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Study on functional properties of protein-rich composite flour utilizing foxtail millet and flaxseed

Abstract

The residue after extraction of oil from flaxseed contains many nutrients and bioactive components, though it is not used that much in the development of food. On the other hand, foxtail millet is a non-conventional source of food, which can be a supplement of wheat flour or flour-made products. The objective of this work is to incorporate flaxseed oil cake into the common food with a non-conventional source of flour and also to check the functionality of differently pre-processed foxtail millet. In utilizing oil industry waste, i.e., de-oiled flaxseed, for developing a value-added product, the concept of green technology was applied. Flaxseed (*Linum usitatissimum*) oil cake flour was mixed in a 1:1 ratio with Foxtail millet (*Setaria italica*) flour, prepared in three forms: raw, germinated, and microwave treated. The study mainly focused on utilizing the non-thermal processes to investigate the functional properties of the flour blends. Also, an analysis of functionality among three forms of foxtail millet has been done in this study. The mixed flours were further evaluated for nutrient composition and functionality. The results of the developed flour blends are compared with the conventional wheat flour and raw foxtail millet flour to determine the importance of Oil Cake Waste and pre-processing of Millet. The functional properties of mixed flour increased compared with raw foxtail millet flour.

Keywords: foxtail millet, flaxseed oil cake, plant-based protein, composite flour, value addition, waste utilization

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Introduction

Food and Agricultural Organization and the United Nations have recognized 2023 as the International Year of Millet (IYM) 2023 for providing awareness about the health and nutritional benefits of millet. A plethora of health benefits of millet has been identified and found to be beneficial for consumption. In addition, the cultivation of millet in an environment with low water conditions will aim or will be aiming to create a non-conventional source of bioactive compounds with its increased production and consumption.[1] The theme of the International Year of Millet is “healthy millet, healthy people”. The high fiber content in millet helps promote digestion and lower cholesterol deposition. In 2018, millets were rebranded as nutri-cereals and declared the ‘National Year of Millet’ in India.[2] Focusing on this popularization of millet and millet-based products catering worldwide is the main purpose of this study.

Millets are among the crops that are cultivated in the arid climate of India and categorized into various types like pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), kodo millet (*Paspalum scrobiculatum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*) and many more. Due to adverse climatic conditions, water scarcity, and insect infestation, millet cultivation has become popularized. Much more research attention has been given to its nutritional and medicinal values in recent years.[3–5] The nutritional value of foxtail millet is mainly comprised of non-gluten and non-acid forming effects, and thereby easily digestible. It can address the health problems of celiac patients and is also helpful for diabetic patients by lowering their glycemic value.[6] Foxtail millet also has some phytochemicals and bioactive compounds, mainly flavonoids, essential amino acids, and various minerals (calcium, potassium, phosphorus, zinc).[7,8] Many researchers have investigated the health benefits of such a category of millet regarding hypertension, heart attack, cancer, and obesity.[9,10] The nutritional benefit of foxtail millet encompasses B vitamins and minerals – micronutrients like phosphorus, calcium, iron, potassium, and zinc.[11]

Though Flaxseed is considered a non-conventional oil seed in India, the current market has focused on extracting flaxseed oil for its bioactive components. It contains a high amount of α- linolenic acid (ALA) from about 50 to 70% of the whole fatty acid. ALA helps in resistance to the process of production and spreading of pro-inflammatory factors and improves cell membrane flexibility.[12] Flaxseed contains approximately 21% protein. It holds all types of amino acids, but lysine is limited.[13] Cysteine and Methionine reduce the risk of cancer by improving antioxidant levels.[14] The protein percentage can be increased after oil extraction, making it a good plant protein source.

Flaxseed oil cake has been utilized in some value-added products for its bioactive components.[1] The flaxseed oil cake was considered a potential source of protein isolate and hydrolysate for its therapeutic effect, and many of the functional food products like bread (10% - 15% blend utilized in flour) and cake have been developed through current research.[12,15,16] The current study has focused on utilizing oil cake residues in the composite flour blends to investigate the formulation of the blend along with a non-conventional source of proteins (millet) to compare the functional properties of the flour blends with conventional flour(wheat flour).

The utilization of oil industry waste having the goodness of bioactive components could be a challenge for making a value-added product, composite flour, which may be used in some functional foods as well as in some designer foods in the bakery and confectionary sectors. The therapeutic effect of phytonutrients in combination with flaxseed oil cake and foxtail millet (processed in different ways) might be the pathway for the development of functional /healthy foods in staple food sectors worldwide.

