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Bioactive metabolites identification of the foxnut and broken millet-based nutritional bar using HR-MS

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ABSTRACT

The by-products of the grain processing industry are a vital resource for the valorization methods in the food industry. In comparison to the whole grain, the broken kernels and seeds own similar nutrient and bioactive compounds having multifaceted health properties. This study aims to develop a nutritional bar by utilizing the by-products from barnyard millet and foxnut with added sweeteners. Furthermore, high-resolution mass spectrometry (HR-MS) metabolomics was carried out in positive and negative both ion modes to identify the major bioactive compounds formed in the matrix of the best-optimized valorized bar. The formulation of the bar having 15 % foxnut flour and the barnyard flour each, was elucidated highest rheological and sensory scores. A sum of 29 bioactive metabolites has been observed in the obtained metabolome. Major metabolites were palmitoyl serinol, glycitein, persin, bufagargarizin, apigenin, carvone, etc. covering a wide area in the mass spectrum. The therapeutic value of these compounds is heart health promotion, anti-inflammatory, anti-carcinogenic, anti-diabetic, anti-microbial, etc. This work highlights the bioactivity of the valorized nutritional bar employing robust and accurate tool of mass spectrometry. The developed snack is a functional food for the consumers.

1. Introduction

Valorized food products have immense potential for being an economical alternative to secondary foods which utilize waste or by-products of food processing. As there is an escalation in the demand for energy-dense and bioactive-rich foods worldwide, the by-product utilization may address issues such as high demand, nutrient deficiency, and hunger (Singh and Krishnaswamy, 2022; Kaur et al., 2023; Singh et al., 2023). Additionally, valorization also aids in the sustainability needs of the food processing facilities by minimizing carbon footprint.

India's position as the world's second-largest producer and consumer of cereals, including wheat, rice, and millet grains (Chandra et al., 2021). The waste and by-products of the grain processing industry are husk, hull, bran, and broken kernels that are rich in many bio-active components such as polysaccharides, polyphenols, peptides, etc. that own multifaceted health benefits (Annapure et al., 2023). The conversion of this waste is an intriguing optimization tool for the novel development of various types of functional products.

Foxnut (*Eurale forex L.*) is a non-cereal food crop, that belongs to the Nymphaeaceae family and is grown in flood-prone regions of Bihar, Assam, and Bengal states of India, and has high nutritional value. Globally it is recognized by several names such as "Gorgon nut", "Prickly water lily", etc. but in the Indian sub-continent, it is known as "Makhana" and their seeds are consumed popped, and roasted (Liaquat et al., 2022). According to a report by Singh et al. (2021), Bihar not only provides 80 % of India's total output of fox nut seeds (23,000 Tonnes), but it also accounts for 85 % of the country's total land area (15,294 Ha) that is used for the crop. It contains ~ 9.7 % protein that is easily digested having all essential amino acids plus phosphorus and potassium are abundant in fox nuts (Rathod et al., 2023). Fox nut is claimed to be a detoxifier and has its role in the treatment of chronic disorders such as renal problems, diarrhea, excessive leucorrhoea, and spleen hypofunction (Vikram and Mishra, 2021). The utilization of broken seeds of foxnut in the production of any value-added product leads to the development of a nutrition bar from it.

Millets are tiny grains that contain various minor and major nutrients along with important bioactive and are popular due to their health-

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improving attributes among consumers. The global millet production scale highlights that India produces 41 % of total data, approximately 10.5 MMT (Tagade and Sawarkar, 2023). These are gluten-free, regulate blood sugar, depress cholesterol, combat colon tumor growth, solve malnourishment, control obesity, and have other health benefits (Tripathi et al., 2021). However, many food processing technologies are doing a broad array of value-added products, but still, the implementation in the food processing industries is skimpy at the commercial level. Millet copious potential has grasped the attention of the research community on these grains for the development of millet-based foods on a commercial scale.

Barnyard (*Echinochloa esculentaL.*) is a popular minor millet that is mostly grown in the USA, Africa, the East Indies, Malaysia, Japan, and China (Priya et al., 2023). Barnyard grain carries polysaccharides (51–65 %), protein (11–12 %), lipids (2.5–6 %), and approximately 5 % ash content and it also has the highest content of dietary fibers (6–10 % insoluble and 3.5–4.6 % soluble) (Sharma et al., 2021). The high polyphenol level of barnyard millet includes caffeic, chlorogenic, gallic, p-hydroxybenzoic, vanillic acid, ferulic, and p-coumaric acids (Akanbi et al., 2019). Research conducted by Chethan Kumar et al. (2022) has shown there is a significant amount of highly soluble protein in the germinated barnyard millet flours, which implies this grain is the source of functional nutrients. The utilization of by-products of millet processing plants for the development of energy bars could be an efficient valorization approach and the assessment of the bioactive compounds formed in the bar is intriguing.

