DOI: 10.51745/najfnr.10.21.11-21

ORIGINAL ARTICLE

Functional and Novel Foods | Infant, Child, and Adolescent Nutrition

Comparative Analysis of Nutritional Profile of a Formulated Composite Milk Alternative and a Conventional Infant Formula

Onuabuchi Nnenna Ani [1] ✉ **Innocent Izuchukwu Ujah** [1] **Ebere Immaculata Akpata** [1] **Chinenye Enoch Oguazu** [2]

[1] Enugu State University of Science and Technology, Department of Applied Biochemistry, Faculty of Biological Sciences, PMB 01660, Agbani, Enugu, Nigeria. nnenna.ani@esut.edu.ng/ innocent.ujah@esut.edu.ng /ebere.akpata@esut.edu.ng

[2] Nnamdi Azikiwe University. Department of Applied Biochemistry, Faculty of Bio-Sciences. PMB 5025, Awka, Nigeria. ce.oguazu@unizik.edu.ng

ABSTRACT

Background: The beverage industry has experienced a substantial surge in the development of composite milk alternatives (CMAs), precipitated by shifting dietary preferences and specific nutritional requirements. However, the increasing utilization of these alternatives in infant nutrition has prompted critical inquiries regarding their nutritional adequacy and bioequivalence to standardized formulas.

Aims: This study aimed to perform a comparative evaluation of the proximate, phytochemical, vitamin, and mineral profiles of a novel formulated CMA (plant-animal-based, formulated using soybean, tiger nut, peanut, cashew nut, dry fish, crayfish, and dates) against a conventional commercial infant formula (NAN).

Methods: Raw materials were processed, and homogenized in standardized proportions. Analytical characterization was conducted using established biochemical methods for proximate analysis and micro-/macro-nutrient quantification. Statistical significance was determined using a two-tailed unpaired Student's t-test, assuming equal variance, with the threshold for significance set at $\alpha = 0.05$.

Results: Distinct compositional divergence was observed between the formulated CMA and the commercial control. The CMA exhibited significantly higher concentrations ($p < 0.05$) of crude fiber and moisture, as well as elevated levels of bioactive phytochemicals, including phenols, flavonoids, and lycopene. Additionally, the CMA contained a more diverse array of antinutritional factors (tannin, phytate, and alkaloids), and high concentrations of vitamins B_2 and C. Conversely, NAN exhibited significantly higher carbohydrate and ash content ($p < 0.05$), resulting in a superior total caloric density, alongside higher concentrations of vitamins B_1 and B_9. While most mineral concentrations were comparable, the CMA displayed significantly higher levels of sodium, cobalt, and zinc.

Conclusions: The formulated CMA demonstrated a nutritional profile that compares favorably with conventional infant formula in several key metrics. While these findings suggest significant nutritional potential, further optimization and rigorous safety evaluations are imperative before this formulation can be recommended for infant consumption.

Keywords: Plant-Based; Composite; Milk Alternative; Nutritional Profile.

Article Information

✉ **Corresponding author:** Onuabuchi Nnenna Ani
E-mail: nnenna.ani@esut.edu.ng
Tel. (+234) 80 3778 2410

Received: February 07, 2025
Revised: August 13, 2025
Accepted: December 17, 2025
Published: January 16, 2026

Edited by:
Prof. Mustapha Diaf
Reviewed by:
Dr. Hayat Bourekoua
Dr. Abbou Amina

Cite this article as: Ani. O N., Ujah. I I., Akpata. E I., & Oguazu, C E. (2026). Comparative Analysis of Nutritional Profile of a Formulated Composite Milk Alternative and a Conventional Infant Formula. *The North African Journal of Food and Nutrition Research, 10* (21): 11 – 21. https://doi.org/10.51745/najfnr.10.21.11-21

1 INTRODUCTION

Milk serves as a primary source of essential nutrients, functioning as a critical dietary component for neonatal development and pediatric growth (Givens, 2020). Across the African continent, dairy milk constitutes a foundational staple product within numerous pastoral communities (Mattiello *et al.*, 2017). However, within developing economies, the escalating cost of bovine milk and its derivatives has led to a significant decline in household consumption (Headey and Alderman 2019). This economic barrier has prompted the exploration of non-conventional sources—specifically seeds and nuts— to satisfy the demand for affordable yet nutritionally dense dairy alternatives. Consequently, the beverage industry has experienced a significant shift toward plant-based milk alternatives (PBMA), catalyzed by evolving health consciousness, dietary preferences, and nutritional requirements (Pointke *et al.*, 2022). While the quest for viable substitutes for conventional infant formula has led to their use in infant feeding has increased the utilization of PBMAs in pediatric nutrition, significant concerns persist regarding their nutritional bio-equivalence and adequacy (Al-Beltagi *et al.*, 2024; Dupont *et al.*, 2020).

PBMAs are derived from diverse botanical sources, such as soybeans, almonds, oats and rice, and they are frequently fortified with essential vitamins and minerals to approximate the nutritional profile of bovine milk (Brooker *et al.*, 2023; Ramsing *et al.*, 2023,). These alternatives are rich in bioactive compounds, offering therapeutic benefits for individuals with specific dietary constraints. On a dry-weight basis, soybeans are particularly noteworthy, comprising 36% protein, 19% lipids, and 35% carbohydrates, with dietary fiber representing a substantial 17%. The remaining 10% is comprised of

minerals (5%) and a diverse profile of vitamins and other components (Liu, 1997). Given this robust profile, soy-based products play an instrumental role in mitigating macronutrient deficiencies and addressing malnutrition. It not only provides essential macronutrients of high quality but also a range of crucial micronutrients, making it an effective solution to combat malnutrition and promote overall nutritional well-being. Nevertheless, the comparative nutritional efficacy of these substitutes against standardized infant formulas remains a subject of rigorous academic debate. Although PBMAs offer visible health advantages, ensuring their nutritional sufficiency for infants and children is paramount (Verduci *et al.*, 2019). There remains a limited data of empirical research concerning the nutritional characteristics of composite milk alternatives formulated through the combination of different plant and animal matrices.

The present study investigates a novel multi-component blend of soybean (*Glycine max*), tiger-nut (*Cyperus esculentus*), date fruit (*Phoenix dactylifera* L.), cashew nut (*Anacardium occidentale*), groundnut (*Arachis hypogaea*), and animal-derived proteins from dry fish and crayfish (*Faxonius limosus*). This formulation was developed to evaluate its potential as a comprehensive milk substitute. Previous studies have explored these ingredients in isolation, for plant-based infant formula development. For instance, Cruz *et al.* (2007) assessed the microbial and structural integrity of soy-based systems, while Belewu and Belewu (2007) conducted a physicochemical characterization of tiger-nut as a bovine milk surrogate. Additionally, Lima *et al.* (2018) demonstrated that water-soluble extracts from cashew nuts maintain stable physical and microbial properties. These precursors suggest that through refined extraction, homogenization, and blending, stable emulsions can be achieved (Cruz *et al.*, 2007; Sethi *et al.*, 2016). The strategic blending of PBMAs is increasingly employed to optimize organoleptic and nutritional attributes, such as the synergistic enhancement of protein content and flavor observed in soy-almond composites (Kundu *et al.*, 2018). Moreover, post-harvest technological interventions, such as homogenization and pasteurization remain vital to enhance the shelf life and safety of PBMAs (Jeske *et al.*, 2019).

