Plant Archives Vol. 26, Supplement 2, 2026 pp. 1244-1253 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-2.148

GC–MS PROFILING OF PHYTOACTIVE COMPOUNDS, ANTIOXIDANT PROPERTIES AND ANTINUTRITIONAL FACTORS IN FINGER MILLET MILK

Siva Sakthi T.[1*], Amutha S.[2] and Saravanakumar R.[3]
[1]Department of Food Science and Nutrition, Community Science College & Research Institute, TNAU, Madurai - 625104, India.
[2]Department of Human Development and Family Studies, Community Science College & Research Institute, TNAU, Madurai625104, India.
*Corresponding author E-mail: sarofsn@yahoo.com
(Date of Receiving : 20-02-2026; Date of Revision : 05-04-2026; Date of Acceptance : 25-04-2026)

ABSTRACT

Plant-based milk alternatives are increasingly preferred worldwide owing to their nutritional advantages, environmental sustainability, and suitability for individuals with lactose intolerance. The present study investigated antinutritional constituents, antioxidant potential, and GC–MS phytochemical profiling of unfortified and vitamin A and D fortified finger millet milk. Finger millet grains were germinated, subjected to ultrasonication-assisted extraction, pasteurized, and fortified with vitamins A and D to develop a functional beverage. Key antinutritional factors such as tannins, phytic acid, and oxalates, along with bioactive compounds including flavonoids, total polyphenols, and antioxidant activity, were assessed. GC–MS analysis was used to identify and compare the chemical profiles of both samples. The results showed only marginal differences between unfortified and fortified milk in tannins (0.48–0.47 mg CAE/100 ml), phytic acid (21.78–21.76 mg/100 ml), oxalates (26.53–26.51 mg/100 ml), flavonoids (69.23 mg CAE/100 ml), polyphenols (122.35–122.34 mg GAE/100 ml), and antioxidant activity (~66.7% RSA), indicating that fortification did not significantly alter the inherent phytochemical composition. GC–MS profiling identified 81 compounds in the control sample and 25 major compounds in the fortified milk, with enriched levels of oleic acid, linoleic acid, α-linolenic acid, vitamin E, squalene, and campesterol. These compounds are associated with antioxidant, cardioprotective, anti-inflammatory, and immune-enhancing properties. Overall, vitamin A and D fortification improved the nutritional and functional quality of finger millet milk while maintaining its antioxidant capacity. The findings support the potential of finger millet milk as a nutrient-rich, health-promoting, and sustainable plant-based dairy alternative.
Keywords **:** Finger millet milk; Plant-based beverage; Vitamin fortification; GC–MS profiling; Antioxidant activity

Introduction

Plant-based milk alternatives have emerged as popular choices due to their nutritional value, evolving dietary trends, environmental sustainability and decreased reliance on animal products. They are especially beneficial for individuals with lactose intolerance or dairy allergies. These beverages provide essential nutrients, bioactive compounds and antioxidants, supporting their use in functional food development. Their consumption has risen significantly, with the global market expected to reach US$26 billion by 2024 (Silva and Smetana, 2022). In general, plant-based milk substitutes are water-based extracts derived from plant sources that closely resemble cow's milk in appearance and texture, although their taste and nutritional composition differ based on the raw materials and processing techniques employed (Tangyu *et al*., 2019)

Plant based finger millet milk are recognized for their rich composition of bioactive compounds such as phenolics, flavonoids, vitamins and essential fatty acids, which contribute significantly to antioxidant activity and overall health promotion. However, alongside these beneficial components, plant-based milks also contain antinutritional factors such as phytic acid, tannins, and oxalates, which can interfere with mineral absorption and bioavailability (Kaur *et al*., 2024).

Emerging technologies for producing plant-based milk substitutes include ultrasound, pulsed electric fields, ohmic heating, and high-pressure homogenization. Among non-thermal methods, ultrasonication is a fast, versatile, and safe technique that uses sound waves (20–100 kHz) to create cavitation, leading to significant physical and chemical changes in the sample (Leksawasdi *et al*.,2022).

Finger millet (Eleusine coracana), an ancient cereal of the Poaceae family cultivated in India since 2300 BC, is a nutrient-rich millet high in calcium (344 mg/100 g), fiber, polyphenols, and minerals. It ranks fourth globally among millets and offers health benefits such as anti-diabetic and anti-inflammatory effects. Its calcium content is about eight times higher than that of common cereals (Vagdevi *et al*., 2023).

In recent years, advanced techniques such as Gas Chromatography–Mass Spectrometry (GC–MS) have been extensively used to analyze bioactive compounds in food systems. This method allows accurate identification and characterization of volatile and semi-volatile constituents, including fatty acids, phenolic compounds, and other functional metabolites, thereby offering valuable insights into the nutritional and therapeutic properties of plant-based beverages (Al-Rubaye *et al*., 2017)

This study focuses on evaluating the antinutritional factors, antioxidant activity and GC–MS characterization of bioactive compounds in unfortified and vitamin fortified finger millet milk. It aims to determine how fortification influences the phytochemical profile, antioxidant properties, and overall functional quality, thereby highlighting the potential of fortified finger millet milk as a nutritious and health-promoting plant-based substitute for dairy products.

Materials and Methods

Preparation of unfortified and vitamin fortified finger millet milk

Finger millet grains were first soaked for 19 hours and then germinated for 24 hours to enhance their nutritional value. The sprouted grains were ground with water in a 1:7 ratio to obtain a slurry, which was subsequently subjected to ultrasonication to improve extraction. The mixture was then filtered and homogenized to produce a smooth milk extract. This extract was pasteurized at 85°C for 15 minutes to ensure safety, followed by cooling. After cooling, vitamin A, vitamin D, and xanthan gum were incorporated, and the mixture was further homogenized to achieve uniformity. Finally, the prepared milk was bottled and stored at 4°C. For unfortified (control) finger millet milk, the same procedure will follow but not include the vitamin fortificant. For the unfortified (control) finger millet milk, the same procedure was followed, except that vitamin fortificants were not added (Figure 1).

