



## Proximate, nutrients and dietary energy profiles of vitamin and mineral fortified predigested soymilk for complementary feeding

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### Abstract

Soymilk is an economic candidate for complementary feeding but deficient in vitamin and minerals. This work therefore evaluated the influence of fortifying soymilk from sprouted soybean with ferric ammonium citrate, calcium carbonate and vitamin C on the nutrients, proximate and calorific composition. Proximate and nutrient as well as energy content of fortified soymilk (FSM) and unfortified soymilk (UFSN) from 12h tap water steeped and 72h sprouted TGX 923-2E soybean variety was evaluated using standard analytical methods and Atwater factor respectively. Oil content obtained (1.8%) was marked up to 3.5%. Ash (i.83%) and crude fiber (0.06%) content of FSM were higher than the respective values of 0.4% and 0.05% from UFSM samples. Moisture (86.77%), fat (3.50%), protein (4.43%) and carbohydrate (4.75%) of UFSM were higher than respective values of 85.66%, 3.42%, 4.42% and 4.63% from FSM. Dietary iron (5.52 mg/100ml), calcium (253.68mg/100ml) and vitamin C (40.60mg/100ml) content of FSM were significantly ( $p < 0.05$ ) higher than respective 0.525, 3.8 and 4.31mg/100ml values from UFSM. Dietary energy value of 363.74Kj (64.03Kcal) from FSM was lower than 373.04Kj (65.39Kcal) from UFSM per 100ml soymilk. Fortification increased dietary iron, calcium vitamin C, ash and fiber content of soymilk samples and lowered MC, fat, protein, carbohydrate and dietary energy. Despite the lowering, dietary energy values of both soymilk samples were within the RDA range (200 to 550Kcal per day) recommended by WHO for infants aged 6 to 23 months and therefore can meet the complementary energy requirement by consuming 50 to 150ml of soymilk per day.

**Keywords:** dietary energy, soymilk fortification, sprouted soymilk and complementary feeding.

### Introduction

Extensive nutritional and complementary studies have confirmed that beyond the critical infancy period of from 4 to 6 months to two years, breast milk alone cannot meet the nutritional and energy needs of infants and young children. Besides, magnitude of breast milk nutrient variations among lactation mothers (Lonnerdal, 1985) <sup>[1]</sup> and their poor sources of iron and ascorbates portend a mandatory introduction of adequate and appropriate nutrition as regards calorie, vitamins and minerals else, the baby will not achieve the expected growth. This period is characterized by tremendous physical growth which oftentimes manifests in increased length and weight as well as physiological, immunological and mental development (Yeung, 2011) <sup>[2]</sup>. Complementary food is therefore introduced to improve or meet both the energy and nutrient demand since the child will no longer gain weight despite appropriate breast feeding, and will be feeling hungry always despite frequent breast feeding (Rarback, 2011) <sup>[3]</sup>.

Complementary foods are non-breast milk or nutritional companion or additional foods like specially prepared foods or modified family meals which may be solid, semi-solid or liquid in texture (Agostoni, 2008; UNICEF, 2010) <sup>[4, 5]</sup>. They are introduced as readily consumed and digested to the diets of breast-or-formula fed infants during their transition to adult diet. The aimed of introduction is to provide the baby with extra nutrients and energy needed during the transition period (Iwe, 2010; UNICEF, 2010) <sup>[6, 5]</sup>.

Soymilk has been reported as a good economic breast milk substitute from vegetable origin (Osuji and Ubbaonu, 2004; Fallon and Enig, 2007) <sup>[7, 8]</sup>, but deficient (lower than RDI) in vitamins and mineral and calls for fortification (STS,

1987) <sup>[9]</sup>. Sprouting or germination of whole soybean and fortification of soymilk have long been adopted to improve the nutritional content of soymilk for school children (STS, 1987; Rafferty *et al.*, 2007) <sup>[9, 10]</sup>. Sprouting increases nutrients, digestibility and bioavailability, reduces trypsin inhibitor (Iwe, 2003) <sup>[11]</sup>, flatulence (Omosaiye *et al.*, 1978) <sup>[12]</sup>, oil content (Nsofor *et al.*, 1997) <sup>[13]</sup> and stabilizes soymilk made from them thus making it a good candidate for infant formula (Osuji and Ubbaonu, 2004) <sup>[7]</sup>. Soymilk has been successfully fortified with vitamin B<sub>12</sub>, fat (Omega fatty acids and fish oil), ascorbate and calcium to prevent development of their deficiency symptoms in infants and young children (Oeveren, 2005; Fallon and Enig, 2007) <sup>[14, 8]</sup>.

Food fortification is a deliberate addition of one or more essential micronutrients to a food, whether or not it is normally contained in the food for the purpose of preventing or correcting demonstrated deficiency of one or more nutrients in the population or specific population group (Clarke, 1995) <sup>[15]</sup>. Food fortification with shortfall essential nutrients has proved to be the optimal strategy in dealing with widespread nutrient deficiencies.

Dietary energy is a non-nutritive food ingredient required by human body but supplied through food consumption and oxidation. Dietary energy is utilized for basal metabolism necessary for life such as cell metabolism, growth, physiological functions, muscular activity, heat production, transport of substances around the body, and synthesis of enzymes and hormones. Dietary energy is usually expressed in kilocalories or kilojoules per person per day. Physical activity is the most variable determinant of energy need and is the second largest user of energy after basal metabolic

rate (NHMRC, 2016) [16]. Infants and toddlers are fast growing and active, require plenty of energy and therefore do not need low energy diets. Early research findings have registered food nutrients like carbohydrates, proteins and fats as major dietary energy sources, in foods. Generally, nutrient densities are index of energy content of food (BNF, 2004; Mullan, 2006) [17, 18], but are affected by some factors (Mullan, 2006) [18]. The aim of this study is to evaluate proximate, nutrient and dietary energy content of some vitamin and mineral fortified soymilk from sprouted soybean for complementary feeding.