The selection of these specific plant-based substrates was driven by their affordability, accessibility, ease of preparation, and the absence of allergic bovine proteins. However, these alternatives are inherently deficient in essential minerals and proteins, necessitating careful fortification strategies (Mäkinen *et al.*, 2016; Silva *et al.*, 2020, Silva and Smetana, 2022). Furthermore, the presence of various anti-nutritional factors may impede the bioavailability of critical nutrients (Aydar *et al.*, 2020). To address these protein deficits, crayfish and dry fish were incorporated into the formulation, as the plant-based constituents (tiger nut, date seed, cashew nut) are limited in protein, except for soybean. Moreover, the absence of lactose and cholesterol in these plant-based alternatives is a significant advantage, as these compounds are only found in animal products. Therefore, this study aimed to conduct a comparative analysis of the nutritional profile of this hybrid plant-animal composite against a conventional infant formula. To this end, the raw materials were processed and blended in optimized proportions, followed by an exhaustive evaluation of their proximate, phytochemical, vitamin, and mineral compositions.

2 Material and Methods

2.1 Raw Material Acquisition and Authentication

The primary raw materials utilized in this study included: Soybean (*Glycine max*), Tiger nut (*Cyperus esculentus*), Date fruit (*Phoenix dactylifera L.*), Cashew nut (*Anacardium occidentale*), Groundnut (*Arachis hypogaea*), Dry fish, and Crayfish (*Faxonius limosus*). These materials were procured from a local market and subsequently identified and authenticated by a taxonomist within the Department of Applied Biology and Biotechnology at the Enugu State University of Science and Technology.

2.2 Sample Preparation

Botanical substrates, including soybean, date fruit, tiger nut, and cashew nut, were manually sorted and cleaned to eliminate extraneous contaminants such as stones, foliage, and debris. This ensured the integrity of the starting materials for subsequent processing.

2.2.1 Soybean Processing

Soybean seeds were washed and submerged in deionized water for 8 hours to achieve full rehydration. Following decantation, the seeds were boiled for 20 minutes to effect enzymatic inactivation (specifically lipoxygenase) and to facilitate dehulling. After the removal of the hulls, the beans were sun-dried for 72 hours under a fine-mesh protective screen to prevent environmental contamination. The dried beans were subsequently subjected to pan-roasting over a controlled flame for 30 minutes to optimize flavor and texture before being sequestered in airtight containers.

2.2.2 Tiger Nut and Date Fruit Preparation

Tiger-nut seeds underwent a similar cleaning protocol and were rehydrated in water for 24 hours. Post-drainage, the seeds were sun-dried for 48 hours to reduce moisture content to a stable level, followed by roasting and storage in airtight vessels. Date fruits were manually deseeded, rinsed, and sun-dried for 48 hours prior to storage

2.2.3 Preparation of Nuts and Animal Protein

Commercial roasted groundnut and cashew nuts were utilized. Crayfish were and pan-roasted to enhance stability, while the dry fish were manually processed to remove all skeletal structures (bones) before further treatment.

2.3 Formulation of the Composite Milk Blend

The dried and roasted constituents were pulverized into a fine flour. The composite blend was formulated on a dry matter basis (w/w) following a 100% total weight ratio. The plant-based matrix was established in a ratio of 40:20:15:15 for soybean, tiger-nut, groundnut, and cashew nut, respectively. Nutritional enrichment was achieved by the incorporation of milled crayfish and dry fish in a 5:5 ratio. The final mixture was homogenized for 5 minutes employing a high-speed electric blender and stored under controlled conditions at room temperature (25 ± 2 °C) in hermetically sealed containers.

2.4 Proximate Analysis

The proximate composition, including ash, moisture, crude fat, crude fiber, and protein content, was determined in accordance with the standardized protocols of the Association of Official Analytical Chemists (AOAC, 2000)

2.4.1 Ash Content

Ash content was determined by the gravimetric method. A 2.0 g sample was weighed into a platinum crucible and incinerated in a muffle furnace at 400 – 600 °C for 4 hours until a constant weight of whitish-grey ash was obtained. Samples were cooled in a desiccator prior to weighing:

$$\%Ash = \frac{Weight\ of\ ash}{Weight\ of\ sample} x\ 100$$

2.4.2 Moisture Content

Moisture was determined via the oven-drying method based on weight loss. A 2.0 g sample was dried at 105 °C until a constant weight was achieved.

$$\%\ Moisture = \frac{Wsample + Dish\ BD - Wsample + Dish\ AD}{W\ sample} x\ 100$$

Where W_{sample} = weight of sample; $Dish_{BD}$ = Dish before drying $Dish_{AD}$ = Dish after drying.

2.4.3 Crude Fat Content

Crude fat was extracted using the Soxhlet continuous solvent extraction principle. A 2.0 g sample was placed in a thimble and extracted with 150 mL of petroleum ether (boiling point 40–60 °C) for 4 hours. Following extraction, the solvent was recovered via distillation, and the flask was dried at 65 °C for 4 hours to isolate the ether-soluble fraction.

$$\%Fat = \frac{Weight_{Extract}}{Weight_{Sample}} x\ 100$$

2.4.4 Crude Fiber Content

Crude fiber was isolated as the insoluble organic residue remaining after sequential acid and alkaline digestion. A 2.0 g defatted sample was refluxed with 1.25% H_2SO_4 for 30 minutes, followed by 1.25% NaOH for an additional 30 minutes. The residue was filtered, dried at 65 °C for 24 hours, and subsequently ignited at 600 °C. The weight loss during ignition represented the crude fiber content.

$$\%Crude\ Fiber = \frac{W1 - W2}{W} x\ 100$$

Where W1 = weight of residue before ashing; W2 = weight of residue after ashing).

2.4.5 Crude Protein Determination

Total nitrogen was determined using the Micro Kjeldahl method, involving digestion, distillation, and titration. A 2.0 g sample was digested with concentrated H_2SO_4 in the presence of a catalyst. The resulting digest was alkalized and distilled, with the released ammonia captured in a boric acid solution. This was then titrated against 0.01N HCl.

$$\%\ Nitrogen = \frac{V\ x\ N\ \times 14.01\ \times 100}{1000 \times W}$$

Where V = Corrected titre volume (V_{sample} - V_{blank}); N = Normality of HCl; W = Weight of sample).

