RESEARCH PAPER

PREPARATION, PHYSICOCHEMICAL AND NUTRITIONAL ANALYSIS OF PRECURSORS OF L-CARNITINE-RICH DETOX FLOUR FOR THERAPEUTIC BENEFITS

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Abstract

Functional foods and nutraceuticals have significantly contributed to treat many noncommunicable diseases such as non-alcoholic fatty liver disease, diabetes and obesity. L-carnitine is one of them, which serves as an ergogenic aid among athletes for muscle building and energy production. In previous studies, L-carnitine supplementation has shown significant reduction in liver enzymes, blood sugar and excess bodyweight of the patients. Beef, lamb, duck and pork are naturally rich in L-carnitine, but they are high in saturated fat too, which could be atherogenic. L-carnitine is synthesised in the human body from two essential amino acids (lysine, methionine) and four cofactors (ascorbate, niacin, pyridoxin and iron). The present study aimed to develop lysine and methionine-rich detox flour (DF) for endogenous synthesis of L-carnitine. The flour was prepared after mixing the germinated, boiled, dried and ground grains, spices, oil-seeds and moringa powder in the required proportion. Chapatis were made with different concentrations of detox flour in wheat flour. The findings indicate that the chapati prepared with a 1:1 ratio showed the most acceptable (7.8%) physicochemical and sensory properties after the control (7.9%). Nutritional analysis of sprouted grains showed 1623.09±0.16 mg Lysine in 100g mung flour, 1017.39±0.12 mg in 100g Bengal gram flour then 105.71±0.06 mg Methionine in 100g finger millet flour, 365.69±0.01 mg in 100g Amaranth flour. Hence, plant-based protein-rich detox flour could provide therapeutic benefits globally if incorporated in the recommended amount in the daily diet.

Keywords: Ergogenic aid, L-carnitine, Lysine, Methionine, Metabolic syndrome, Non-alcoholic fatty liver disease, Nutraceuticals.

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Introduction

Obese people with metabolic syndrome have key risk factors for chronic non-communicable diseases (NCDs) such as cardiovascular disease, type-2 diabetes, non-alcoholic fatty liver disease (NAFLD) and PCOD. More than 10% of liver fat progresses from NAFLD to NASH (non-alcoholic steatohepatitis) and an inflamed liver further progresses to Liver cirrhosis and carcinoma.[1] When fat and toxins accumulate in the liver, metabolism slows down, resulting in obesity. Lifestyle modification with proper diet, exercise, and sustained weight loss is the only solution. Functional foods containing Nutraceuticals and many bioactive compounds can significantly prevent NAFLD and manage many lifestyle-related diseases.[2] Many molecules such as milk thistle, tea polyphenol, CoQ_{10}, vitamin E, L-carnitine, moringa oleifera and insulin sensitizers like metformin have showed potency to reverse NCDs at various clinical trials.[1] L-carnitine is gaining importance as an ergogenic aid for athletes and as a therapeutic supplement for patients with NCDs. L-carnitine helps body turn Fat into Energy by transporting long-chain fatty acids into the inner mitochondrial membrane for β-oxidation to generate energy.[3]

L-carnitine (β-hydroxy-γ-N-trimethylammonium butyrate) is a water-soluble amino acid, derived from the Latin word

caro/*carnis* meaning flesh/meat. It is synthesized in the liver, kidneys and brain from the two amino acids - Lysine and Methionine in the presence of cofactors (ascorbic acid, iron, vitamin B6 and niacin) and then transported to cardiac and skeletal muscles.[4] The rate of synthesis declines with age, chronic disease, obesity, poor diet and prolonged use of steroids or antibiotics. This makes L-carnitine a conditionally essential nutrient.[5] In case of deficiency, one should take either carnitine-rich foods or a supplement. Furthermore, L-carnitine is produced by plants and can be manufactured in laboratories to create therapeutic pharmaceuticals. While non-vegetarians can easily meet their daily carnitine requirements through chicken, fish, beef, lamb, duck and pork, the richest sources are lamb (190 mg/100 g) and beef (143 mg/100 g).[6] However, because these red meats are also high in saturated fat, regular consumption may increase the risk of atherosclerosis.