### Material and methods

The TGX923-2E variety of soybean used for the study was procured from the National Cereal Research Institute (NCRI) outstation, Amakama Olokoro while Food processing Laboratory of Department of Food Science and Technology of Michael Okpara University of Agriculture both in Abia State, Nigeria provided analytical space and reagents used.

### Preparation of soymilk

Soymilk was prepared according to Okwunodulu and Okwunodulu (2016) [19]. Cleaned and weighed soybeans were soaked in tap water for 12hrs, drained thereafter, spread on a clean jute sack on the floor, covered with black polyethylene and allowed to sprout for 72h at room temperature. The beans were sprinkled with water as soon as their surfaces dry. Sprouted beans were boiled in

0.5%NaHCO<sub>3</sub> solution for 20min, drained, hand-dehulled and the hulls with the shoots were removed by water floatation to obtain cleaned soybean cotyledons. Cleaned cotyledons were milled in QLink (Japan) variable speed kitchen blender with hot water (93°C) in a ratio of 2.7 parts hot water to one part cotyledons to obtain sprouted bean slurry. Soy extract was obtained by screening the slurry through a double layered muslin cloth. Oil content (1.8%) determined was marked up to 3.5% with soybean oil before fortification.

### Soy milk fortification

Soymilk obtained was fortified with ferric ammonium citrate, calcium carbonate and vitamin C according to Box Wilson (1951) [20] Experimental Design Matrix (Table 1). The three independent process variables (Table 2) used were chosen based on their functions, growth enhancement and health benefits (Rafferty *et al.*, 2007; Fallon and Enig, 2007) [10, 8]. Ferric ammonium citrate is the best fortificant for milk (Clarke, 1995) [15]. Iron regulates growth and cell differentiation (Fallon and Enig, 2007) [8]. Calcium carbonate is an economical source of calcium and efficiently absorbed by infants. Vitamin C enhances calcium absorption and stabilizes nutrients (Fallon and Enig, 2007) [8]. Five variable fortificant level combinations (Table 2) were used in the fortification. Each column with different combinations of the three fortificants represents a sample. Fortified samples were sterilized in autoclave at 121°C for 5min, cooled at room temperature and stored for analyses.

**Table 1:** Box-Wilson (1951) experimental design matrix for coded, real independent process variables and responses (mg/100g).

Runs	Coded Independent Processes Variables			Real Independent Process Variables			Responses		
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Fe (X <sub>1</sub> )	Ca(X <sub>2</sub> )	C (X <sub>3</sub> )	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>
0	0	0	0	0	0	0	0.525	3.8	4.31
1	-1	-1	-1	2	100	16	2.514	103.617	14.84
2	-1	-1	1	2	100	32	2.516	103.606	30.30
3	-1	1	-1	2	200	16	2.518	203.612	14.93
4	-1	1	1	2	200	32	2.518	203.625	31.13
5	1	-1	-1	4	100	16	4.518	103.609	14.79
6	1	-1	1	4	100	32	4.517	103.637	14.89
7	1	1	-1	4	200	16	4.516	203.606	14.90
8	1	1	1	4	200	32	4.516	203.623	30.01
9	1.682	0	0	5	150	24	5.517	153.600	24.80
10	-1.682	0	0	1	150	24	1.516	153.617	23.03
11	0	1.682	0	3	250	24	3.516	253.675	23.80
12	0	-1.682	0	3	50	24	3.518	53.725	23.50
13	0	0	1.682	3	150	40	3.517	153.611	40.60
14	0	0	-1.682	3	150	8	3.517	153.621	8.20
15	0	0	0	3	150	24	3.516	153.604	23.30
16	0	0	0	3	150	24	2.514	103.617	14.84
17	0	0	0	3	150	24	2.516	103.606	30.30
18	0	0	0	3	150	24	2.518	203.612	14.93
19	0	0	0	3	150	24	2.518	203.625	31.13
20	0	0	0	3	150	24	4.518	103.609	14.79
21	0	0	0	3	150	24	4.517	103.637	14.89

X<sub>1</sub>(Fe) = iron fortificant, X<sub>2</sub>(Ca) = calcium fortificant, X<sub>3</sub>(Vit C) = vitamin C fortificant used in the fortification trials while Y<sub>1</sub> = iron response, Y<sub>2</sub> = calcium response and Y<sub>3</sub> vitamin C response. Each row represents a fortification trial run or adjustment levels of the process variable combination at one run. The experimental design has the upper limit of +1.682, intermediate limit of zero (0) and a

lower limit of -1.682 values of process variable combinations. The three process variables (Fe, Ca, and Vitamin C) and the five experimental levels coded -1., -1.682, 0, 1 and +1.682 gave 15 variable combination which when replicated six times at center point (0) for estimation of error gave a total of 21 experimental runs shown in Table 3.2.

**Table 2:** Variable levels of fortificants used in the fortification.

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

Fe – Ferric ammonium citrate, Ca - Calcium carbonate and C- vitamin C. k-3 – the total number of fortificants (variables) used.

### Experimental analyses

All the analyses were carried out in triplicate and the mean used for statistical analyses.

### Moisture content determination

The moisture content of the samples was determined by the gravimetric method described by AOAC (2005) [21]. Five grams (5g) of milk sample was placed into each washed, dried and weighed moisture can. The cans and their samples were dried in the oven at 105°C for 3 hours cooled in desiccators and reweighed. Their weights were recorded and the samples were returned to the oven for further drying, cooling, and weighing until their constant weights were obtained. By difference the weight of moisture lost was determined and expressed as percent thus:

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where: W<sub>1</sub> = Weight of empty can, W<sub>2</sub> = Weight of can + sample before drying, W<sub>3</sub> = Weight of can + sample after drying to a constant weight.

### Ash content determination

Furnace incineration gravimetric protocol of AOAC (2005) [21] was adopted. Five grams (5g) of each soymilk sample were measured into a previously washed and weighed porcelain crucible, put in a muffle furnace preset at 550°C and allowed to ash for 3 hours (grey in colour). Thereafter, the samples in crucible were carefully removed from the furnace, cooled in a desiccators and reweighed. The weight of ash obtained was calculated and expressed in percent thus:

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where: W<sub>1</sub> = weight of empty crucible, W<sub>2</sub> = weight of crucible + milk before ashing and W<sub>3</sub> = weight of crucible + milk after ashing.