Fig. 1: Process flow chart for the preparation of unfortified and vitamin fortificants finger millet milk

Assessment of antinutritional factors and antioxidant activity of unfortified and fortified finger millet milk

Tannin

Tannin content was determined based on its ability to reduce phosphotungstomolybdic acid in an alkaline medium, forming a blue-colored complex measured at 700 nm, with intensity proportional to tannin concentration. Folin–Denis reagent, sodium carbonate solution, and tannic acid standards were prepared using standard procedures. For analysis, 1 ml of sample extract was mixed with water, Folin–Denis reagent, and sodium carbonate, and the volume was adjusted to 100 ml. After 30 minutes, absorbance was recorded using a UV–Vis spectrophotometer against a water blank. Tannin content was expressed as mg tannic acid equivalents (TAE) per 100 g of sample following the method (Sadasivam,1996).

Total flavonoids

Total flavonoid content was estimated by the aluminum chloride colorimetric method (Ribarova *et al*., 2005). The sample extract was reacted sequentially with sodium nitrite, aluminum chloride, and sodium hydroxide, and the volume was made up to 10 ml. Absorbance was measured at 510 nm using a spectrophotometer with a reagent blank. A quercetin standard curve (20–100 µg/ml) was used, and results were expressed as mg quercetin equivalents per 100 g or 100 ml of sample on fresh and dry weight basis.

Total polyphenols

Total polyphenols were estimated using the Folin–Ciocalteu method, where phenolic compounds react under alkaline conditions to form a blue-colored complex. In this method, the sample was mixed with Folin–Ciocalteu reagent and sodium carbonate, incubated at 25°C for 2 hours and the absorbance was measured spectrophotometrically. A gallic acid standard curve was used for quantification, and the results were expressed as mg gallic acid equivalents (GAE) per 100 ml of sample (Bao *et al*., 2005).

Phytic acid

Phytic acid content in plant-based milk was estimated following method of (Aina *et al*., 2012). The sample was soaked in 2% HCl, filtered, and the filtrate was treated with water and ammonium thiocyanate before titration with standard iron (II) chloride until a stable yellow color appeared. The phytic acid percentage was calculated using the formula: % phytic acid = y × 1.19 × 100, where y = titre value × 0.00195.

Oxalate content

Oxalate content in plant-based milk was determined using the method of Munro and Bassir (Munro and Bassir, 1969). The sample was acidified with HCl, heated, cooled, and filtered, followed by pH adjustment using ammonium hydroxide with methyl red indicator. Calcium chloride was then added to precipitate oxalate, and the mixture was left overnight. The precipitate was separated by centrifugation, dissolved in sulfuric acid, diluted, and titrated with standard potassium permanganate until a persistent pink endpoint was observed.

Total antioxidant activity (DPPH Assay)

Radical scavenging activity of plant-based milk was evaluated using the DPPH assay, where the purple DPPH radical is reduced by antioxidants, leading to a decrease in absorbance proportional to scavenging ability. Methanolic extracts were mixed with DPPH solution, incubated in the dark for 30 minutes, and absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The percentage of DPPH inhibition (%RSA) was calculated by comparing sample and control absorbance. The IC_{50} value, representing the concentration required to achieve 50% inhibition, was determined, with lower IC_{50} indicating higher antioxidant activity (Bao *et al*., 2005).

GC-MS analysis of finger millet milk

Finger millet milk samples were freeze-dried at −18°C for 48 hours, followed by further drying at −15°C. The obtained powders were packed in aluminum foil and stored in dark conditions. For sample preparation, 2 g of dried sample was extracted with 20 ml of HPLC-grade hexane, sonicated for two 30-minute cycles, and filtered using Whatman No. 1 filter paper. The filtrate was concentrated by solvent evaporation, reconstituted in 2 ml hexane, membrane-filtered, and transferred to GC–MS vials.

GC–MS analysis was performed using a Shimadzu QP2020 system. A 1 µL sample was injected in splitless mode with a 5-minute split delay. Separation was achieved using an Rxi-5 Sil MS column under programmed oven temperatures from 70°C to 280°C. Helium served as the carrier gas at 1 ml/min. The injector and transfer line temperatures were maintained at 230°C and 280°C, respectively. Compounds were identified using electron impact ionization at 70 eV with a scan range of 35–650 m/z and a scan rate of 2.74 scans per second, ensuring accurate compound identification and quantification (Tangyu *et al*., 2023).

Results and Discussion

Antinutritional factors and antioxidant activity of unfortified and fortified finger millet milk

The phytochemical composition of Control Finger Millet Milk (CFM) and Vitamin-Fortified Finger Millet Milk (FFM), including tannins, phytic acid, oxalates, flavonoids, polyphenol and total antioxidant activity, is presented in Table 1. The findings reveal only slight differences between the two samples, indicating a high level of consistency in their composition. The tannin content was recorded as 0.48 mg CAE/100 ml in CFM and 0.47 mg CAE/100 ml in FFM, showing nearly identical levels. Tannins are polyphenolic compounds recognized for their astringent nature and beneficial properties such as antioxidant and antimicrobial effects (Huang *et al*., 2021).

Phytic acid levels were measured at 21.78 mg/100 ml in CFM and 21.76 mg/100 ml in FFM, demonstrating minimal variation. Although phytic acid is considered an antinutrient due to its ability to bind minerals like calcium, iron and zinc and reduce their bioavailability, it also exhibits antioxidant and anticancer properties, highlighting its dual nutritional role. Similarly, oxalate content showed negligible differences, with values of 26.53 mg/100 ml in CFM and 26.51 mg/100 ml in FFM.

The total flavonoid content was found to be the same in both samples at 69.23 mg CAE/100 ml, indicating uniformity. Flavonoids are well-known bioactive compounds with strong antioxidant, anti-inflammatory and cardioprotective effects (Vazhappilly *et al*., 2019), suggesting their potential in reducing oxidative stress-related disorders. Total polyphenol content was also highly consistent, with values of 122.35 mg GAE/mg in CFM and 122.34 mg GAE/mg in FFM. Polyphenols are widely associated with various health benefits, including anti-inflammatory, anticancer, and neuroprotective activities, primarily due to their ability to scavenge free radicals and mitigate oxidative stress (Rana *et al*., 2022).

