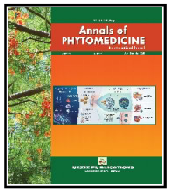
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Nutraceutical potential and shelf stability of an iron and anthocyanin rich health mix formulated from the traditional red rice Kuzhiadichan (*Oryza sativa* L.)

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Abstract

The present study aimed to formulate and evaluate a nutritionally superior and shelf-stable health mix utilizing the traditional kuzhiadichan red rice (*Oryza sativa* L.) in combination with green gram and pearl millet. The proximate composition of kuzhiadichan rice revealed high levels of protein (9.64 g/100 g), fiber (4.69 g/100 g) and essential minerals including phosphorus (498 mg/100 g), calcium (45.13 mg/100 g) and iron (6.26 mg/100 g) highlights its nutritional richness. The rice also exhibited strong antioxidant potential with total polyphenols (460 mg GAE/100 g), anthocyanins (56.8 mg C3G/100 g) and antioxidant activity (62.51%). Gas chromatography mass spectrometry (GC-MS) profiling identified major bioactive compounds such as methyl elaidate (37.19%), methyl erucate (22.35%) and methyl palmitate (9.54%), which are known for their antioxidant, anti-inflammatory and cardioprotective effects. Four formulations (T_1 to T_4) of the health mix were developed with varying proportions of kuzhiadichan rice, green gram and pearl millet. Among these, T_4 (50% kuzhiadichan: 20% green gram: 30% pearl millet) exhibited the most balanced nutritional profile with significant carbohydrate (74.12 g/100 g), stable mineral content and superior sensory acceptability (8.8 ± 0.22). After 90 days of storage, the T_4 formulation maintained its nutritional integrity, microbial safety and organoleptic quality confirming excellent shelf stability. The iron and anthocyanin rich T_4 health mix demonstrates high antioxidant potential, improved hemoglobin synthesis and protection against oxidative stress related disorders such as anemia, diabetes and cardiovascular diseases. Thus, the kuzhiadichan based health mix serves as a functional and nutraceutical food that effectively bridges traditional nutritional wisdom with modern dietary innovation.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops and serves as the primary source of dietary energy for over half of the world's population providing about 60 to 70% of the daily caloric intake in Asia (Puri *et al.,* 2014). Despite its global importance most rice is consumed in the polished or milled form due to its soft texture, appealing flavor and long shelf-life (Upadhyay and Karn, 2018). However, the polishing process removes the bran, husk and germ layers, which are rich in essential nutrients and phytochemicals leaving only the starchy endosperm (Roy *et al.,* 2011). Consequently, significant nutrient losses occur during milling like calcium, phosphorus, iron and zinc contents are drastically reduced along with B-complex vitamins such as thiamine, niacin and biotin (Abbas *et al.,* 2011). This depletion contributes to micronutrient malnutrition or "hidden hunger" particularly in rice dependent populations (Maclean *et al.,* 2002).

In contrast, traditional pigmented rice landraces including red, black and purple rice retain the bran and germ layers making them superior in nutritional and functional qualities. These pigmented varieties are rich in secondary metabolites such as phenolic acids, flavonoids, anthocyanins and proanthocyanidins that exhibit antioxidant, anti-inflammatory and cytoprotective activities (Berni *et al.,* 2018; Wang *et al.,* 2018). Regular consumption of such rice varieties has been associated with reduced oxidative stress and a lower incidence of chronic diseases including diabetes, cancer and cardiovascular disorders (Setyaningsih *et al.,* 2015).

Among the pigmented varieties, red rice holds particular nutritional and functional significance. The red coloration of its bran is attributed to proanthocyanidin and anthocyanin pigments, which are potent antioxidants (Wirjahardja *et al.,* 1983; Oki *et al.,* 2005). Nutritionally, red rice contains higher amounts of protein 10.49 g/100 g, fat 1.81 g/100 g and crude fiber 2.71 g/100 g compared with white rice 7.6 g/100 g protein; 0.62 g/100 g fat and 0.23 g/100 g fiber (Raghuvanshi *et al.,* 2017). It is also richer in essential minerals such as calcium 8.71 mg/100 g; iron 13.45 mg/100 g; magnesium 192.27 mg/100 g and zinc 1.91 mg/100 g (Reddy *et al.,* 2025). Moreover, its total phenolic 143.38 mg GAE/100 g and flavonoid 120.0 mg/100 g contents are several times higher than those in polished rice (Wang *et al.,* 2018). These bioactive compounds impart antioxidant, anticarcinogenic and hypoglycaemic properties making red rice a promising functional food ingredient.

Kuzhiadichan is a traditional red rice landrace of Tamil Nadu recognized for its exceptional nutritional and nutraceutical attributes.

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Among the traditional varieties studied, kuzhiadichan exhibited the highest protein 9.65 ± 0.67 g/100 g and fat 2.53 ± 0.17g/100 g contents as well as superior mineral composition like phosphorus 359 ± 24.7 mg/100 g, calcium 46.8 ± 3.23 mg/100 g, magnesium 135 ± 9.30 mg/100 g, iron 5.70 ± 0.39 mg/100 g and zinc 2.70 ± 0.19 mg/100 g. It also contains considerable anthocyanins 13.50 ± 0.69 mg/100 g and flavonoids 3.92 ± 0.20 mg/100 g, contributing to a total antioxidant capacity of 416 ± 22.9 µm AAE/100 g. The high iron and anthocyanin levels indicate strong potential for combating anemia and oxidative stress related disorders. These attributes make kuzhiadichan a nutritionally dense, iron and anthocyanin rich landrace ideal for functional food development (Udhaya Nandhini *et al.,* 2024).