### Fat content Determination

Continuous solvent extraction methods using soxhlet apparatus described by AOAC (2005) [21] was used. Five grams (5g) of each soymilk sample were wrapped in a filter paper (Whatman number one) and put in a soxhlet flask containing 200ml of petroleum ether. The upper end of the reflux flask was connected to a condenser. By heating the solvent in the flask through electro-thermal heater, it vaporized and condensed into the reflux flask. Soon the wrapped samples were completely immersed in the solvent and remained in contact with it until the flask filled up and siphoned over those carrying the oil extract from the sample down to the boiling flask. The process was allowed to go on repeatedly for about 48 hours before the defatted sample was removed and reserved for crude fiber analysis. The solvent

was recovered and the extracting flask with its oil content was dried in the oven at 60°C for 3 min in order to remove any residual solvent. After cooling in desiccators, the flask was reweighed.

The weight of fat (oil) extraction was determined and expressed as a percent of the sample weight as follows:

$$\% \text{ Fat} = \frac{W_2 - W_1}{W_3 - W_1} \times \frac{100}{1}$$

Where W<sub>1</sub> = weight of empty extraction flask. W<sub>2</sub> = Weight of flask and sample before extraction and W<sub>3</sub> = weight of flask and oil extract after extraction.

### Protein content Determination

Micro Kjeldahl method described by the AOAC (2005) [21] was used to determine the protein content of soymilk samples. Each sample was mixed with 10ml of concentrated H<sub>2</sub>SO<sub>4</sub> along with one tablet of selenium catalyst in a heating tube and the mixture was heated under a fume cupboard (digestion) until a clear solution was obtained. Ten milliliter (10ml) portion of the digester was mixed with equal volume of 45% sodium hydroxide solution in a Kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 10ml of 4% boric acid solution containing 3 drops of mixed indicator (methyl red and bromo cresol green). A total of 50ml distillate was obtained and titrated as well. The nitrogen content was calculated and multiplied by 6.25 to obtain the protein as shown below.

$$\text{Percentage Nitrogen (\%N)} = \frac{100 \times N \times 14 \times V_f T}{W \times 1000 \times V_a}$$

Where W = Weight of sample analyzed, N = Concentrated H<sub>2</sub>SO<sub>4</sub> titrant, V<sub>f</sub> = total volume of digest

V<sub>a</sub> = Volume of digest analyzed, T = titre value – blank.

Protein content was calculated using the formula below:

$$\% \text{ Protein} = \% N_2 \times 6.25$$

### Crude fiber determination

Crude fiber determination protocol of Onwuka (2005) [22] was adopted. Two grams (2g) of each sample were digested with 200ml of solution containing 1.25g of H<sub>2</sub>SO<sub>4</sub> per 100ml of solution under reflux for 30 min. The digest was then filtered with Buckner funnel equipped with muslin cloth. The filtrate was washed with hot water until no longer acidic, scooped into a conical flask and boiled for 30 min with 200 ml of solution containing 1.25% of carbonate free NaOH per 100ml. The final residue was filtered, scooped into a previously dried and weighed crucible and dried in electric oven at 105°C to a constant weight. The dish with its content was reweighed after drying and then ashed in the muffle furnace at 550°C for 3 h. The ash was removed from

the muffle furnace cool and reweighed. The weight of fiber was calculated as a percent weight of sample analyzed as below:

$$\% \text{ Crude fiber} = \frac{W_2 - W_3}{\text{Weight of sample}} \times 100$$

Where:  $W_2$  = weight of crucible + sample after boiling, washing and drying and  $W_3$  = weight of crucible + sample as ash.

### Carbohydrate determination

Carbohydrate content was calculated by difference as the free nitrogen extract (NFE) according to Rampersad *et al.* (2003) [23] protocol as.

$$\% \text{ Carbohydrate} = 100\% - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ fat} + \% \text{ Crude fibre} + \% \text{ Crude protein}).$$

### Nutrient analyses

Vitamin C was determined according to Rahman Khan *et al.* (2006) [24] while calcium and dietary iron were determined using AAS-ashing method of Tee *et al.* (1989a and b) [25, 26] using Buck Model 200A AAS. All the analyses were carried out in triplicates and the average recorded for both the control and stored samples.

### Energy content

The energy levels of the samples were determined by calculation with the energy substrates (crude fat, protein, crude fiber and carbohydrate) using Atwater factor (Mullan, 2006) [18].

### Statistical analysis

Data obtained from the samples were subjected to analysis of variance. Completely randomized design using the SPSS version 20 was used for proximate and energy analyses. T-test was used for nutrient analyses. Means were separated with Duncan multiple range test at 95% confidence level ( $p < 0.05$ ).

## Results and Discussion

### Proximate composition

Table 3 presents the proximate compositions of the soymilk as affected by fortification.

### Moisture content (MC)

Though there was no significant ( $p < 0.05$ ) moisture content difference between FSM and UFSM, but UFSM (86.87%) was slightly higher than FSM (85.66%). The difference could be due to fortificants, most especially calcium carbonate ( $\text{CaCO}_3$ ) which may have increased the total solids (TS) thereby decreased MC. Inverse relationship between TS and MC in soymilk had been established (Nsofor *et al.*, 1997) [13]. Higher MC from both FSM and UFSM samples than other nutrients indicated that soymilk in this study was predominantly water which agrees with soymilk definition Iwe (2003) [11]. Higher MC levels in this study could be due to sprouting which minimizes TS and maximizes MC of soymilk (Nsofor *et al.*, 1997; Nsofor and Maduakor, 1992) [13, 27]. Though their MC was below 88.50% according to definition, the difference could be due to bean: water ratio (1kg: 2.7 liter), soybean variety and level of calcium carbonate fortification (50mg to 250mg/100ml) used in this study. These higher levels used

were justified due to poor calcium content (0.47mg /100ml) of soymilk that demands relatively more calcium fortificants than others (STS, 1987; Onweluzo and Nwakalor, 2009) [9, 30]. This agreed with the report of MI (2011) [28] that larger amount of calcium is required in fortification than other nutrients. Higher moisture content of soymilk will aid swallowing by the baby whose suckling reflexes is still high within the complementary age.