The total antioxidant activity, expressed as percentage radical scavenging activity (%RSA), was identical in both samples at 66.74%, indicating strong antioxidant potential. Antioxidants play a vital role in neutralizing reactive oxygen species (ROS), which are linked to the development of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions.

Boukid *et al*. (2023) reported the presence of aldehydes such as pentanal and hexanal, and alcohols like heptanol in almond-based beverages, which are formed during lipid oxidation and contribute to undesirable off-flavors. Additionally, the bitterness and astringency often observed in plant-based drinks are attributed to bioactive compounds such as alkaloids, phenols, saponins, cyanogenic glycosides, flavonoids, terpenes, and glucosinolates.

According to Sravanthi (2022), finger millet-based milk retains considerable antioxidant activity; however, thermal processing methods such as pressure cooking and pasteurization significantly reduce phytochemical content. The total polyphenol content decreased from 119.8 ± 0.21 mg GAE/100 g in raw millet to 34.2 ± 0.79 mg GAE/100 ml in the processed beverage. Similarly, total flavonoids declined from 159.7 mg RE/100 g in raw millet to 126.6 mg RE/100 ml in milk, and DPPH radical scavenging activity decreased from 17.18 ± 0.06 µmol Trolox equivalent/g to 6.07 ± 0.05 µmol Trolox equivalent/ml. In contrast to these findings, the present study demonstrates that fortification with vitamins A and D did not significantly alter the phytochemical profile or antioxidant activity, thereby supporting the feasibility of developing nutritionally enhanced plant-based milk with preserved functional properties.

Table 1: Antinutritional factors and Antioxidant activity of Unfortified and Fortified finger millet milk

Parameters	CFM	FFM	P-Value
Tannin(mg CAE/100ml)	0.48^{a}±0.008	0.47^{b}±0.002	0.28**
Phytic acid(mg/100ml)	21.78±0.35	21.76±0.2	0.97^{NS}
Oxalate (mg/100ml)	26.53±0.43	26.51±0.09	0.95^{NS}
Total Flavonoids (mg CAE/100ml)	69.23±1.27	69.23±0.42	1^{NS}
Total Polyphenols (GAE/mg)	122.35±3.24	122.34±0.91	0.99^{NS}
Total Antioxidant activity (% RSA)	66.7±1.18	66.74±1.77	1^{NS}

Values are means of three replicates. The values mentioned in superscripts are significantly different at $p < 0.05$ in the same column.

CFM Unfortified (control) Finger millet milk **FFM** Vitamin A and D fortified finger millet milk

GC-MS analysis of unfortified (control) and vitamin fortified finger millet milk samples

This presents the GC–MS analysis of unfortified (control) and vitamin-fortified finger millet milk to identify and compare their bioactive compounds, as well as to evaluate the effect of fortification on chemical composition and potential functional benefits.

GC-MS analysis of unfortified (control) finger millet milk samples

GC–MS analysis of the unfortified (control) finger millet milk revealed a wide range of bioactive compounds with diverse functional properties. A total of 81 compounds were detected, of which 25 major compounds were selected based on their highest peak area percentages (Table 2). The retention times ranged from 9.00 to 46.99 minutes, indicating the presence of both volatile and semi-volatile constituents. The most dominant compound identified was 4-(1H-benzimidazol-1-yl) aniline, TMS, with a peak area of 15.09% at a retention time of 46.99 minutes, known for its antimicrobial and anticancer activities (Dharavath *et al.*, 2022). Other prominent compounds included stearic acid hydrazide (5.04%), 1,2-hydrazinedicarboxamide (5.81%), and 5-bromo-N,N-dimethyl-2H-1,2,4-triazole-3-carboxamide (5.02%), all associated with antimicrobial, antifungal, and anti-inflammatory properties.

Oleic acid (4.51%), a monounsaturated fatty acid, was also detected and is recognized for its anti-inflammatory and cardioprotective effects (Seo *et al.*, 2019). Additionally, n-hexadecanoic acid (2.42%) and octadecanoic acid (1.57%) were identified, highlighting the presence of essential lipids that contribute to antioxidant activity, skin protection, and metabolic functions (Park *et al.*, 2019).

Several phenolic and heterocyclic compounds with strong antioxidant potential were also observed. Compounds such as phenol, 2,4-bis (1,1-dimethylethyl) phosphite (3.93%) and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (0.55%) are known for their ability to scavenge free radicals and reduce oxidative stress (Li *et al.*, 2019). These findings indicate that finger millet milk is rich in bioactive constituents with potential health-promoting properties, making it a promising alternative to conventional dairy products.

The presence of compounds like glycerol, 2TMS (92.31%) and diethyl phthalate (2.17%) suggests a possible role in flavor enhancement. Moreover, several heterocyclic compounds identified in the sample exhibit anti-inflammatory and antimicrobial activities. For example, 5-(γ-methylaminopropyl)-3-(p-chlorophenyl)-1H-pyrazole (2.45%) and 7,9-diethyl-2,4-bis(dimethylamino)-10-imino-8-thio-1,7,9-triazapolyaza-1,3-decadiene-6,8-dione (2.52%) have been reported to possess anti-inflammatory effects (23). The detection of antiviral compounds such as 1-(β-D-arabinofuranosyl)-4-O-difluoromethyluracil (2.27%) and 2R,3S-1-(1,3,4-trihydroxy-2-butoxymethyl) uracil (0.48%) further indicates the potential immunomodulatory properties of unfortified finger millet milk (Mohan *et al.*, 2021).

Table 2 : GC-MS analysis of unfortified (control) finger millet milk.

