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Formulation and evaluation of phytomilk from soybean (*Glycine max*_ L) as an alternative to dairy milk

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ABSTRACT

Phytomilk made from soybean was prepared in distilled water and in 4% aqueous gum Arabic solution. The two products, as well as peak milk (standard dairy milk product) were analyzed for their physical and chemical characteristics and compared. Taste enhancement with sweeteners, Vanilla and Banana flavours were tested. The shelf life of the product with, and without the addition of ascorbic acid and selenium salt were also tested. Relative viscosity and emulsion stability were higher in samples that contained gum Arabic solution, than in those made with distilled water only. The gum Arabic preparation also has higher levels of free reducing sugars, soluble peptides, total lipids, and amino acids. Result of the sensory evaluation of the samples; indicated that the preparation containing banana flavour appeared to be most acceptable to the panelist compared to that containing vanilla flavour and that without any flavour. Addition of ascorbic acid (0.05%) or selenium (0.01%) to the pasteurized sample significantly reduced the rate of change in pH irrespective of storage condition, whether ambient or at 4°C

Key words: Gum Arabic, Ambient, Phytomilk, Muslin cloth, Hedonic scale.
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INTRODUCTION

Milk and milk products, meat and meat products, fruits and vegetables and the cereal groups are the four basic food groups for sources of essential nutrients needed by the body. The milk and milk products food group is usually lacking from the diets of people in most developing countries. This is due to the fact that, the product comes from animals such as cows, goats and sheep, and statistics have shown that these livestock are inadequate to meet the demand for milk as well as for meat to feed the growing population in most developing countries of which Nigeria is one [1]. Hence the problem of malnutrition, due to lack of certain nutrients obtainable from milk sources as observed in these countries.

As a result of the shortage of livestock in these countries, milk and milk products and meat are expensive. Foods and milk of plant origin are easily available, cheaper and within the reach of most citizens of these countries hence they tend to be vegetarians [2]. Some people who take animal or dairy milk have problem with the digestion of lactose, the carbohydrate in milk. Therefore, there is a need to formulate a milk-like product that will be easily available, sensorily and physiologically acceptable and affordable. An example is the milk product called "phytomilk" [3] produced from protein rich plant seeds – such as, soybean (*Glycine max* L), melon seeds (*Citrullis colocythis* L) and tiger nuts (*Cyprus esculentus*).

Soybeans was chosen for this work because it is readily available and is the cheapest of the three seeds mentioned above. Some of the advantages of soybean are as follows. It has protein of exceptionally high biological value, and high oil content which could be of importance in Nigeria, especially in areas with high cases of malnutrition. It was reported by Balmir [4] that dietary soybean increased plasma thyroxin, an advantage for areas with endemic goiter. Phytomilk from soybeans could be used by individuals that are lactase deficient [5]. It can conveniently be used as a weaning formula instead of cow's milk for infants. This is because infants have limited capacity to excrete the high solute load in cow milk or cow-milk based formula with increased water required for the maintenance of water balance, which could be a problem [6]. Akinrele and Edwards [7], found that the biological value of maize "Ogi" (pap) was improved when supplemented with soybean, the protein efficiency ratio (PER) increasing three fold, and making the protein almost as good as casein. Soon *et al*. [8], observed that the soy protein lipid film (SPLF) was very rich in protein, lipids, and free sugars. Soy protein and soy Isoflavones, have been observed to have hypocholesterolemic effects

in chemical trials as reported by Xing-Gang and Shaw [9].

There are however some major problems associated with soybean phytomilk. These problems have made it impossible for some people to use the product. Newberne [10], reported the presence of some toxic substances in soybean, which have been chemically characterized. Nunomura and Sasaki [11], reported the presence of trypsin inhibitors commonly found in varieties of soybean. The presence of other toxic components, such as hemagglutinins or lectins and saponins, was reported by Wogan [12]. These toxic and antinutritional factors can be neutralized in the digestive tract, or greatly reduce by proper cooking [10].

