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Rheological behaviour of fortified soymilk from sprouted soybean for complementary feeding: a response surface analysis

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ABSTRACT

Viscosity of soymilk extracted from 12hrs tap water steeped and 72hrs sprouted TGX 923-2E soybean variety fortified according to Box-Wilson (1951) experimental design matrix using ferric ammonium citrate (fe), calcium carbonate (Ca) and vitamin C (vC) fortificants was optimized. Central composite design (k = 3) was used to develop a prediction model to investigate the influence of these fortificants on the viscosity. Data on each run were statistically regressed with Minitab (version 11.21) computer software. Regression models showed that calcium carbonate ($CaCO_3$) had significant ($P \leq 0.05$) increasing effects on viscosity. Fortified soymilk exhibited an apparent non-ideal Newtonian viscosity with alternating pseudoplastic and dilatant flow behaviours. Maximum viscosity was obtained from fortificant combinations 2, 250, 24 for ferric ammonium citrate, calcium carbonate and vitamin C.

Key words: *Rheological behaviour, fortified soymilk, sprouted soybean, complementary feeding and response surface methodology*

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I. Introduction

Rheology is defined by Rubalya & Neelameagam (2008) as the study of the change in the form and flow of viscosity, elasticity and plasticity. Rheological properties of foods deal with the science concerned with the deformation and flow of matter when force is applied to a food material. Similarly**,** viscosity is defined as an aspect of rheology that deals with the study of deformation and flow of matter (Rao, 2003). However, viscosity is the internal friction of a fluid, that is, the resistance of one part of the fluid to move relative to another one which is closely related with the structural parameters of the fluid particles (Rubalya & Neelameagam, 2008).

Many brands of commercial preparatory weaning foods developed and marketed in most developing countries like Nigeria are scarce and expansive. Preparation of CFs from locally available staples like cereals, legumes and tubers becomes an available option for the survival of the infants and young children. Complementary foods prepared from these staples like porridge prepared from cereal flour

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such as maize are associated with high viscosity, low energy, low nutrient density, malnutrition and infestations when reconstituted. High viscosity CFs impart undesirable sensory properties and malnutrition which affects physical growth, morbidity, mortality, cognitive development and others in children (Uwaegbute, 2008; Elemo et al., 2011). High viscosity CF results in the child not eating enough per meal to meet the energy demand. Diluting with water involves low energy and nutrient density which is not suitable for growing children.

To lower the viscosity and increase both energy and nutrient density of complementary foods so as to prevent malnutrition, several preparatory traditional technologies were employed by some researchers. Elemo et al. (2011) employed a combination of germinated cereals and legumes with various sources of rich protein from animal, legumes, and oil seeds. Other viscosity lowering strategies in CFs include incorporation of adequate food grade amylase, or malted soybean as a good source of amylase (Kimmons et al., 2004), toasting (Udensi et al., 2012), combination of extrusion processing and traditional processing technology (germination) by Magala-Nyago (2010) and food formulation technology (Oti & Akobundu, 2007).

Extensive study on germination of legumes and cereal was found useful and advantageous in increasing energy and nutrient density of infant diets. During germination, the endogenous enzymes released digest some of the starch into dextrin-maltose which does not swell when cooked into gruel (Tizazu et al., 2010; Elemo et al., 2011). Bean germination (Mostafa et al., 1987) decreases total oil content, increases the quality and bioavailability of many essential nutrients of soymilk made from them. Germination also eliminates soymilk anti-nutrients and endows soymilk with some desirable healthy benefits (Iwe, 2003; Osuji & Ubbaonu, 2004).

Soymilk also known as vegetable or imitation milk is produced from whole soybean (*Glycine max*) with very high research attention as reference vegetable milk (Onweluzo & Nwakalor, 2009). Soymilk resembles cow's milk in appearance, flavour and nutritive value when properly processed (Wassef et al., 1988; Iwe, 2003) and breast milk in health benefits (Osuji & Ubbaonu, 2004). However, the vitamin and minerals content of soymilk are lower (STS, 1987) than human milk and recommended daily intake (RDI). Successful fortification of soymilk with adequate fat (including omega fatty acids and fish oil), ascobates and calcium had been acknowledged (Oeveren, 2005; Fallon & Enig, 2007). Soymilk fortification with vitamins and minerals for complementary feeding complements breast milk compositions (Lonnerdal, 1985; Rarback, 2011) beyond exclusive breast feeding period of 6 months so as to prevent nutrient and energy deficiency symptoms, promotes growth (Fallon & Enig, 2007), physiological, immunological and mental development among breast feeding infants (Yeung, 2011).