Different robust and sophisticated omics technologies are available these days that are highly involved in the advanced search for bioactive compounds, contaminants, anti-nutritional factors, etc. in several foods. Efficient analytical strategies for the identification of bioactive components in food products are elucidating the use of chromatography coupled with mass spectrometry detectors as a preferential option owing to better sensitivity and specificity. The metabolomics analysis of the valorized nutritional bar with a high-resolution mass spectrometer (HR-MS) will facilitate the bioactive compound detection on an accurate level in real-time proving its functional value.

This study aimed to develop a nutritional bar with the potential utilization of by-products derived from foxnut pseudo-cereal and barnyard millet. Additionally, a comprehensive exploration of the metabolite composition of the developed bar was done to highlight bioactivity and functionality in the product.

2. Materials and methods

2.1. Materials and reagents

All reagents and chemicals were purchased from Sigma-Aldrich (Bangaluru, India) and Hi-Media (Mumbai, India). Other equipment and consumables were procured from Fisher Scientific (Bangaluru, India). Broken fox nut seeds were procured from the local market of Darbhanga, Bihar. Broken barnyard millet, coconut, sucrose, and palm sugar were bought from the local market of Varanasi. Liquid glucose was purchased from Alka Foods Private Limited, Varanasi, India.

2.2. Development of nutritional bar

The development of the valorized bar starts with a heated pan at a medium flame in which coconut milk (30 g w/v) is added, then after three minutes sucrose and palm sugar were also added to the pan and stirred for 15 min. Then roasted fox nut flour (FNF) and broken barnyard millet powder (BMP) were added to the mixture and mixed well until the brown color appeared. Lastly, liquid glucose was added to the mixture, and the bar mixture was poured into the mold and cooled to room temperature.

A sum of five treatments was prepared following the procedure suggested by Selokar et al. (2022) with slight modifications, and each formulation was prepared thrice. The different formulations for 100 g bar were as follows: A: 5 g FNF+25 g BMP, B: 10 g FNF+20 g BMP, C: 15 g FNF+15 g BMP, D: 20 g FNF+10 g BMP, E: 25 g FNF+5g BMP and two control samples F1 and F2 were prepared from 30 g FNF and 30 g BMP, respectively. The concentration of the different sweeteners in the nutritional bar was maintained at a maximum level of 35 % so that the taste and texture were not affected (Mridula et al., 2013). Samples were undergone through a similar electric oven cooking temperature of 120 °C for 30 min to control this parameter. The molded samples were kept at room temperature for a uniform setting followed by being stored under refrigerated conditions at 5 °C until analysis. The nutrition bars were analyzed for different sensory characteristics like color and appearance, body and texture, aroma and taste, and overall acceptability by 9 semi-trained judges. After the sensorial analysis and triplicates textural studies of the independent samples using XT plus texture profile analyzer, (stable micro systems, UK) employment cylindrical probe (TA-35) as the attachment was calibrated at 10 mm distance using data acquisition software (Exponent Lite XT PLUS, version 4.0.13.0 lite). With a 5 g trigger force, the test speed was set at 1.00 mm/s, and the post-test speed was 10 mm/s (Kumar et al., 2018). The force at which the sample crumbles, cracks, or shatters is known as fracturability. Hardness is defined as the maximal peak force during the first compression cycle or the first bite, and it was expressed in g.s (gram*-second). Sample C having 15 g FNF and 15 g of BMP was found acceptable among all.

2.3. HR-MS analysis of bar

After adding 200 μL of a 1:1 methanol: acetonitrile solvent to each of the three technical replicates of the optimized nutritional bar samples (20 mg), zirconia beads were used to homogenize the mixture in a shaker for 20 min. After that, the homogenates are centrifuged for 10 min at 3500 rpm. After filtering the supernatant via a 0.22 mm syringe filter to remove the hydrophobic component, 2 μL of the extract was used in the LC–orbitrap–MS analysis. The LC-MS system consisted of at tribrid high-resolution accurate mass spectrometer "Orbitrap Eclipse" (Thermo Fisher Scientific) coupled with ultra-high pressure liquid chromatography (DionexUltiMate 3000) system with an electrospray source operating in both positive and negative ion mode. Bajya et al. (2024) report that the spray voltage was 3.2 kV and the capillary temperature was 300 °C. The high-resolution mass spectrometer was operated in full scan MS and collision-induced dissociation (CID)-based data-dependent MS collection mode in addition to single survey scan mode (scan range: *m/z* 100–1000; resolution: 60,000 at *m/z*). 2 μL of the isolated sample were placed onto a Hypersil GOLD™C18 RP-HPLC column (1.9 μm × 2.1 mm × 100 mm particle size). The solvent A comprises 99.9 % water and 0.1 % formic acid, while solvent B contains 99.9 % methanol and 0.1 % formic acid, and a gradient of 2 %–98 % was used. The temperature was kept at 40 °C and the flow rate at 300 μL/min, respectively. The compound discoverer software (Version 3.3.2.31) was used for both the data collection and peak annotation.