Therefore, there is a need for a plant-protein-based detox flour, rich in precursors of L-carnitine so that carnitine is produced in the human body. Enriching cereal-based flour (wheat or rice) with legumes and pulses could be a highly effective solution for improving bioavailability of essential amino acids and overall nutrition. In a study[7] incorporation and optimization of millet flour to wheat flour from 0 to 50% significantly alters the nutritional, physical and sensory profile of multigrain chapatis. Finger millet contains 220 mg/100 g of lysine and 210 to 310 mg/100 g of Methionine which is much higher than other millet varieties.[8] Amaranth flour, being a good source of iron (7.61 mg/100 g), lysine (750 mg/100 g) and methionine (230 mg/100 g), significantly reduced cholesterol levels and hepatic lipid accumulation in rats.[9] Amla, one of the richest sources of vitamin C, has a beneficial effect on liver function, hyperlipidaemia and metabolic syndrome.[10] 100 g of Moringa leaves contain 2.22 mg of niacin, 1.2 mg of pyridoxine, 204 mg of vitamin C and 28 mg of iron (more than spinach-2.7 mg/100 g). Incorporating 30% to 50% barley, sorghum, Bengal-gram, amaranth, soybean, and finger millet into wheat flour significantly increases the protein and dietary fibre content of chapatis.[11]Chapatis are a staple food for millions of people across India and Southeast Asia. Since chapatis are eaten two to three times daily, they serve as an ideal vehicle for fortification.

The present study aimed to design and develop a functional detox flour (DF) for patients managing obesity, NAFLD and type 2 diabetes. To create this specialized detox flour, grains rich in L-carnitine precursor, were selected. Blending this detox flour with standard wheat flour can offers a wholesome, highly acceptable option for making chapatis with enhanced protein, vitamin and mineral contents. This study evaluated the effects of different blending ratios of detox flour to wheat flour **(**3:0, 2:1, 1:1, 1:2, and 0:3**)** on the physicochemical properties and texture profiles of the resulting chapatis. Finally, sensory analysis was conducted using standard procedures to determine the overall acceptability and identify the optimal chapati formulation for targeted therapeutic benefits.

Materials and methods

a. Materials: Grains, legumes and spices were bought from the local grocery store, while amla, moringa, raw banana and kulekhara were bought from a local vegetable vendor in Kolkata, West Bengal.

Chemicals used: All the reagents and chemicals used in the investigation were of analytical grade and were procured from Sigma, BDH Chemicals and Merck India.

Study Setting: The current study was carried out in the research laboratory of the Food and Nutrition Department of Swami Vivekananda University, Barrackpore, West Bengal.

b. Methods:

Preparation of Raw Materials: The flow chart for the preparation of detox flour from its various ingredients is shown in Figure 1.

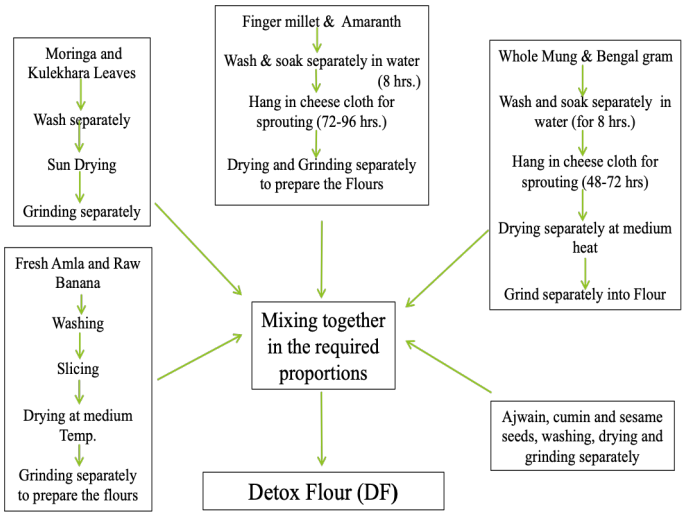


Figure 1: Flow Chart of the Preparation of Detox Flour

Each ingredient was cleaned, washed, dried or roasted, then ground into powder using an electric grinder.

Germination of Legumes: Bengal gram, whole Mung, finger millet and amaranth seeds were soaked overnight separately in water and then kept for 72, 48, 72 and 96 hours respectively to sprout, as this process increases their lysine and methionine content. The process of germination, boiling and drying significantly ($p < 0.05$) reduces phytate, tannin and saponin contents and improve bioavailability of iron, zinc, phosphorus and calcium.[12]

Boiling and Drying of legumes: The grains were boiled, dried under the sun to remove moisture, roasted (at low flame to preserve nutritional quality) and ground separately in an electric grinder to prepare the flours.