Soymilk viscosity is the major quality attribute that is affected by solid content which in turn is influenced by processing methods and bean variety (FSTA, 2009). Liquid soymilk are classified according to its rheological behavior as Newtonian, pseudoplastic, dilatant, thixotropic, among others and some of their rheological data have been summarized in the literature (Dankor et al., 2007). As a suspension, there is a marked influence of storage, temperature, constituents and structure in the rheological behavior. The flow properties of soymilk could be measured and have been reported to be related to mouth feel and viscosity (Oguntunde & Akintoye, 1999; Chougrani et al., 2009). This study aims at optimizing the effects of fortification on soymilk viscosity behaviour.

II. Materials and Methods

TGX923-2E variety of soybean was procured from the National Cereal Research Institute (NCRI) outstation, Amakama Olokoro while preparation and analyses of samples were carried out at the National Root Crop Research Institute (NRCRI) Umudike both in Abia State, Nigeria.

Preparation of soymilk: Previously cleaned, 12hrs steeped and 72hrs sprouted TGX 923-2E soybean variety (Mostafa et al., 1987; Nsofor & Maduakor, 1992) was boiled in 0.5%$NaHCO_3$ solution for 20min, drained (Omosaiye et al., 1978), cooled to room temperature and hand-dehulled. The hulls and the shoots were removed by water floatation. Soybean cotyledons obtained were milled in QLink (Japan) variable speed kitchen blender with hot water (93^oc) in a ratio of 2.7 parts hot water to one

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part cotyledons (v/w) to obtain sprouted bean slurry (STS, 1987; Nsofor & Maduakor, 1992). Soy extract was obtained by screening the slurry through a double layered muslin cloth (Osuji & Ubbaonu, 2004). The oil content of soymilk (1.8%) was evaluated by soxhlet method and marked up to 3.5% (Iwe, 2003). Soymilk obtained was fortified according to Box & Wilson (1951) experimental design matrix (Table 02) and sterilized at 121°C for 5min. Viscosity study was carried out at different speeds (6, 12, 30 and 60) on 200ml of each run at room temperature with bench rotary viscometer, NDS-55 Surgifriend Medical England with spindle no 1 and the results were expressed in Pas (Chongrani et al., 2009).

Experimental design and statistical analysis: Central composite response surface design (CCRSD) for k=3 was employed to optimize the linear, quadratic and interactive effects of the fortificants on the viscosity of fortified soymilk. The experimental variables namely ferric ammonium citrate, calcium carbonate and vitamin C were of five coded levels (Table 01). Box & Wilson (1951) experimental design matrix (Table 02) was employed for fortification. Six times replications only at center point (0, 0, 0) according to Iwe et al. (2003) generated a total of 21 experimental runs (Table 02). Each row shows the fortification levels of the variables at one trial run. Analytical determinations were carried out in triplicate and mean data obtained on each run was statistically regressed and analyzed for variance using Minitab software (version 11.21). Matlab (version R2012) software was used to develop plots to have a clear mental picture of the effects more clearly. Precision of the model was checked using coefficient of determination (R^2) and correlation coefficient (R). Statistical significance was acceptable at 5% probability levels ($p \leq 0.05$).

Table 01. Coded and actual levels of the independent variables for the experimental design

Independent process variables (mg/100ml)	Code	K = 3	Variable levels				
			-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	C	X_3	8	16	24	32	40

Table 02. Experimental design matrix for coded, real independent process variables and the responses

Expt. runs	Coded independent processes variables			Real independent process variables			Viscosity (pas) speed			
	X_1	X_2	X_3	Fe(X_1)	Ca(X_2)	C(X_3)	6	12	30	60
0	0	0	0	0	0	0	196.0	98.2	196	98.3
1	-1	-1	-1	2	100	16	196.27	98.28	196.28	98.27
2	-1	- 1	1	2	100	32	196.28	98.28	196.27	98.28
3	-1	1	-1	2	200	16	197.39	98.39	197.40	98.49
4	-1	1	1	2	200	32	197.36	98.40	197.41	98.42
5	1	-1	-1	4	100	16	196.28	98.28	196.27	98.27
6	1	-1	1	4	100	32	196.29	98.29	196.28	98.23
7	1	1	-1	4	200	16	197.28	98.40	197.39	98.41
8	1	1	1	4	200	32	197.30	98.41	197.29	98.42
9	1.682	0	0	5	150	24	196.35	98.31	196.34	98.32
10	-1.682	0	0	1	150	24	196.34	98.34	196.32	98.32
11	0	1.682	0	3	250	24	197.88	98.75	197.84	98.78
12	0	-1.682	0	3	50	24	196.22	98.23	196.23	98.28
13	0	0	1.682	3	150	40	196.34	98.34	196.33	98.33
14	0	0	-1.682	3	150	8	196.32	98.31	196.30	98.31
15	0	0	0	3	150	24	196.33	98.33	196.32	98.33
16	0	0	0	3	150	24	196.32	98.32	196.32	98.32
17	0	0	0	3	150	24	196.33	98.33	196.33	98.32
18	0	0	0	3	150	24	196.34	98.34	196.33	98.34
19	0	0	0	3	150	24	196.32	98.32	196.31	98.33
20	0	0	0	3	150	24	196.34	98.34	196.32	98.34
21	0	0	0	3	150	24	196.33	98.33	196.31	98.33