2.4. Statistical analysis

Three separate statistical computations were performed using Microsoft Excel, and the mean of the total data was used to show the results. The one-way ANOVA was applied and the significance value (p-value <0.05) was used to compare the means.

3. Results and discussion

The present investigation was done to formulate a nutritional bar based on by-products of foxnut and millet manufacturing and then the detailed metabolome profile of the bar was studied highlighting the major metabolites showing bioactivity. The work started with the development of the nutritional bar with different ingredients such as coconut milk and some sweeteners and the addition of different concentrations of BMP and FNF. Adjustment of the major ingredient ratio was done to balance the taste and functional value of the bar in terms of bioactivity. It has been observed that the amount of both raw ingredients (*i.e.*, BMP and FNF) is the primary factor that influences the manufacturing of this valorized bar. The optimization was based on organoleptic parameters (color and appearance, flavor, texture, and overall acceptability) and textural attributes (cohesiveness, hardness, gumminess, fracture ability, and chewiness). Results revealed that sample C having 15 % BMP and FNF, was the best optimized showing an overall acceptability value of 8.1 ± 1.21 on the hedonic scale. The rheological attributes of formulation C (hardness 42,723.39 ± 268.49 g.s and fracturability 19.05 ± 0.31 s) were similar to the control samples (F1: hardness 42,939.36 ± 119.56 g.s; fracturability 20.17 ± 0.87 s) and (F2: hardness 42,267.42 ± 113.61 g.s and fracturability 19.33 ± 0.89 s). A similar ratio of FNF and BMP could be responsible for the desirable textural parameters of sample C because all the sweeteners were added in equal amounts in each formulation (Kumar et al., 2018; Li et al., 2023b). The findings of the HR-MS untargeted metabolomics for the valorized bar revealed the raw data of 2028 significant metabolites that were created as a result of various interactions between the elements of the ingredients in the matrix. Metabolome displays the 29 significantly expressed compounds out of the total number of annotated peaks in the obtained mass spectrum (Fig. 1), which represent distinct compounds encompassing the principal and secondary categories of metabolites.

A detailed description of the predominant metabolites identified in the foxnut and broken millet-based nutritional bar is given in Table 1 with their chemical formula, molecular weight, retention time (RT), the total area covered in the mass spectrum, and their corresponding functions. These bioactive metabolites are listed in Table 1 in accordance to their presence in the obtained metabolome. Among the major 29 metabolites that were identified in the valorized millet-based nutritional bar, nine were lipids/lipid-like molecules, four were amino acids and derivatives, four were phenolic acids, and some other important metabolites including organic acids, sugar alcohols, sterols, terpenoids, flavonoids, alkaloids, nucleotides, and derivatives.

Metabolomics has been employed extensively in numerous comprehensive researches to analyze the metabolites present in food products. Metabolomics analysis of various cereal crops including millets have revealed several important metabolites with various physiological and bioactive functions. (Padhiyar et al., 2022) identified a total of 35 metabolites throughout the development stages of the barnyard millet. Among these metabolites, the highest were sugar/sugar alcohols followed by organic acids, amino acids, and sterols respectively. Untargeted metabolomics of two different varieties of foxnut collected from several different geographical regions revealed putative metabolites belonging to 15 different classes of compounds including organic acids, amino acids/derivatives, and flavonoids among others (Bao et al., 2022).

Several of these metabolites including the ones that were identified in the present study have shown multiple physiological effects on the human body such as antioxidative, anti-microbial, anti-diabetic, anti-inflammatory, anti-depressive, anti-septic, anti-biotic, anti-angiogenic, neuro-protective, anti-carcinogenic, antipyretic and immunomodulatory activities, etc. Sedanolide is a phthalide-like compound found in plant products and is known to exhibit anti-inflammatory, anti-microbial, anti-diabetic, and anti-tumor activities (Lin et al., 2005). A recent study reported that sedanolide activates the NRF2-mediated antioxidant response by promoting nuclear translocation of NRF2, upregulating antioxidant enzyme transcription, and enhancing glutathione metabolism, as revealed by RNA sequencing. Additionally, sedanolide exhibits cytoprotective effects against H_2O_2-induced cell death by attenuating reactive oxygen species (ROS) generation, maintaining mitochondrial membrane potential, and reducing caspase-3/7 activity, suggesting its potential as a therapeutic agent for oxidative stress-related conditions (Tabei et al., 2023). Palmitoyl Serinol is a derivative of the fatty acid palmitic acid and the amino acid serine. It is found in cell membranes, particularly in the skin where they help in maintaining the skin barrier function and overall skin health (Shin et al., 2021). Palmitoleic acid, also known as omega-7 fatty acid, is a monounsaturated fatty acid. While it is not considered an essential fatty acid since the body can synthesize it, it still plays several important physiological roles in human health such as prevention and amelioration of several skin conditions, lowering levels of LDL (low-density lipoprotein) or "bad" cholesterol, regulation of glucose and insulin metabolism, anti-inflammatory effects, cognitive function and brain health (Frigolet and Gutiérrez-Aguilar, 2017).