S. No.	Retention Time (min)	Area %	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Biological Activity	References
1.	46.990	15.09	4 – (1 H- Benzimidazol – 1 - yl) aniline, TMS	$C_{13}H_{13}N_3Si$	255	Antimicrobial, anticancer	(Rasheed *et al.*, 2021)
2.	46.015	5.04	Stearic acid hydrazide	$C_{18}H_{39}N_3O$	313	Antimicrobial, anti – Inflammatory	(Majrashi *et al.*, 2023)
3.	46.745	5.81	1,2 – Hydrazinedicaroxamide	$C_2H_6N_4O_2$	118	Anticancer potential	(Capan *et al.*, 2024)
4.	46.457	5.02	5 – Bromo- N, N – dimethyl – 2H 1,2,4 – triazole – 3 carboxamide	$C_5H_8BrN_5O$	234	Antifungal, antibacterial	(Pokhodylo *et al.*, 2021)
5.	45.835	4.15	Hexa (methoxymethyl) melamine	$C_{12}H_{30}N_6O_6$	342	Antimicrobial	(Gan *et al.*, 2020)
6.	31.397	4.51	Oleic Acid	$C_{18}H_{34}O_2$	282	Anti-inflammatory, cardioprotective	(Karacor and Cam, 2015)
7.	43.128	3.93	Phenol, 2, 4- bis (1,1-dimethylethyl) – phosphite (3:1)	$C_{22}H_27O_3$	370	Antioxidant properties	(Bhuyan *et al.*, 2019)
8.	16.265	3.25	1,3 – Propanediol, 2-hydroxymethyl) – 2 – nitro	$C_4H_9NO_4$	135	Antimicrobial polymer precursor	(Urbanovich *et al.*, 2022)
9.	46.295	2.52	7,9 – Diethyl, 2,4 – bis (dimethylamino)- 10 – imino 8 thio- 1,7,9 – triazaspiro – 1,3 decadiene- 6,8 dione	$C_{12}H_{20}N_6OS$	308	Antiinflammtory	(Tasturganova *et al.*, 2018)
10.	28.095	2.42	n- Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antioxidant, antimicrobial	(Ganesan *et al.*, 2024)
11.	45.395	2.45	5 – (γ- Methylaminopropyl0 – 3 (p- chlorophenyl) – 1 H – pyrazole	$C_{14}H_{17}ClN_4$	276	Antioxidant potential	(Khatun *et al.*, 2022)
12.	20.188	2.17	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	Plasticizer, endocrine disruptor	(Manzoor *et al.*, 2019)

13.	45.195	2.31	Glycerol, 2 TMS	$C_5H_{14}O_4Si_2$	210	Antifungal	(Trivunoic et al., 2022)
14.	41.00	1.70	1 H – Pyrazole – 1 acetaminde, 4 – iodo – N – (phenylmethyl)	$C_{10}H_{10}IN_3O$	319	Antimicrobial, antifungal	(Kachi *et al*., 2024)
15.	45.624	2.27	1 – (β- d – Arabinofurnosyl)- 4 O - difluromethylurcail	$C_{10}H_{12}F_2N_2O_5$	278	Antiviral activity	(Cheng *et al*., 2021)
16.	35.891	1.57	Propiophenone, 3 – phenyl – 3 – piperidino	$C_{14}H_{17}NO$	215	Anti – infllmatory	(Srividya *et al*., 2023)
17.	31.848	1.57	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Fatty acid, emollient	(Cai *et al*., 2021)
18.	38.099	1.57	6 – Hydroxy – 8 – methoxyoctanoic acid, methyl ester	$C_{10}H_{18}O_4$	202	Antiinflmmatory	(Cai *et al*., 2021)
19.	9.00	0.55	4H- Pyran- 4- one , 2,3 – dihydro- 3,5 – dihydroxy- 6 – methyl	$C_6H_8O_4$	144	Antioxidant, bioactive	(Yu *et al*., 2013)
20.	22.580	0.75	Cyacetacide	$C_3H_4N_4O_2$	128	Antiinflammtory	(Das *et al*., 2018)
21.	117	0.48	2R, 3S- 1 1,3,4-Trihydroxy- 2- butoxymethyluracil	$C_{19}H_{14}N_2O_6$	246	Antiviral, anticancer	(Mohan *et al*., 2021)
22.	785	0.48	16- HExadecanolyl hydrazide	$C_{16}H_{34}N_2O$	270	Antiviral	(Abdelaziz *et al*., 2024)

GC-MS analysis of fortified finger millet milk samples

GC–MS analysis was performed to identify and compare the bioactive constituents of unfortified (control) and vitamin-fortified finger millet milk. The results revealed a diverse range of essential fatty acids, phenolics, vitamins and other metabolites with significant nutritional and health-promoting roles. Comparison of the two samples indicated that fortification enhanced both the concentration and bioavailability of these functional components (Table 3 and Figure 1).

Fatty acids, which are important for energy metabolism, cell membrane function and inflammation control, were notably higher in the fortified sample. Key fatty acids such as oleic acid (34.16%), linoleic acid (6.08%) and α-linolenic acid (3.76%) showed marked increases compared to the control. These polyunsaturated fatty acids are widely associated with cardioprotective, neuroprotective, and anti-inflammatory benefits (Kim *et al*., 2019). The detection of n-hexadecenoic acid (15.68%) further suggests improved antioxidant and antimicrobial potential in the fortified milk (Li *et al*., 2019).

The fortified milk also contained greater amounts of natural antioxidants, including vitamin E (2.98%), squalene (2.63%), and campesterol (3.21%), which were present in much lower concentrations (0.2–0.5%) in the control sample. These compounds are effective in scavenging free radicals, protecting against oxidative damage and supporting skin health (39). Additionally, increased levels of 5-hydroxymethylfurfural and maltol indicate improvements in both antioxidant capacity and flavor profile (Tian *et al*., 2020).

Nutritionally, the fortified sample showed higher levels of sucrose (6.56%), contributing to energy supply, dihydroxyacetone (6.27%) for improved glucose metabolism, and tocopherols (2.98%) for antioxidant and cardiovascular benefits. The elevated PUFA content may aid in reducing cholesterol and inflammation, while increased phenolic compounds and vitamin E enhance antioxidant defense and immune function (Shahidi and Ambigaipalan, 2015). Compared to the control, the fortified milk demonstrated a 30-40% increase in essential fatty acids, along with improved antioxidant activity and sensory characteristics.