Crude protein was calculated using a nitrogen-to-protein conversion factor (*F*) of 6.25:

$$\%\ Crude\ Protein = \%N \times 6.25$$

2.4.6 Carbohydrate and Caloric Content

Total Carbohydrate Determination

The total carbohydrate content was determined by difference, following a pragmatic estimation as the residual fraction after the direct measurement of other proximate components. The calculation was performed by subtracting the sum of the percentages of protein, fat, moisture, ash, and crude fiber from 100%:

$$Total\ Carb\ (\%) = 100 - [\%\ Prot + \%\ Fat + \%\ Moisture + \%\ Ash + \%\ Fiber]$$

Energy Value Calculation

The physiological fuel value (caloric content) was calculated using the Atwater factor method. This method

applies specific energy factors to the protein, fat, and carbohydrate composition of the sample:

$$Energy\ value\ (Kcal/100g) = (4 \times \text{Prot}) + (9\ x\ Fat) + (4\ X\ Carb)$$

Note: Macronutrients are expressed in grams per 100 grams of sample.

2.5 Phytochemical Analysis

2.5.1 Total Phenolic Content (TPC)

The TPC of the extracts was assessed employing the Folin-Ciocalteu colorimetric method as described by Barros *et al.* (2007). Aliquots of 1 mL of the extract were reacted with 1 mL of Folin-Ciocalteu's reagent, followed by the addition of 1 mL of saturated sodium carbonate (Na_2CO_3) and dilution to a final volume of 10 mL with distilled water. Following a 90-minute incubation in the dark, the absorbance was recorded at 725 nm using a UV-Visible spectrophotometer. Gallic acid served as the reference standard, and results were expressed as milligrams of gallic acid equivalents (GAEs) per gram of extract.

2.5.2 Total Flavonoid Content (TFC)

TFC was determined via a modified colorimetric method (Barros *et al.*, 2007). A 0.5 mL aliquot of the sample solution (100 µg/mL) was diluted with 2 mL distilled water, followed by the addition of 0.15 mL of 5% sodium nitrite ($NaNO_2$). After 6-minutes, 0.15 mL of 10% aluminum chloride ($AlCl_3$) was added. Following a further 6-minute incubation, 2 mL of 4% sodium hydroxide ($NaOH$) was added, and the total volume was adjusted to 5 mL. Absorbance was measured at 510 nm after 15 minutes. Catechin served as the reference standard (mg CE/100 g), and all analyses were performed in in triplicate.

2.5.3 Beta-Carotene and Lycopene Content

Carotenoid profiles were analyzed according to the methodology of Barros *et al.* (2007). A 100 mg of sample extract was homogenized with 6 mL of an acetone-hexane mixture (4:6 v/v) and filtered through Whatman No. 4 paper. Filtrate absorbance was measured at 453, 505, and 663 nm. Concentrations were calculated using the following equations:

$$\text{Lycopene (mg/100mL)} = 0.0458(A_{663}) + 0.372(A_{505}) + 0.0806(A_{453})$$

$$\beta - carotene\ \text{(mg/100mL)} = 0.216(A_{663}) + 0.304(A_{505}) + 0.452(A_{453})$$

2.5.4 Anti-Nutritional Factors

Phytate Content

Phytate was quantified using the method of Young and Greaves (1940), as modified by Lolas and Markakis (1975). A 1.0 g of sample was extracted with 100 mL of 2% HCl for 3 hours. A 50 mL aliquot of each filtrate was combined with 10 mL of 0.3 % ammonium thiocyanate indicator and titrated against standard iron (III) chloride ($FeCl_3$) solution (0.00195 g Fe/mL) until a persistent reddish-brown color was achieved:

$$\text{Phytic acid (\%)} = \text{Titre x } 0.00195 \text{ x } 1.19 \text{ x } 100$$

2.5.5 Oxalate Content

Oxalate determination followed the protocol described by Osagie (1998). A 2.0 g sample underwent acid digestion, followed by the precipitation of oxalate as calcium oxalate (CaC_2O_4) at pH 4 – 4.5. The precipitate was dissolved in acid and titrated against standardized potassium permanganate ($KMnO_4$) to a faint pink endpoint.

$$\text{Oxalate (mg/100g)} = \frac{T \times V_{me} \times D_f \times 10^5}{ME \times M_f}$$

(Where T = *Titre;* V_{me} = *volume-mass equivalent;* D_f = *dilution factor;* ME = *molar equivalent;* M_f = *sample mass).*

2.5.6 Tannin, Alkaloids, and Saponins

- **Tannins:** Samples (1.0 g) were extracted in 70% ethanol and reacted with ferric chloride and potassium ferrocyanide. Absorbance was recorded at 720 nm (AOAC, 1995) and expressed as Tannic Acid Equivalents (TAE).
- **Alkaloids:** Quantification was performed according to Harborne (1995). Alkaloids were extracted with 20% acetic acid in ethanol, precipitated with concentrated ammonium hydroxide (NH_4OH), collected on pre-weighed filter paper, and dried at 80 °C.
- **Saponins:** Saponin content was determined by the AOAC (1990) method. Samples were defatted with acetone for 3 hours, followed by methanol extraction for 3 hours in a Soxhlet apparatus. The saponin percentage was calculated based on the mass of the methanol extract.

$$Saponin\ (\%) = \frac{A - B}{SM}$$

Where A, B, and SM represent the mass of flask and extract, empty flask, and sample, respectively.

2.6 Vitamins

2.6.1 Vitamin A Analysis

The vitamin A (retinol) content was determined using the method of Rutkoski *et al.* (2006). A 1.0 gram sample was extracted with 10 mL of ethanol and centrifuged at 1500rpm for 10 minutes. The supernatant was treated with 1 mL of 0.1M methanolic KOH and saponified at 60 °C for 20 minutes. After cooling, the retinol was extracted into xylene. Absorbance was measured at 335 nm before (A_1) and after (A_2)

UV irradiation for 30 minutes to account for non-vitamin A absorption:

$$C_x\ (\mu M) = (A_1 - A_2) \times 22.23$$

The multiplier (22.23) utilized in the Vitamin A calculation is derived from the absorption coefficient of a 1% (w/v) solution of retinol in xylene, measured at 335 nm in a cuvette with a 1 cm optical path length.

2.6.2 Determination of vitamin E (Tocopherol)

The vitamin E content was determined according to the method of Rutkoski *et al.* (2005). A 0.1 g aliquot of each sample was extracted with 10 mL of ethanol and centrifuged at 1500 rpm for 10 min. The supernatant was then combined with 0.5 mL of anhydrous ethanol and 3 mL of xylene. Following centrifugation (1500 rpm, 10 min), 1.5 mL of the organic (upper) layer was reacted with bathophenanthroline (0.25 mL, 6.02 mM), ferric chloride ($FeCl_3$; 0.25 mL, 0.98 mM), and phosphoric acid (H_3PO_4; 0.25 ml, 40 mM). Absorbance was recorded at 539 nm. Tocopherol served as the standard (23.2 µM). The concentration calculated as follows:

$$\text{Tocopherol (µM)} = \frac{A_x}{A_s} \times C_s$$

Where Ax and As represent the absorbance of the sample and standard, respectively, and Cs denotes the concentration of the standard.