**Table 3:** Proximate composition (%) of fortified and unfortified soymilk

Sample	MC	Ash	Crude fat	Protein	Crude fiber	CHO
FSM	85.66 <sup>a</sup> ± 0.93	1.83 <sup>a</sup> ± 0.10	3.40 <sup>a</sup> ± 0.07	4.42 <sup>a</sup> ± 0.10	0.06 <sup>a</sup> ± 0.01	4.63 <sup>a</sup> ± 0.10
UFSM	86.87 <sup>a</sup> ± 0.52	0.4 <sup>b</sup> ± 0.04	3.50 <sup>a</sup> ± 0.04	4.43 <sup>a</sup> ± 0.01	0.05 <sup>a</sup> ± 0.01	4.75 <sup>a</sup> ± 0.26

Values are means of triplicate determinations ± standard deviations. Values with different letter subscripts had significant difference ( $p < 0.05$ ) between them. FSM-fortified soymilk, UFSM- un-fortified soymilk CHO- carbohydrate

### Ash

Ash content of FSM (1.83%) was significantly ( $p < 0.05$ ) higher than UFSM with 0.4% probably due to higher calcium carbonate levels (50mg to 250mg/100ml) than other fortificants used. Similar calcium increase had been reported in calcium carbonate fortified flour (MI, 2011) [28]. Besides, sprouting may have hydrolyzed most of soymilk macromolecules (Nsofor *et al.*, 1997; Nsofor and Maduakor, 1992) [13, 27] thereby increased the nutrient content unlike in un-sprouted soybeans with relatively larger macromolecules (Omosaiye *et al.*, 1978) [12]. The ash content is an index of mineral content of soymilk which implies that calcium carbonate fortification had positive influence in increasing soymilk calcium content. Ash content of both FSM and UFSM were higher than 0.27% reported by Soya (2009) in database for standard reference and 0.19% from Omosaiye *et al.* (1978) [12], but only UFSM was lower than 0.5% reported in Wikipedia (2011) [39] and 0.98% by Onweluzo and Nwakalor (2009) [30]. In infants, calcium is an important nutrient throughout their lives as it helps in the healthy development and growth of the babies' bones and teeth. Calcium is useful to the muscle and the heart (Christine, 2009) [31]. Iron works in synergy with protein and copper to produce red blood cells that transport oxygen from lungs to all the tissues where they are needed for maintaining all body's life functions like fuelling the cell division and growth of a developing body (Fallon and Enig, 2007) [8].

### Crude fat

Oil content (1.8%) obtained from soymilk before fortification was slightly lower than 2.0% and 2.35% reported respectively by Wikipedia (2011) [29] and BNF (2004) [17] probably due to bean variety and sprouting (Mostafa *et al.*, 1987; Youssef *et al.*, 1987) [32, 33]. Though there was no significant ( $p < 0.05$ ) difference between fat content of FSM and UFSM samples, but UFSM was slight higher than FSM probably due to calcium-fat interaction (Igyor *et al.*, 2011) [34] which may have lowered FSM oil content than UFSM. Making soymilk oil (1.8%) up to 3.5% was in consonance with soymilk definition (Iwe, 2003) [11] and in line with the recommendation of inclusion of

vegetable oil in infant and children foods to increase the energy density and as transport vehicle of fat-soluble vitamins (Zakpan, *et al.*, 2010) [35]. Also, fat is an indispensable energy substrate in infants' diet because they need energy for activity and growth.

### Crude protein

Fortification never significantly ( $p>0.05$ ) affected soymilk protein content despite slight higher protein value of UFSM (4.43%) than FSM (4.42%) probably due to soymilk protein interactions with  $\text{CaCO}_3$  (Rafferty *et al.*, 2007; MI, 2011) [10, 28]. Calcium carbonate is an economical and bioavailable calcium source recommended in infant foods. Protein values of both samples were higher than 3.6% reported by STS (1987), 3.2% by Onweluzo and Nwakalor (2009) [30] and 3.5% by Wikipedia (2011) [29]. The differential higher values may stem from soybean variety used and processing technique employed (Iwe, 2003) [11]. Though the protein values of both soymilk samples cannot meet the recommended daily intake of 11g for infants aged 7 to 12 months by Institute of Medicine per serving, consumption of 200ml per day will meet the need which is feasible considering infants' stomach capacity of 200ml (Uwaeghute, 2008) [36]. Protein is essential for proper growth and development, a major structural component of muscle tissues, helps repair, increase and maintain muscle mass in children. Insufficient protein intake result in stunted growth, malnutrition, permanent mental and physical problems in children (SFGATE, 2018b) [37]. Protein is also one of the three major energy substrates and enhances calcium absorption (Fallon and Enig, 2007) [8].

### Crude fiber

Crude fiber content of FSM and UFSM were not significantly ( $p>0.05$ ) different from each other, but FSM (0.06%) was slightly higher than UFSM (0.05%) which may be due to fortificants. Crude fiber contents of both soymilk samples were lower than 3.2% reported by Youssef *et al.* (1987) [33] from dehulled beans which may be due to variety and preparation method used. Crude fiber content of both FSM and UFSM can only meet the recommended fiber intake of 5g/d for infants up to 1yr by consuming 84ml of FSM and 100ml of UFSM. Therefore, crude fiber content levels of both soymilk samples were not only good sources, but are acceptable (SFGATE, 2017) [38] for infants so as not to prevent them from meeting their energy needs (BNF, 2004) [17]. Fiber is one of sub type of carbohydrate contents which consists of soluble and insoluble portions responsible in normalizing infants' bowel movements, prevents constipation and helps to control blood sugar (SFGATE, 2017) [38].