Other problems associated with phytomilk are the presence of beany-flavour, attributed to a mixture of many volatile compounds and low calcium levels [13,14]. Some chemicals such as phenolic compounds are known to develop and cause off-flavour during thermal processing of phytomilk and even dairy milk [15]. Phytomilk does not remain in colloidal suspension for long after straining the milk from whole soybean. The particles, which make up this suspension, tend to settle with time at the bottom of the container leaving a clear liquid at the top. This is due to the difference in relative density of the water and these particles.

The objective of this work, therefore, is to produce phytomilk from soybean, which will either reduce or eliminate some of the problems mentioned above and to assess the physicochemical properties as well as its acceptability, while making a comparison with a standard dairy milk (peak milk).

MATERIALS AND METHODS

The soybean (Glycine max L) (white variety) was purchased from "Monday Market", Maiduguri, Borno State, Nigeria. Sugar, gum Arabic, peak milk, vanilla and Banana flavours (propylene glycol, water and flavouring colour) (made by Rayner and Co. Ltd. England) were also purchased from the same market.Ascorbic acid (Mulchemic,Germany),Selenium (Thompson and Capper,Ltd.England).

Preparation of phytomilk (soybean milk)

One hundred grams of cleaned soybeans were boiled in 200ml of 2% (W/V) sodium carbonate solution for fifteen minutes. This is to hasten the softening of the husk for easy removal. The boiled seeds were washed twice with hot water and dehusked. The seeds were further washed twice in cold water and blended to a smooth paste using a Monlenex blender (South Korea). The paste was then mixed with twice the volume of distilled water or 4% (W/V) aqueous gum Arabic solution to obtain a suspension, which was sieved through a muslin cloth (pore size, 125um) into a dry bowl. The filtrate was boiled while stirring with a glass rod for twenty five minutes and allowed to cool at room temperature (37^{o}c). The phytomilk was then divided into two batches, one was dispensed into sterilized bottles and stored at 4^{o}c until required, while the other half was treated with sugar (6g/100ml), calcium carbonate (between 288 to 360 mg) vanilla and Banana flavours (0.1ml/100ml), for further evaluation.

Proximate analysis

Proximate Analysis of phytomilk was done according to the method described by the American Association of cereal Chemist [16].

Emulsion stability

Phytomilk exhibited unstable emulsion when left standing at ambient temperature and at 4^{o}C, hence the need to improve on the emulsion stability. The emulsion stability of the phytomilk was determined by the method of Aoki and Nagano [17].

In a preliminary study, phytomilk was prepared in distilled water and in various concentrations (0.5 to 7% W/V) of aqueous gum Arabic solution as base. The preparation (in 4% gum Arabic) with the highest emulsion stability was chosen for subsequent work. Phytomilk prepared in distilled water was always used as a control for comparison. Peak milk (liquid) was used as the standard throughout the work.

Other comparative analysis

Comparative test carried out include; Titratable Acidity by the A.O.A.C [18]; Relative Viscosity by the method of Reid and Ugwu [19]: Total Ash, A.A.C.C. [16]; Crude Fat by the method described by the A.O.C.S [20]; Crude Protein. A.O.A.C. [20]; Reducing Sugars by the ferricyanide-molybdate method of Alan, and Austen [23]; Total Amino acid by the method of Moore and Stein [22]; and Total Protein by the method of Layne [23].

Shelf life analysis

The presence of certain compounds in phytomilk has been reported to be responsible for its short shelf life [13]. Heat treatment reduces these compounds and the shelf life is affected. Two different anti-oxidants: Ascorbic acid and Selenium were added as follows, 0.05% solution of Ascorbic acid and 0.01% Selenium .

Statistical analysis

All chemical analysis was done in triplicate and the data presented as mean ± standard Deviation (SD). Comparison between paired means was done by the standard student t test. The comparisons were done between the following:

(a) distilled water extract of soybean, fortified with $CaCO_3$, referred to as control.