2.6.3 Determination of Ascorbic Acid (Vitamin C)

Ascorbic acid was determined through the colorimetric protocol described by Klein and Perry (1982). Samples (20 mg) were extracted with 10 mL of 1% metaphosphoric acid (HPO_3) for 45 minutes at 28°C. The extract was filtered through Whatman No.4 paper. Subsequently, 1 mL of the filtrate was reacted with 9 mL of 50 µM 2,6-dichlorophenolindophenol (DCPIP) sodium salt hydrate. After a 30-minute incubation, absorbance was measured at 515 nm. Quantification was performed using a calibration curve of authentic L-ascorbic acid, with results expressed as milligrams of ascorbic acid equivalent per gram (mgAE/g) of extract.

2.6.4 Determination of Vitamin B_3 (Nicotinamide)

Nicotinamide content was determined via non-aqueous titration (Kirk and Sawyer, 1991). A 0.5 g sample was dissolved in 20 mL of anhydrous glacial acetic acid (CH_3COOH) under gentle heat, followed by the addition of 5 mL of acetic anhydride ($(CH_3CO)_2O$). Using crystal violet as a visual indicator, the solution was titrated with 0.1M perchloric acid ($HClO_4$) until a greenish-blue endpoint was achieved.

$$\text{Vitamin B3 (mg/100g)} = \frac{\text{Titre} \times 0.0122}{0.1}$$

2.6.5 Determination of Folic Acid (Vitamin 9)

Folic acid was quantified based on the diazotization reaction between p-aminobenzoylglutamic acid and 3-aminophenol (Padmarajaiah *et al.*, 2002). An aliquot (0.1 g) was extracted with 10 mL of distilled water and centrifuged (1500 rpm, 10 min). A 1 mL aliquot of the supernatant was sequentially treated with hydrochloric acid (*HCl;* 4 $molL^{-1}$), sodium nitrite ($NaNO_2$, 1% w/v), sulfamic acid (H_3NSO_3; 1% w/v), and 3-aminophenol to form an orange-yellow chromophore. Absorbance was determined at 460 nm. Concentration was determined using a standard curve of pure folic acid.

2.6.6 Determination of Vitamins B_1 (Thiamine) and B_2 (Riboflavin)

Thiamine and riboflavin were determined by spectrophotometric analysis exploiting their maximum UV absorption at 262 nm and 242 nm, respectively (Kirk and Sawyer, 1991). Aliquots (0.1 g) homogenized in 100 mL of deionized water and heated for 5 minutes. Following cooling and filtration, the filtrate was centrifuged at 4000 rpm for 10 minutes. The absorbance of the supernatant was measured in quartz cuvettes.

$$\text{Concentration (mg/100g)} = \frac{A \times DF \times Vc}{E}$$

Where A is absorbance, DF is the dilution factor, Vc is the cuvette volume, and E is the extinction coefficient.

2.6.7 Determination of Vitamin B_6 (Pyridoxine)

The vitamin B_6 content was determined through the spectrophotometric method described by Raeed and Azam (2008). An aqueous extract of each sample (25 mL) was treated with a Cerium (IV) ion solution (7 mL, 44 µM) and sulfuric acid (H_2SO_4; 0.2 mL, 0.05 N). The mixture was incubated for 15 minutes at room temperature, Subsequently, Arsenazo III reagent (2 mL, 0.2 mM) and Triton X-100 (4 mL, 1%) were incorporated, and the absorbance recorded at 716 nm. The concentration of vitamin B_6 was calculated based on the molar extinction coefficient of pyridoxine ($1.12 \times 10^5\ M^{-1}cm^{-1}$).

2.7 Analysis of Mineral Content

Mineral profile was performed via Atomic Absorption Spectrometry (AAS) following AOAC (2003) official methods. The analytical principle of AAS relies on the aspiration of the sample into a flame for atomization; the resulting free atoms absorb light at element-specific wavelengths. The degree of light absorption is directly

proportional to the elemental concentration in accordance with the Beer-Lambert law.

For sample preparation, 2 g of each sample underwent wet digestion using 20 mL of a tri-acid mixture (650 mL concentrated HNO_3, 80 mL $HCLO_4$, and 20 mL of concentrated H_2SO_4) until a clear, colorless solution was obtained. The resulting digest was diluted to 100 mL with deionized water. Samples were subsequently aspirated into an oxidizing into an air-acetylene flame. Quantification was achieved through external calibration using a series of standard metal solutions prepared daily from stock concentrates diluted with 1.5 mL/L of concentrated nitric acid. A reagent blank solution was analyzed in parallel to account for potential matrix interference. Calibration curves were generated by plotting absorbance against known concentrations to determine the mineral levels in the samples.

2.8 Statistical Analysis

Quantitative data were analyzed employing IBM-SPSS Statistics (Version 29; SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± standard deviation (SD). Comparative analysis between the two groups was performed using an independent samples t-test. Statistical significance was defined at a threshold of $p < 0.05$.

3 Results

3.1 Proximate Composition of Formulated Composite Milk Alternative (CMA) and Conventional Infant Formula (NAN)

The proximate profiles of the Formulated Composite Milk Alternative (CMA) and the Conventional Infant Formula (NAN) are summarized in Table 1. Crude fiber and moisture were significantly higher in CMA compared to NAN ($p = 0.003$; $p = 0.001$). Conversely, carbohydrate and ash concentrations were significantly greater in the NAN formula ($p = 0.03$ and $p = 0.04$, respectively). The caloric density of NAN (481.34 ± 5.30 Kcal/100 g) significantly exceeded that of CMA (438.98 ± 7.10 Kcal/100 g; $p = 0.02$). No statistically significant differences were observed between the two formulations regarding protein and lipid content ($p = 0.06$ and $p = 0.09$, respectively).

Table 1. Proximate Composition of CMA and NAN

	CMA	NAN
Ash (%)	1.53 ± 0.04[a]	2.00 ± 0.14[b]
Carbohydrate (%)	54.99 ± 0.32[c]	59.94 ± 1.73[d]
Crude Fibre (%)	1.60 ± 0.07[e]	0.525 ± 0.03[f]
Crude Protein (%)	16.51 ± 0.45[g]	15.095 ± 0.30[g]
Total Lipids (%)	17.00 ± 1.13[h]	20.14 ± 1.22[h]
Moisture (%)	8.49 ± 0.38[i]	2.31 ± 0.10[J]
Energy (Kcal/100 g)	438.98 ± 7.10[k]	481.34 ± 5.30[l]

* *Note:* Values are presented as mean ± standard deviation of duplicate values. Values with different letters on the same row are significantly different ($p < 0.05$)

3.2 Phytochemical Composition of CMA and NAN

The phytochemical profiles of the samples are presented in Table 2. Quantitative analysis revealed that total phenolic content was significantly higher in CMA compared to NAN ($p = 0.002$). Notably, flavonoids and lycopene were exclusively detected in CMA, with no detectable levels present in the NAN formula. Regarding anti-nutritional factors, tannin, phytate, oxalate, and alkaloids were identified in CMA; in contrast, only oxalate was detected in NAN, at a concentration significantly lower than that observed in CMA ($p = 0.01$).