The functional properties of foxtail millet flour and the flour composites with de-oiled flaxseed flour have been investigated in the current research. The effect of thermal and non-thermal processing of foxtail millet has been investigated in this study. Selected processing conditions are focussed on finding out better output in nutritional effect with the reduction concept of some anti-nutrients found in millet. Protein enrichment is the main target of the development of composite flours that can be an alternative product for household and industrial use. The gluten-free flour development is also one of the objectives of the current study. The flour, as such, can be a product to be sold in the market for its health and functional benefits.

Materials and method

Preparation of composite flour

Foxtail millet and flaxseed were collected from the local market of Kolkata in West Bengal, India, and were characterized by the botanical picture of *Setaria italica.*

The millet was then washed, cleaned, and dried keeping it in open air. Next, the foxtail millet was fractionated into three parts. The first part was ground in a grinder, done in the food processing laboratory, Techno Main Salt Lake, to make flour. The second part was germinated and then ground to flour and the third part was treated in the microwave (Samsung; M/C: MW73AD-B/XTL, 800 W) for 2 minutes at 300°C and then ground to flour. All three parts were then passed through a 100-mesh sieve to get the flour.

On the other hand, flaxseed was first roasted at medium heat for 5 minutes then ground to make it powder and then de-oiled in a Soxhlet apparatus (Pelican Equipment India, M/C: SLS6) using n-hexane as a solvent for extraction. The de-oiled cake was taken and kept in the open air for 12 hours to evaporate out the solvent residues and then passed through a 100-mesh sieve to get the flour.

The formulation for making the composite flour was primarily chosen as a 1:1 ratio to find out the corresponding functional properties of differently processed foxtail millet flour.

Flow chart of preparation of composite flours

Sample 1 – Composite flour 1 (CF1)

Sample 2 – Composite flour 2 (CF2)

Sample 3 – Composite flour 3 (CF3) (Figure 1)