(b) 4% gum Arabic extract of soybean fortified with $CaCO_3$ referred to as "Test".

(c) Commercially available dairy milk (Peak milk) referred to as standard.

Sensory evaluation

Sensory evaluation was conducted with 120 volunteer panelists who were asked to score various samples separately using various sensory parameters from a minimum of 1 to a maximum of 6 points on a score sheet provided for each panelist. The milk samples were noted for appearance, taste, flavour, and overall acceptability on a six point Hedonic scale on the sheet. The acceptability for each milk sample, ranging from 'I like it very much – 6 point' to 'I dislike it – 1 point', was calculated using the formula below:

$$\text{Acceptability} = \frac{\text{Sum of No. of respondents corresponding score}}{\text{Sum of the Responded score.}}$$

The score of each sample was compared with that of the standard, peak milk (commercial dairy milk) from the result obtained, the following needed to be improved upon (i) Flavour (ii) Taste (iii) Overall acceptability.

RESULTS

Proximate composition

Phytomilk preparation in 4% gum Arabic solution had no significant effect on the percentage ash, crude protein and carbohydrate content (Table 1) of the milk. However, the percentage total lipids of the phytomilk preparation in 4% gum Arabic was significantly higher ($P < 0.05$) than that of the milk preparation in distilled water and the standard milk.

Chemical parameters of phytomilk

Results of the chemical parameters that were analyzed in the phytomilk as presented in Table 4. The phytomilk preparation in gum Arabic had no significant effect ($P< 0.05$) on the Titratable acidity and calcium level. The moisture content of the gum Arabic preparation was significantly lower ($P< 0.05$) than that of the distilled water preparation. The levels of these parameters were however still significantly lower than those in the standard dairy milk. The free reducing sugars, amino acids, and soluble peptides in gum Arabic preparation were 42%, 42%, and 47% respectively of the values in the standard dairy milk. The calcium content of the 4% gum Arabic phytomilk preparation was found to be only 3% of that found in the dairy milk. This was however raised to 75% by the addition of 288 mg of calcium carbonate powder to 100 ml of the phytomilk sample.

DISCUSSION

The results of this study showed that the viscosity and emulsion stability of phytomilk increased when prepared in 4% gum Arabic solution than in distilled water. This may be attributed to the pectin like properties of gum Arabic [24]. Aqueous solutions of pectin have been shown to be more viscous than water [25].

Apparently the increased viscosity also caused an increase in emulsion stability of the samples containing gum Arabic. Amongst other factors, the viscosity of a suspension is known to affect the emulsion stability [26]. Thus, the more viscous an emulsion is, the more stable it becomes. However, in spite of these improvements the phytomilk preparation in gum Arabic is still less stable than the standard commercial diary milk with which it was compared.

The levels of total free reducing sugar and amino acids as well as soluble peptides were higher in the phytomilk samples prepared in gum Arabic than most

Table 1: Proximate analysis of standard dairy milk and phytomilk prepared in distilled water and 4% aqueous gum arabic solution

Parameters* C%	Control	4% Gum Arabic Test	Standard
Ash	1.58 ± 0.12^a	1.62 ± 0.08^a	5.10 ± 0.25^b
Crude proteins	19.60 ± 0.86^a	21.10 ± 0.54^a	26.16 ± 1.15^b
Total lipids	13.50 ± 1.62^a	16.50 ± 0.97^b	9.00 ± 8.81^c
Crude Carbohydrate	65.10 ± 3.71^a	60.40 ± 5.86^a	58.17 ± 3.80^b

of the samples prepared in distilled water. These increases are attributable to the fact that, gum Arabic being a plant material contained some amount of these substances in it. A lower moisture content was observed in the gum Arabic preparation (Table 4) than the distilled water preparation. These may have also affected the concentration of substances in the gum Arabic preparation.

Proximate analysis of the phytomilk sample (Table 1) indicates that, the presence of gum Arabic

Table 2: Sensory evaluation of standard dairy milk and phytomilk in 4% agueous gum arabic solution flavored with banana or vanilla.

