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Research Article

Response Surface Analysis for Acceptability Assessment of Micronutrient Fortified Un-Stabilized Soymilk from Sprouted Soybeans

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Abstract

Soymilk is deficient in vitamins and mineral which calls for fortification. Such fortification may affect soymilk appearance, consistency and general acceptability depending on the types and levels of fortimicins used. This study tends to optimize the effect of ferric ammonium citrate, calcium carbonate and vitamin C on the acceptability of predigested soymilk. Soymilk prepared from 72h sprouted TGX 923 - 2E soybean variety after steeping for 12h was fortified with ferric ammonium citrate (Fe), calcium carbonate (Ca) and vitamin C (Vit. C) as fortify cant variables according to Box-Wilson (1951) experimental design matrix. Fifteen samples generated were separately subjected to consumer preference test using twenty five semi-trained panelists. A central composite response surface design (CCRSD) for K = 3 was employed to study the linear, quadratic and cross product effect of appearance and viscosity (consistency) ratings of the panelists on the general acceptability of the fortified soymilk. Response surface data for each run was regressed and analyzed for variance using Minitab computer software (version 11.21). Results obtained showed that ferric ammonium citrate had significant increase effect on appearance and decrease in general acceptability while calcium carbonate had significant increase in viscosity. Sample with fortification levels of 2, 100 and 32 for ferric ammonium citrate, calcium carbonate and vitamin C respectively had the highest acceptability of 6.74 (Like moderately).

Keywords: Acceptability; Fortified Soymilk; Sprouted Soybean; Response Surface Analysis

Introduction

Soymilk, an aqueous extract of soybean (*Glycine max*) is endowed with useful essential human nutrients [1,2] and proven evidence of a good substitute for cow`s milk [2]. Soymilk acceptability when fortified with deficient nutrients like vitamins and minerals [1,3] for human consumption [4,5] is best assessed by subjecting it to consumer preference test using a 9 - point Hedonic scale which measures the degree of liking and disliking [6].

Food fortification, a deliberate addition of one or more nutrients to a food whether or not it was normally contained in the food has been adopted as an intervention program for preventing or correcting demonstrated deficiency of one or more nutrients in the population or specific population groups [7,8]. Fortification and formulation of foods have been applied in the manufacture of complementary foods from local staples to meet the nutrients and energy needs of infants and young children [9,10]. Besides, varieties of calcium and vitamins B and D fortified beverages have been advanced with the sole aim of providing reasonable percent of their RDI to prevent their deficiency symptoms [11]. Similarly, legume sprouting has been an age long method used by pioneer researchers to increase the nutrient content and bioavailability of essential nutrients, eliminate anti-nutrients and endow soymilk with some health benefits [12]. Sprouting also reduces flatulence [13] and trypsin inhibitor [14,1], and stabilizes soymilk [15].

Response surface methodology (RSM) has been adapted into food process optimization to predict the values of dependent response variables by determining the optimum process variable combinations that maximize or minimize the product response [16]. In this study, RSM was employed to evaluate the magnitude of influence of the process variables (fortify cants) on the response variable (acceptability). The aim of this study is to subjectively optimize the general acceptability of micronutrient fortified un-stabilized soymilk from sprouted soybean.

Material and Methods

TGX923 - 2E variety of soybean was procured from the National Cereal Research Institute (NCRI) outstation, Amakama Olokoro in Abia State Nigeria.

Preparation of soymilk

Cleaned whole TGX 923 - 2Esoybean variety, steeped in tap water for 12h and drained was sprouted on a cleaned jute sack on the floor for 72 h. The beans were sprinkled with water regularly, as soon as their surfaces dry [12,17]. The sprouted beans were boiled in 0.5% $NaHCO_3$ solution for 20min, drained [13,18], cooled to room temperature (23°C to 26°C) and hand-dehulled. The hulls and the shoots were removed by water floatation to obtain cleaned soybean cotyledons which were milled in Power Deluxe variable speed electric blender (model PDB – 8231 - F) with hot water (93°C) in a ratio of 2.7 parts hot water to one part cotyledons (v/w) to form sprouted bean slurry [3,17]. Soy extract was obtained by sieving the slurry through a double layered muslin cloth. The oil content of soymilk was evaluated and made up to3.5% [1]. Soymilk obtained was fortified according to Box-Wilson [19] experimental design matrix (Table 2) and each row in the design matrix was bottled separately as a run and sterilized at121°C for 5min.