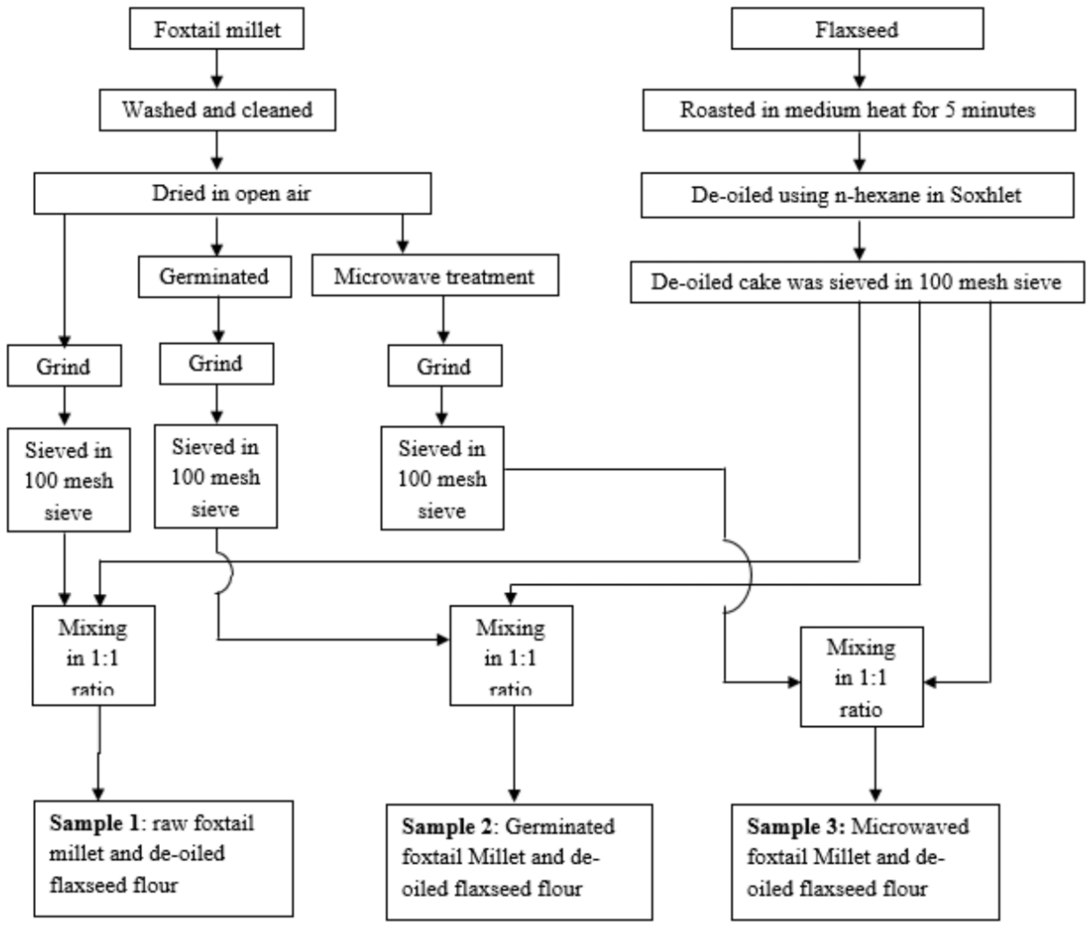

Figure 1 Flowchart of composite flour.

Proximate analysis

Analyses of these three composite flours were done as per AOAC, 2010, a standard method for moisture content, Ash, fat, protein, and crude fiber.[17] Moisture content was determined by drying the samples in a Hot Air Oven (Instruments of India, indigenous) at 105° C until constant weight of mass was seen. Ash was determined by incinerating the sample in Muffle Furnace (Instruments of India, Indigenous) at 650° C to 700° C for 6 hours. Fat Content was estimated by Soxhlet apparatus (Pelican Instrument, M/C: SLS6). Protein content was determined by analyzing the total nitrogen content by the Kjeldahl method (KEL PLUS, M/C: KES06L). The crude fiber was estimated by acid and alkali digestion processes.

The proximate analysis was done for all three samples (CF1, CF2, CF3) along with the control wheat flour (WF), Foxtail Millet flour (FMF), and de-oiled flaxseed flour (DFF).

Functional properties

Bulk density (BD): Bulk density was determined by placing flour samples in various measuring cylinders and filling them up to a predetermined level. The sample's initial weight and ultimate volume were measured at equal intervals on twenty consecutive tapes.[18]

Water holding capacity (WHC), Water absorption index (WAI), Water solubility index (WSI): The Water Holding Capacity and Water Absorption Index are two different things. Water Holding Capacity measures the amount of water that a specific measured substance can hold and Water Absorption Index measures the volume occupied by the granule after swelling in excess water.[19,20]

In a centrifuge tube 1 g of flour was added with 25 ml of distilled water and mixed using a vortex (TARSONS, M/C: 1901751, 50 Watt) thoroughly. Next, the sample was centrifuged (REMI, R–4) at 2500 g for 30 min followed by 30 min of rest. Then the soluble fraction was decanted off obtaining a residue left in the centrifugal tube.

The Water Holding Capacity was calculated by WHC = $(W_2 – W_1)/W_0$, Where W_0 = The weight of the dry sample (gm), W_1 = The weight of the tube with the dry sample (gm), and W_2 = The weight of the tube with sediment

The Water Absorption Index was expressed as WAI = Weight of sediment/Weight of dry solid.

The Water Solubility Index was determined as WSI = (Weight of dissolved solid in supernatant/weight of dry solid) ×100

Oil absorption capacity: Oil absorption capacity measures the volume of oil absorbed by the sample and it was determined using 1g of sample with 10 ml of refined soyabean oil (Fortune, Adani brand. Density 0.91 g/ml) in a centrifuge tube. Then the sample was mixed using vortex and centrifuged at 2500 g for 30 min. The supernatant was measured using a 10 ml volumetric measuring cylinder as ml of oil absorbed by 1gm of the sample.[19]

Foaming capacity: Foaming capacity was determined by a little modification of Lawhon et al., as 0.5 g of the sample was dissolved in 20 ml water and stirred in a Magnetic stirrer (Eltek, M/C: 1DM1114, RPM - 5000) initiated with 5 min and continued for 15 min. With 5 min intervals, the observations were recorded.[18]

Gelation property: The gelling property was checked using approximately 0.7 g of sample and 5 ml of water. Mixed properly then heated in a water bath at 60° centigrade for 1 h and froze at 4° C immediately for 12 h and checked the gelling properties.[18]

Estimation of protein by Lowry method: The estimation of soluble protein content was measured using the Lowry Method.[31] The peptide bonds in the polypeptide chain will react with copper sulfate (in alkali) and give rise to a blue-colored complex. Folin-ciocalteau reagent is used to give a bluish product which contributes to the enhanced sensitivity of this method.