Pearl millet (*Pennisetum glaucum*) is a highly nutritious, gluten-free cereal containing 12.4% protein, 13.1% dietary fiber and essential minerals such as iron (7.5 mg/100 g), zinc (4.0 mg/100 g), calcium (57.8 mg/100 g) and magnesium (142.7 mg/100 g). Rich in phenolics and antioxidants, pearl millet helps reduce oxidative stress, supports digestive health and lowers the risk of chronic diseases such as diabetes and cardiovascular disorders, while its high iron content aids in combating anemia (Jamuna *et al.,* 2025 and Aditi *et al.,* 2025). According to Oghbaei and Prakash (2017), the whole green gram (*Vigna radiata*) exhibited an excellent nutritional profile containing protein (27.24%), starch (53.76%), ash (3.71%) and notable mineral contents such as iron (6.56 mg/100 g), zinc (4.00 mg/100 g) and calcium (72.76 mg/100 g). It was also rich in dietary fiber with insoluble fiber (18.05 g/100 g), soluble fiber (1.77 g/100 g) and total dietary fiber (19.82 g/100 g) along with phytic acid (261.62 mg/100 g).

Despite their superior nutritional composition traditional rice landraces such as red rice are declining in cultivation due to their coarse texture, longer cooking time and lower yield compared with modern high yielding varieties (Mau *et al.,* 2017). However, they represent valuable genetic and nutritional resources for developing biofortified and value added food products. With the rising consumer demand for functional foods those providing physiological benefits beyond basic nutrition traditional grains have gained renewed importance (Srivastava *et al.,* 2022).

One promising strategy for utilizing such landraces is the formulation of health mixes composite blends of cereals, pulses and millets that provide balanced macronutrients and micronutrients. When red rice serves as the base ingredient these blends demonstrate improved protein quality, mineral density and antioxidant potential. Incorporating red rice into cereal pulse combinations has been reported to enhance total phenolic content by up to 30%, iron by 40% and protein by 15% over conventional formulations (Semmarath *et al.,* 2023). Such nutrient rich products are particularly beneficial for children, pregnant women and the elderly due to their balanced composition and low glycemic index (Gunaratne *et al.,* 2013).

Considering these advantages the present study was undertaken to formulate and evaluate a nutrient rich, iron and anthocyanin enriched health mix using the traditional kuzhiadichan red rice. The study aimed to determine its proximate composition, mineral profile, phytochemical content, antioxidant activity and storage stability thereby establishing the potential of traditional red rice as a functional ingredient for health promoting food formulations that integrate traditional knowledge with modern nutritional science.

2. Materials and Methods

2.1 Plant material authentication

The present research was conducted at the Department of Food Science and Nutrition, Community Science College and Research Institute, Tamil Nadu Agricultural University (TNAU), Madurai. The traditional red rice landrace Kuzhiyadichan (*Oryza sativa* L.) was sourced from the Central Farm, Agricultural College and Research Institute, TNAU, Madurai. This indigenous landrace native to Tamil Nadu has been officially documented and registered by the ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi under the National Rice Germplasm Exploration and Conservation Programme. Collected and evaluated in 2019, Kuzhiyadichan is registered under the Accession Identity: IC632698 (Vishnu Kumar *et al.,* 2024). The kuzhiyadichan rice obtained was subsequently employed in the formulation of health mix samples. All chemicals and reagents utilized throughout the experimental procedures were of analytical grade ensuring the accuracy, precision and reliability of the analytical outcomes.



Kuzhiadichan paddy **Kuzhiadichan rice**

Figure 1: Kuzhiadichan paddy and rice.

2.2 Procedure for the preparation of health mix

The raw materials including kuzhiadichan rice, green gram and pearl millet were procured, cleaned and processed for the formulation of the health mix. The kuzhiadichan rice landrace was dehulled manually to remove the outer husk and all ingredients were thoroughly cleaned to eliminate dust and foreign materials. The cleaned grains were then subjected to roasting at 50^{0}C for 10 min to enhance flavor, improve digestibility and reduce moisture content. After roasting the ingredients were cooled to ambient temperature and subsequently pulverized into fine flour using a laboratory grinder. The resulting flour samples were blended in predetermined proportions as per the formulations (T_1 to T_4) and then packed in polypropylene pouches to prevent moisture absorption and maintain product stability. The packaged samples were stored under ambient conditions for further analysis of their nutritional and functional characteristics.

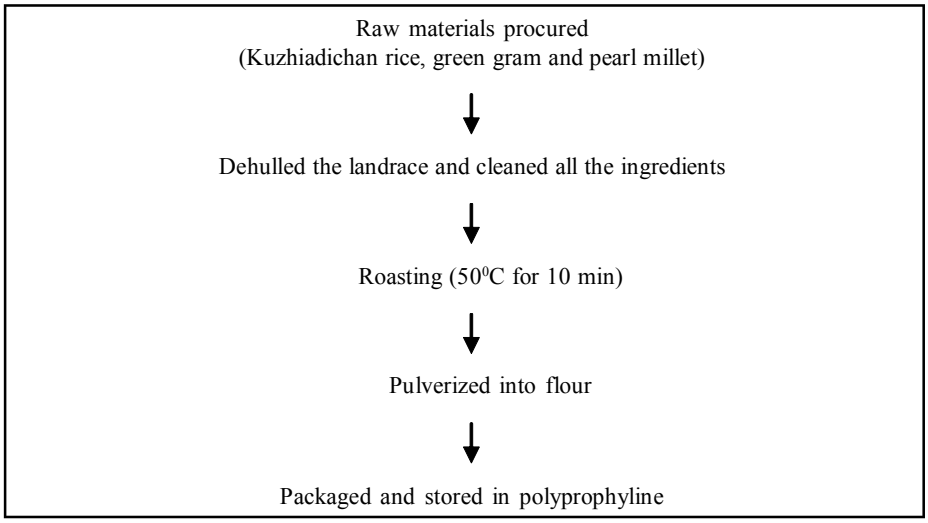


Figure 2: Process flowchart for preparation of kuzhiadichan red rice-based health mix.