Oleamide, which is a derivative of fatty acid and amide has shown potential as a signaling molecule in the central nervous system. It is known to interact with cannabinoid receptors and is believed to modulate neurotransmission, sleep, and other neurological functions (Roy et al., 2021). Similar biological activity has been reported in allenic-apo-aldehyde, an aldehyde derivative that is generated as an intermediate in lipid metabolic pathways in plants and other organisms (Endo et al., 2014). Oleanolic acid, a pentacyclic triterpenoid abundantly present in various plant-derived products, is gaining recognition for its diverse biological effects against several physiological conditions. These effects include antibacterial, antifungal, anticarcinogenic, anti-inflammatory, hepato-protective, gastro-protective, hypo-lipidemic, and anti-atherosclerotic activities (Castellano et al., 2022). Persin is a fatty acid derivative metabolite that has anti-carcinogenic attributes of combating cancer cell proliferation (Zhang and Kanakkanthara, 2020).

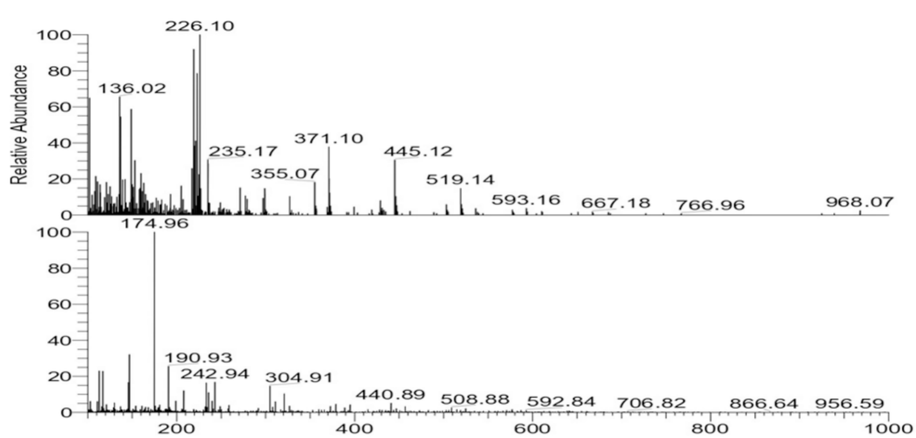

Fig. 1. The representative mass spectrum obtained after HR-MS metabolomics of the optimized nutritional bar sample. The upper and lower mass spectrum obtained from the positive and negative ion mode of electrospray ionization, respectively.

Table 1
The major metabolites from the metabolomics of valorized nutritional bars using high-resolution mass spectrometry.