Experimental design and statistical analysis

Central composite response surface design (CCRSD) for k = 3was employed to study the linear, quadratic and interactive effects of the independent experimental variables on general acceptability of micronutrient fortified soymilk from sprouted soybeans. The experimental variables namely ferric ammonium citrate, calcium carbonate and vitamin C were of five levels (Table 1). Box-Wilson [19] experimental design matrix (Table 2) was employed for the fortification. The center point (0, 0, 0) was only replicated six times [20] for estimation of errors. Analytical determinations were carried out in triplicate on appearance and viscosity. Mean data obtained on each run were regressed and analyzed for variance using Minitab software (version 11.21). Precision of the model was checked using coefficient of determination (R^2) and correlation coefficient (R) obtained from Minitab software. Statistical significance was acceptable at 5% probability levels ($P < 0.05$).

Independent process variable	Code	K=3	Variable levels				
			-1.682	-1	0	+1	+1.682
Dietary iron (mg/100ml)	Fe	X_1	1	2	3	4	5
Calcium (mg/100ml)	Ca	X_2	50	100	150	200	250
Vitamin C (mg/100ml)	C	X_3	8	16	24	32	40

Table 1: Five independent process variable combinations used in the design (k = 3)

Fe (X_1) = ferric ammonium citrate, Ca (X_2) =Caicium carbonate, C (X_3) = vitamin C.

Experimental Runs	Coded Independent Processes Variables			Real Independent Process Variables			Responses		
	X_1	X_2	X_3	X_1(Fe)	X_2(Ca)	X_3(Vit. C)	Y_1	Y_2	Y_3
1	-1	-1	-1	2	100	16	6.71	6.88	6.59
2	-1	- 1	1	2	100	32	6.60	7.00	6.61
3	-1	1	-1	2	200	16	6.30	6.32	6.65
4	-1	1	1	2	200	32	6.21	6.41	6.74
5	1	-1	-1	4	100	16	6.11	6.61	6.25
6	1	-1	1	4	100	32	5.92	6.70	6.12
7	1	1	-1	4	200	16	5.91	5.95	6.21
8	1	1	1	4	200	32	6.00	6.04	6.25
9	1.682	0	0	5	150	24	5.90	5.62	5.99
10	-1.682	0	0	1	150	24	6.85	6.88	6.48
11	0	1.682	0	3	250	24	7.11	5.91	6.08
12	0	-1.682	0	3	50	24	5.97	6.96	6.41
13	0	0	1.682	3	150	40	6.72	6.01	6.22
14	0	0	-1.682	3	150	8	6.80	6.18	6.10
15	0	0	0	3	150	24	5.91	6.13	6.29
0	0	0	0	0	0	0	6.89	7.01	6.48

Table 2: Box-Wilson (1951) experimental design matrix for coded and real independent process variables and their rating responses. X_2, X_3 and X_3 are the coded values of the respective independent variables. X_1(Fe), X_2(Ca) and X_3 (VC) each represents respective concentrations (mg/100ml) of ferric ammonium citrate, calcium carbonate and vitamin C fortificants used in the fortification trials. Y_1, Y_2 and Y_3 each represent respective response on appearance, viscosity and general acceptability. 0 represents unfortified sample. Each row represents the coded variables, real variable values and the ratings of each sample or run.