Detox Flour Preparation: To prepare the detox flour (DF**)**, 10 g each of grain and raw banana flours were mixed with 2 g each of powdered moringa leaves, kulekhara leaves, cumin seeds, sesame seeds, amla, and ajwain. The DF was then stored at room temperature in an air-tight container.

Formulation of mixed flour Chapati Samples: Five types of chapatis samples were made by blending detox flour and wheat flour in ratios of 3:0, 2:1, 1:1, 1:2, 0:3. Additionally, a cheela sample was prepared using a 1:1 mix of DF and dehydrated curd.

Chapati Samples Preparation: Each 30 g flour mixture was kneaded with water and rested for 15 minutes then rolled into a round shape of 150 mm diameter (2-3 mm thickness) and baked on a hot pan at 230 °C for 1–2 minutes on each side.[13] The best chapati sample will be standardised for an 'optimal recipe' after the acceptance tests through physicochemical and sensory analysis.

Physicochemical analysis of the chapati: Moisture and ash contents of all the flour samples were estimated using the AOAC[14] method. Protein, carbohydrate and Fat were estimated using Lowry[15], Anthrone and Soxhlet methods, respectively. Crude fibre and energy were calculated using these formulae:

$$\text{Crude Fiber (g)} = 100 - (\text{Carbohydrate} + \text{moisture} + \text{Ash} + \text{protein} + \text{fat})$$

$$\text{Energy (kJ)} = (\text{Carbohydrate} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)$$

All the measurements were taken in triplicate.

Sensory evaluation: Thirty semi-trained panellists (faculty, scholars and students aged 19-45) provided their informed consent and evaluated the chapati samples. They rated appearance, aroma, taste, texture, pliability, mouthfeel, and overall acceptability using a 9-point hedonic scale (1 for disliked extremely, 5 for neutral, 9 for liked extremely).[16] The evaluation was conducted in a well-lit, quiet, odour-free room**.** The chapati samples with codes were served in a randomized order to prevent carryover effects, and panellists rinsed their mouths with water between samples. After data collection, statistical analysis was performed to identify any significant differences among the samples.

Textural characteristics of chapati: The Texture Profile Analysis (TPA) method is used for texture analysis of food products. Simulating human chewing action, the TPA test performs two compression and decompression cycles (bites).[17] In this study, 16 prepared chapati samples were tested at room temperature using a Texture Analyser (Model CT 3-50K, Brookfield Engineering Laboratories, Middleboro, Massachusetts, USA). Data acquisition, graph plotting, and report generation were done using Texture Pro CT software. Texture profile analysis was performed using a chapati sample (4 cm x 2 cm), which was compressed twice using a TA-5 (cylindrical compression probe) where the load cell was 50 kg and the Warner blade speed was set at 1 mm/sec. Readings were taken in triplicate (in Newton), and a graph was plotted.

Analysis of 2 Amino Acids in the selected sprouted grains flour using HPLC method:

Acid hydrolysis**,** pre-column derivatization and Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) three step method suggested by ICMR-National Institute of Nutrition (ICMR-NIN), was performed[18] to separate and estimate the amounts of lysine and methionine in the selected grain's flours. The reversed phase high performance liquid chromatography (RP-HPLC) method was described by Varzaru et al.[19]

Statistical Analysis: Percentages, means and standard deviations were calculated for physicochemical and sensory scores of the developed chapatis. Radar, Error Bar graphs were plotted to know the most preferred flour mix. Data evaluation and statistical significance was determined using one-way analysis of variance (ANOVA), t-test and Tukey's test ($p < 0.05$) using IBM SPSS Statistics 29.0 software.

Results and Discussion:

Results of the analysis of chapati samples are summarized and listed below:

Physicochemical Analysis of the Chapati Samples-

All the chapati samples were tested and analysed for proximate composition, using the standard Association of Official Analytical Chemists (AOAC)[13] procedure. Table 1 shows the results of moisture, ash, protein, carbohydrate and fat content all the chapati samples. All mixed flour samples showed higher ash, crude fibre and protein compared to control. Amaranth was included in a significant amount in the detox flour because, it contains a good amount of ash (2.53%) and protein (17%) compared to protein in other cereals (8-14%).[20]

Table 1 Proximate Composition of the samples: Three measurements for each combination were made and the mean result values are tabulated below