Metabolite Name	Classification	Formula	Annot. Delta Mass [ppm]	Calc. MW	*m/Z*	RT [min]	Area (Max.)	Bioactive Function	References
Palmitoyl serinol	Lipid	$C_{19}H_{39}NO_3$	0.25	329.2931	330.3004	22.319	1,501,371	Anti dermatosis	Shin et al., 2021
Sedanolide	Isobenzofuran	$C_{12}H_{18}O_2$	−0.48	194.1306	195.1379	12.937	24,806,411	AIF, anti-tumor activities	Tabei et al., 2023
Dracocephalumoid	Diterpenoid	$C_{20}H_{24}O_5$	−3.03	344.1613	343.1541	22.432	8,763,817	Brain activity	Nie et al., 2021
Palmitoleic acid	Fatty acid	$C_{16}H_{30}O_2$	0.15	254.2246	237.2213	22.707	16,046,746	AIF	Frigolet and Gutiérrez-Aguilar, 2017
Ferulic acid	Phenylpropanoids	$C_{22}H_{38}O_4\ Si_2$	1.92	422.2317	421.2244	22.809	32,460,555	Anti-viral, anti-cancer, AIF	Li et al., 2023°
Docosahexaenoic acid	Fatty acid	$C_{22}H_{32}O_2$	0.06	328.2403	329.2475	23.321	9,167,774	Anti-CVD	Li et al., 2021
alpha-Tocotrienol	Prenol lipid	$C_{29}H_{44}O_2$	0.13	424.3342	425.3415	26.114	7,001,575	Antioxidant	Thompson and Cooney, 2020
Oleanolic acid	Prenol lipid	$C_{30}H_{48}O_3$	4.02	456.3585	437.3406	25.801	6,513,095	Anti-diabetic, antiviral, anti-HIV, antibacterial, antifungal, anticarcinogenic, AIF, hepatoprotective, gastroprotective, hypolipidemic, anti-atherosclerotic activities	Castellano et al., 2022
Ambiguine isonitrile	Alkaloid	$C_{26}H_{30}N_2\ O_3$	3.77	418.2272	419.2345	24.723	2,869,169	Anti-fungal	Al-Yousef and Amina, 2021
Kammogenin	Steroidal saponin	$C_{27}H_{40}O_5$	4.11	444.2858	443.2785	24.205	4,826,807	Anti-microbial activity	Shahidi and Chandrasekara, 2013
Persin	Fatty acid derivative	$C_{23}H_{40}O_4$	0.3	380.2928	381.3001	24.149	8,962,168	Anti-cancer	Zhang and Kanakkanthara, 2020
Oleamide	Fatty acid derivative	$C_{18}H_{35}NO$	0.23	281.2719	282.2792	23.956	25,224,399	Biological activity	Roy et al., 2021
Muscone	Cyclic ketone	$C_{16}H_{30}O$	0.19	238.2296	239.2369	23.823	8,814,079	Anti-ischemic effect	Wang et al., 2020
Cholinephosphate	Fatty acid derivative	$C_5H_{14}NO_4P$	0.24	183.0661	184.0734	23.813	14,975,102	Anti-bacterial	Lu et al., 2020
Bartericin	Amino acid derivative	$C_{25}H_{28}O_5$	−1.86	408.1929	407.1856	23.813	81,161,204	Anti-microbial	Zhai et al., 2022
Bufogargarizin	Steroid	$C_{24}H_{26}O_3$	2.83	362.1892	385.1785	23.807	29,756,128	Cardiotonic, anesthetic, antineoplastic	Liu et al., 2013
Geldanamycin	Polyketide	$C_{29}H_{42}N_2O_{11}$	1.44	594.278	595.2853	23.806	3,912,989	Anti-cancer	Fukuyo et al., 2010
Alverine	Phenylpropylamines	$C_{20}H_{27}N$	0.19	281.2144	282.2217	23.76	1,253,573	Anti-depressant	Gupta et al., 2014
Xestoaminol	Amine	$C_{14}H_{31}NO$	0.17	229.2406	230.2479	22.374	590742.2	Anti-cancer	Chen et al., 2011
Padanamide	Fatty acid amide	$C_{31}H_{46}N_6\ O_9$	1.94	646.3314	645.3241	22.352	8,078,809	Cytotoxic	Williamset al., 2011
Allenic-apo-aldehyde	Aldehyde	$C_{25}H_{34}O_3$	4.02	382.2523	381.2451	24.98	8,812,270	Anti-cancer, AIF, anti-viral, anti-bacterial	Endo et al., 2014
Piceatannol	Phenylpropanoids	$C_{14}H_{12}O_4$	0.16	244.0735	245.0808	19.277	53,279,671	Anti-cancer	Banik et al., 2020
Dicyclomine	Carboxylic acid derivative	$C_{19}H_{35}NO_2$	0.07	309.2668	310.2741	21.58	11,755,262	Anti-cholinergic, AIF	Akbari and Forotan,2009
Xanthoangelol	Phenylpropanoids	$C_{22}H_{22}O_5$	3.7	366.1454	365.1381	21.53	29,776,283	Anti-allergic	Kimura, 2005
Glycitein	Isoflavonoids	$C_{16}H_{12}O_5$	0.34	284.0684	285.0757	19.701	6,100,141	Anti-mutagenic activity, Antioxidant activity	Shahidi and Chandrasekara, 2013
Dracocephalumoid	Diterpenoid	$C_{20}H_{24}O_5$	3.03	344.1613	343.1541	22.432	8,763,817	AIF	Nie et al., 2021
Spisulosine	Amine	$C_{18}H_{39}NO$	0.37	285.3033	286.3106	21.056	4,320,798	Anti-cancer	Ganesher et al., 2020
Carvone	Prenol lipids	$C_{10}H_{14}O$	0.22	150.1045	133.1012	18.922	25,590,221	Anti-microbial, antioxidant, insecticidal, antitumor, AIF, antidiabetic activities	Bouyahya et al., 2021
Apigenin	Flavonoids	$C_{15}H_{10}O_5$	3.69	270.0538	271.0611	11.447	6,418,740	AIF, anti-oxidant, anti-tumor	Shahidi and Chandrasekara, 2013

*Calc. MW: Calculated molecular weight; M/Z: Mass/Charge; RT: Retention time; CVD: Cardiovascular disease; AIF: Anti-inflammatory activities.