Milk samples	Appearance	Taste	Flour (%)	Overall	Percentage
Soymilk with Vanilla	4.46± 091[b]	4.53 ± 1.04[b]	4.77 ± 1.17[b]	4.391[b]	75.63
Soymilk with Banana	5.41 ± 0.46[a]	5.61 ± 0.87[a]	45. 48 ±1.23[a]	4.48 ± 1.63[b]	87.63
Standard peak milk	5.72 ± 0.72[a]	5.83 ± 1.63[a]	5.51 ± 0.98[a]	5.91 ± 0.3[a]	95.70

Table 3: Physical perameters of standard dairy milk and phytomilk prepared in distilled water and 4% aqueous gum Arabic solution.

Parameters	Standard	Control	Taste
pH	8.38 ± 0.03[b]	7.40 ± 0.02[a]	7.30 ± 0.06c
Relative Viscosity	13.99 ± 0.00[b]	3.05 ± 0.07[a]	4.08 ±0.02[c]
Emulsion Stability	99.80 ± 0.10[b]	89.50 ± 2.36[a]	96.50 ± 2.80[b]

Each value is the mean of three determination = SD: values with different subscripts within a row are statistically different (P 0.05). 'Control' and 'Taste' refer to samples prepared in distilled water and 4% gum Arabic, respectively.

Table 4: Chemical parameters of standard dairy milk and phytomilk prepared in distilled water and 4% aqueous gum Arabic solution

Parameters	Standard	Control	Test
Moisture content	87.72 ±1.58[b]	92.59 ± 1.20[a]	89.29 ± 1.00c
Titratable Acidity (mgacid/100ml)	468.00 ±25.83[b]	125. ± 10.40[a]	143.78 ± 14.41[a]
Free reducing sugar (mg/100 ml)	18.00 ± 50.30[b]	315.00 ± 15.68[a]	760.00 ± 25.98[a]
Soluble peptides (mg/100ml)	16.50 ± 43.61[b]	570.00 ± 20.00[a]	780.00 ±25.98[a]
Free Amino Acids (mg/100ml)	2100 ± 25.00[b]	750.00 ± 25.00[a]	890.00 ± 35.18[a]

Each value is the mean of three determination v = SD, values with different superscripts within a now are statistically different in water (0.05%) 'control' and 'taste' to samples prepared in water and 4 gum Arabic, respectively.

Table 5: Shelf life of phytomilk treated and untreated

	Control		Treated with Ascorbic Acid		Treated with Selenium	
Parameter	4ºC	R/Temp	4ºC	R/Temp	4ºC	R/Temp
D_pH	0.7	2.6	1.8	2.7	2	2.1
Time taken (days)	9	2	`6	5	24	4
Rate of change in P_H (unit/days)	0.08	1.3	0.11	0.54	0.08	0.53

DpH = Change in pH during the period indicated

R/Temp = Room Temperature

significantly (P< 0.05) affected only the total lipid level. Lipid level was 13.5% in water preparation and 16.5% in gum Arabic. Gum Arabic behaves like dietary fibre by binding to lipids in solution thus, 4% gum Arabic solution probably bound to soy lipids than distilled water leading to higher lipid content in phytomilk [26].

The phytomilk prepared in 4% aqueous gum Arabic sweetened and flavoured with Vanilla or Banana flavours had similar acceptance compared with the standard commercial dairy milk indicating that phytomilk product will have market acceptability.

CONCLUSION

The results of this study showed that the viscosity and emulsion stability of phytomilk were increased when prepared in 4% gum Arabic solution than in distilled water. The percentage acceptability of the phytomilk flavoured with synthetic banana was similar to that of peak milk. The addition of 0.01% selenium solution to soymilk effectively preserved it for about twenty four days at 4ºc (Table 5). However, as a result the fact that selenium is toxic in nature, it would not be

wise to handle it at the local level but rather at the industrial level since it is recommended as an additive in baby formulae.

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