Sl. No.	Chapati Samples	Moisture		Ash		Protein		Carbohydrate		Fat		Crude Fibre.		Energy	
		(g/100g) Mean ± SD	%	(g/100g) Mean ± SD	%	(g/100g) Mean ± SD	%	(g/100g) Mean ± SD	%	(g/100 g) Mean ± SD	%	(g/100g) Mean ± SD	%	(kJ/100 g) Mean ± SD	%
1	Control	9.05± 0.27	9.1	1.65± 0.01	1.7	9.69±0.03	9.7	71.07± 0.06	71.1	1.09 ±0.01	1.1	7.45± 0.24	8	333± 0.63[a]	333
2	DFC-1	7.58± 0.15[ab]	7.6	2.71± 0.06	2.7	13.60± 0.10	13.6	66.06± 0.06	66.1	1.26 ±0.02	1.3	8.79± 0.36	9	330± 0.58[a]	330
3	DFC-2	7.33± 0.18[a]	7.3	3.03± 0.01[b]	3	16.37± 0.05	16.4	52.09± 0.17	52.1	2.51 ±0.02	2.5	*18.67 ±0.47	19	297± 0.64	297
4	DFC-3	7.84± 0.27[b]	7.8	2.38± 0.03	2.4	19.71± 0.09	19.7	49.03± 0.03	49	3.01 ±0.01	3	18.03 ±0.75	18	302± 0.37	302
5	DFC-4	6.91± 0.22	6.9	3.05± 0.01[b]	3.1	*27.76 ±0.08	27.7	**41.79±0.05	41.8	3.62 ±0.03	3.6	16.87 ±0.57	17	311± 0.58	311

(*highest protein and Fibre in DFC-2,**lowest carbohydrate in DFC-4) [The values represent Mean ± Standard deviation (SD) of triplicate measurements. Mean values with the same letter (a-c) in superscript within the same column are not significantly different from each other at $p \leq 0.05$] {Abbreviations: Control/WF-Wheat Flour, DF-Detox Flour, DFC1-Detox flour chapati(WF+DF1:2), DFC 2 Detox flour chapati(WF+DF1:1), DFC 3-Detox flour chapati (WF+DF1:3), DFC 4-Detox flour chapati (WF+DF 0:3), Detox flour chapati 4c(CF+DF1:1), CF-Curd Flour.}

Moisture content: The moisture content of the samples varied from 6-9% and was statistically different at $p \leq 0.05$ from each other, and it was highest in control. Flours containing >12% moisture have lower storage stability. Rice-Soya flour (rice 40%, soya 60%) had less moisture compared to Rice-Millet flour (rice 40%, millet 60%), which was due to the high protein content in soya-based flour.[21]A similar trend was observed in Table 1 where the protein-rich flour sample DFC-4, showed the lowest moisture content (6.91%) among all other samples.

Ash content: Heating the samples at 600°C removed moisture and organic macronutrients, leaving behind inorganic mineral ash. Table 1 shows that as the ratio of wheat flour decreased, the ash content in the chapati samples gradually increased. Similar results were found in a study, where the highest ash content of 4.02% was found in millet-maize mix (70:30), and the lowest content of 0.35% was in wheat-rice mix (50:50). [22,23] Here, higher ash content in DFC 4 and DFC 2 indicates a greater amount of minerals in the detox flour.

Protein Content: Protein content of all the samples was significantly different at $p \leq 0.05$. Chapati sample DFC 4 contained the highest protein at 27.7% compared to 9.7% in the control, which could be due to the addition of protein-rich chickpeas, mung and millet sprouts in the detox flour. A similar study found that incorporating protein-rich green mung significantly enhanced the flavour and overall acceptability of biscuits fortified with 20% mung.[24] In a study, multigrain mix prepared from finger millet, maize, Bengal gram and soybean in the proportion of 40 : 25 : 25 : 10, was organoleptically acceptable with higher protein content.[25]

Fat content: In this study, detox flour chapati sample (DFC 4) showed the highest fat content (3.6%) which could be due to the incorporation of sesame seeds and millet flour. A study revealed that multigrain composite Mix-1 (with ground nut) had the highest fat content (13%) and Mix 4 (with soya flour) had lowest fat content (9%), this could be due to higher presence of total fats in groundnuts (25% more monounsaturated fatty acids).[26]

Carbohydrate Content: The highest carbohydrate content was found in wheat flour (71.1%) and the least (41.8%) was in DFC 4 which was significantly different at $p < 0.05$. There was decrease in carbohydrate content as the ratio of the wheat flour decreases in the flour mix.[27]

Effect of Soaking, Germination and Roasting:

In this study there was a significant increase in ash, protein, fibre and decrease in fat, and carbohydrates compared to control, which could be due to germination of selected grains. In a study there was a significant increase in ash, protein, fibre, iron, ascorbate and folic acid and decrease in

anti-nutritional factors after 48, 72 and 96 hours of sprouting of mung bean.[28,29] Germination of mung bean significantly increases methionine[30], protein from 19.5 to 21.63%, ascorbic acid from 2.71 to 9.16%, folic acid from 168.83 to 172.89%, amino acid from 80.92 to 90.50% and decreased crude fat from 1.79 to 1.4% with an increase in germination time.[31,32]

There was decrease in the fat content of chickpeas during soaking which could be due to use of fat as an energy source in sprouting process.[33] Soaking and roasting of finger millet resulted in an increase in protein digestibility and reduction in phytic acid, tannins which interfere with protein and mineral absorption. [34]

Sensory analysis- Results of sensory evaluation are shown in Table 2.

Table 2 Average Sensory Scores of Control and Different Chapati samples:

Chapati Samples using different proportions of the two flours (DF : WF)		Sensory Evaluation and Mean Scores of chapati samples on a 9-point Hedonic Scale						
		Appearance	**Aroma**	**Pliability**	**Mouthfeel**	**Taste**	**Eating Quality**	**Overall Acceptability**
		Score (9)	**Score (9)**	**Score (9)**	**Score (9)**	**Score (9)**	**Score (9)**	**Score (9)**
		round/smooth/dark/cracked/charred	Nutty/rancid/earthy/spicy/burnt/fermented	Soft/Flexible/ semi-soft/hard/ Very hard	Smooth/ Grainy/ Moist/ Chewy	Acidic/bitter/salty/spicy/buttery/ burned	Good Quality/ Acceptable /borderline /Poor Q	Acceptable /Slightly Acc./ not Acceptable
Samples	**Ratio**	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control	(0 : 3)	8.10±0.20^{a}	7.07±0.15^{c}	8.17±0.31^{ab}	7.33±0.29^{a}	7.50±0.26^{b}	8.34±0.15^{a}	7.93±0.26^{a}
DFC 1	(1 : 2)	7.50±0.10^{b}	7.47±0.29^{b}	8.03±0.12^{a}	7.19±0.40^{b}	6.71±0.31^{a}	7.63±0.15^{c}	7.56±0.20^{b}
DFC 2	(1 : 1)	6.87±0.21^{d}	**7.68±0.32^{b}	7.13±0.12^{c}	7.08±0.15^{bc}	**7.93±0.25^{bc}	**7.87±0.4^{b}	**7.80±0.10^{a}
DFC 3	(2 : 1)	5.53±0.21^{c}	6.73±0.21^{a}	6.27±0.15^{d}	6.43±0.45^{d}	6.87±0.32^{a}	6.70±0.10^{d}	6.83±0.25^{c}
DFC 4	(3 : 0)	6.03±0.21^{f}	6.80±0.60^{a}	6.80±0.60^{e}	6.20±0.26^{e}	5.93±0.23^{d}	5.73±0.45^{f}	6.33±0.25^{d}
DFC 4C	(3 : 0)	5.76±0.22^{e}	*7.82±0.32^{d}	*8.40±0.21^{b}	7.02±0.21^{c}	*8.13±0.24^{c}	7.24±0.36^{e}	7.28±0.15^{e}

(DFC-2**- most acceptable with >3 sensory characteristics, DFC-4 C*- acceptable with 3 sensory characteristics, (Q-Qality, Acc.- Acceptable) [The values represent Mean ± Standard deviation (SD) of triplicate measurements. The values with the same letter (a-f) in superscript within the same column are not significantly different from each other at $p \leq 0.05$] {Abbreviations: DFC 1-Detox flour chapati(WF+DF1:2), Control:WF-Wheat Flour, DF-Detox Flour, DFC 2 Detox flour chapati(WF+DF1:1), DFC 3-Detox flour chapati (WF+DF1:3), DFC 4-Detox flour chapati (WF+DF 0:3), Detox flour chapati 4c(CF+DF1:1), CF-Curd Flour.}.