Analyses

Sensory evaluation

This was carried out (Table 2) using consumer preference test on fortified samples (n = 15) [21,22]. Sensory evaluation was carried out at Food Processing Laboratory of Michael Okpara University of Agriculture Umudike, Abia State Nigeria. Twenty five semi-trained panelists drowned from student and staff of Food Science and Technology Department of same University aged between 20 to 40 yr were used to rate the samples. Each panelist was presented with 15 coded samples at different times in glass tumblers along with a glass of water. They were to rinse their mouth before and after tasting each sample and score them according to a 9 - point Hedonic Scale, where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely [6]. The parameters rated were appearance, viscosity (consistency) and general acceptability. Data obtained were regressed (Table 3).

Results and Discussion

Results of sensory scores of the fortified and unfortified soymilk samples were presented in table 2 as Y_1, Y_2 and Y_3 while those of regression analyses were presented in (Tables 3, 4 and 5) respectively for appearance, viscosity and general acceptability.

Vb.	Coefficient	Linear	Quadratic	CP	R	R^2 (%)	ANOVA
X_1	0.07162	0.004*	0.458	0.786	0.93	87.0	0.081
X_2	0.02465	0.138	0.994	0.885	NA	NA	0.081
X_3	-0.00357	8.808	0.996	0.421	NA	NA	0.081

Table 3: Regression analysis results showing significant effects ($P \leq 0.05$) of fortificant variables coded (X_1, X_2 and X_3) on appearance (Y_1). Vb= variables, Y_1 = Appearance, of fortified soymilk. CP= cross product.$X_{1,}$ X_2 and X_3 are respective dietary iron, calcium and vitamin C fortificants, R is the correlation coefficient and $R^2$is coefficient of determination. ANOVA is the P-values of themodel. * indicates significant ($p<0.05$) effects, NA indicates no account.

Vb.	Coefficient	Linear	Quadratic	CP	R	R^2 (%)	ANOVA
X_1	-0.05700	0.588	0.832	0.200	NA	NA	0.197
X_2	0.26582	0.043*	0.267	0.179	0.89	80.0	0.197
X_3	0.5044	0.630	0.282	0.840	NA	NA	0.197

Table 4: Regression analysis results showing significant effects ($P \leq 0.05$) of fortificant variables coded (X_1, X_2 and X_3) on viscosity (Y_2). Vb= variables, Y_2 = viscosity of fortified soymilk. CP= cross product. $X_{1,}$ X_2 and X_3 are respective dietary iron, calcium and vitamin C fortificants, R is the correlation coefficient and R^2 is coefficient of determination. ANOVA is the P-values of the model. * indicates significant (0<05) effect, NA indicates no account.

Vb.	Coefficient	Linear	Quadratic	CP	R	R^2 (%)	ANOVA
X_1	-0.18602	0.028*	0.716	0.838	0.83	68.8	0.434
X_2	-0.02332	0.832	0.720	0.820	NA	NA	0.434
X_3	0.01306	0.671	0.645	0.885	NA	NA	0.434

Table 5: Regression analysis results showing significant effects ($P \leq 0.05$) of fortificant variables coded (X_1, X_2 and X_3) on acceptability (Y_3).

Vb= variables, $Y_{3,}$ = General acceptability of fortified soymilk. CP= cross product.X_1 X_2 and X_3 are respective dietary iron, calcium and vitamin C fortificants, R is the correlation coefficient and R^2 is coefficient of determination. ANOVA is the P-values of the model.* indicates significant ($p<0.05$) difference, NA indicates no account.