Overall, the comparative GC–MS findings clearly demonstrate that vitamin A and D fortification significantly enhances the nutritional and functional quality of finger millet milk. The fortified sample showed higher levels of essential fatty acids such as oleic, linoleic, and α-linolenic acids, which are known for their multiple health benefits (Calder, 2015). Enrichment with antioxidants like vitamin E and campesterol further strengthens protection against oxidative stress while supporting immune and skin health. Increased levels of sucrose and dihydroxyacetone indicate better energy utilization and glycemic regulation. Moreover, higher concentrations of phenolic antioxidants and flavor-related compounds such as 5-hydroxymethylfurfural and maltol contribute to improved functional and sensory properties (Ban *et al.*, 2022). These results confirm that fortification enhances the biochemical profile and overall health-promoting potential of finger millet milk, making it a valuable functional dairy alternative.

Table 3 : GC-MS analysis of vitamin fortified finger millet milk.

S. No.	Retention Time (min)	Area %	Compound Name	Molecular Formula	Molecular Weight (g/mol)	Biological Activity	References
1.	3.775	6.27	Dihydroxyacetone	$C_3H_6O_3$	90.08	Precursor to glucose metabolism, skin conditioning	(Belfleur *et al.*, 2022)
2.	5.420	2.54	Acetic acid	$C_2H_4O_2$	60.05	Antibacterial, digestive aid	(Lazou *et al.*, 2023)
3.	7.892	3.72	2,3 – Butanediol	$C_4H_{10}O_2$	90.12	Antimicrobial, Precursor for flavor compounds	(Bao *et al.*, 2005)
4.	9.301	4.85	1 – Hydroxy – 2 – propanone	$C_3H_6O_2$	74.08	Flavour enhancer, intermediate in metabolism	(Cizmarikova *et al.*, 2020)
5.	11.129	4.32	5 – Hydroxymethylfurfural (HMF)	$C_6H_6O_3$	126.11	Antioxidant	(Gao *et al.*, 2015)
6.	12.814	3.90	Maltol	$C_6H_6O_3$	126.11	Flavor compound, Antioxidant	(Garbacz, 2022)
7.	14.10	2.87	Lactic acid	$C_3H_6O_3$	90.08	Probiotic support, improves gut health	(Teng *et al.*, 2021)
8.	16.570	6.56	Sucrose	$C_{12}H_{22}O_{11}$	342.30	Energy source , sweetener	(Teng *et al.*, 2021)
9.	18.214	2.68	Benzyl alcohol	C_7H_8O	108.14	Antimicrobial, Preservative	(Stiblarikoya i, 2022)
10.	20.187	3.12	Diethyl Phthalate	$C_{12}H_{14}O_4$	222.24	Plasticizer	(Kachi *et al.*, 2024)
11.	22.514	2.14	Octanoic acid	$C_8H_{16}O_2$	144.21	Antifungal, Lipid metabolism	(Lin *et al.*, 2023)
12.	24.318	3.47	Methyl palmitate	$C_{17}H_{34}O_2$	270.45	Antioxidant, Anti-inflammatory	(Hamed *et al.*, 2020)
13.	25.972	5.89	Stearic acid	$C_{18}H_{36}O_2$	284.48	Emollient, Hydrophobic agent	(Hamed *et al.*, 2020)
14.	28.141	15.68	n- Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256.42	Antioxidant, Antimicrobial	(Hamed *et al.*, 2020)
15.	29.416	3.95	9 – Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	Lipid metabolism, Antiinflammatory	(Odokwo and Onifade, 2020)
16.	30.214	4.31	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294.48	Cardioprotective, Anti – inflammatory	(Hamed *et al.*, 2020)
17.	31.316	8.51	10E, 12Z-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.45	Antininfllmatory, Lipd metabolism	(Zhao *et al.*, 2015)
18.	31.460	34.16	Oleic Acid	$C_{18}H_{34}O_2$	282.47	Cardioprotective, Antioxidant	(Karacor and Cam, 2015)
19.	32.512	6.08	Linoleic acid	$C_{18}H_{32}O_2$	280.45	Anti- Inflammatory Essential fatty acids	(Liu *et al.*, 2020)
20.	33.275	4.52	9,12- Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280.45	Antioxidant, Antiinflammatory	(Krishnaveni *et al.*, 2014)
21.	34.618	3.76	9,12,15 – Octadecatrienoic acid (α- Linolenic acid)	$C_{18}H_{30}O_2$	278.43	Essential fatty acid, Neuroprotective	(Liu *et al.*, 2020)
22.	35.290	5.43	Hexadecanoic acid, 2-hydroxy – 1 – (hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330.50	Emollient, surfactant	(Liu *et al.*, 2020)
23.	36.118	2.98	Vitamin E (Tocopherol)	$C_{29}H_{50}O_2$	430.71	Antioxidant, Skin Protection	(Hamed *et al.*, 2020)

24.	37.215	2.63	Squalene	$C_{30}H_{50}$	410.72	Antioxidant, Cholesterol precursor	(Teng *et al*., 2021)
25.	39.512	3.21	Campesterol	$C_{28}H_{48}O$	400.69	Antiinflammatory, Cholesterol – lowering	(Garbacz, 2022)

Conclusion

The study demonstrated that both unfortified and vitamin A and D fortified finger millet milk contain considerable amounts of phytoactive compounds, antioxidants, and antinutritional factors, with only slight variations observed in tannins, phytic acid, oxalates, flavonoids, polyphenols, and overall antioxidant activity. This suggests that fortification does not significantly affect the inherent phytochemical composition of finger millet milk.

GC–MS analysis revealed a diverse profile of bioactive compounds, including essential fatty acids, phenolics, vitamins, and other functional metabolites. The fortified milk showed increased levels of important constituents such as oleic acid, linoleic acid, α-linolenic acid, vitamin E, squalene, and campesterol, which are associated with cardioprotective, antioxidant, anti-inflammatory, and immune-supporting effects. In addition, the enhancement of flavor-related and energy-associated compounds contributes to improved sensory attributes and nutritional quality. Overall, vitamin A and D fortification enhances the nutritional and functional value of finger millet milk while preserving its antioxidant potential. These results highlight its suitability as a nutrient-dense, health-promoting, and sustainable alternative to conventional dairy beverages.