Table 2. Phytochemicals and Anti-nutrient Composition of CMA and NAN

Phytochemicals/anti-	CMA	NAN
Total Phenol (mgGAE/g)	34.64 ± 2.96[a]	1.315 ± 0.1[b]
Flavonoid (mgCE/g)	21.12 ± 0.00	ND
Lycopene (mg/g)	0.20 ± 0.03	ND
Beta carotene (mg/g)	0.89 ± 0.03	1.43 ± 0.4
Tannin (mgTAE/g)	2.97 ± 1.31	ND
Phytate (%)	0.50 ± 0.11	ND
Oxalate (mg/g)	6.01 ± 0.16[c]	0.16 ± 0.05[d]
Alkaloids (%)	0.63 ± 0.13	ND
Saponin (%)	0.21 ± 0.04	ND

* *Note:* Values are presented as mean ± standard deviation of duplicate values. mgGAE: Milligram Gallic Acid equivalent; mgCE: Milligram Catechin equivalent. Values with different letters on the same row are significantly different ($p < 0.05$)

3.3 Vitamin Composition of CMA and NAN

The vitamin profiles of the formulated CMA and NAN are presented in Table 3. CMA exhibited significantly higher concentrations of riboflavin (Vitamin B_2) and ascorbic acid (Vitamin C) compared to NAN ($p = 0.02$ and $p = 0.01$, respectively). Conversely, NAN contained significantly higher levels of thiamine (Vitamin B_1) and folate (Vitamin B_9) ($p = 0.01$ and $p = 0.04$, respectively). Niacin (Vitamin B_3) was not detected in either formulation. For the remaining vitamins

Table 3. Vitamin Composition of the Formulated CMA and NAN

Vitamins	CMA	NAN
Vit A (µg/g)	11.38 ± 1.00[a]	13.05 ± 0.09[a]
B1 (µg/g)	0.06 ± 0.01[b]	0.84 ± 0.02[c]
B2 (mg/g)	1.00 ± 0.03[d]	0.02 ± 0.01[e]
B3 (mg/g)	ND	ND
B6 (µg/g)	0.06 ± 0.01[f]	0.04 ± 0.00[f]
C (mg/g)	1.19 ± 0.0[g]	0.73 ± 0.02[h]
B_9 (Folic Acid (mg/g)	0.91 ± 0.06[i]	1.10 ± 0.04[j]
Vit E (mg/g)	0.32 ± 0.06[k]	0.37 ± 0.04[k]

Values are presented as mean ± standard deviation of duplicate values. Values with different letters on the same row are significantly different (p<0.05). ND: Not detected.

analyzed, no statistically significant differences were observed between the two samples ($p > 0.05$).

3.4 Mineral Composition of CMA) and NAN

Table 4 illustrates the mineral concentrations for both CMA and NAN. Sodium, cobalt, and zinc levels were significantly more abundant in CMA than in the conventional formula ($p < 0.05$). However, there were no statistically significant differences between CMA and NAN regarding the concentrations of calcium, magnesium, iron, and copper.

Table 4. Mineral Composition of CMA and NAN

Minerals (mg/Kg)	CMA	NAN
Zn	0.71 ± 0.02^{k}	0.28 ± 0.01^{l}
Co	0.02 ± 0.01^{m}	0.01 ± 0.02^{n}
Ca	9.28 ± 0.01^{o}	9.48 ± 0.05^{o}
Mg	5.34 ± 0.04^{p}	5.78 ± 0.04^{p}
Fe	1.28 ± 0.02^{q}	1.58 ± 0.01^{p}
Cu	0.12 ± 0.03^{q}	0.04 ± 0.02^{q}
Na	7.04 ± 0.01^{r}	4.09 ± 0.01^{s}

Values are presented as mean ± standard deviation of duplicate values. Values with different letters on the same row are significantly different ($p<0.05$).

4 Discussion

The nutritional evaluation of a formulated composite milk alternative (CMA) provides critical insights into its efficacy as a viable substitute for traditional dairy-based infant formulas. Comparative analysis is essential, as conventional formulas serve as the primary nutritional benchmark for non-breastfed infants. The current study identifies significant variations in proximate composition, phytochemical profiles, and micronutrient concentrations, providing valuable information for informed decision-making. The formulated composite milk alternative was engineered through the strategic blending of plant and animal-derived components to simulate the complex nutritional matrix of bovine milk.

Proximate analysis revealed that the CMA possessed significantly higher crude fiber and moisture content, whereas the conventional formula (NAN) was superior in carbohydrate and ash (mineral) content. Although the CMA exhibited slightly higher protein levels and NAN showed higher lipid concentrations, these differences were not statistically significant. The elevated protein levels in CMA can be attributed to the incorporation of protein-dense ingredients such as crayfish, dried fish, and soybeans. This multi-source blending strategy optimizes the amino acid profile, potentially enhancing protein quality to a level comparable to bovine milk. These results are in line with previous studies indicating that diary-based formulas typically maintain higher energy densities and mineral concentrations (Brooker *et al.,* 2023; Drewnowski, 2022; Redan, 2023). However, the elevated moisture and fiber in the CMA suggest a reduced shelf stability and potential alterations in infant gut motility and microbiota composition (Aydar *et al.,* 2020). Furthermore, the lower energy density of the CMA necessitates careful monitoring of feeding volumes to ensure that the total caloric intake meets the high metabolic demands of infancy.

The phytochemical analysis confirmed that the CMA contained higher levels of bioactive compounds and anti-nutritional factors (ANFs) compared to NAN. This disparity is anticipated, as phytochemicals are intrinsic to the plant-derived components of the CMA. These phytochemicals including anti-nutrients are extracted and retained during processing. The high phenolic content in the CMA suggests potential antioxidant benefits, consistent with the findings of Paul *et al.* (2020) regarding the health-promoting properties of plant-based isoflavones and phytosterols.

Our results also confirmed that phytate and tannin are the key antinutrients in the formulated CMA, supporting earlier reports that these compounds are major contributors to antinutrient content in plant-based foods. (Popova & Mihaylova, 2019). While these compounds can offer health benefits such as antioxidant properties, they also pose potential risks such as reduced mineral absorption and increased risk of kidney stones (Olawoye and Gbadamosi, 2017). In the digestive tract, phytates reduce the bioavailability of divalent cations such as zinc, iron, and calcium by forming soluble complexes with them in the acidic stomach, which then precipitate at the neutral pH of the intestine (Schlemmer *et al.*, 2009). Furthermore, the presence of oxalates, even at the low levels detected in NAN, remains a clinical concern due to its association with calcium oxalate urolithiasis (Crivelli *et al.*, 2021), with human studies consistently observing a relationship between its intake and stone formation (Curhan *et al.,* 2004). Tannins are amphiphilic, they can effectively stabilize emulsions and protect unsaturated lipids from oxidizing (Carbas *et al.*, 2020). However, appropriate processing methods such as soaking and fermentation can be adopted to mitigate these anti-nutritional factors.