The sensory evaluation and reformulation of flour mixes was carried until a satisfactory level of acceptance for all the chapati samples by the panel members. After mixing the detox flour, overall eating quality and aroma of the chapati increases due to the presence of herbs, multigrain and spices.[35]

Aroma, Taste and Mouthfeel of sample: The aroma and taste attributes of control chapati was a typical nutty and earthy but as the amount of detox flour increased, the aroma became more spicy and flavourful. At a higher level of incorporation of detox flour in DFC 3 (75%) and DFC 4 (100%), there was no significant difference between them in terms of aroma, as they both gave strong palatable, baked aroma. According to Table 2, no significant difference were observed in taste ($p = 0.841$), mouthfeel ($p = 0.58$) between DFC 2 and DFC 4C as both of them were flavourful and tasty. Furthermore the taste attribute of Control and DFC 2 chapati samples didn't differ significantly ($p = 0.145$) at ($p \leq 0.05$) but DFC 2 was the most acceptable formulation in terms of aroma, taste and overall acceptability. Similarly, adding varying proportions of pumpkin or sesame seeds to the biscuit dough enhanced the flavour, taste and protein content.[24]

Colour and Appearance of the sample:

All the samples were significantly different in terms of eating quality and appearance at $p \leq 0.05$. Increasing the detox flour ratio from control to DFC 4, there was a significant decrease in colour and appearance of the chapatis**,** primarily due to the added grains and spices. A mix of wheat and Bengal gram flour gives yellowish colour and ragi into wheat gives dark brown colour to chapati but improved protein content and higher satiety value.[21,36]

Softness and pliability: Softness and pliability decreases with an increase in detox flour which could be due to decrease in gluten and an increase in protein.[37] There was no significant difference in terms of pliability between Control and DFC 1 ($p = 0.186$), Control and DFC4C ($p = 0.146$) at ($p \leq 0.05$). However, DFC 2 and DFC 4C were softer, more flavourful and tasted better than other samples. Conversely, DFC 3 and DFC 4 showed low pliability (6.27±0.15 and 6.80±0.60 respectively) and brittle texture, caused by the addition of detox flour.

Eating Quality and Overall acceptability score: Statistical analysis from Table 2 shows that all chapati samples differed significantly in eating quality and overall acceptability, except for DFC 2 and the Control. Chapati sample DFC 2 showed most acceptable sensory score of 7.8% after control 7.9% which could be due to 50% detox flour. Similar results were found in a study where out of the eight fortified mixtures, the most acceptable composite flour blend was 51.09% wheat combined with 48.91% millets (20% finger + 20% foxtail + 8.91% barnyard millet) and 23.19 mg/g protein with highest desirability of 0.845.[22,38] Hence, optimal use of composite flours improves not only the nutritional and rheological properties of the chapati but also improves its texture and sensory attributes of chapati.

Texture profile Analysis (TPA)

Table 3 shows the results of Texture Analyzer for the 5 types of chapati samples and 1 cheela sample.

Table 3 Results of Texture Profile Analysis using TA-5 probe:

Chapati Samples	Hardness (N)	Cohesiveness	Springiness	Gumminess	Chewiness	Adhesiveness	Resilience
Control	12.95±2.35^{a}	0.91±0.14^{abc}	1.57±0.52^{d}	12.4±1.32^{b}	18.70±2.74^{b}	0.03±0.06^{ab}	0.36±0.10^{def}
DFC 1	10.3±0.64^{b}	0.95±0.08^{a}	1.12±0.12^{a}	9.84±0.76^{a}	11.00±1.51^{a}	0.07±0.06^{ac}	0.39±0.18^{cdg}
DFC 2	5.15±0.99^{d}	0.88±0.19^{bd}	1.46±0.48^{bc}	4.50±1.04^{c}	8.40±0.90^{c}	0.17±0.21^{e}	0.40±0.08^{bc}
DFC 3	9.35±0.92^{c}	0.85±0.09^{cd}	1.41±0.15^{b}	7.48±0.79^{f}	10.50±1.28^{a}	0.07±0.06^{bc}	0.33±0.07^{ae}
DFC 4	28.98±1.14^{f}	0.61±0.35^{e}	1.05±0.04^{a}	20.58±1.04^{e}	24.57±2.10^{d}	0.27±0.29^{d}	0.42±0.14^{b}
DFC 4c	2.75±0.57^{e}	0.76±0.16^{f}	1.49±0.15^{cd}	2.07±0.53^{d}	3.03±0.50^{e}	0.23±0.15^{d}	0.34±0.04^{afg}

[The values represent Mean± Standard deviation (SD) of triplicate measurements. The values with the same letter (a-g) in superscript within the same column are not significantly different from each other at $p \leq 0.05$] {Abbreviations: DFC 1-Detox flour chapati(WF+DF1:2), Control: WF-Wheat Flour, DF-Detox Flour, DFC 2 Detox flour chapati(WF+DF1:1), DFC 3-Detox flour chapati (WF+DF1:3), DFC 4-Detox flour chapati (WF+DF 0:3), Detox flour chapati 4c(CF+DF1:1), CF-Curd Flour, N=Newton.}