2.3 Health mix proportions

The Table 1 and Figure 3 indicated the formulation of health mix prepared in four treatments (T_1 to T_4) with varying ratios of kuzhiadichan rice, green gram and pearl millet. In formulation T_1 kuzhiadichan rice constituted the highest proportion (80%), combined with 20% green gram. In T_2 the kuzhiadichan rice proportion was reduced to 70%, while green gram remained constant at 20% and 10% pearl millet was incorporated. The T_3 formulation further decreased kuzhiadichan rice to 60%, maintaining 20% green gram and increasing pearl millet to 20%. Finally, T_4 contained the lowest proportion of kuzhiadichan rice (50%), a consistent 20% green gram and the highest level of pearl millet (30%). This gradual reduction of kuzhiadichan rice with a corresponding increase in pearl millet content, while keeping green gram constant was designed to assess the impact of incremental pearl millet substitution on the nutritional, functional and storage characteristics of the developed health mix.

Table 1: Formulation of kuzhiadichan red rice-based health mix

Ingredients	T_1	T_2	T_3	T_4
Kuzhiadichan (%)	80	70	60	50
Green gram (%)	20	20	20	20
Pear millet (%)	-	10	20	30

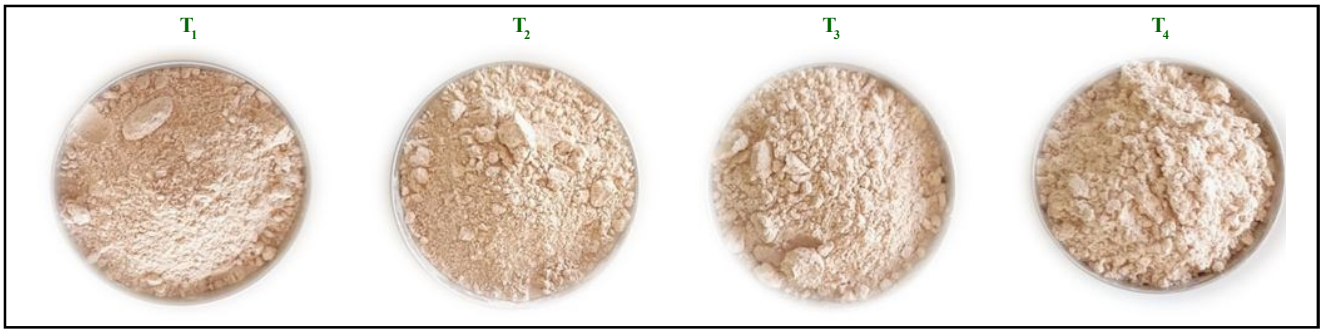


Figure 3: Different combinations (T_1 to T_4) of kuzhiadichan red rice-based health mix formulations.

2.4 Proximate analysis

The physical characteristics of kuzhiadichan rice including kernel length (mm), width (mm) and thickness (mm) were measured using a vernier caliper with an accuracy of 0.01 mm. The 1,000 kernel weight was determined according to the method used by Devi *et al.* (2025). The proximate composition of kuzhiadichan rice and the formulated health mix was analyzed following standard procedures. The moisture content was determined by the hot air oven drying method as per AOAC (1995) guidelines. Protein content was estimated using the Kjeldahl method (AOAC, 2005), while fat content was assessed following the procedure outlined by Carpenter (2010). Carbohydrate content was calculated based on the method proposed by Kurzyna Szklarek *et al.* (2022), and crude fiber was determined according to Sadasivam and Manickam (1996). The mineral composition including calcium, phosphorus, iron, sodium, potassium and zinc was analyzed using the triple acid digestion method described by Renganna (1986). Total phenolic content was quantified following the protocol of Sadasivam and Manickam (2008), while total anthocyanin content was determined using the pH differential method (AOAC, 2005), which measures absorbance changes at two pH levels to calculate total monomeric anthocyanins expressed as cyanidin-3-glucoside equivalents. Furthermore, gas chromatography-mass spectrometry (GC-MS) analysis was performed using a shimadzu QP2020 system for the identification and quantification of bioactive compounds present in the samples following the method developed by Sathish *et al.* (2025).

2.5 Packing and storage studies of health mix

The formulated health mix samples were packed in airtight polypropylene pouches and stored under ambient conditions for a period of 90 days to assess their storage stability. The samples were periodically analyzed to evaluate changes in quality parameters including nutritional, microbial and sensory characteristics throughout the storage duration.

2.6 Statistical analysis

Statistical analysis of the experimental data was performed using AGRES software. The data were analyzed following a completely randomized design (CRD) to determine the influence of different treatments on the measured parameters. A one way analysis of variance (ANOVA) was employed to identify statistically significant differences among the treatment means at a 5% probability level (p<0.05). Wherever significant differences were observed mean separation was carried out using the least significant difference (LSD) test to accurately compare treatment effects.

3. Results

3.1 Physical properties of kuzhiadichan rice

The physical characteristics of the kuzhiadichan rice variety revealed distinct morphological features. The pericarp color was observed to be red indicating the presence of natural pigments such as anthocyanins. The grain length measured 6.25 ± 0.08 mm, while the breadth and thickness were 2.75 ± 0.003 mm and 1.98 ± 0.03 mm, respectively, suggesting a medium slender grain type. The 1000 grain weight was recorded as 26.25 ± 0.26 g reflecting a moderate grain density typical of traditional landraces. These parameters collectively highlight the characteristic grain morphology and physical attributes of the kuzhiadichan rice variety (Table 2).