Another compound, alpha-tocopherol, known for its well-established anti-inflammatory properties, was also identified in the current study. Findings from various research studies involving cell cultures, animal models, and human subjects suggest that alpha-tocopherol exhibits considerable benefits by preventing cellular DNA damage over time (Thompson and Cooney, 2020). Carvone is a monoterpene ketone found in many essential oils and is commonly used as a flavoring agent in the food and beverage industry due to its distinctive aroma and flavor. In addition, recent investigations have revealed that carvone possesses various pharmacological properties, including antibacterial, antifungal, antiparasitic, anti-neuraminidase, antioxidant, anti-inflammatory, and anticancer activities (Bouyahya et al., 2021).

Some of the phenolic compounds identified in the metabolome of the nutritional bar such as ferulic acid, glycitein, kammogenin, and apigenin have been comprehensively studied for their antioxidant and anti-inflammatory effects. Being present in bound form in cereals including millets, phenolic compounds can resist gastro-intestinal digestion and remain intact as they reach the colon. Upon release via microbial fermentation, these compounds may exert protective effects within the colon. This phenomenon potentially elucidates how whole grains contribute to preventing colon cancer (Shahidi and Chandrasekara, 2013). Li et al. (2023a) reported that ferulic acid significantly reduced the production and expression of inflammatory markers such as nitric oxide (NO), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) while increasing levels of the anti-inflammatory factor β-endorphin (β-EP) in a human HMC-3 microglial cell model.

This valorized foxnut and broken millet-based nutritional bar developed in the present study seems to not only provide essential nutrition but also contain metabolites with multiple bioactive properties, as identified by metabolomic analysis. Thus, the valorized bar is a functional snack for consumers of all age groups.

4. Conclusion

The study aims to develop a novel energy bar by utilizing the by-products of the foxnut and millet processing industry. With the application of high-resolution mass spectrometer-equipped metabolomics, this work highlights the major bioactive metabolites formed in the valorized nutritional bar. The formulation of the bar having 15 g each, foxnut, and broken barnyard millet floor had having best textural and sensorial characteristics. Various lipids, amino acids, and phenolics observed in the HR-MS metabolome reveal the developed nutritional snack is also a functional food. Valorization of the waste and by-products from the grain processing plants into different functional food products could be an economical and environmentally sustainable option for the food industry. The application of robust and sophisticated technology of LC-MS omics in the characterization of bioactive ingredients is an intriguing approach. For the future scope, detailed *in-vitro* and *in-vivo* studies could reveal the concerned bioactivity of such valorized bars.

CRediT authorship contribution statement

Vishal Kumar: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Arvind Kumar:** Supervision, Resources. **Manish Kumar Singh:** Writing – review & editing, Visualization. **Priya Dhyani:** Writing – review & editing. **Himanshu Mishra:** Writing – review & editing. **Dinesh Chandra Rai:** Writing – review & editing, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Akanbi, T. O., Timilsena, Y., & Dhital, S. (2019). Bioactives from millet: Properties and effects of processing on bioavailability. *Bioactive factors and processing technology for cereal foods*, 171–183.

Akbari, E., & Forotan, S. A. (2009). The effect of dicyclomine hydrochloride, an anticholinergic agent, on heart rate variability and squatting test in healthy volunteers. *Journal of Mazandaran University of Medical Sciences, 19*(72), 10–17.

Al-Yousef, H. M., & Amina, M. (2021). Phytoconstituents and pharmacological activities of cyanobacterium Fischerella ambigua. *Arabian Journal of Chemistry, 14*(6), Article 103153.

Annapure, U. S., Kalaivendan, R. G. T., Mishra, A., & Eazhumalai, G. (2023). Processing technologies of nutri-cereals. In *Nutri-Cereals* (pp. 305–324). CRC Press.

Bajya, S. L., Bunkar, D. S., Goyal, S. K., Singh, M. K., Paswan, V. K., Lal, S., & Dhyani, P. (2024). Foodomics-based metabolites profiling of the Greek yogurt incorporated with unripened papaya peel powder. *Food Chemistry: Molecular Sciences, 8*, Article 100199.

Banik, K., Ranaware, A. M., Harsha, C., Nitesh, T., Girisa, S., Deshpande, V., & Kunnumakkara, A. B. (2020). Piceatannol: A natural stilbene for the prevention and treatment of cancer. *Pharmacological Research, 153*, Article 104635.

Bao, K., Jing, Z., Wang, Q., Huang, Z., Han, D., Dai, S., & Xu, F. (2022). Quality analysis of Euryales Semen from different origins and varieties based on untargeted metabolomics. *Journal of Chromatography B, 1191*, Article 123114.

Bouyahya, A., Mechchate, H., Benali, T., Ghchime, R., Charfi, S., Balahbib, A., & Omari, N. E. (2021). Health benefits and pharmacological properties of carvone. *Biomolecules, 11*(12), 1803.

Castellano, J. M., Ramos-Romero, S., & Perona, J. S. (2022). Oleanolic acid: Extraction, characterization and biological activity. *Nutrients, 14*(3), 623.