### Carbohydrate

Carbohydrate contents of UFSM (4.7%) was significantly ( $p<0.05$ ) higher than 4.63% from FSM may be in part due to fortificants which had insignificant ( $p>0.05$ ) decrease in MC, protein and fat in FSM and increased in MC and protein in UFSM. Though the carbohydrate values of FSM (4.63%) and UFSM (4.73%) obtained in this study was higher than 3.4% reported by STS (1987) and 3.13% from Onweluzo and Nwakalor (2009) [30], they were generally low due to bean steeping and sprouting. Low carbohydrate content of both samples will only meet the daily recommended carbohydrate content of 130g by

complementing with other carbohydrate rich foods. Carbohydrates provide energy that fuels children's metabolism, supports growth, keeps their brain and nervous system working and maintains overall health (SFGATE, 2018a) [39].

### Nutrient composition

Nutrient content of UFSM and FSM were presented in Table 4.

#### Dietary iron

Significant ( $p<0.05$ ) higher iron content (5.52 mg/100ml) of FSM (Table 1) than 0.525 mg/100ml from UFSM projected ferric ammonium citrate fortification as a major iron contributor. Therefore, fortification is a better way of enhancing iron concentration of soymilk. The FSM proved to be good source of iron and can meet iron RDI of 11 mg/d for infants of both sexes aged from 0 to 2years and above (ODS, 2007) [40] upon consumption of 200 - 300mg/100ml of the samples along with breast milk and other iron containing foods. This is possible considering the infant stomach capacity of 200ml (Uwaegbute, 2008) [36]. The aim of iron fortification in this study, to deliver greater percentage of RDI or RDA of calcium (50.18%) was achieved (Clarke, 1995; Barclay, 2011) [15, 41]. Lower iron content recorded (less than the RDI) by UFSM may be traced to poor iron source of soymilk (Yeung *et al.*, 2005) [42], while general low levels of the dietary iron from UFSM and FSM depended on fortificant levels used (1 to 5mg/100ml). However, healthy full-term infants are born with a supply of iron from the mother that lasts for 4 to 6 months (Oeveren, 2005; Uwaegbute, 2008) [14, 36]. Hence iron fortification is inevitable in iron deficient infant foods. Iron regulates growth, transfers oxygen to the muscles, metabolizes protein for energy with the aid of calcium and copper only, forms muscle myoglobin, and contraction (Fallon and Enig, 2007; ODS, 2007) [8, 40]. Advanced stage of the depletion leads to anemia which is characterized with fatigue as a result of low intake and absorption of iron or excessive blood loss.

**Table 4:** Nutrient composition of fortified and unfortified soy milk (mg/100ml)

Sample	Fe	Ca	Vit. C
UFSM	0.525 <sup>b</sup> ±0.014	3.80 <sup>b</sup> ±0.14	4.31 <sup>b</sup> ±0.01
FSM	5.52 <sup>a</sup> ±0.14	253.68 <sup>a</sup> ±0.11	40.60 <sup>a</sup> ±0.14

Values a-b are mean triplicate determinations ± standard deviation. Values in same column with different superscripts have significantly ( $p<0.05$ ) different between them. FSM – fortified soymilk, UFSM – un-fortified soymilk. Fe –ferric ammonium citrate, Ca – calcium carbonate and vit C – vitamin C.

#### Calcium content

Significant ( $p<0.05$ ) higher calcium content (253.68 mg/100ml) of FSM (Table 1) than UFSM (0.525 mg/100ml) no doubt stemmed from calcium carbonate ( $\text{CaCO}_3$ ) fortification. Calcium carbonate is one of the highest levels calcium compounds as small amount will deliver the desired level of calcium in foods. Also,  $\text{CaCO}_3$  is as an economical source and bioavailable form of calcium salt (STS, 1987) [9]. However, lower levels of calcium concentration in unfortified soymilk is in consonant with the reports of STS

(1987)<sup>[9]</sup> and Wikipedia (2010)<sup>[29]</sup> that soymilk is naturally deficient in calcium, lower than the RDI, and therefore poor source of calcium. Calcium fortification of soymilk for infant diets is therefore imperative as breast milk calcium is also low out of which 67% is absorbed by babies (Donovan, 2009)<sup>[43]</sup> despite efficient calcium absorption by infants (Bhatia, 2008)<sup>[44]</sup>.

Primary aim of calcium fortification, providing a reasonable percentage (41.65 to 92.55%) of calcium RDA or RDI per serving (Soya, 2009; SMI, 2009)<sup>[46, 46]</sup> was achieved in this study. Consumption of 106 to 236.51 mg/100ml of the fortified soymilk will meet calcium RDI (270mg/d – 600mg/day) for infants aged between 7 to 12 months (WhereinCity, 2009; BHC 2009)<sup>[47, 48]</sup>. The FSM samples are excellent sources of calcium as they contained more than 20% of daily value (Rafferty *et al.*, 2007)<sup>[10]</sup>. Calcium helps nutrient flow across the cell wall, (Fallon and Enig, 2007; Wherein City, 2009)<sup>[8, 47]</sup>, helps in healthy development and growth of babies' bones, teeth (IMF, 2004)<sup>[49]</sup>, muscle and heart (Chritine, 2009)<sup>[31]</sup>.