Table 2: Physical properties of kuzhiadichan red rice

S.No.	Parameters	Kuzhiadichan rice
1.	Pericorp color	Red
2.	Length (mm)	6.25 ± 0.08
3.	Breadth (mm)	2.75 ± 0.003
4.	Thickness (mm)	1.98 ± 0.03
5.	1000 grain weight (g)	26.25 ± 0.26

3.2 Nutritional properties of kuzhiadichan rice

The nutritional composition of kuzhiadichan rice revealed its richness in essential nutrients and minerals. The grain exhibited a moisture content of 8.26 ± 0.11%, protein level of 9.64 ± 0.16 g/100 g, fat content of 2.89 ± 0.01 g/100 g and fiber content of 4.69 ± 0.14 g/100 g indicating its balanced macronutrient profile. The carbohydrate content was notably high at 73.51 ± 1.01 g/100 g confirming its role as a good energy source. Among the minerals-phosphorus was recorded at 498 ± 13.21 mg/100 g, potassium at 238 ± 5.82 mg/100 g, sodium at 330 ± 8.98 mg/100 g, calcium at 45.13 ± 1.23 mg/100 g, iron at 6.26 ± 0.17 mg/100 g and zinc at 2.78 ± 0.03 mg/100 g. These results indicate that kuzhiadichan rice is nutritionally dense offering a valuable source of macro and micronutrients beneficial for dietary and health applications (Table 3).

Table 3: Proximate and mineral composition of kuzhiadichan red rice

S.No.	Parameters	Kuzhiadichan rice
1.	Moisture %	8.26 ± 0.11
2.	Protein (g/100 g)	9.64 ± 0.16
3.	Fat (g/100 g)	2.89 ± 0.01
4.	Fiber (g/100 g)	4.69 ± 0.14
5.	Carbohydrate (g/100 g)	73.51 ± 1.01
6.	Phosphorus (mg/100 g)	498 ± 13.21
7.	Potassium (mg/100 g)	238 ± 5.82
8.	Sodium (mg/100 g)	330 ± 8.98
9.	Calcium (mg/100 g)	45.13 ± 1.23
10.	Iron (mg/100 g)	6.26 ± 0.17
11.	Zinc (mg/100 g)	2.78 ± 0.03

Table 4: Antioxidant properties of kuzhiadichan red rice

S.No.	Parameters	Kuzhiadichan rice
1.	Antioxidant activity (%)	62.51 ± 0.51
2.	Flavonoid content (mg CE/ 100 g)	3.88 ± 0.01
3.	Polyphenol (mg GAE/100 g)	460 ± 10.02
4.	Total anthocyanin (mg C3G/100 g)	56.8 ± 1.73

3.3 Antioxidants of kuzhiadichan rice

The antioxidant profile of kuzhiadichan rice demonstrated its strong free radical scavenging potential and rich phytochemical content. The antioxidant activity was recorded at 62.51 ± 0.51% indicating

high oxidative stability. The flavonoid content was found to be 3.88 ± 0.01 mg CE/100 g, while the total polyphenol content measured 460 ± 10.02 mg GAE/100 g reflecting the presence of abundant phenolic compounds contributing to antioxidant efficacy. Additionally, the total anthocyanin content was 56.8 ± 1.73 mg C3G/100 g, which is responsible for the red pericarp color and notable antioxidant properties. These findings suggest that kuzhiadichan rice is a potent source of natural antioxidants valuable for promoting health and preventing oxidative stress related disorders (Table 4).

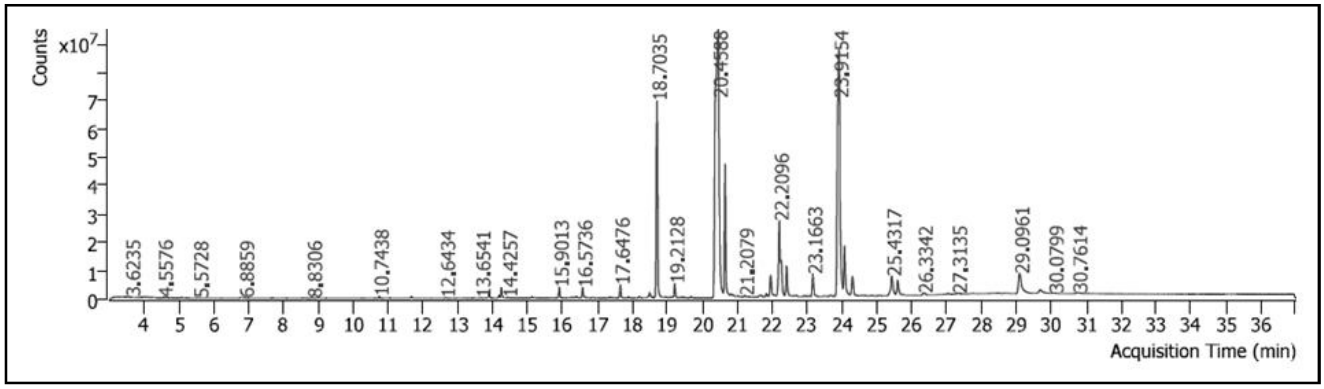


Figure 4: Gas chromatography mass spectrometry (GC-MS) chromatogram of kuzhiadichan rice.