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X_1, X_2 and X_3 are respective coded concentrations of ferric ammonium citrate, calcium carbonate and vitamin C while X_1(Fe), X_2(Ca) and X_3(Vit C) represents their real concentrations used in the fortification trials. Each row represents a fortification trial run or adjustment levels of the process variable combination at one run. Y_1, Y_2, Y_3 and Y_4 are the viscosity responses at the respective speeds.

III. Results and Discussion

Viscosity results (Table 02) showed slight changes among the fortified samples and between fortified and unfortified samples. The samples exhibited non-Newtonian with alternating pseudoplastic and dilatant flow behaviours when subjected to varying shear rates (Figure 01) which simulated the work of Sivanandan et al. (2008). Apparent viscosity decreased with increase in share rate from 6 to 12 and 30 to 60 rps (rounds per second) and increased from 12 to 30 rps (Table 02 and Figure 01). Also, there were slight increases in apparent viscosity with increase in $CaCO_3$ fortificant in all the fortified samples. Number 6 and 11, each represents soymilk sample containing fortificant concentrations of 4, 100, 32mg/100ml and 3, 250, 24mg/100ml while No 0 represents unfortified sample with 0, 0, 0mg/100ml for fe, ca and vc respectively.

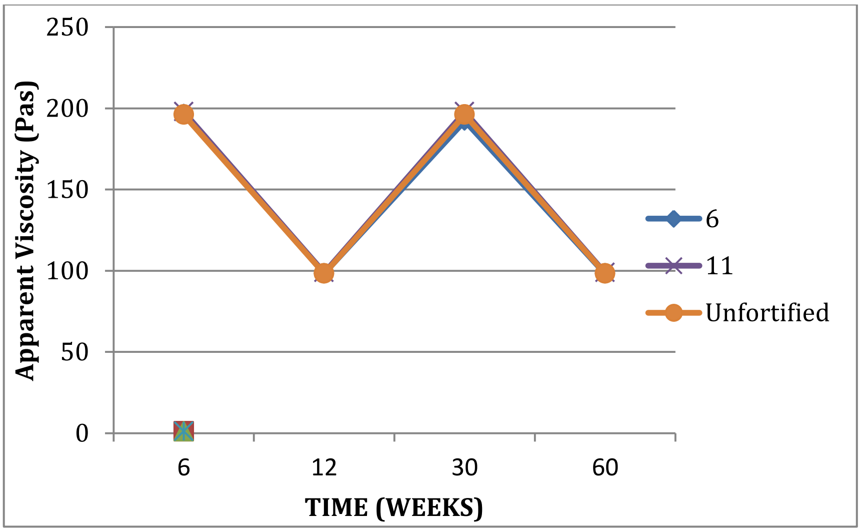

Figure 01. Plots demonstrating apparent alternation of pseudoplastic and dilatant viscosity behaviours of some selected fortified and unfortified soymilk samples due to increasing shear rate.