### Vitamin C content

Vitamin C content of the soymilk increased (89.38%) significantly ( $p < 0.05$ ) from 4.31 mg/100ml in UFSM to 40.60 mg/100ml in FSM (Table 1) was due to vitamin C fortification. Vitamin C is lost through light, Ph and interaction with minerals, mineral and food components (Barclay, 2011)<sup>[41]</sup> which calls for overage in multiple food fortifications to maintain label claims (Hurrell *et al.*, 2004)<sup>[50]</sup>. Similar low vitamin C content (4.21mg/100ml) was recorded by Onweluzo and Nwakalor (2009)<sup>[30]</sup> from unfortified soymilk. General low vitamin C content for both FSM and UFSM could be traced to steeping (Sathithon and Yan-bin, 2012)<sup>[51]</sup>, lack of protection (Clarke, 1995)<sup>[15]</sup>, and processing losses during bicarbonate blanch, hot grind and sterilization. Despite the losses, vitamin C content of most FSM was between good and excellent sources of vitamin C (NFH, 2001; Rafferty *et al.*, 2007)<sup>[52, 10]</sup>. The aim of vitamin C fortification to deliver greater percentage of the RDI or RDA ( $>100\%$ ) was achieved in this study (Clarke, 1995; Barclay, 2011)<sup>[15, 41]</sup>. The FSM can meet vitamin C RDI of 30 - 35mg/serving for infants (NFH, 2001; MedicineNet, 2011)<sup>[52, 53]</sup> upon consumption of between 96 – 427 mg/100ml while UFSM will meet by consuming between 696 – 812mg/100ml. Only soymilk from run 13 (40.60mg/100ml) can meet the RDI per serving (100ml). As vitamin C is needed in trace amount for proper biological functions (Onwuka, 2005)<sup>[22]</sup>, vitamin C levels of FSM obtained in this study may likely meet the biological needs of infants and young children more than 4.32 mg/100ml from UFSM. Lack of vitamin C results in anemia (DSM, 2011)<sup>[54]</sup>. Vitamin C stabilizes micronutrients in multiple fortified foods and beverage (Wikipedia, 2011; DSM, 2011)<sup>[29, 54]</sup> and it is required by the body for formation and

maintenance of bones blood vessels and skin (MedicineNet, 2011)<sup>[53]</sup>. Vitamin C synergizes with enzymes to release energy from digested foods and regulates chemical reactions in the body (Onwuka, 2005)<sup>[22]</sup>.

### Energy level

Results of calculated energy profiles of FSM and UFSM soymilk by Atwater factor were presented in Table 5. Energy value (373.04Kj or 65.39Kcal) of UFSM was significantly ( $p < 0.05$ ) higher than 363.74Kj (64.03Kcal) from FSM probably due to fortificants' interactions with soymilk components. Calcium carbonate ( $\text{CaCO}_3$ ) interacts weakly with protein without coagulation (SMI, 2009)<sup>[46]</sup> thereby preventing their availability. Metabolized soymilk protein by iron fortificant for absorption (Fallon and Enig, 2007)<sup>[8]</sup> and sprouting (Osuji and Ubaonu, 2004)<sup>[7]</sup> may have made more protein available for interactions with calcium. Also, calcium interacts with fat (Igyor *et al.*, 2011)<sup>[34]</sup>. Barclay (2011)<sup>[41]</sup> had reported that interactions results in nutrient loss which may have been responsible for lower protein and fat content in FSM. Despite the difference, energy values obtained per 100ml from FSM and UFSM were within the RDA range (200 to 550 Kcal per day) recommended by WHO (2002)<sup>[55]</sup>, for infants aged 6 to 23 months.

Lower energy values of both soymilk samples than the upper limit of 550Kcal could be traced to rapid depletion of energy components of soymilk like oil, protein and carbohydrate during the sprouting (Nsofor *et al.*, 1997)<sup>[13]</sup> and soymilk nutrient-fortificant interactions. The growing sprouts (embryo) absorbed protein hydrolyzates for growth, and carbohydrate hydrolyzates for respiration, incorporation into cell walls and translocation to growing embryo. In same vein, the growing embryo used fat for respiratory energy. Very low fiber content recorded could be due to variety and dehulling. Youssef *et al.* (1987)<sup>[33]</sup> had earlier reported a reduction of 89.9% of fiber by dehulling. These notwithstanding, consumption of 55ml to 151.21ml of FSM and 53ml to 147.44ml of UFSM soymilk per day will meet infants' WHO (2002)<sup>[55]</sup> recommended daily energy need. Furthermore, consumption of such adjunct foods like biscuits, fruits and vegetable (Agostoni *et al.*, 2008)<sup>[4]</sup> alongside breast milk with calorific value of 0.7Kcal/g (Uwaegbute, 2008)<sup>[36]</sup> and avoidance of high fiber foods that will prevent the child from meeting their energy need (BNF, 2004)<sup>[17]</sup> will help as well. Dietary energy is needed in the body for metabolic processes, physiological functions, muscular activity, heat production, growth, transport of substances around the body, synthesis of enzymes and hormones. American Academy of Pediatrics (AAP) recommended that young children need calories from fat for growth and brain development in the first 2 years of life as they are active.

**Table 5:** Calculated energy values of fortified and un-fortified soymilk by Atwater factor per 100ml

Sample	Protein	Fat	Fiber	Carbohydrate	Energy values Kj/g	Kcal/g
FSM	4.42 <sup>a</sup> +0.10	3.40 <sup>a</sup> +0.07	0.06 <sup>a</sup> +0.01	4.63 <sup>b</sup> +0.01	363.74 <sup>b</sup> +0.3	64.03 <sup>b</sup> +0.13
UFSM	4.43 <sup>a</sup> +0.01	3.50 <sup>a</sup> +0.04	0.05 <sup>a</sup> +0.01	4.75 <sup>a</sup> +0.06	373.04 <sup>a</sup> +0.04	65.39 <sup>a</sup> +0.01

Values are mean triplicate determination  $\pm$  standard deviation. Values in same column with same superscript have no significant difference ( $P < 0.05$ ) while those with

different superscript have significant ( $P \geq 0.05$ ). FSM = Fortified soymilk. UFSM = Unfortified soymilk.

## Conclusion

Soy milk fortification had only significant higher effects on ash, but insignificant effect on other components presumably due to the levels of fortificants used. Fortified soy milk had significant increase than un-fortified soy milk in dietary iron, calcium and vitamin C in all levels of fortificants used in this study. Soy milk components are therefore compatible to the fortificants used as nutrient increase is a function of fortificant levels. Consumption of 50 to 150ml of these FSM and UFSM daily alongside other adjunct energy substrate foods with adequate breast feeding will meet the WHO daily energy requirement for infants aged 6 to 23m provided their fiber level does not exceed 0.06%. Consumption of up to 150ml soy milk daily is possible considering infant stomach capacity of 200ml. Therefore, soy milk fortification for complementary feeding is feasible as it will offer an economic complementary food liable to prevent weaning deficiency in infants.