3.4 Quantification of bioactive compounds in kuzhiadichan rice

Figure 4 and Table 5 showed the GC-MS profiling of the kuzhiadichan rice landrace revealed a diverse range of bioactive lipid derived compounds primarily composed of fatty acid methyl esters (FAMEs) and organosiloxane derivatives, which contribute to the nutritional, functional and therapeutic potential of this traditional variety. Among the identified compounds, methyl elaidate ($C_{10}H_{30}O_2$; 296.49 g/mol) was found to be the major component, accounting for the highest peak area of 37.19% suggesting it as the predominant constituent responsible for the sample's lipid profile. The next abundant compound was methyl erucate ($C_{23}H_{40}O_2$; 354.61 g/mol), contributing 22.36%, followed by methyl palmitate ($C_{17}H_{34}O_2$; 270.45 g/mol) with 9.54% both of which are known for their roles in maintaining membrane fluidity and potential bioactivities such as antioxidant and antimicrobial effects. Other notable components included methyl stearate (5.64%) a saturated fatty acid ester commonly associated with emollient and surfactant properties and methyl cis-13-eicosenoate (5.77%), an unsaturated fatty acid methyl ester with known bioactive potential. Methyl behenate (3.49%) and 2,5-dihydroxyacetophenone (3.47%) were also identified in moderate proportions the latter indicating the possible presence of a phenolic derivative contributing to antioxidant activity. Additionally, minor compounds such as methyl behenate derivative (1.66%), methyl 19-methyleicosanoate (1.51%), methyl nervonate (1.58%) and methyl lignocerate (0.84%) were detected, representing long-chain fatty acid methyl esters typically involved in cell signaling, structural lipid formation, and potential neuroprotective effects. The results demonstrate that the sample is rich in long-chain methyl esters of fatty acids particularly methyl elaidate and methyl erucate, which dominate the chromatographic profile. The composition indicates a predominance of unsaturated fatty acids contributing to the biological significance of the sample due to their known roles in maintaining membrane integrity, modulating lipid metabolism and exhibiting various pharmacological properties such as anti-inflammatory, antimicrobial and antioxidant activities.

Table 5: Quantification of bioactive compounds in kuzhiadichan rice

Retention time (min)	Area (%)	Compound name	Molecular formula	Molecular weight (g/mol)	Biological activity	References
20.45	37.194	Methyl elaidate	$C_{19}H_{36}O_2$	296.49	Anticancer activity Antimicrobial Antibacterial	Adebayo *et al.*, 2018
23.91	22.359	Methyl erucate	$C_{23}H_{46}O_2$	354.61	Anti-inflammatory, Neuroprotective and lipid metabolism regulation	
18.70	9.537	Methyl palmitate	$C_{17}H_{34}O_2$	270.45	Antifibrotic and Anti-infla-mmatory	Ayadi *et al.*, 2022
20.65	5.641	Methyl stearate	$C_{19}H_{38}O_2$	298.51	Energy source and Mild antimicrobi activity	Elghaffar *et al.*, 2022
22.20	5.769	Methyl cis-13-eicosenoate	$C_{21}H_{40}O_2$	324.55	Antibacterial and Anti-inflammatory	Marashiyan *et al.*, 2020
24.07	3.487	Methyl behenate	$C_{23}H_{46}O_2$	354.61	Anti-inflammatory	Marashiyan *et al.*, 2020

23.66	3.472	2,5-Dihydroxyace-tophenone,	$C_{20}H_{60}O_{10}Si_{10}$	740.34	Antioxidant, Antimicrobial, Anti-inflammatory and Anticancer potential	Cushnie and Lamb 2005
21.96	1.664	Methyl behenate	$C_{18}H_{54}O_9Si_9$	667.40	Caloric lipid source	Shahidi and Zhong, 2010
22.25	1.505	Methyl 19-methyleico-sanoate	$C_{21}H_{42}O_2$	326.56	Anti-inflammation	Mao *et al.*, 2023
25.43	1.578	Methyl nervonate	$C_{25}H_{48}O_2$	380.65	Neuroprotective and Myelin -enhancing effects	Cao *et al.*, 2020
25.59	0.838	Methyl lignocerate	$C_{25}H_{48}O_2$	380.65	Antidiabetic activity	Shilpa *et al.*, 2009

3.5 Shelf-life evaluation of kuzhiadichan rice health mix

A comparison of the nutritional properties of the health mix formulated using the traditional kuzhiadichan rice variety before and after 90 days of storage period Tables 6 and 7 revealed slight but scientifically relevant variations in its compositional and sensory characteristics.

Before storage the moisture content of the samples ranged from 6.25 ± 0.04% (T_4) to 6.73 ± 0.06% (T_1), while after 90 days, it increased marginally to 6.32 ± 0.19% and 6.81 ± 0.02%, respectively, showing a statistically significant difference (p<0.05). This slight rise in moisture content may be attributed to environmental humidity and the hygroscopic nature of the product, which can influence shelf-life and storage stability. Despite this increase the moisture levels remained well within acceptable limits for dry health mixes indicating that the product maintained its physical integrity during storage.

Table 6: Nutritional properties of kuzhiadichan red rice-based health mix before storage