The control chapati required a higher tear force of 12.95 N, likely due to less fibre, high gluten and gelatinized starch molecules. Chapati sample DFC 2 had lesser gluten, requiring a lower tear force of 5.15 N. Conversely, DFC 4 contained no gluten but had the highest fibre and protein content, resulting in the highest tear force of 28.98 N. In contrast, the DFC 4c cheela was significantly softer than control, requiring the lowest tear force of 2.75 N, which could be due to addition of dehydrated curd powder to the DF in 1:1 ratio and no gluten. In the Table 3, when the amount of detox flour in Control to DFC-2 chapatis increased from 0% to 50%, a gradual decrease in hardness, cohesiveness, gumminess, and chewiness was observed. A study reported that addition of millet flour by 30% into wheat flour showed reduced level of chewiness, elasticity and extensibility with slightly brittle texture while 50% addition led to increased tear force, hardness and gumminess in the chapati samples.[39] Dried Moringa leaf powder is a good source of iron and protein (20%) but its high fibre content (12%) can increase the hardness of bread, chapatis, and biscuits if added in excess.[40] Tukey HSD test results of 'cohesiveness' shown in Figure 2 revealed that DFC 2 was significantly different at $p \leq 0.05$ with all the chapati samples except with control ($p=0.71$) and DFC 3 ($p=0.63$), which could be due to higher amount of gluten in control and fibre in later.

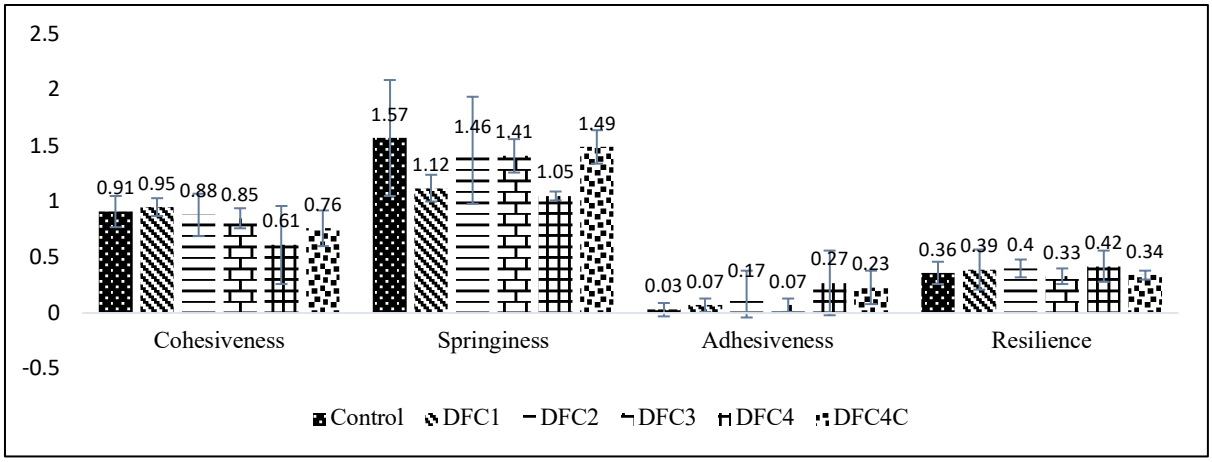


Figure 2: Texture profile analysis (TPA) of chapati samples

The springiness of Control chapati was highest at 1.57 mm shown in the Figure 2, has the capacity to regain its original shape post deformation compared to lowest springiness recorded at 1.05 mm in DFC 4 chapati. DFC 2 with ≤ 50% detox flour, showed a significant difference ($p=0.00001$) at $p > 0.05$ with all the chapati samples in terms of adhesiveness profile.

The results of Tukey HSD test for resilience profile revealed that DFC 2 was significantly different with all the chapati samples except with DFC 1 and DFC 4. TPA results revealed that DFC 2 sample, containing 50% detox flour, achieved the highest acceptability score with moderate hardness, good springiness, and optimal cohesiveness and chewiness.