## Acknowledgement

The authors are grateful to National Cereal Research Institute (NCRI) outstation, Amakama Olokoro for provision of soybean variety, and food processing laboratory of Michael Okpara University of Agriculture for research space and reagents both in Abia state Nigeria. Also, our regards go to World Bank Assisted Step B Project for their financial assistance.

## References

1. Lonnerdal B. Effects of Maternal Dietary Intake on Human Milk Composition, 1985. [www.researchDiets.com](http://www.researchDiets.com).
2. Yeung LD. Iron and Complementary Food Fortification. United Nations University <http://unu.edu>.
3. Rarback S. Infant nutrition In: Nutrition and well-being A to Z. Powered by JRank, 2011. <http://www.fags.org/nutrition/hea-Irr/infact-Nutrition.html> 2011.
4. Agostoni C, Decsi T, Fewtrell M, Goulet O, Kolasek S, Koletzko B, *et al.* ESPGHAN Committee on Nutrition J. *Pediatr. Gastroenterol. Nutri.* 2008; 46(1):99-110.
4. UNICEF. Complementary Foods and Feeding: Nutritional Companion to Breast feeding Infants after six months, 2010. <http://www.unicef.org/programmer/breast-feeding/food.htm>.
5. Iwe MO. Principles of Complementary Foods Formulation. An Invited Paper Presented at the Zonal Consultation on Breast Feeding and Complementary Feeding Held at the Marble Arch Hotel, Awka Anambra State, 2010.
6. Osuji CM, Ubaonu CN. Chemical and Physical Properties of Predigested Soy milk Concentrates Developed by Enzyme Hydrolysis of Soybean Extracts. Nutritional Society of Nigeria 34<sup>th</sup> Annual Conference and Scientific Meeting. Theme: Child Survival and the Right of Adequate Nutrition, 2004, 116-119. York San Francisco London. 2004 219 - 246.
7. Fallon S, Enig MG. Nourishing tradition In: The cookbook that challenges politically correct nutrition and the Diet Dictocrates. New Trends Publishing Inc. [www.Newtrendpublishing.com](http://www.Newtrendpublishing.com), 2007.
8. Soya Technology Systems Ltd (STS). Soy milk in brief – A case that makes science 11. Dhoby Ghaut. No.11-06 Gathay Building Singapore 0922, 1987, 9-47.
9. Rafferty K, Walters G, Heaney RP. Calcium Fortificants: Overview and Strategies for Improving Calcium Nutrition of US population. *J Fd. Sci.* 2007; 72(9):R152-R158.
10. Iwe MO. The Science and Technology of Soybean. Published by Rojoint Communication Services Ltd, 2003, 27-262.
11. Omosaiye O, Cheryan M, Matthews ME. Removal of oligosaccharides from soybean water extracts by ultrafiltration. *J Fd. Sci.* 1978; 43:353-360.
12. Nsofor LM, Okpara BU, Osuji CM. Tropical storage stability and chemical properties of soy milk from sprouted soybeans *J Fd. Sci. Technol.* 1997; 34(6):377-384.
13. Oeveren VM. Vitamin B12 for Vegan Mothers and their Babies In: Public Health Service. Nelson Marlborough District Health Board, 2005, 1-3.
14. Clarke RI. Micronutrient Fortification of Foods: Technology and Quality Control FAO Corporate Document Repository, 1995. <http://www.fao.org/docrep/w284oe/w284oeob.htm>.
15. Nutritional Health and Medical Research Council (NHMRC). Dietary energy. [https://www.nrv.gov.au/sites/default/files/page\\_pdf](https://www.nrv.gov.au/sites/default/files/page_pdf) 2016.
16. British Nutrition Foundation (BNF). Nutrient requirement and recommendations. <http://www.britishnutrition.org.uk/home.asp?sited=438>. section id=4148 subsection id =3208 parent section, 2004.
17. Mullan WMA. Labeling determination of the energy content of food (On-line). <http://www.dairyscience.info/packing-/119-labelling-determination-of-the-energy-content-of-food-html.2006>.
18. Okwunodulu IN, Okwunodulu FU. Physicochemical properties of vitamin and minerals fortified soy milk for complementary feeding. A response surface analysis. *International Journal of Applied Research and Technology.* 2016; 5(7):94-101.
19. Box GEP, Wilso KB. On the experimental attainment of optimum Conditions. *J Roy Stat.* 1951; 13:1-45.
20. AOAC Official Methods of analysis, Association of Official Analytical Chemist. 18th edition. Washington DC. USA, 2005
21. Onwuka GI. Food Analysis and Instrumentation. Theory and Practice. Published by Naphthali Prints, 2005, 104-121.
22. Rampersad R, Badrie N, Comisciong E. Physico-chemical and sensory characteristics of flavoured snacks from extruded cassava/pigeon pea flour. *J Ed. Sci.* 2003; 68(1):363-367.
23. Rahman Khan MM, Rahman MM, Islam MS, Begum SA. A simple UV-Spectrophotometric Method for the Determination of Vitamin C Content in Various Fruits and Vegetables at Sylhet Areas in Bangladesh. *J. Biological Sci.* 2006; 6(2):388-392.
24. Tee ES, Khor SC, Siti MS. Determination of Calcium in Foods by the Atomic Absorption Spectrophotometric and Titrimetric Methods. *Pertanika.* 1989a; 12(3):303-311.
25. Tee ES, Khor SC, Siti MS. Determination of Iron in Foods by the Atomic Absorption Spectrophotometric