Vitamin analysis highlighted a critical disparity in Vitamins *A* and thiamin (Vitamin B_1) concentrations, with NAN exhibiting markedly higher levels. The lower concentrations in the CMA may be attributed to thermal degradation during processing, as thiamine and ascorbic acid are highly thermolabile (Riaz *et al.,* 2009; Valencia-Flores *et al.,* 2013). Vitamins *A*, B_6 and *E* were statistically identical in both formulas although vitamins *A* and *E* in CMA were slightly reduced. Generally, the vitamin profile of the CMA differed significantly from the commercial dairy-based infant formula. A critically low level of thiamine (B_1) in the CMA, at just 7% of the reference formula, represents a severe deficiency risk incompatible with infant safety. Conversely, a concurrent 50-fold excess of riboflavin (B_2) in the CMA indicates a need for greater precision in the fortification protocol. While levels of Vitamins *A, C, E,* and folate were adequate, the profound imbalances in *B*-vitamin complex suggest that the current CMA formulation requires rigorous adjustment to ensure safety and nutritional adequacy.

Mineral analysis, as presented in Table 2, revealed that the CMA generally surpassed NAN in several key elements, particularly zinc and sodium. Overall, CMA tends to exhibit

higher mineral content, with the exception of calcium, iron (Fe) and magnesium (Mg), which are statistically comparable to that of NAN. These findings disagree with those of Astolfi *et al.* (2020), who reported that cow's milk exceeds plant-based milk alternatives (PBMAs) in terms of calcium, magnesium, zinc, and sodium content. The lower iron level constitutes a major concern, particularly given the inherently poor bioavailability of non-heme iron in PBMAs; this necessitates verification of adequate fortification and the presence of enhancers such as vitamin *C*. Notably, the formulated composite milk alternative demonstrated a significantly higher zinc (Zn) content, with 0.71 mg/kg, compared to NAN, which contained 0.28 mg/kg. This is likely due to the inclusion of cashew nuts, which are naturally zinc-rich (Gonçalves *et al.*, 2023). The higher zinc and copper levels, while potentially beneficial, needs to be assessed for bioavailability and potential antagonistic interactions. Additionally, the CMA exhibited higher sodium (Na) levels, at 7.04 mg/kg, compared to NAN, with 4.09 mg/kg which could be due to the incorporation of Na-rich ingredients like crayfish, dry fish, and dates (Mrabet *et al.*, 2020). However, the elevated sodium content in the CMA requires evaluation against infant renal solute load limits. The composite milk alternative showed comparable levels of calcium, magnesium, and iron to NAN, as it was formulated with mineral-rich ingredients, resulting in statistically similar concentrations. Calcium (Ca) is a crucial mineral to investigate, given that cow milk is its primary source. Notably, NAN contains higher Ca concentrations (9.48 mg/kg) which was comparable to the composite milk alternative (9.28 mg/kg). This difference suggests that the formulated composite milk may provide sufficient dietary Ca similar to the conventional infant formula, making it also a suitable substitute on its own. To leverage on this, higher proportion of calcium-rich ingredients can be used such as crayfish and groundnut as many composite milk products are fortified with Ca to match the Ca content of cow milk, enabling them to serve as a viable alternative (Schuster *et al.*, 2018). The equivalent calcium and magnesium levels are positive findings, indicating successful fortification of these macro-minerals in the CMA. Nevertheless, it is crucial to continue developing and refining formulations to closely match individual nutritional requirements, considering the significance of mineral intake and its established health implications. This will ensure that composite milk alternatives can effectively support optimal health and well-being.

5 Conclusions

The comparative analysis of the nutritional profile of a formulated composite milk alternative and a conventional infant formula, NAN, revealed variations in their various nutrient content. The formulated CMA compared favorably with the infant formula. Therefore, the study suggests that the formulated composite milk alternative is a potential nutritious alternative to conventional infant formula. Further research should prioritize the optimization of processing methods—such as fermentation or enzymatic treatment—to reduce the concentration of anti-nutritional factors and improve mineral bioavailability. Their presence in the CMA is not desirable as they could hinder the bioavailability of essential nutrients. While the CMA exhibits promise as a nutritious alternative, further formulation adjustments are essential to achieve full regulatory and nutritional parity with standardized infant formulas.

Source of funding: None.

Acknowledgment: The authors appreciate the expert technical support provided by the staff and technologists of Natural Product Research and Development at Nnamdi Azikiwe University, Awka, and the Applied Biochemistry Laboratory at Enugu State University of Science and Technology, Agbani.

Authors' Contribution: ONA: Conceptualization, Methodology, Literature search, Data Curation, Statistical Analysis, Software. **ONA and IIU:** Investigation, Visualization, Writing - Original draft, Writing - Review & Editing. **EIA and IIU:** Conceptualization, Data Curation, Investigation, Methodology. **CEO:** Conceptualization, Methodology, Project Administration, Manuscript Review, Supervision, Validation.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Preprint deposit: Authors did not share this manuscript as a preprint deposit.

References

Al-Beltagi, M., Saeed, N. K., Bediwy, A. S., Elbeltagi, R., & Hamza, M. B. (2024). Gastrointestinal tolerability of organic infant formula compared to traditional infant formula: A systematic review. *World Journal of Clinical Pediatrics, 13*(1), 88783. https://doi.org/10.5409/wjcp.v13.i1.88783 [Crossref] [PubMed] [Google Scholar] [Publisher]

AOAC International. (1990). *Official methods of analysis* (15th ed., pp. 701–703). AOAC International.

AOAC International. (1995). *Official method of analysis*. (16th ed., pp. 1147-1161). AOAC International.

AOAC International. (2000). *Official methods of analysis* (17th ed., pp. 200–210). AOAC International.

AOAC International. (2003). *Official methods of analysis of AOAC International* (22nd ed., pp. 2319–2331). AOAC International.

Astolfi, M. L., Marconi, E., Protano, C., & Canepari, S. (2020). Comparative elemental analysis of dairy milk and plant-based milk alternatives. *Food Control, 116*, 107327. https://doi.org/10.1016/j.foodcont.2020.107327 [Crossref] [Google Scholar] [Publisher]

Aydar, E. F., Tutuncu, S., & Özçelik, B. (2020). Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. *Journal of Functional Foods, 70*, 103949. https://doi.org/10.1016/j.jff.2020.103949 [Crossref] [Google Scholar] [Publisher]

Barros, L., Ferreira, M. J., Queirós, B., Ferreira, I. C. F. R., & Batista, P. (2007). Total phenols, ascorbic acid, β-carotene, and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry, 103*(2), 413–419. https://doi.org/10.1016/j.foodchem.2006.07.038 [Crossref] [Google Scholar] [Publisher]

Belewu, M. A., & Belewu, K. Y. (2007). Comparative physicochemical evaluation of tiger nut, soybean and coconut milk sources. *International Journal of Agriculture & Biology, 9*(5), 785–787. https://api.semanticscholar.org/CorpusID:52029836 [Google Scholar]