Chandra, A. K., Chandora, R., Sood, S., & Malhotra, N. (2021). Global production, demand, and supply. In *Millets and pseudo cereals* (pp. 7–18). Woodhead Publishing.

Chen, B. S., Yang, L. H., Ye, J. L., Huang, T., Ruan, Y. P., Fu, J., & Huang, P. Q. (2011). Diastereoselective synthesis and bioactivity of long-chain anti-2-amino-3-alkanols. *European Journal of Medicinal Chemistry, 46*(11), 5480–5486.

Chethan Kumar, P., Amutha, S., Oberoi, H. S., Kanchana, S., Azeez, S., & Rupa, T. R. (2022). Germination induced changes in bioactive compounds and nutritional components of millets. *Journal of Food Science and Technology, 59*(11), 4244–4252.

Endo, A., Okamoto, M., & Koshiba, T. (2014). ABA biosynthetic and catabolic pathways. *Abscisic Acid: Metabolism, Transport and Signaling*, 21–45.

Frigolet, M. E., & Gutiérrez-Aguilar, R. (2017). The role of the novel lipokine palmitoleic acid in health and disease. *Advances in Nutrition, 8*(1), 173S–S181.

Fukuyo, Y., Hunt, C. R., & Horikoshi, N. (2010). Geldanamycin and its anti-cancer activities. *Cancer letters, 290*(1), 24–35.

Ganesher, A., Chaturvedi, P., Sahai, R., Meena, S., Mitra, K., Datta, D., & Panda, G. (2020). New Spisulosine Derivative promotes robust autophagic response to cancer cells. *European Journal of Medicinal Chemistry, 188*, Article 112011.

Gupta, D., Radhakrishnan, M., & Kurhe, Y. (2014). Anxiolytic-like effects of alverine citrate in experimental mouse models of anxiety. *European Journal of Pharmacology, 742*, 94–101.

Kaur, J., Brar, D. S., & Nanda, V. (2023). Exploring the potential of osmotic dehydration with honey to develop value-addedfood from watermelon (Citrullus lanatus) rind. *Turkish Journal of Agriculture and Forestry, 47*(6), 1058–1077.

Kimura, Y. (2005). Antitumor and vascular physiological effects of natural products. *Studies in Natural Products Chemistry, 30*, 55–78.

Kumar, A., Mohanty, V., & Yashaswini, P. (2018). Development of high protein nutrition bar enriched with Spirulina plantensis for undernourished children. *Current Research in Nutrition and Food Science Journal, 6*(3), 835–844.

Li, J., Pora, B. L., Dong, K., & Hasjim, J. (2021). Health benefits of docosahexaenoic acid and its bioavailability: A review. *Food Science & Nutrition, 9*(9), 5229–5243.

Li, P. L., Zhai, X. X., Wang, J., Zhu, X., Zhao, L., You, S., & Yang, J. L. (2023). Two ferulic acid derivatives inhibit neuroinflammatory response in human HMC3 microglial cells via NF-κ B signaling pathway. *Molecules, 28*(5), 2080.

Li, S., Chen, W., Zongo, A. W. S., Chen, Y., Liang, H., Li, J., & Li, B. (2023). Effects of non-starch polysaccharide on starch gelatinization and digestibility: A review. *Food Innovation and Advances, 2*(4), 302–312.

Liaquat, M., Pasha, I., Ahsin, M., & Salik, A. (2022). Roasted fox nuts (Euryale Ferox L.) contain higher concentration of phenolics, flavonoids, minerals and antioxidants, and exhibit lower Glycemic Index (GI) in human subjects. *Food Production, Processing and Nutrition, 4*(1), 1.

Lin, G., Chan, S. S. K., Chung, H. S., & Li, S. L. (2005). Chemistry and biological activities of naturally occurring phthalides. *Studies in natural products chemistry, 32*, 611–669.

Liu, J., Zhang, D., Li, Y., Chen, W., Ruan, Z., Deng, L., & Ye, W. (2013). Discovery of bufadienolides as a novel class of ClC-3 chloride channel activators with antitumor activities. *Journal of Medicinal Chemistry, 56*(14), 5734–5743.

Lu, M., Yu, S., Wang, Z., Xin, Q., Sun, T., Chen, X., & Li, J. (2020). Zwitterionic choline phosphate functionalized chitosan with antibacterial property and superior water solubility. *European Polymer Journal, 134*, Article 109821.

Mridula, D., Singh, K. K., & Barnwal, P. (2013). Development of omega-3 rich energy bar with flaxseed. *Journal of Food Science and Technology, 50*(5), 950–957.

Nie, L., Li, R., Huang, J., Wang, L., Ma, M., Huang, C., & Hu, X. (2021). Abietane diterpenoids from Dracocephalum moldavica L. and their anti-inflammatory activities in vitro. *Phytochemistry, 184*, Article 112680.