Parameters	T_1	T_2	T_3	T_4	CD @ 5%	CV %
Moisture (%)	6.73 ± 0.06	6.68 ± 0.08	6.45 ± 0.09	6.25 ± 0.04	0.269**	2.19
Protein (g/100 g)	12.39 ± 0.36	12.26 ± 0.06	12.14 ± 0.02	12.02 ± 0.008	0.56	1.70
Fat (g/100 g)	3.63 ± 0.09	3.58 ± 0.10	3.56 ± 0.001	3.54 ± 0.09	0.15	2.26
Fiber (g/100 g)	4.23 ± 0.02	4.18 ± 0.07	4.11 ± 0.07	4.02 ± 0.13	0.17	2.26
Carbohydrate (g/100 g)	68.99 ± 2.11	70.11 ± 2.14	72.24 ± 0.19	74.12 ± 2.42	2.17**	2.02
Phosphorus (mg/100 g)	446 ± 5.76	449 ± 14.05	452 ± 6.15	457 ± 14.92	22.67	2.67
Potassium (mg/100 g)	228 ± 5.89	231 ± 6.12	235 ± 0.95	238 ± 2.75	7.89	1.80
Sodium (mg/100 g)	312 ± 2.33	316 ± 10.53	324 ± 0.88	329 ± 7.61	12.98	2.15
Calcium (mg/100 g)	48.23 ± 0.03	48.44 ± 0.06	48.82 ± 0.13	48.98 ± 1.36	2.69	2.95
Iron (mg/100 g)	5.69 ± 0.08	5.71 ± 0.13	5.73 ± 0.10	5.76 ± 0.05	0.27	2.51
Zinc (mg/100 g)	3.15 ± 0.02	3.18 ± 0.04	3.26 ± 0.002	3.33 ± 0.10	0.12**	2.11
Overall acceptability (1 to 9 Hedonic scale)	8.2 ± 0.03	8.5 ± 0.27	8.4 ± 0.18	8.8 ± 0.22	0.32**	2.01

Note: T_1- kuzhiadichan- 80%:green gram 20%; T_2 - kuzhiadichan- 70% :green gram 20% :pear millet 10%; T_3 - kuzhiadichan- 60% :green gram 20% :pear millet 20%; T_4 - kuzhiadichan- 50%: green gram 20% :pear millet 30%.

Table 7: Nutritional properties of kuzhiadichan red rice-based health mix after storage period (90 days)

Parameters	T_1	T_2	T_3	T_4	CD @ 5%	CV %
Moisture (%)	6.81 ± 0.02	6.83 ± 0.21	6.51 ± 0.19	6.32 ± 0.19	0.35*	2.85
Protein (g/100 g)	12.35 ± 0.24	12.24 ± 0.34	12.10 ± 0.23	11.98 ± 0.08	0.29	1.31
Fat (g/100 g)	3.66 ± 0.002	3.61 ± 0.12	3.59 ± 0.004	3.56 ± 0.11	0.11	1.71
Fiber (g/100 g)	4.21 ± 0.02	4.17 ± 0.10	4.09 ± 0.09	4.01 ± 0.03	0.26	3.39
Carbohydrate (g/100 g)	68.95 ± 0.93	70.07 ± 0.57	72.20 ± 1.17	74.07 ± 0.75	1.88**	1.40
Phosphorus (mg/100 g)	445 ± 4.84	448 ± 5.30	451 ± 9.81	456 ± 10.23	21.18	2.50
Potassium (mg/100 g)	227 ± 3.07	230 ± 0.46	234 ± 0.47	237 ± 2.09	12.71	2.91
Sodium (mg/100 g)	311 ± 5.50	315 ± 3.64	324 ± 3.48	328 ± 5.13	13.97	2.32

Calcium (mg/100 g)	48.21 ± 1.18	48.40 ± 0.55	48.79 ± 0.62	48.96 ± 0.76	2.89	3.16
Iron (mg/100 g)	5.66 ± 0.14	5.68 ± 0.04	5.70 ± 0.07	5.74 ± 0.12	0.20	1.94
Zinc (mg/100 g)	3.14 ± 0.01	3.15 ± 0.08	3.24 ± 0.05	3.31 ± 0.10	0.10*	1.76
Overall acceptability (1 to 9 Hedonic scale)	8.0 ± 0.11	8.3 ± 0.11	8.2 ± 0.25	8.6 ± 0.22	0.48	3.12

Note: T_1- kuzhiadichan- 80%:green gram 20%; T_2 - kuzhiadichan- 70% :green gram 20% :pear millet 10%; T_3 - kuzhiadichan- 60% :green gram 20% :pear millet 20%; T_4 - kuzhiadichan- 50%: green gram 20% :pear millet 30%.

Protein content exhibited only minor fluctuations across treatments ranging from 12.02 ± 0.008 to 12.39 ± 0.36 g/100 g initially and from 11.98 ± 0.08 to 12.35 ± 0.24 g/100 g after storage showing no significant difference (p>0.05). This stability demonstrates that the protein matrix of the formulation was not adversely affected by storage conditions, which is a positive indicator for the preservation of the products nutritional quality. Similarly, fat content remained nearly constant (3.54 to 3.63 g/100 g before and 3.56 to 3.66 g/100 g after storage) implying minimal oxidation or rancidity during the 90 day period. This suggests that the packaging and storage environment provided adequate protection against lipid degradation, which is critical for maintaining product flavor and safety.

Fiber content also showed negligible reduction (from 4.02 ± 0.13 to 4.23 ± 0.02 g/100 g initially and from 4.01 ± 0.03 to 4.21 ± 0.02 g/100 g after storage) with differences remaining statistically non-significant. The retention of fiber indicates that the structural carbohydrates were largely unaffected by storage duration. Carbohydrate content on the other hand, showed a significant difference (p<0.05) increasing slightly from 68.99 ± 2.11 to 74.12 ± 2.42 g/100 g before storage and 68.95 ± 0.93 to 74.07 ± 0.75 g/100 g after storage. This relative increase may be linked to minor moisture loss and concentration effects, a typical phenomenon observed during dry storage of cereal based foods.