Nutritional Analysis:

Analysis of 3 Amino Acids in the selected sprouted grains using HPLC method: Complex proteins break down into smaller peptides and free amino acids, upgrading protein quality and digestibility. A study by Rahman[41] observed that the amount of free amino acids such as lysine, methionine, tryptophan, and cysteine increases after germination of wheat, legumes and millets. In the Table 4, the amount of lysine in the germinated Mung flour (1623%) was significantly higher than boiled (1465%) Mung flour. Germinated mung flour showed lesser lysine than the soaked and dried mung flour(1956%) which could be due to removal of antinutrients, break down and utilization of stored nutrients, proteins and amino acids to fuel the growth of the new sprouts. Germination activates the protein breakdown into free amino acids and provides highest level of ready-to-absorb lysine. Boiling can degrade certain heat-sensitive nutrients and reduce the bioavailability of free lysine. Combining sprouted legumes and millets flour maximizes lysine and methionine because sprouting synthesizes and enhances their bioavailability. Again due to low glycaemic index, this blend also slows blood sugar spikes, making it excellent for diabetes management.[42,43]

The lysine content in amaranth millet is twice that of wheat and three times higher than maize.[44] Amaranth was included because it is a nutritional powerhouse and a complete plant protein as it contains both lysine and methionine in good quantity which makes it very close to an animal protein.

Table 4: Available Lysine and Methionine in the sprouted & Boiled grains flours

	Grains Type and their Flours after Germination and Boiling		Lysine (mg/100g)	Methionine (mg/100g)
1	Mung	So MF	1956.17±0.12	-
		Sp. MF	1623.09±0.16	-
		Sp. &B MF	1465.75±0.01	-
2	Bengal Gram	So BGF	1053.98±0.13[a]	
		Sp. BGF	1017.39±0.12[a]	-
		Sp.&B BGF	971.03±0.09[a]	-
3	Finger Millet	So FMF	84.49±0.12[b]	93.97±0.04
		Sp. FMF	94.84±0.08[b]	105.71±0.06
4	Amaranth Millet	So AMF	110.18±0.12	119.16±0.04
		Sp. AMF	180.51±0.16	365.69±0.01
5	C (Wheat Flour)	WF	242.98±0.05	85.06±0.12[b]

[So.MF: Soaked Mung Flour, Sp.MF: Sprouted Mung Flour, Sp.&BMF: Sprouted and boiled mung flour, So BGF: Spoaked Bengal gram flour, Sp.BBGF: Sprouted Bengal gram flour, Sp.&B BBGF: Sprouted & Boiled Bengal gram flour, So.FMF: Soaked Finger millet flour , Sp.FMF: Sprouted Finger Millet Flour, So.AMF: Soaked Amaranth Millet Flour, Sp.AMF: Sprouted Amaranth Millet Flour], [The values represent Mean±Standard deviation (SD) of triplicate measurements. The values with the same letter (a-b) in superscript within the same column are not significantly different from each other at $p \leq 0.05$]

The HPLC results showed decreased amounts of amino acids after boiling and drying. In this study to utilize the maximum amount of lysine and methionine, germinated and dried flours of mung, Bengal gram, finger millet and amaranth were taken not the flours after boiling the grains.

Conclusion

Wheat is naturally deficient in the essential amino acid lysine. To address this nutritional gap, a plant-based detox flour was formulated using a blend of amaranth, finger millet, pulses, legumes, oilseeds, herbs, and spices. Blending this functional mix with traditional wheat flour produced chapatis with significantly higher protein and fibre content, alongside reduced carbohydrate levels compared to standard wheat chapatis. Furthermore, sensory panellists reported that all mixed-flour chapati samples tasted better than the control. Specifically, the DFC 2 sample (a 50:50 ratio) received a highly satisfactory overall acceptability score of 7.8%. DFC 2 (50% detox flour) is the most acceptable chapati sample, offering optimal texture (moderate hardness, good springiness, cohesiveness, and chewiness) despite significant textural differences from the control ($p < 0.05$). Nutritionally, it contains 16.4% protein, 19% fibre, 3.03% ash, 7.3% moisture, and 297 kcal energy. However, increasing detox flour beyond 50% (DFC 3 and DFC 4) degrades quality, creating a firmer, harder, and gummier texture with reduced cohesiveness and elasticity due to higher fibre and lower moisture, starch and gluten. DFC 4 (100% detox flour) yields the highest protein (27.7%) and lowest moisture (6.91%). This study highlighted the health benefits of a plant-protein-rich, gluten-free detox flour that could promote endogenous carnitine synthesis if consumed in the recommended amount. In the future, incorporating detox flour into chapatis could enhance their nutritional profile as well as could treat patients with various chronic diseases.

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Conflict of Interest

The authors do not have any conflict of interest.

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