- and Colorimetric Methods. Pertenika. 1989b; 12(3):313-322.
26. Nsofor LM, Maduako O. Stabilized Soymilk for Ambient Tropical Storage: A Preliminary Report. *Int. J. Fd. Sci. Technol*, 1992; 27:573-576.
  27. MI (Mineral Incorporated). Properties of minerals used in cereal fortification (Section 6 in MI's Fortification Handbook) <http://www.fortisf.org/files/propertiesofminerals.pdf> 2011.
  28. Wikipedia, Ascorbic acid. Wikipedia, the free Encyclopedia. <http://en.wikipedia.org/wiki/Ascorbicacid>, 2011.
  29. Onweluzo JC, Nwakalor C. Development and evaluation of vegetable milk from *Treculia africana* (Decne) seeds. *Pakistan J of Nutri*. 2009; 8(3):233-238.
  30. Christine. List of calcium rich foods – Building healthy teeth and bones. The homemade baby food recipes Blog. <http://blog.homemade-baby-food-recipes.com/list-of-calcium-rich-foods/>.2009.
  31. Mostafa MM, Rahma EH, Rady AH. Chemical and nutritional changes in soybeans during germination. *Fd. Chem*, 1987; 23:257-275.
  32. Youssef MM, Abd EL-Aal MH, Laila AE, Ziena HM. Effects of dehulling, soaking and germination on chemical composition, mineral elements and protein patterns of faba beans (*Vicia Faba L.*) *Food Chemistry*, 1987; 23:129-138.
  33. Igyor MA, Yusuf PA, Sengev IA. Functional and sensory properties of fermented fura powder supplemented with soy. *Nig. Food J*, 2011; 29:113-121.
  34. Zakpan HD, Mak-Mensah EE, Adubofour J. Production and characterization of flour produced from ripe "apem" plantain (*Musa sapientum L. var. paradisiacal*; French horn) grown in Ghana. *J Agric. Biotech. Sustainable Dev*. 2010; 2(6):92-99.
  35. Uwaegbute AC. Adequate infant feeding: bed rock for national development, poverty alleviation and empowerment. Sixth Inaugural Lecture at Michael Okpara University of Agriculture Umudikem, 2008, 1-47.
  36. SFGATE. How much protein should a child have? <http://healthyeating.sfgate.com/much-protein-should-child-have-422>, 2018b.
  37. SFGATE. Normal fiber intake for children. <http://healthyeating.com/normal-fiber-intake-children-4548.html>, 2017.
  38. SFGATE. Carbohydrate needs in children. <http://healthyeating.sfgate.com/carbohydrate-needs-children-8050.html> 2018a.
  39. Office of Dietary Supplements (ODS). Dietary Supplements Fact Sheet: Iron Office of Dietary Supplements National Institutes for health, 2007.
  40. Barclay D. Multiple fortification of beverages. <http://unu.edu/unupress/food/v192e/ch13.htm> 2011.
  41. Yeung CK, Glahn RP, Welch RM, Miller DD. Prebiotics and Iron Bioavailability – Is There a Connection? *J Food Science*. 2005; 70(5):R88-R92.
  42. Donovan D. Concerned about Baby's Calcium Intake. Common Concerns. Published by: Village Inc, 2009. <http://parenting.ivillage.com/baby/bbreastfeed/0,,3x9,,00.html>.
  43. Bhatia V. Dietary Calcium Intake – A Critical Reappraisal. *Indian J Med. Res*, 2008; 127:269-273.
  44. Soya. Nutritional values of soymilk. home soy foods reviews soya health soya recipes soy candles. E:/Nutritional values of soymilk.htm, 2009.
  45. Specialty Mineral Incorporated (SMI). Interactions of precipitated calcium carbonate (PCC) with proteins in the calcium fortification of dairy and non-dairy beverages ([www.specialtymineral.com/specialty-applications/specialty-markets-mineral/food-fortification/beverage](http://www.specialtymineral.com/specialty-applications/specialty-markets-mineral/food-fortification/beverage)). 2009.
  46. Wherein City Medical (WM) Calcium. Wherein City Medical, 2009. <http://www.whereincity.com/medical/minerals/cdcium.php>.
  47. Better Health Channel (B.H.C.) Calcium-factSheet [www.betterhealth.vic.gov.au](http://www.betterhealth.vic.gov.au/state_of_Victoria). state of Victoria (Australia). All Right Reserved, 2009, 1-3.
  48. International Association of Infant Food Manufacturers (IMF) Alpha-linolenic acid in infant formula: is there an optimum level. IFM's Advisory Committee, 2004. [www.ifm.net/industry/cow\\_milk.htm](http://www.ifm.net/industry/cow_milk.htm).
  49. Hurrell RF, Lynch S, Bothwell T, Cori H, Glahn R, Hertramp FE, *et al.* Enhancing the absorption of fortification of iron. A SUSTAIN Task Force Report. *Int. J. Vitam. Nutr. Res*. 2004; 74(9):387-401. Abstract. <http://www.ncbi.nlm.nih.gov/pubmed/15743016>.
  50. Sathithon P, Yan-bin X. Effect of sprouting on the chemical and nutritive quality and phenolic alkaloid content of lotus (*Nelumbo nucifera Geartn.*) seeds. *Afr. J Fd. Sci*. 2012; 6(7):204-211.
  51. Natural Food Hub [NFH]. Natural Food-- Fruit vitamin C content [http://www.naturalhub.com/natural\\_food\\_guide\\_fruit\\_vitamin\\_c.htm](http://www.naturalhub.com/natural_food_guide_fruit_vitamin_c.htm), 2001.
  52. Medicine Net (MN). Definition of ascorbic acid <http://www.medterms.com/script/main/art.asp?articlekey=12536>, 2011.
  53. Dutch State Mines (DSM). DSM nutritional products' vitamin C – Our portfolio of vitamin C products. Copyright DSM Nutritional Products. [www.quali-c.com](http://www.quali-c.com), 2011.
  54. World Health Organization (WHO). Complementary feeding: report of the global consultation, and summary of guiding principles for complementary feeding of the breastfed child. World Health Organization Switzerland. Email: [bookorders@who.int](mailto:bookorders@who.int), 2002.