The sample preparation was done by using a 0.2 g composite flour sample with varied solvents like water, water, and salt solution to have a view of the soluble protein character of the flour composites. The samples were kept for soaking in the said solvent for 1 h then filtered and filtrates were collected for analysis. The final estimation was done using a known concentration of BSA (Bovine Serum Albumin) protein in preparing a standard calibration chart, wherein the X axis of the known concentrations of BSA were plotted, and against each plot in the Y axis the absorbance of the corresponding plot was measured using UV visible spectrophotometer following.[21]

Sensory analysis: All three samples of flour were served to 5-panel members of the Food Technology Department of Techno Main Salt Lake for sensory evaluation as per the standard hedonic scale rating from 9 (like extremely) to 1 (dislike extremely), according to Saleem-ur-Rehman et al.[22]

Results and discussion

The moisture percentage of raw foxtail millet was estimated as 11.12% whereas the moisture percentage of de-oiled flaxseed flour was found to be 7.5%. The flour blends (1:1 mix) were shown with variations of moisture with reduced content. This might be due to the different operational processes that were carried out for making foxtail and de-oiled flaxseed flour such as microwave treatment, roasting operation, soaking, and germination followed by a trey drying operation. The proximate analysis of determining crude protein percentage in flour blends showed heterogeneous values in comparison with foxtail flour and de-oiled flaxseed flour. The composite flour blend CF1, CF2, and CF3 showed significantly higher values concerning crude protein content compared with wheat flour (8.2%). The result may be compared with the flour blend made up of wheat flour with seed protein as referred by Roy Chowdhury et al.,[18] All the composite flour blends are rich in crude fiber and also contain an admissible amount of fat and ash content as shown in Table 1 and 2.

Table 1 Proximate analysis of wheat flour, foxtail millet flour and flaxseed oil cake

Parameter	Wheat flour	Foxtail millet	Flaxseed oil cake
Moisture content (%)	10.8	11.12	7.5
Ash content (%)	0.54	2.1	3.9
Fat content (%)	1.2	4.2	5.2
Protein content (%)	8.2	12.19	34.32
Crude fibre (%)	0.75	4.25	4.5

Table 2 Proximate analysis of composite flour (CF1, CF2, CF3)

Parameter	CF 1(wet basis)	CF1 (Dry basis)	CF 2(wet basis)	CF2 (Dry basis)	CF 3 (wet basis)	CF3 (Dry basis)
Moisture content (%)	4.8	5.1	5.1	5.46	4.9	5.17
Ash content (%)	3.1	3.41	2.9	3.21	2.73	2.95
Fat content (%)	4.6	4.96	4	4.3	4.5	4.8
Protein content (%)	22.66	24.44	22.63	24.54	26.95	29.1
Crude Fibre (%)	6.5	7.01	6.6	7.15	7.1	7.6
Carbohydrates	58.34	55.08	58.77	55.34	53.82	50.38

Table 3 illustrates the fundamental properties of the foxtail millet along with their composite flours concerning water absorption index, water solubility index, and water holding capacity. The water holding capacity, water absorption index, water solubility index, and oil absorption capacity are analyzed following A dwiani et al[19]. and Nargis Yousf et al.[20] Functional properties are physicochemical properties that reflect the complex interactions among the molecules in composition, their structures, and related molecular conformations. The food components or the food matrix and their behaviour with water, were investigated following some standard methods, that will be associated with industrial use. The characterization of food components in a composite flour is needed as it will predict how the new matrix with the combination of new protein, fat, fiber, carbohydrate, and minerals and their behavior in a specified environment might have some changes due to variations of process parameters.[23,24] The food properties related to structure, nutritional value, and overall quality and acceptability of a food product also can be judged in relation to the functional property of the flour blend. The water absorption index is defined as the amount of water taken up by the flour to get consistency and to create a quality product.[25] The high-water absorption value indicates the presence of more hydrophilic components in the flour matrix. Polysaccharides, a hydrophilic protein, can interact with water in foods. The increased value of WAI is associated with a high amount of starch (amylose with a higher solubility). The high-water absorption values sometimes can lead to loss of starch crystalline structure. Thilagavathi et al.[26] has shown the water absorption capacity of various types of millets. The comparison data of millet, wheat, and soybean flour was investigated and found as 74.08ml/100, 74.08 to 78.83 ml/100 g, and (58.17-60.02 ml/100 g) for millet, wheat, and soybean flour, respectively. The observed WAI values for foxtail millet in all categories of flours are shown in Table 3, which are comparable more or less with the data shown in T. Thilagavathi et al.[26] The varying data for composite flour is mostly due to the different protein combinations and their variation in concentration level, which is affected by the degree of interaction with water. The conformational behavior of protein and its structure are reflected in water absorption capacity. The results are in tally with Table 3.