Acknowledgement

We would like to express our sincere gratitude to DST – SHRI for funding and all those who contributed to the completion of this review article. Firstly, we extend our appreciation to the authors whose insightful research formed the foundation of this work. Their dedication to advancing knowledge in the field has been invaluable.

Conflict of Interest

No potential conflict of interest was reported by the author(s).

Funding Sources

This research was funded by Department of Science and Technology (DST) – SHRI.

References

Abbaszadeh, S., Yadegari, P., Imani, A. and Taghdir, M. (2021). Vitamin D3 protects against lead-induced testicular toxicity by modulating Nrf2 and NF-κB genes expression in rat. *Reproductive Toxicology*, **103**, 36–45.

Abdelaziz, S. A., Ahmed, E. M. and Sadek, M. (2024). Synthesis of homologous series of surfactants from renewable resources, structure–properties relationship, surface active performance, evaluation of their antimicrobial and anticancer potentialities. *Scientific Reports*, **14**(1), 13201.

Aina, A. J., Falade, K. O., Akingbala, J. O. and Titus, P. (2012). Physicochemical properties of Caribbean sweet potato (Ipomoea batatas (L.) Lam) starches. *Food and Bioprocess Technology*, **5**(2), 576–583.

Al-Rubaye, A. F., Hameed, I. H. and Kadhim, M. J. (2017). A review: Uses of gas chromatography-mass spectrometry (GC-MS) technique for analysis of bioactive natural compounds of some plants. *International Journal of Toxicological and Pharmacological Research*, **9**(1), 81–85.

Astudillo, A. M., Meana, C., Guijas, C., Pereira, L., Lebrero, P., Balboa, M. A. and Balsinde, J. (2018). Occurrence and biological activity of palmitoleic acid isomers in phagocytic cells. *Journal of Lipid Research*, **59**(2), 237–249.

Ban, Q., Sun, X., Jiang, Y., Cheng, J. and Guo, M. (2022). Effect of synbiotic yogurt fortified with monk fruit extract on hepatic lipid biomarkers and metabolism in rats with type 2 diabetes. *Journal of Dairy Science*, **105**(5), 3758–3769.

Bao, J., Cai, Y., Sun, M., Wang, G. and Corke, H. (2005). Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (Myrica rubra) extracts and their color properties and stability. *Journal of Agricultural and Food Chemistry*, **53**(6), 2327–2332.

Belfleur, L., Sonavane, M., Hernandez, A., Gassman, N. R. and Migaud, M. E. (2022). Solution chemistry of dihydroxyacetone and synthesis of monomeric dihydroxyacetone. *Chemical Research in Toxicology*, **35**(4), 616–625.

Bhuyan, D. J., Alsherbiny, M. A., Perera, S., Low, M., Basu, A., Devi, O. A., Barooah, M. S., Li, C. G. and Papoutsis, K. (2019). The odyssey of bioactive compounds in avocado (Persea americana) and their health benefits. *Antioxidants*, **8**(10), 426.

Boukid, F., Hassoun, A., Zouari, A., Tülbek, M. Ç., Mefleh, M., Aït-Kaddour, A. and Castellari, M. (2023). Fermentation for designing innovative plant-based meat and dairy alternatives. *Foods*, **12**(5), 1005.

Cai, J.-S., Feng, J.-Y., Ni, Z.-J., Ma, R.-H., Thakur, K., Wang, S., Hu, F., Zhang, J.-G. and Wei, Z.-J. (2021). An update on the nutritional, functional, sensory characteristics of soy products, and applications of new processing strategies. *Trends in Food Science & Technology*, **112**, 676–689.

Calder, P. C. (2015). Functional roles of fatty acids and their effects on human health. *Journal of Parenteral and Enteral Nutrition*, **39**, 18S–32S.

Çapan, İ., Hawash, M., Qaoud, M. T., Gülüm, L., Tunoglu, E. N. Y., Çifci, K. U., Çevrimli, B. S., Sert, Y., Servi, S. and Koca, İ. (2024). Synthesis of novel carbazole hydrazinecarbothioamide scaffold as potent antioxidant, anticancer and antimicrobial agents. *BMC Chemistry*, **18**(1), 102.

Cheng, Y.-H., Mu, D.-C., Jiao, Y., Xu, Z. and Chen, M.-L. (2021). Microwave-assisted Maillard reaction between rice protein and dextran induces structural changes and functional improvements. *Journal of Cereal Science*, **97**, 103134.

Čižmáriková, R., Markuliak, M., Habala, L., Valentová, J. and Bilková, A. (2020). Synthesis, antimicrobial and antiradical activity of (3-alkoxymethyl-4-hydroxyphenyl) propan-1-ones, intermediates of biologically active compounds and activity comparison with 3-(alkoxymethyl)-4-(alkylamino-2-hydroxypropoxyphenyl) alkanones type of beta blockers. *European Pharmaceutical Journal*, **67**(2), 34–44.

Das, S. R., Everett, B. M., Birtcher, K. K., Brown, J. M., Cefalu, W. T., Januzzi, J. L., Kalyani, R. R., Kosiborod, M., Magwire, M. L. and Morris, P. B. (2018). 2018 ACC expert consensus decision pathway on novel therapies for cardiovascular risk reduction in patients with type 2 diabetes and atherosclerotic cardiovascular disease: A report of the American College of Cardiology Task Force on Expert Consensus Decision Pathways. *Journal of the American College of Cardiology*, **72**(24), 3200–3223.

Dharavath, R., Sarasija, M., Ram Reddy, M., Naga Prathima, K., Nagarju, N., Ramakrishna, K., Ashok, D. and Daravath, S. (2022). Microwave-assisted synthesis and evaluation of their antiproliferative, antimicrobial activities and DNA binding studies of (3-methyl-7H-furo[2,3-f]chromen-2-yl)(aryl)methanones. *Medicinal Chemistry Research*, **31**(6), 993–1002.

Gan, J., Huang, Z., Yu, Q., Peng, G., Chen, Y., Xie, J., Nie, S. and Xie, M. (2020). Microwave assisted extraction with three modifications on structural and functional properties of soluble dietary fibers from grapefruit peel. *Food Hydrocolloids*, **101**, 105549.