Padhiyar, S. M., Kheni, J., & Singh, H. V. D. R. (2022). Metabolomic characterization of barnyard millet (Echinochloa frumentacea L.) involved in different stages of spike development. *Pharma innov, 11*, 604–611.

Priya, Verma, R. K., Lakhawat, S., Yadav, V. K., Gacem, A., Abbas, M., & Mishra, S. (2023). Millets: Sustainable treasure house of bioactive components. *International Journal of Food Properties, 26*(1), 1822–1840.

Rathod, R. V., Neve, G., Jain, A., & Giri, P. (2023). A comprehensive review on health benefits and nutritional aspects of foxnut (Makhana). *The Pharma Innovation Journal, 12*(6), 4432–4438.

Roy, A., Kundu, M., Chakrabarti, S., Patel, D. R., & Pahan, K. (2021). Oleamide, a sleep-inducing supplement, upregulates doublecortin in hippocampal progenitor cells via PPAR α. *Journal of Alzheimer's Disease, 84*(4), 1747–1762.

Selokar, N., Vidyalakshmi, R., Thiviya, P., Sinija, V. R. N., & Hema, V. (2022). Assessment of nutritional quality of non-conventional millet malt enriched bar. *Journal of Food Processing and Preservation, 46*(12), e17271.

Shahidi, F., & Chandrasekara, A. (2013). Millet grain phenolics and their role in disease risk reduction and health promotion: A review. *Journal of Functional Foods, 5*(2), 570–581.

Sharma, R., Sharma, S., Dar, B. N., & Singh, B. (2021). Millets as potential nutri-cereals: A review of nutrient composition, phytochemical profile and techno-functionality. *International Journal of Food Science & Technology, 56*(8), 3703–3718.

Shin, K. O., Kim, S., Park, B. D., Uchida, Y., & Park, K. (2021). N-palmitoyl serinol stimulates ceramide production through a CB1-Dependent mechanism in in vitro model of skin inflammation. *International Journal of Molecular Sciences, 22*(15), 8302.

Singh, D. K., Kumar, A., Kumar, U., & Singh, I. S. (2021). Production and profitability of makhana (Euryale ferox salisb.) in aquatic ecosystem of North Bihar, India. *Indian Journal of Extension Education, 57*(2), 62–66.

Singh, P., & Krishnaswamy, K. (2022). Sustainable zero-waste processing system for soybeans and soy by-product valorization. *Trends in Food Science & Technology, 128*, 331–344.

Singh, J., Rasane, P., Kaur, R., Kaur, H., Garg, R., Kaur, S., & Mlcek, J. (2023). Valorization of grape (Vitis vinifera) leaves for bioactive compounds: Novel green extraction technologies and food-pharma applications. *Frontiers in Chemistry, 11*, 1290619.

Tabei, Y., Abe, H., Suzuki, S., Takeda, N., Arai, J. I., & Nakajima, Y. (2023). Sedanolide activates KEAP1–NRF2 pathway and ameliorates hydrogen peroxide-induced apoptotic cell death. *International Journal of Molecular Sciences, 24*(22), 16532.

Tagade, A., & Sawarkar, A. N. (2023). Valorization of millet agro-residues for bioenergy production through pyrolysis: Recent inroads, technological bottlenecks, possible remedies, and future directions. *Bioresource Technology, 384*, Article 129335.

Thompson, M. D., & Cooney, R. V. (2020). The potential physiological role of γ-tocopherol in human health: A qualitative review. *Nutrition and cancer, 72*(5), 808–825.

Tripathi, M. K., Mohapatra, D., Jadam, R. S., Pandey, S., Singh, V., Kumar, V., & Kumar, A. (2021). Nutritional composition of millets. *Millets and millet technology*, 101–119.

Vikram, V., & Mishra, S. (2021). A review on Euryale ferox: Daily dietary regime for Covid− 19 patients. *International Journal of Applied Research, 7*(6S), 29–34.

Wang, J., Xing, H., Qin, X., Ren, Q., Yang, J., & Li, L. (2020). Pharmacological effects and mechanisms of muscone. *Journal of Ethnopharmacology, 262*, Article 113120.

Williams, D. E., Dalisay, D. S., Patrick, B. O., Matainaho, T., Andrusiak, K., Deshpande, R., & Andersen, R. J. (2011). Padanamides A and B, highly modified linear tetrapeptides produced in culture by a Streptomyces sp. isolated from a marine sediment. *Organic letters, 13*(15), 3936–3939.

Zhai, J., Sun, B., & Sang, F. (2022). Progress of isolation, chemical synthesis and biological activities of natural chalcones bearing 2-hydroxy-3-methyl-3-butenyl group. *Frontiers in Chemistry, 10*, Article 964089.

Zhang, D., & Kanakkanthara, A. (2020). Beyond the paclitaxel and vinca alkaloids: Next generation of plant-derived microtubule-targeting agents with potential anticancer activity. *Cancers, 12*(7), 1721.