Table 3 Functional properties: bulk density (BD), water absorption index (WAI), water solubility index (WSI), water holding capacity (WHC), oil absorption capacity (OAC)

Parameter	Bulk density	Water absorption index	Water solubility index	Water holding capacity	Oil absorption index
CF1	0.42	3.6	10	2.6	1.99
CF2	0.41	3.7	8.51	2.7	2.08
CF3	0.44	3.8	9.68	2.85	2.2
Raw foxtail flour	0.62	2.303	4.95	1.3	2.13
Germinated foxtail flour	0.64	2.325	1.768	1.32	1.54
Microwave foxtail flour	0.65	2.369	5.047	1.37	2.3

The entrapment of oil in a flour matrix is an important physical attribute since oil acts as a flavor retainer.[27] Oil increases the test of food for consumers. The mellowing effect of a finished food product can be achieved with a good oil absorption capacity. The investigation of the oil absorption capacity of millet flour and composite flour has been conducted by various researchers with varied results. According to Amir et al.[28] Finger millet flour showed oil absorption of 1.93 gm/gm while Pearl millet showed 1.60gm/gm. In our investigation the result obtained as CF1 is 1.99, CF2 is 2.08 and CF3 is 2.20 and 2.13, 1.54, and 2.3, respectively for raw foxtail millet flour, germinated foxtail millet flour, and microwaved foxtail millet flour which are comparable with the data provided by Amir et al.[28] The organoleptic characterization of the flour and flour blends can influence the flavor and fat content that is directly related to the high value of oil absorption.

Generally, the bulk density of the flour is measured by the heaviness of the flour along with the particle size. For industrial purposes, the bulk density is to be determined for packaging requirements, handling of raw materials concerning process operation, handling wet processing in the food industry, and many more. The bulk density investigated in the present study can be reported as CF1 0.42, CF2 0.41, and CF3 0.44 and 0.62, 0.64, and 0.65, respectively for raw foxtail millet flour, germinated foxtail millet flour, microwaved foxtail millet flour. The recorded bulk density values are comparable with pearl millet and finger millet with slight deviation, pearl millet 0.67 g/ml to 0.54 g/ml. Our observed data for bulk density shows a little difference in value for different varieties of millet which may be correlated with the observed data of Krishnan et al.[29] The microwave treatment (100 mesh) of foxtail millet showed a higher value than the composite flours.

Foam capacity and foam stability both are calculated by the entrapment of air into the flour slurry which results in the destabilization of the liquid surface where the flour has been held. As the surface tension of the water matrix decreases, foam formation will be increasing. The foam formation and its stability will be guided by the air-water interface. Good foaming can be formed if the soluble protein content becomes higher and protein can unfold or rearrange its structure in the water interface. As a result, the foam formation will be enhanced.[30] The investigation was done to find out the foam capacity and foam stability for the above-said millet and their composites following the method of Roy Chowdhury et al.[18] The distinct observation of the formation of foam was found to be generated, but bubbles were unstable to sustain in the form of foam even though the time duration for agitation was varied starting from 5 to 15 min with 250 rpm. The observation is tabulated in Table 4. Foxtail millet contains a high amount of protein and flour blends contained an appreciable amount of protein, still, the foam formation was hard to form which indicates the water-soluble fraction of foxtail millet protein is not in good amount with their hydrophilic characters as per the methodology followed by. Hence the foam-forming ability and the process parameter by which the protein could be solubilized with their changed conformation (hydrophilic group to be exposed) could lead to better foam capacity. This has to be focused more thoroughly.

Gelatinization temperature for all flours and flour samples was investigated with different percentages of flour to study the least gelation property. A significant level of variation was observed for composite flour at a temperature range of 70° -75° C. The freeze and thawing study were undergone to have a better view of gelatinization and post-gelatinization effect followed by the gel stability. The gelatinization temp mostly characterizes the fate of starch molecules in a food matrix and their out pattern from the granular to the swollen phase, followed by their bursting out to form and normalize the gel network along with the other components present in the matrix.[31] According to Chandra et al.[32] flour that has higher starch content takes a lower temperature for gelatinization than those with lower starch content takes a higher temperature (Table 5).

