nutrient availability as nutrient interactions are nutrient depletion [32].

Conclusion

Food nutrients work in synergy for positive significant growth effect as obtained in this work where maximum growth was obtained due to synergetic effect of calcium and vitamin C. No single nutrient can effect a significant growth effect. Balance diets with essential micronutrient levels up to their RDI will go a long way to effect significant growth effect.

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