The mineral composition of the health mix demonstrated commendable stability throughout the storage period. Phosphorus content ranged from 446 ± 5.76 to 457 ± 14.92 mg/100 g before storage and from 445 ± 4.84 to 456 ± 10.23 mg/100 g after storage indicating no significant change. Similarly, potassium (228 to 238 mg/100 g before; 227 to 237 mg/100 g after), sodium (312 to 329 mg/100 g before; 311 to 328 mg/100 g after), calcium (48.23 to 48.98 mg/100 g before; 48.21 to 48.96 mg/100 g after) and iron (5.69 to 5.76 mg/100 g before; 5.66 to 5.74 mg/100 g after) showed only minimal variation. These findings suggest that mineral nutrients in the kuzhiadichan rice based health mix are stable and not prone to degradation during storage under controlled conditions. Zinc, however, showed a slight but significant (p<0.05) difference, increasing from 3.15 ± 0.02-3.33 ± 0.10 mg/100 g before storage to 3.14 ± 0.01-3.31 ± 0.10 mg/100 g after storage. This could be due to concentration effects linked to minor variations in moisture levels or analytical measurement sensitivity.

Sensory evaluation results revealed that the overall acceptability of the health mix remained consistently high across both stages. Before storage, the scores ranged from 8.2 ± 0.03 (T_1) to 8.8 ± 0.22 (T_4) while after 90 days, they ranged from 8.0 ± 0.11 to 8.6 ± 0.22. Although there was a marginal reduction in sensory scores over time, the difference was not statistically significant (p>0.05). This indicates that the product retained its desirable organoleptic qualities such as color, flavor, texture and taste throughout the storage period, which is essential for consumer acceptance and marketability.

In summary, the comparative analysis highlights that the kuzhiadichan rice based health mix demonstrated remarkable nutritional and sensory stability during 90 days of storage. The minor changes observed in moisture and carbohydrate levels did not compromise the overall quality of the product. The retention of protein, fat, minerals and sensory properties underscores the formulations robustness and suitability for long term storage. These findings confirm that the health mix is a nutritionally balanced, shelf-stable product with potential as a functional food ingredient that leverages the traditional nutritional richness of kuzhiadichan rice.

Table 8: Changes in the microbial quality of kuzhiadichan red rice-based health mix during storage

Health mix	Storage period	TPC x 10^3	Bacteria x 10^3	Fungi x 10^5
T_1	0 day	0	0	0
	90 day	0	0	0
T_2	0 day	0	0	0
	90 day	1	1	0
T_3	0 day	0	0	0
	90 day	1	1	0
T_4	0 day	0	0	0
	90 day	1	1	0

3.6 Microbial quality

Table 8 shows the microbial quality evaluation of the formulated health mix samples demonstrated excellent stability and safety during the 90 day storage period. Initial day all formulations (T_1, T_2, T_3 and T_4) exhibited complete absence of microbial growth as reflected by zero values recorded for total plate count (TPC), bacterial and fungal populations. This indicates that the preparation, processing and packaging of the health mix were carried out under hygienic conditions effectively preventing microbial contamination at the beginning of the storage period.

After 90 days of storage, slight variations were observed in microbial counts among the treatments. Formulations T_2 , T_3 and T_4 showed a marginal increase in TPC and bacterial load each registering 1 × 10^3 CFU/g, while the fungal count remained undetectable (0 × 10u CFU/g) in all samples. This minor bacterial presence could be attributed to gradual moisture absorption or exposure to environmental factors during storage, which may have supported minimal bacterial survival. However, the absence of fungal growth across all formulations signifies that the product maintained an adequately low moisture level and was resistant to spoilage by molds and yeasts.

Interestingly, the T_1 formulation maintained complete microbial absence (0×10^3 CFU/g for TPC and bacteria and 0×10^5 CFU/g for fungi) even after 90 days, highlighting its superior microbial stability compared to the other formulations. This could be due to differences in ingredient composition, processing conditions or packaging integrity that limited microbial proliferation.

Overall, the microbial assessment confirms that the health mix formulations were microbiologically safe and stable during the 90 day storage period. The minimal bacterial count observed in certain treatments remained well within acceptable safety limits for dry powdered products, emphasizing that the product retained its quality and suitability for consumption throughout the storage duration.

4. Discussion

The present study demonstrates that the traditional kuzhiadichan red rice possesses remarkable nutritional, functional and nutraceutical potential confirming its suitability for developing value-added health food formulations. The proximate and mineral composition analyses revealed that kuzhiadichan rice is a nutrient-dense grain containing high levels of protein (9.64 g/100 g), dietary fiber (4.69 g/100 g) and essential minerals such as phosphorus (498 mg/100 g), calcium (45.13 mg/100 g) and iron (6.26 mg/100 g). These results align with previous reports highlighting that traditional pigmented rice varieties are superior to polished rice in terms of macro and micronutrient content (Raghuvanshi *et al.,* 2017; Srivastava *et al.,* 2022). The elevated iron content is of particular importance in addressing anemia a prevalent micronutrient deficiency in developing countries (FAO, 2004).

The antioxidant profile of kuzhiadichan rice characterized by high polyphenol (460 mg GAE/100 g) and anthocyanin (56.8 mg C3G/ 100 g) contents substantiates its strong free radical scavenging potential (62.51%) comparable to other red rice varieties documented by Oki *et al.* (2005) and Berni *et al.* (2018). Anthocyanins are potent bioactive pigments responsible for the red pericarp color and are known to enhance vascular integrity, modulate oxidative stress and protect against chronic diseases (Finocchiaro *et al.,* 2010). The presence of abundant flavonoids and polyphenols further contributes to the rices antioxidant defense system, which can delay lipid oxidation and promote cellular protection against oxidative damage (Wang *et al.,* 2018). The high antioxidant capacity of kuzhiadichan thus provides a biochemical basis for its traditional recognition as"healing rice".