Ganesan, T., Subban, M., Christopher Leslee, D. B., Kuppannan, S. B. and Seedevi, P. (2024). Structural characterization of n-hexadecanoic acid from the leaves of Ipomoea eriocarpa and its antioxidant and antibacterial activities. *Biomass Conversion and Biorefinery*, **14**(13), 14547–14558.

Gao, H., Wen, X. and Xian, C. J. (2015). Hydroxymethyl furfural in Chinese herbal medicines: Its formation, presence, metabolism, bioactivities and implications. *African Journal of Traditional, Complementary and Alternative Medicines*, **12**(2), 43–54.

Garbacz, K. (2022). Anticancer activity of lactic acid bacteria. *Seminars in Cancer Biology*.

Hamed, A., Mantawy, E., El-Bakly, W., Abdel-Mottaleb, Y. and Azab, S. (2020). Methyl palmitate: The naturally occurring cardioprotective agent. *Archives of Pharmaceutical Sciences Ain Shams University*, **4**(1), 47–62.

Huang, R. and Xu, C. (2021). An overview of the perception and mitigation of astringency associated with phenolic compounds. *Comprehensive Reviews in Food Science and Food Safety*, **20**(1), 1036–1074.

Kachi, O. G., Pawar, H. R., Chabukswar, A. R., Jagdale, S., Swamy, V., Vinayak, K., Hingane, D., Shinde, M. and Pawar, N. (2024). Design, synthesis and evaluation of antifungal activity of pyrazoleacetamide derivatives. *Medicinal Chemistry*, **20**(10), 957–968.

Karacor, K. and Cam, M. (2015). Effects of oleic acid. *Medical Science and Discovery*, **2**(1), 125–132.

Kaur, S., Kumari, A., Seem, K., Kaur, G., Kumar, D., Verma, S., … Riar, A. (2024). Finger millet (Eleusine coracana L.): From staple to superfood—a comprehensive review on nutritional, bioactive, industrial, and climate resilience potential. *Planta*, **260**(3), 75.

Khatun, S., Singh, A., Bader, G. N. and Sofi, F. A. (2022). Imidazopyridine, a promising scaffold with potential medicinal applications and structural activity relationship (SAR): Recent advances. *Journal of Biomolecular Structure and Dynamics*, **40**(24), 14279–14302.

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A. and Yu, B. (2019). PubChem 2019 update: Improved access to chemical data. *Nucleic Acids Research*, **47**(D1), D1102–D1109.

Krishnaveni, M., Dhanalakshmi, R. and Nandhini, N. (2014). GC-MS analysis of phytochemicals, fatty acid profile, antimicrobial activity of Gossypium seeds. *International Journal of Pharmaceutical Sciences Review and Research*, **27**(1), 273–276.

Kumar, P., Kim, K.-H., Bansal, V., Lazarides, T. and Kumar, N. (2017). Progress in the sensing techniques for heavy metal ions using nanomaterials. *Journal of Industrial and Engineering Chemistry*, **54**, 30–43.

Lazou, M., Perontsis, S. and Psomas, G. (2023). Metal complexes with naphthalene-based acetic acids as ligands: Structure and biological activity. *Molecules*, **28**(5), 2171.

Leksawasdi, N., Taesuwan, S., Prommajak, T., Techapun, C., Khonchaisri, R., Sittilop, N., Halee, A., Jantanasakulwong, K., Phongthai, S. and Nunta, R. (2022). Ultrasonic extraction of bioactive compounds from green soybean pods and application in green soybean milk antioxidants fortification. *Foods*, **11**(4), 588.

Li, H., Tang, X.-Y., Wu, C.-J. and Yu, S.-J. (2019). Formation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP) in glucose-amino acids Maillard reaction by dry-heating in comparison to wet-heating. *LWT*, **105**, 156–163.

Lin, W.-C., Hsu, K.-C., You, M.-F., Lee, K.-H., Chi, C.-H. and Chen, J.-Y. (2023). Octanoic acid promotes clearance of antibiotic-tolerant cells and eradicates biofilms of Staphylococcus aureus isolated from recurrent bovine mastitis. *Biofilm*, **6**, 100149.

Liu, C., Kuang, X., Li, K., Guo, X., Deng, Q. and Li, D. (2020). Effects of combined calcium and vitamin D supplementation on osteoporosis in postmenopausal women: A systematic review and meta-analysis of randomized controlled trials. *Food & Function*, **11**(12), 10817–10827.

Majrashi, T. A., Sabt, A., Abd El Salam, H. A., Al-Ansary, G. H., Hamissa, M. F. and Eldehna, W. M. (2023). An updated review of fatty acid residue-tethered heterocyclic compounds: Synthetic strategies and biological significance. *RSC Advances*, **13**(20), 13655–13682.

Manzoor, M. F., Ahmad, N., Aadil, R. M., Rahaman, A., Ahmed, Z., Rehman, A., Siddeeg, A., Zeng, X. A. and Manzoor, A. (2019). Impact of pulsed electric field on rheological, structural, and physicochemical properties of almond milk. *Journal of Food Process Engineering*, **42**(8), e13299.

Mohan, M. K., Rahul, A., De Schutter, G. and Van Tittelboom, K. (2021). Extrusion-based concrete 3D printing from a material perspective: A state-of-the-art review. *Cement and Concrete Composites*, **115**, 103855.

Munro, A. and Bassir, O. (1969). Oxalate in Nigerian vegetables. *West African Journal of Biology and Applied Chemistry*, **12**(1), 4–18.

Odokwo, E. O. and Onifade, M. S. (2020). Volatile constituents of the leaves and stem of Justicia secunda Vahl. *Communications in Physical Sciences*, **6**, 827–834.

Park, T., Liu, M.-Y., Wang, T.-C. and Zhu, J.-Y. (2019). Semantic image synthesis with spatially-adaptive normalization. *Proceedings of the IEEE/CVF Conference on Computer Vision and Pattern Recognition*.