Appearance

Fortified soymilk appearance showed linear positive significant ($p < 0.05$) effect (increasing effect) of ferric ammonium citrate (X_1) which accounted for 87.0% of total soymilk appearance increase. This increase projected ferric ammonium citrate as a major contributor to soymilk appearance increase. First order is well fitted in this study as ferric ammonium citrate was only significant at linear level. Precision of the model was reflected in the respective R and R^2 values of 0.93 and 87% with 13% response variation not explained (Table 3). The ANOVA with p - value of 0.081 (Table 3) indicated that the model can only predict the cumulative effects of the fortify cants on appearance at 1% ($p > 0.05$) level. Increase in appearance observed could be due to brown colour of ferric ammonium citrate and concentration used. Dietary iron had been reported to contribute to off - colour in white food materials like milk and soymilk [7,23]. Despite the off - colour contribution, soymilk acceptability may be traced to vitamin C (X_3) inclusion which had been reported to stabilize colour [24]. Besides, Anjum., *et al* [8] and DSM, [24] have earlier reported that vitamin C (X_3) improves aesthetic appeal in food products. Also, calcium carbonate had been reported to give soymilk a whiter and less yellow colour [11]. Maximum appearance of 7.11 (Run 11) rating (Table 2) at 3mg/100ml concentration of iron fortify cant was in agreement with the above reports. Calcium carbonate and vitamin C may have cancelled the off - colour effect of ferric ammonium citrate at 3mg/100ml level.

Viscosity

Estimated regression coefficient (Table 3) implicated calcium carbonate (X_2) with positive linear significant ($P < 0.05$) effect on apparent viscosity (Y_2) of soymilk by contributing 80% (R^2) of total viscosity increase due to variables and 0.89 (R^2) which is robust. ANOVA also affirmed the significance ($p > 0.05$) of the model to predict the contribution of the variables at 1% level. The linear model was selected in this study as $CaCO_3$ was significant at linear level. This linear significant ($p < 0.05$) viscosity increase may be attributed to concentration of $CaCO_3$(X2), type used, bean variety (STS, 1987) and processing methods adopted [12,25]. Hydrolyzed protein due to sprouting and $CaCO_3$ (X_2) added may have bonded and held soymilk water [26] thereby not only stabilized but also increased the viscosity. Also, hydrolysis of soybean macromolecules by sprouting may have prevented interaction among soymilk components and fortii cants which in turn may have prevents significant viscosity increase that could have affected acceptability. This slight viscosity increase confirmed soymilk component and fortify cant compatibility which portends product stability, consistent and acceptability. Viscosity decides consistency, appearance, quality and stability of soymilk [27]. Knowledge of soymilk viscosity will therefore help for a better understanding of the complex relationship between overall acceptability, soymilk viscosity, and concentration of ingredients [28]. Soy milk viscosity decides acceptability.

General acceptability

Maximum acceptability rating of 6.74 (Like slightly) was obtained from run 4 (Table 2).Negative linear significant ($p < 0.05$) effect of ferric ammonium citrate (X_1)contributed 68.8% to general acceptability (Y_3) decrease of the fortified soymilk (Table 3). Ferric ammonium citrate (X_1) is therefore the primary contributor to this decreasing soymilk acceptability. First order is well adjusted in this study to explain this significant ($p < 0.05$) decreasing effect as a function of ferric ammonium citrate. The model is statistically adequate to explain 68.8% acceptability variation with coefficient of determination (R^2) of 68.8% and R of 0.83 at 1% level. The model regression equation on general acceptability is:

$$Y = 6.25 - 0.186 X_1 - 0.0233 X_2 + 0.0131 X_3 - 0.0179 X_1*X_2 - 0.0304 X_1*X_3 + 0.0217 X_2*X_3 + 0.0400 X_1\text{^}2 + 0.0436 X_2\text{^}2 + 0.0135 X_3\text{^}2 \quad (1).$$

On removing the non-significant terms from the regression analysis, the resulting polynomial became: $GA = 6.247-0.186 X_1$ (2).

The ANOVA with p-value of 0.434 (Table 5) showed non-significant ($p > 0.05$) effect of the model to predict the cumulative effects of the fortify cants on general acceptability (Y_3). This also confirmed model's significant ($p < 0.05$) lack of fit to statistically predict general acceptability as a function of the tested attributes at 5% confidence level. This indicated that other factors not explained by the model may have increasing effect on acceptability. Addition of stabilizer may have improved general acceptability [11]. Therefore, ferric ammonium citrate (X_1) had an inverse linear relationship with general acceptability (Y_3)most especially on appearance(Y_1)as reflected in least acceptability (5.99) at highest iron concentration (5mg/100ml) as shown in Table 2.