Table 4 Foaming property

Parameter	Foaming property			
	5 min	10 min	15 min	0.5gm of salt
CF1	Bubble formation observed but not very stable	No layer was formed along with the foam	Proper foam formation not observed	Foam was observed but not satisfactorily measured, and foam was not stable
CF2	Bubble formation observed but not stable	No distinct layer was formed along with the foam	Proper foam formation not observed	Distinct layer of Foam was not observed in a quantifiable value.
CF3	Initiated with bubbles but could not stay.	No persistent layer was formed along with the foam	Proper foam formation not observed	No persistent foam was observed.
Raw foxtail flour	Bubble formation observed, not very stable	No layer was formed along with the foam	Proper foam formation not observed	Foam was observed not satisfactorily measured, and foam was not stable
Germinated foxtail flour	Bubble formation observed, not very stable	No layer was formed along with the foam	Proper foam formation not observed	Foam was observed not satisfactorily measured, and foam was not stable
Microwave foxtail flour	Bubble formation observed, not very stable	No layer was formed along with the foam	Proper foam formation not observed	Foam was observed not satisfactorily measured, and foam was not stable

Table 5 Gelation property of composite flours (+ indicates formation, - indicates not formed)

Flour type	Gelation property	Gel stability
CF1	+	+
CF2	+	+
CF3	+	+
Raw Foxtail flour	-	-
Germinated foxtail flour	-	-
Microwave Foxtail flour	-	-

The soluble protein content of the composite flours is shown in Table 6 and the corresponding data for CF1, CF2, and CF3 are in close proximity with each other. This reflects that the soluble protein components (hydrophilic character of protein in water and salt soluble fraction) are not in very significant amounts. The conformational part of the composite protein flours is much more rigid in characters concerning their hydrophobicity.

Table 6 Estimation of soluble protein content

Sample name	Weight of sample	Soluble protein content(µg/ml)
CF1	0.205	136
CF2	0.207	136.8
CF3	0.216	138.2

The organoleptic characters of the composite flour were evaluated with respect to the sensory scores based on the parameters of color appearance, flavor, texture, and taste which have been reported in Table 7. The sample CF3 which is microwave-processed flour showed the best character among all. There were not any prominent differences in those parameters for example CF1 and CF2. The non-thermal processing (microwaved processed foxtail millet flour gave a better sensory quality when blended with de-oiled flaxseed flour. The protein content of the plant-based product along with its flavor and taste may be utilized in the development of protein-rich plant-based products along with all organoleptic goodness. Thus, the non-thermal processing could be the alternative process methodology which may raise the better composite flours for designing future food. Among the above said processes microwave processed flour would be a good option to develop the flour composite.

*All the data are taken in triplicate and their mean is shown in the table.

Table 7 Sensory analysis

Parameter	Colour	Appearance	Flavour	Texture	Taste
CF 1	7	7	6.5	7	6
CF 2	7.5	7	6	6	6.5
CF 3	7.5	8	7	8	6.5

Conclusion

The investigation of the current study reveals that the processing has an effect on composite flour-making concerning the protein content, protein solubility, water absorption index, water solubility index, water holding capacity, foam capacity, gelling property, and gel stability. The investigation suggests that the composite flour CF3 may be rated as the best variety among three processed flour CF1, CF2, and CF3 with respect to all the said functional properties. The non-thermal technique, microwave processing, which has been applied for the current research could be the alternative processing method for the development of composite flours with foxtail millet and de-oiled flaxseed flour. The flour as such can be sold in the market as protein-rich blended flour for developing or designing future food for protein nutrition with all sensory attributes.

Though flaxseed and foxtail millet both are more costly compared to other conventional sources of flour, they are more nutrient-rich as well. The importance of the cultivation of such non-conventional sources can lead to generating protein-rich plant-based products which may reduce the cost of the production of final finished products. The unaccountable change in climate and fickle weather conditions are affecting the regular cultivation process, whereas these non-conventional sources are much more tolerant of harsh weather and easy cultivation. The utilization of food industry waste in the value-added product (CF1, CF2, CF3) would be a newer choice for protein nutrition for the sustainable world and its economy.

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None.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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