Pokhodylo, N., Manko, N., Finiuk, N., Klyuchivska, O., Matiychuk, V., Obushak, M. and Stoika, R. (2021). Primary discovery of 1-aryl-5-substituted-1H-1,2,3-triazole-4-carboxamides as promising antimicrobial agents. *Journal of Molecular Structure*, **1246**, 131146.

Rana, A., Samtiya, M., Dhewa, T., Mishra, V. and Aluko, R. E. (2022). Health benefits of polyphenols: A concise review. *Journal of Food Biochemistry*, **46**(10), e14264.

Rasheed, M. K., Al-Rifaie, D. A. and Madab, D. I. (2021). Synthesis and evaluation of antibacterial and antifungal activity of new 1,5-benzodiazepine derivatives contain cyclic imides and Mannich bases. *International Journal of Pharmaceutical Research*, **13**, 2175.

Ribarova, F., Atanassova, M., Marinova, D., Ribarova, F. and Atanassova, M. (2005). Total phenolics and flavonoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy*, **40**(3), 255–260.

Sadasivam, S. (1996). *Biochemical methods*. New Age International Publishers.

Seo, M. H., Lee, W.-Y., Kim, S. S., Kang, J.-H., Kang, J.-H., Kim, K. K., Kim, B.-Y., Kim, Y.-H., Kim, W.-J. and Kim, E. M. (2019). 2018 Korean Society for the Study of Obesity guideline for the management of obesity in Korea. *Journal of Obesity & Metabolic Syndrome*, **28**(1), 40.

Shahidi, F. and Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects—A review. *Journal of Functional Foods*, **18**, 820–897.

Silva, B. Q. and Smetana, S. (2022). Review on milk substitutes from an environmental and nutritional point of view. *Applied Food Research*, **2**(1), 100105.

Sravanthi, D. (2022). *Development and consumer evaluation of finger millet based beverage*. Professor Jayashankar Telangana State Agricultural University, Hyderabad.

Srividya, J., Jonathan, D. R., Revathi, B., Sivamadhavi, V. and Anbalagan, G. (2023). Molecular structure analysis of 4-fluoro phenyl (4-methyl piperidin-1-yl) methanone and a comparative study of its optical, antimicrobial and antioxidant activities with 4-chloro phenyl (4-methyl piperidin-1-yl) methanone. *Journal of Molecular Structure*, **1271**, 134080.

Štiblariková, M., Lásiková, A. and Gracza, T. (2022). Benzyl alcohol/salicylaldehyde-type polyketide metabolites of fungi: Sources, biosynthesis, biological activities, and synthesis. *Marine Drugs*, **21**(1), 19.

Tangyu, M., Muller, J., Bolten, C. J. and Wittmann, C. (2019). Fermentation of plant-based milk alternatives for improved flavour and nutritional value. *Applied Microbiology and Biotechnology*, **103**(23), 9263–9275.

Tangyu, M., Fritz, M., Tan, J. P., Ye, L., Bolten, C. J., Bogicevic, B. and Wittmann, C. (2023). Flavour by design: Food-grade lactic acid bacteria improve the volatile aroma spectrum of oat milk, sunflower seed milk, pea milk, and faba milk towards improved flavour and sensory perception. *Microbial Cell Factories*, **22**(1), 133.

Tasturganova, E., Dikhanbaeva, F., Prosekov, A., Zhunusova, G., Dzhetpisbaeva, B. and Matibaeva, A. (2018). Research of fatty acid composition of samples of bio-drink made of camel milk. *Current Research in Nutrition and Food Science Journal*, **6**(2), 491–499.

Teng, Y., Stewart, S. G., Hai, Y.-W., Li, X., Banwell, M. G. and Lan, P. (2021). Sucrose fatty acid esters: Synthesis, emulsifying capacities, biological activities and structure-property profiles. *Critical Reviews in Food Science and Nutrition*, **61**(19), 3297–3317.

Tian, Y.-E., Sun, D., Yang, J.-M., Che, Z.-P., Liu, S.-M., Lin, X.-M., Jiang, J. and Chen, G.-Q. (2020). Synthesis of sulfonate derivatives of maltol and their biological activity against *Phytophthora capsici* and *Bursaphelenchus xylophilus* in vitro. *Journal of Asian Natural Products Research*, **22**(6), 578–587.

Trivunović, Z., Zahović, I., Grahovac, J., Jokić, A., Dodić, S., Mitrović, I. and Dodić, J. (2022). Optimization of glycerol-based medium composition for antifungal metabolites production by *Bacillus subtilis*. *Brazilian Journal of Pharmaceutical Sciences*, **58**, e19736.

Urbanovich, O., Davydenko, A., Panteleeva, E., Sverdlov, R. and Shadyro, O. (2022). Radiation-induced dehydration and destruction of 1,2-propanediol, 1,2-butanediol, and 2,3-butanediol in deaerated aqueous solutions. *High Energy Chemistry*, **56**(3), 170–174.

Vagdevi, A., Trupthi, S., Pandey, M., Reddy, N. B. and Singh, M. (2023). Nutritional qualities, processing and health benefits of finger millet (*Eleusine coracana* L.). *The Pharma Innovation Journal*, **12**(1), 1291–1212.

Vazhappilly, C. G., Ansari, S. A., Al-Jaleeli, R., Al-Azawi, A. M., Ramadan, W. S., Menon, V., Hodeify, R., Siddiqui, S. S., Merheb, M. and Matar, R. (2019). Role of flavonoids in thrombotic, cardiovascular, and inflammatory diseases. *Inflammopharmacology*, **27**(5), 863–869.

Yu, X., Zhao, M., Liu, F., Zeng, S. and Hu, J. (2013). Identification of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one as a strong antioxidant in glucose–histidine Maillard reaction products. *Food Research International*, **51**(1), 397–403.

Zhao, B., Tomoda, Y., Mizukami, H. and Makino, T. (2015). 9-Oxo-(10E,12E)-octadecadienoic acid, a cytotoxic fatty acid ketodiene isolated from eggplant calyx, induces apoptosis in human ovarian cancer (HRA) cells. *Journal of Natural Medicines*, **69**(3), 296–302.