Decreasing effect of dietary iron (X_1) on acceptability (Y_3) may be traced in part to its deep brown colour as reflected in the strong positive linear significant ($p < 0.05$) effect on soymilk appearance (Table 5). Lower acceptability recorded (5.99, neither like nor dislike) at higher concentration (5mg/100ml) of ferric ammonium citrate (X_1) compared to that (6.48, like slightly) at lower concentration (Table 2) is in line with the reports of Clarke, [7] and Barclay, [23] that dietary iron has lowering effect on acceptability. More so, at highest concentration of $CaCO_3$ in run 11 (Table 2), appearance was scored 7.11, consistency was scored least (5.91), and general acceptability (Y_3) was scored second to the least (6.08, like slightly). These results could be traced to absence of stabilizer which works best with calcium fortification [7] and agreed with the report of SMI, [11] that $CaCO_3$ had an inverse relationship with consistency and acceptability unless stabilized. Choice of $CaCO_3$ (X_2) which is less reactive with food matrix and a high level fortify cant that delivers the required level of calcium in fortified soymilk with small amount to leave soymilk with desirable mouth feel and clarity [11] may have improved acceptability Anjum., *et al*. [8] and DSM, [24] had earlier reported that vitamin C improves aesthetic appeal in food products. Positive coefficient of vitamin C in this study indicated slight ability to increase general acceptability probably due to its antioxidant property [24]. Earlier reports have confirmed that vitamin C protects oxidation of fats and oil, improves the stability of other added nutrients, aids in dietary iron absorption [7,24] and increases acceptability.

Ranking of soymilk attributes by the panelists despite absence of stabilizer observed in this study which fell between like slightly and like moderately according to 9 - point Hedonic scale may be ascribed in part to choice of fortify cants, combinations used, and the unit operations employed. Sorting of broken beans eliminates rancid flavour and undesirable appearance [29]. Bean steeping prior to sprouting removes off-flavour associated with un-steeped beans [30]. Sprouting which had been reported by Nsofor and Madukor, [17] to hydrolyze soybean oligosaccharides must have contributed to palatability of the soymilk due to increased glucose and fructose. Various heat treatments during bicarbonate blanching, hot grinding and boiling of extracts may have eliminated some enzymes responsible for off - flavour and acid forming bacteria responsible for spoilage [1,3]. Also dehulling may have removed off-flavour [31] associated with soymilk from underholed beans. The choice of using unsaturated soybean oil in the fortification may have contributed to the desirable flavour and good mouth feel [3] thereby increased acceptability. Also, choice and concentrations of the chemically micronized insoluble iron salt (ferric ammonium citrate) used in this study must have reduced sensory problems due to soluble iron [32]. These acceptability enhancement notwithstanding, the ratings could have been higher than what was obtained if soymilk had been adequately and appropriately stabilized and homogenized [11,32].

Conclusion

General acceptability of vitamin and mineral fortified soymilk which rated between liked slightly and moderately in this study depended on the types and levels of fortify cants used. Ferric ammonium citrate and calcium carbonate had linear increasing effects on the fortified soymilk appearance but ferric ammonium citrate was more significant ($p < 0.05$). Both in turn decreased acceptability slightly. Calcium carbonate had linear significant ($p < 0.05$) increasing effect on viscosity (consistency) of the fortified soymilk while vitamin C had non-significant ($p > 0.05$) increasing effect. Only ferric ammonium citrate had non-significant decreasing effect. Significant ($p < 0.05$) linear decreasing effect of ferric ammonium citrate on acceptability along non-significant ($p>0.05$) decreasing effect of calcium carbonate may have over shadowed the increasing effect of vitamin C to reduce the acceptability. Maximum acceptability level of 6.74 obtained in fortify cant combination of 2: 200:32 for ferric ammonium citrate, calcium carbonate and vitamin C had lower iron content. Therefore, choice and concentration of ferric ammonium citrate should be visited with caution. General acceptability of the fortified soymilk could have been higher if the viscosity had been increased through stabilization.

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