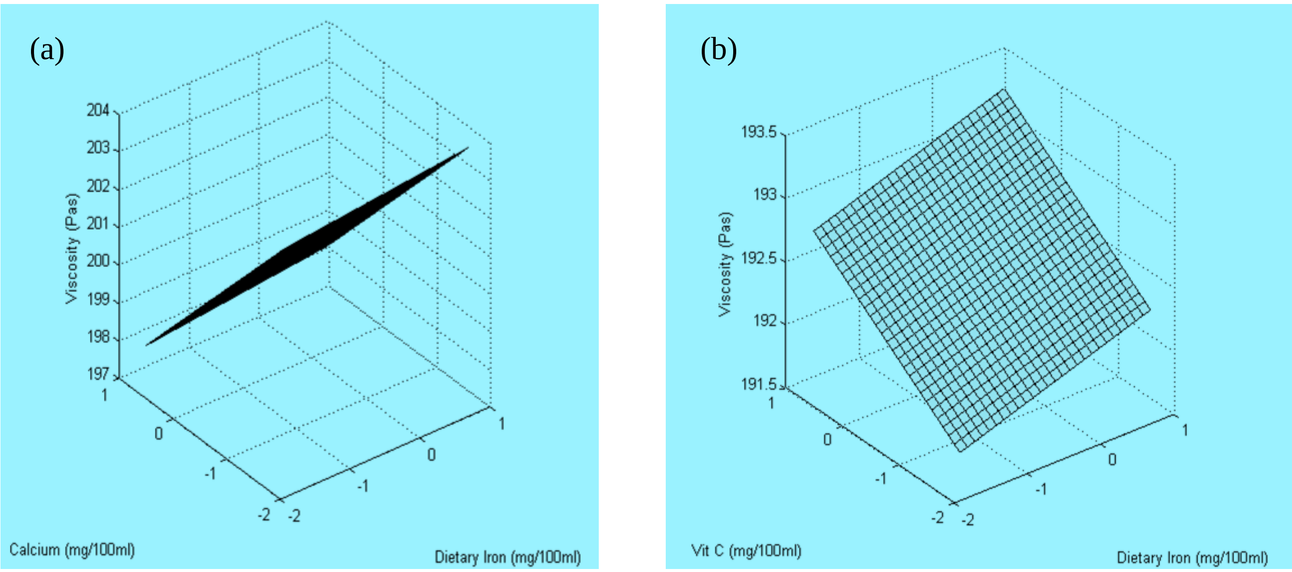

Figure 02. Effects of dietary iron and calcium (a) and dietary iron and vitamin C (b) on viscosity of fortified soymilk

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Table 03. Estimated regression coefficient and ANOVA for viscosity of fortified soymilk

Constant	X1	X2	X3	X1X2	X1X3	X2X3	X_1^2	X_2^2	X_3^2	R	R2	ANOVA
196.352	-0.057	0.266*	0.050	-0.193	-0.163	0.177	-0.026	0.226	-0.031	0.89%	0.80%	0.197

X_1, X_2, X_3 are the coded coefficients for ferric ammonium citrate, calcium carbonate and vitamin C, R is the correlation coefficient, R^2 is the coefficient of determination, * significant level ($P \leq 0.05$)

Estimated regression coefficient (Table 03) indicated that only $CaCO_3$ had linear significant ($p \leq 0.05$) effect which accounted for 80% of total variations in soymilk apparent viscosity due to variables with positive coefficient indication to increase the viscosity. Coefficient of determination (R^2) of 80% indicated that only 20% variability was not explained by the model. Henika (1982) had reported that R^2 more than 75% is relatively adequate for prediction purposes. Therefore, the fitted model is adequate to predict soymilk viscosity in this study. Also, the correlation coefficient (R) of 0.89 indicated a close relationship between the experimental and theoretical values predicted by the model (Table 03). This showed that the fitted regression model equation is good and the CCD model with the experimental design can be effectively applied for the optimization. The model regression equation on viscosity is:

$$Y = 196 - 0.0570 X_1 + 0.266 X_2 + 0.0504 X_3 - 0.193 X_1{*}X_2 - 0.163 X_1{*}X_3 + 0.177 X_2{*}X_3 - 0.025 X_1{\wedge}2 + 0.226 X_2{\wedge}2 - 0.031 X_3{\wedge}2 \quad (1)$$

Deleting the non-significant terms from the regression analysis the prediction equation became:

$$Vis = 196.352 + 0.266 X_2 \quad (2)$$

Equation (2) indicated that the viscosity of fortified soymilk is a function of linear effect of $CaCO_3$ which also is the primary contributor. Linear model will best predict viscosity in this study. ANOVA indicated significant ($p > 0.05$) effect of $CaCO_3$ on apparent viscosity (Table 03). Therefore, the model is adequate to predict viscosity at 1% level. Response surface plots of calcium and dietary iron on viscosity (Figure 02a) affirmed respective decreasing and increasing contributions of dietary iron and calcium carbonate on viscosity while Figure 2b illustrated same respective contributions by dietary iron and vitamin C on soymilk viscosity.