The GC-MS analysis of kuzhiyadichan rice identified several bioactive fatty acid methyl esters and related compounds highlighting its rich nutritional and therapeutic profile. The dominant compound, methyl elaidate (37.19%), exhibited potent anticancer, antibacterial and anti-inflammatory properties (Adebayo *et al.,* 2018), while methyl erucate (22.35%) showed neuroprotective and lipid metabolism regulating activities (Sayed *et al.,* 2022). Methyl palmitate (9.54%) demonstrated antioxidant and anti-inflammatory functions (Shaikh *et al.,* 2020) and methyl stearate (5.64%) contributed to energy provision and mild antibacterial action (Elghaffar *et al.,* 2020). Similarly, methyl cis-13-eicosenoate (5.77%) and methyl behenate (3.49%) exhibited antibacterial and anti-inflammatory effects (Marashlyan *et al.,* 2020). The phenolic compound 2,5-dihydroxyacetophenone (3.47%) showed antioxidant, antimicrobial and anticancer potential (Lushbue and Lamb, 2005), whereas methyl behenate derivative (1.66%) and methyl 19-methyleicosanoate (1.51%) were associated with caloric lipid metabolism and anti-inflammatory roles (Mao *et al.,* 2023). Minor constituents like methyl nervonate (1.58%) and methyl lignocerate (0.84%) were linked to neuroprotective, myelin-enhancing and antidiabetic effects (Cao *et al.,* 2020; Shilpa *et al.,* 2009). Finally, the presence of these bioactive compounds in kuzhiyadichan rice indicates its potential as a functional food with antioxidant, anti-inflammatory, neuroprotective and antidiabetic properties, underscoring its significance as a traditional landrace with considerable health-promoting and nutraceutical value.

When kuzhiadichan rice was blended with green gram and pearl millet to develop four formulations (T_1 to T_4) progressive substitution with pearl millet improved the carbohydrate and mineral profile of the mix without compromising protein or fat content. Among all, the T_4 formulation (50% kuzhiadichan:20% green gram:30% pearl millet) exhibited superior nutritional balance, highest carbohydrate content (74.12 g/100 g) and excellent sensory acceptability (8.8 ± 0.22). This synergistic combination integrates the complementary nutritional strengths of cereals, millets and pulses offering enhanced amino acid balance, improved digestibility and higher micronutrient bioavailability (Semmarath *et al.,* 2023). The results are consistent with previous findings that incorporation of millets into rice-based formulations improves iron, zinc and antioxidant retention (Gunaratne *et al.,* 2013).

Storage stability analysis over 90 days revealed minimal deterioration in the nutritional and sensory properties of the formulations. The minor increase in moisture content (from 6.25% to 6.32%) was within acceptable limits for dry health mixes, while protein, fat and mineral levels remained largely stable indicating resistance to oxidative and enzymatic degradation. The microbial analysis confirmed the product's safety with no fungal growth and only negligible bacterial load (1×10^3 CFU/g) after 90 days. Similar trends of stability in composite flour blends during extended storage were reported by Arise *et al.* (2018) confirming that proper roasting, low moisture and airtight packaging are critical for maintaining product safety and quality. The stability of the kuzhiadichan-based health mix underscores its suitability for commercialization and long-term storage under ambient conditions.

From a functional standpoint the high iron and anthocyanin contents of the T_4 health mix enhance its potential for addressing iron-deficiency anemia and oxidative stress related metabolic disorders. Anthocyanins facilitate iron absorption by modulating gut microflora and chelating reactive oxygen species, thus preventing oxidative damage to erythrocytes (Wiczkowski *et al.,* 2015). Furthermore, the presence of bioactive lipids and phenolics contributes to cardiovascular protection by improving lipid metabolism and endothelial function (Ravichanthiran *et al.,* 2018). Therefore, the T_4 health mix represents a functional food matrix with broad spectrum health benefits including antioxidant defense, hemoglobin synthesis and metabolic regulation.

In summary, the results validate kuzhiadichan red rice as a valuable traditional landrace with high nutraceutical potential. Its integration into a composite cereal-pulse-millet health mix successfully enhances nutritional quality, functional stability and consumer acceptability. The findings support the repositioning of traditional

pigmented rice varieties within the modern functional food sector promoting sustainable utilization of indigenous genetic resources and contributing to dietary diversification and health promotion.

5. Conclusion

The present study successfully formulated and evaluated a nutritionally superior and shelf-stable health mix based on the traditional kuzhiadichan red rice landrace blended with green gram and pearl millet. The kuzhiadichan variety exhibited exceptional nutritional richness being particularly high in protein (9.64 g/100 g), fiber (4.69 g/100 g) and essential minerals such as phosphorus (498 mg/100 g), calcium (45.13 mg/100 g) and iron (6.26 mg/100 g). Its elevated total polyphenol (460 mg GAE/100 g) and anthocyanin (56.8 mg C3G/100 g) contents highlight its potent antioxidant potential and functional value. GC-MS profiling confirmed the presence of bioactive lipid compounds such as methyl elaidate, methyl erucate and methyl palmitate, which contribute to antioxidant, anti-inflammatory and cardioprotective functions. Among the developed formulations T_4 (50% kuzhiadichan rice:20% green gram:30% pearl millet) was identified as the most nutritionally balanced, functionally enriched and organoleptically preferred blend, maintaining its stability and acceptability even after 90 days of storage. The iron and anthocyanin rich T_4 health mix provides significant health benefits including enhanced antioxidant defense, improved hemoglobin synthesis and protection against oxidative stress related disorders such as cardiovascular diseases, diabetes and anemia. With its balanced macronutrient composition, high mineral density and excellent shelf stability, the kuzhiadichan-based health mix represents a promising functional food or nutraceutical suitable for all age groups particularly beneficial for children, women and the elderly, thereby bridging traditional nutritional wisdom with modern dietary innovation and offering a sustainable, health-promoting food alternative.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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