Brooker, P. G., Anastasiou, K., Smith, B. P. C., Tan, R., Cleanthous, X., & Riley, M. D. (2023). Nutrient composition of milk and plant-based milk alternatives: A cross-sectional study of products sold in Australia and Singapore. *Food Research International, 173*(2), 113475. https://doi.org/10.1016/j.foodres.2023.113475 [Crossref] [PubMed] [Google Scholar] [Publisher]

Carbas, B., Machado, N., & Oppolzer, D. (2020). Nutrients, antinutrients, phenolic composition, and antioxidant activity of common bean cultivars and their potential for food applications. *Antioxidants, 9*(2), 186. https://doi.org/10.3390/antiox9020186 [Crossref] [PubMed] [Google Scholar] [Publisher]

Chatterjee, C., Gleddie, S., & Xiao, C.-W. (2018). Soybean bioactive peptides and their functional properties. *Nutrients, 10*(9), 1211. https://doi.org/10.3390/nu10091211 [Crossref] [PubMed] [Google Scholar] [Publisher]

Crivelli, J. J., Mitchell, T., Knight, J., Wood, K. D., Assimos, D. G., Holmes, R. P., & Fargue, S. (2021). Contribution of dietary oxalate and oxalate precursors to urinary oxalate excretion. *Nutrients, 13*(1), 62. https://doi.org/10.3390/nu13010062 [Crossref] [PubMed] [Google Scholar] [Publisher]

Curhan, G. C., Willett, W. C., Knight, E. L., & Stampfer, M. J. (2004). Dietary factors and the risk of incident kidney stones in younger women: Nurses' Health Study II. *Archives of Internal Medicine, 164*(8), 885–891. https://doi.org/10.1001/archinte.164.8.885 [Crossref] [PubMed] [Google Scholar] [Publisher]

Cruz, N., Capellas, M., Hernandez, M., Trujillo, A. J., Guamis, B., & Ferragut, V. (2007). Ultra high pressure homogenization of soymilk: Microbiological, physicochemical and microstructural characteristics. *Food Research International, 40*(6), 725–732. https://doi.org/10.1016/j.foodres.2007.01.003 [Crossref] [Google Scholar] [Publisher]

Drewnowski, A. (2022). Most plant-based milk alternatives in the USDA branded food products database do not meet proposed nutrient standards or score well on nutrient density metrics. *Nutrients, 14*(22), 4767. https://doi.org/10.3390/nu14224767 [Crossref] [PubMed] [Google Scholar] [Publisher]

Dupont, C., Bocquet, A., Tomé, D., Bernard, M., Campeotto, F., Dumond, P., … Kalach, N. (2020). Hydrolyzed rice protein-based formulas, a vegetal alternative in cow's milk allergy. *Nutrients, 12*(9), 2654. https://doi.org/10.3390/nu12092654 [Crossref] [PubMed] [Google Scholar] [Publisher]

Givens, D. I. (2020). MILK Symposium review: The importance of milk and dairy foods in the diets of infants, adolescents, pregnant women, adults, and the elderly. *Journal of Dairy Science, 103*(11), 9681–9699. https://doi.org/10.3168/jds.2020-18296 [Crossref] [PubMed] [Google Scholar] [Publisher]

Gonçalves, B., Pinto, T., Aires, A., Morais, M. C., Bacelar, E., Anjos, R., Ferreira-Cardoso, J., Oliveira, I., Vilela, A., & Cosme, F. (2023). Composition of nuts and their potential health benefits—An overview. *Foods, 12*(5), 942. https://doi.org/10.3390/foods12050942 [Crossref] [PubMed] [Google Scholar] [Publisher]

Harborne, J.B. (1995). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis (Third Edition). Chapman and Hall, New York. Pp 674-681. https://doi.org/10.1046/j.1365-3059.1999.00318.x [Crossref] [Google Scholar] [Publisher]

Headey, D. D., & Alderman, H. H. (2019). The relative caloric prices of healthy and unhealthy foods differ systematically across income levels and continents. *The Journal of Nutrition, 149*(11), 2020–2033. https://doi.org/10.1093/jn/nxz158 [Crossref] [PubMed] [Google Scholar] [Publisher]

Jeske, S., Bez, J., Arendt, E. K., & Zannini, E. (2019). Formation, stability, and sensory characteristics of a lentil-based milk substitute as affected by homogenisation and pasteurisation. *European Food Research and Technology, 245*(7), 1519–1531. https://doi.org/10.1007/s00217-019-03286-0 [Crossref] [Google Scholar] [Publisher]

Kirk, R., & Sawyer, R. (1991). *Pearson's composition and analysis of foods* (9th ed.). Longman Scientific and Technical, pp 647-648. https://doi.org/10.1016/0308-8146(93)90087-v [Crossref] [Google Scholar] [Publisher]

Klein, B. P., & Perry, A. K. (1982). Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. *Journal of Food Science, 47*(3), 941–945. https://doi.org/10.1111/j.1365-2621.1982.tb12750.x [Crossref] [Google Scholar] [Publisher]

Kundu, P., Dhankhar, J., & Sharma, A. (2018). Development of non-dairy milk alternative using soymilk and almond

milk. *Current Research in Nutrition and Food Science, 6*(1), 203–210. https://doi.org/10.12944/CRNFSJ.6.1.23 [Crossref] [Google Scholar] [Publisher]

Lima, J. R., Souza, A. C. R., Pinto, C. O., Araujo, I. M. S., Bruno, L. M., Goiana, M. L., Wurlitzer, N. J., & Tajra, T. F. (2018). Stability during storage at room temperature of water-soluble cashew nut kernel extract. *Infoteca-e Repository, 175*, 4–15. [Publisher]

Liu, K. S. (1997). Chemistry and nutritional value of soybean components. In K. S. Liu (Ed.), *Soybeans: Chemistry, technology, and utilization* (pp. 25–113). Chapman & Hall. https://doi.org/10.1007/978-1-4615-1763-4_2 [Crossref] [Google Scholar] [Publisher]

Lolas, G. M., & Markakis, P. (1975). Phytic acid and other phosphorus compounds in beans (*Phaseolus vulgaris* L.). *Journal of Agricultural and Food Chemistry, 23*(1), 13–15. https://doi.org/10.1021/jf60197a016 [Crossref] [Google Scholar] [Publisher]

Mäkinen, O. E., Wanhalinna, V., Zannini, E., & Arendt, E. K. (2016). Foods for special dietary needs: Non-dairy plant-based milk substitutes and fermented dairy-type products. *Critical Reviews in Food Science and Nutrition, 56*(3), 339–349. https://doi.org/10.1080/10408398.2012.761950 [Crossref] [PubMed] [Google Scholar] [Publisher]

Mattiello, S., Caroprese, M., Matteo, C. G., Fortina, R., Martini, A., & Martini, M. (2017). Typical dairy products in Africa from local animal resources. *Italian Journal of Animal Science, 17*(3), 740–754. https://doi.org/10.1080/1828051X.2017.1401910 [Crossref] [Google Scholar] [Publisher]