Slight variations in soymilk viscosity (Table 02) among the fortified samples may be attributed to different levels of $CaCO_3$ fortification, increased pH due to bicarbonate blanch that must have prevented sedimentation, low TS due to sprouting (MI, 2011) and absence of stabilizer (SMI, 2009) and generally low soymilk viscosity (Sivanandan et al., 2008) due to bean: water ratio (STS, 1987; Osuji & Ubbaonu, 2004). This viscosity variation is in line with the report of Iwe (2007) and Barclay, (2011) that soymilk undergoes both physiological and rheological changes that may be due to ingredients supplementation, interaction and processing methods during storage. Slight variation signified little or no undesirable interactions among the fortificants and between same and soymilk components which could have increased the soymilk solids and viscosity more. Slight increase in viscosity of fortified samples observed over unfortified could be traced to fortificants most especially $CaCO_3$ concentration levels used (Table 02). Igyor et al. (2011) had reported that calcium and protein bind and hold water. This may have increased soymilk total solids and viscosity. However, FSTA (2009) had earlier reported that soymilk viscosity is the major quality attribute that is affected by solid content which in turn is influenced by the processing methods and bean variety. Besides, Oguntunde & Akintoye (1991) reported that soymilk viscosity has an exponential relationship with TS. More still, Kawada et al. (2002) further confirmed that soymilk viscosity increases significantly as TS increases. Viscosity is closely related with the structural parameters of the fluid particles (Rubalya & Neelameagan, 2008). Even at constant TS, the soymilk viscosity varies greatly depending on the production process and operation conditions.

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Pseudoplastic behavior (shear thinning) exhibited by soymilk (Figure 01) when the shear force (spindle speed) was increased (6 to 12rps) could be attributed in part to very low resistance of soymilk particles to the increased shear rate. Rao (2003) and SMI (2009) had reported that shear flow properties (viscosities) of fluid foods depends on shear rate and shear stress data as mathematically expressed in equation 3:

$$\text{Viscosity} = \frac{stress}{Rate\ of\ strain} \quad \text{or} \quad \eta = \frac{\sigma}{y} \qquad (3)$$

Where, σ = stress and y = rate of strain which is proportional to stress or force per unit area (applied force)

Here, the denominator (rate of strain) decides the fate of viscosity, increase or decrease (SMI, 2009). Furthermore, SMI (2009) reported that when soymilk is standing, their particles are evenly suspended because the package viscosity (soymilk medium viscosity when only very small gravitational force is applied) is very low. Due to non-application of stabilizer in this study which could have increased soymilk medium viscosity, increased shear rate (package viscosity plus applied shear force) from 6 to12 (Figure 01) broke down soymilk medium viscosity that suspended soymilk particles and soymilk viscosity appears or 'feels' thin. This is pseudoplastic or shears thinning flow. Similar flow properties were obtained by Monia et al. (2005) when the rheological properties of prickly pear seed oil were evaluated at constant temperature with increasing and immediate decreasing of shear rates. Also, SMI (2009) reported same flow property in soymilk fortified with $CaCO_3$.

However, as the shear force was increased further (>12 to 30rps), soymilk solids way likely be subjected to more increased frequency of collision which in turn may have resisted the applied shear force and the viscosity increased (Rao, 2003; SMI, 2009). This is shear thickening or dilatant behavior (SMI, 2009). With further increase in shear rate (>30 to 60rps), the soymilk viscosity may thin down due to less resistance of the particles to the increased applied shear force and this is shear thinning. This agreed with SMI (2009) who reported that medium viscosity is inversely related to settling rates of the particles. This alternating shears thinning and thickening, as a result of increased shear rate (Figure 01) portends soymilk stability (Osuji & Ubbaonu, 2004). Soymilk in this study proved to be non-ideal Newtonian liquid, changes with shear rates, with alternating pseudoplastic and dilatants flow behaviors (Figure 01) when subjected to increasing share force. Viscosity of soymilk in this study was therefore apparent as it depended on shear rate, therefore the knowledge of soymilk viscosity will help for a better understanding of the complex relationship between the overall viscosity of soymilk and the type and concentration of ingredients (Rao, 2003). Besides, the viscosity will decide consistency, appearance, acceptability, quality and stability of soymilk (FSTA, 2009).

IV. Conclusion

Calcium carbonate is the major contributor to fortified soymilk viscosity although vitamin C had an insignificant contribution to increase viscosity unlike ferric ammonium citrate. Dependency of calcium carbonate fortified soymilk viscosity on medium viscosity which in turn depended on total solids and processing methods implies that stabilization and bean sprouting will offer a better soymilk stability, consistency, acceptability and appearance.

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