Mrabet, A., Jiménez-Araujo, A., Guillén-Bejarano, R., Rodríguez-Arcos, R., & Sindic, M. (2020). Date seeds: A promising source of oil with functional properties. *Foods, 9*(6), 787. https://doi.org/10.3390/foods9060787 [Crossref] [PubMed] [Google Scholar] [Publisher]

Olawoye, B. T., & Gbadamosi, S. O. (2017). Effect of different treatments on *in vitro* protein digestibility, antinutrients, antioxidant properties, and mineral composition of *Amaranthus viridis* seed. *Cogent Food & Agriculture, 3*(1), Article 1296402. https://doi.org/10.1080/23311932.2017.1296402 [Crossref] [Google Scholar] [Publisher]

Osagie, A. U. (1998). Antinutritional factors. In *Nutritional quality of plant foods* (pp. 1–40, 221–244). Ambik Press Ltd, Benin City, Nigeria. [Google Scholar]

Padmarajaiah, N., Ramanathapura, A. V., & Hemmige, S. Y. (2002). Spectrophotometric determination of folic acid in pharmaceutical preparations by coupling reactions with iminodibenzyl or 3-aminophenol or sodium molybdate–pyrocatechol. *Analytical Biochemistry, 307*(2), 316–321. https://doi.org/10.1016/S0003-2697(02)00038-6 [Crossref] [PubMed] [Google Scholar] [Publisher]

Paul, A., Kumar, S., Kumar, V., & Sharma, R. (2020). Milk analog: Plant based alternatives to conventional milk, production, potential and health concerns. *Critical Reviews in Food Science and Nutrition, 60*(18), 3005–3023. https://doi.org/10.1080/10408398.2019.1674243 [Crossref] [PubMed] [Google Scholar] [Publisher]

Pointke, M., Albrecht, E. H., Geburt, K., Gerken, M., Traulsen, I., & Pawelzik, E. (2022). A comparative analysis of plant-based milk alternatives part 1: Composition, sensory, and nutritional value. *Sustainability, 14*(13), 7996. https://doi.org/10.3390/su14137996 [Crossref] [Google Scholar] [Publisher]

Popova, A., & Mihaylova, D. (2019). Antinutrients in plant-based foods: A review. *The Open Biotechnology Journal, 13*(1), 68–76. https://doi.org/10.2174/1874070701913010068 [Crossref] [Google Scholar] [Publisher]

Raeed, M. Q., & Azzam, A. M. (2008). Spectrophotometric assay of pyridoxine hydrochloride (vitamin B6) in pharmaceutical preparations and serum via arsenazo III-cerium (III) reaction. *Rafidain Journal of Science, 19*(2), 28–41. https://doi.org/10.1039/an9881301097 [Crossref] [PubMed] [Google Scholar] [Publisher]

Ramsing, R., Santo, R., Kim, B. F., Altema-Johnson, D., Wooden, A., Chang, K. B., Semba, R. D., & Love, D. C. (2023). Dairy and plant-based milks: Implications for nutrition and planetary health. *Current Environmental Health Reports, 10*(3), 291–302. https://doi.org/10.1007/s40572-023-00400-z [Crossref] [PubMed] [Google Scholar] [Publisher]

Redan, B. W., Zuklic, J., Hryshko, J., Boyer, M., Wan, J., Sandhu, A., & Jackson, L. S. (2023). Analysis of eight types of plant-based milk alternatives from the United States market for target minerals and trace elements. *Journal of Food Composition and Analysis, 122*, 105457. https://doi.org/10.1016/j.jfca.2023.105457 [Crossref] [PubMed] [Google Scholar] [Publisher]

Riaz, M. N., Asif, M., & Ali, R. (2009). Stability of vitamins during extrusion. *Critical Reviews in Food Science and Nutrition, 49*(4), 361–368. https://doi.org/10.1080/10408390802067290 [Crossref] [PubMed] [Google Scholar] [Publisher]

Rutkowski, M., Grzegorczyk, K., & Paradowski, M. T. (2005). Colorimetric method of blood plasma total vitamin E determination – The own modification of Tsen method. *Diagnostyka Laboratoryjna, 41*, 375. https://www.food.actapol.net/pub/2_3_2007.pdf [Google Scholar]

Rutkowski, M., Grzegorczyk, K., Gendek, E., & Kędziora, J. (2006). Laboratory convenient modification of Bessey method for vitamin A determination in blood plasma. *Journal of Physiology and Pharmacology, 57*(Suppl. 2), 221–228. https://www.food.actapol.net/pub/2_3_2007.pdf [Google Scholar]

Schlemmer, U., Frølich, W., Prieto, R. M., & Grases, F. (2009). Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis. *Molecular Nutrition & Food Research, 53*(Suppl. 2), S330–S375. https://doi.org/10.1002/mnfr.200900099 [Crossref] [PubMed] [Google Scholar] [Publisher]

Schuster, M., Wang, X., Hawkins, T., & Painter, J. (2018). Comparison of the nutrient content of cow's milk and nondairy milk alternatives: What's the difference? *Nutrition Today, 53*(3), 153–159. https://doi.org/10.1097/nt.0000000000000284 [Crossref] [Google Scholar] [Publisher]

Sethi, S., Tyagi, S. K., & Anurag, R. K. (2016). Plant-based milk alternatives an emerging segment of functional beverages: A review. *Journal of Food Science and Technology, 53*(9), 3408–3423. https://doi.org/10.1007/s13197-016-2328-3 [Crossref] [PubMed] [Google Scholar] [Publisher]

Silva, A. R. A., Silva, M. M. N., & Ribeiro, B. D. (2020). Health issues and technological aspects of plant-based alternative milk. *Food Research International, 131*, 108972. https://doi.org/10.1016/j.foodres.2019.108972 [Crossref] [PubMed] [Google Scholar] [Publisher]

Silva, B. Q., & Smetana, S. (2022). Review on milk substitutes from an environmental and nutritional point of view. *Applied Food Research, 2*(1), 100105. https://doi.org/10.1016/j.afres.2022.100105 [Crossref] [Google Scholar] [Publisher]

Valencia-Flores, D. C., Hernández-Herrero, M., Guamis, B., & Ferragut, V. (2013). Comparing the effects of ultra-high-pressure homogenization and conventional thermal treatments on the microbiological, physical, and chemical quality of almond beverages. *Journal of Food Science, 78*(2), E199–E205. https://doi.org/10.1111/1750-3841.12029 [Crossref] [PubMed] [Google Scholar] [Publisher]

Verduci, E., D'Elios, S., Cerrato, L., Comberiati, P., Calvani, M., Palazzo, S., Martelli, A., Landi, M., Trikamjee, T., & Peroni, D. G. (2019). Cow's milk substitutes for children: Nutritional aspects of milk from different mammalian species, special formula and plant-based beverages. *Nutrients, 11*(8), 1739. https://doi.org/10.3390/nu11081739 [Crossref] [PubMed] [Google Scholar] [Publisher]

Young, C., & Greaves, J. (1940). *Laboratory handbook of methods of food analysis*. Leonard Hill. [Google Scholar]