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Optimization of soaking duration to improve mineral bioavailability, functional properties, and product development potential of sorghum

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Abstract
Sorghum is a climate- resilient and gluten- free millet with high nutritional potential. The present study evaluated the effect of soaking on the nutritional quality, antinutritional factors and functional properties of sorghum grains, followed by the development of value- added products. Sorghum grains soaked for 24 hours exhibited improved protein and fat content, enhanced extractable calcium and iron levels, and reduced phytate and tannin content compared to grains soaked for 12 and 36 hours. Soaking for 24 hours reduced water and oil absorption and slightly lowered swelling power due to changes in starch-protein interactions and protein structure. Functional properties were favourably modified, indicating suitability for product formulation. The flour of 24hours treated sorghum was used for developing two products: tomato khakra *s* and beetroot *muthiya*. Sorghum incorporation at 50% was found most acceptable and selected as the base formulation. Tomato and beetroot powders were added in the products at 5%, 10% and 15% levels. Sensory evaluation, based on 9- point hedonic scale, revealed khakra *s* and *muthiya* with 10% tomato and beetroot respectively, incorporation to have highest mean overall acceptability mean score (>8.0), while higher levels slightly reduced sensory scores. Optimized soaking combined with moderate vegetable incorporation enhanced nutritional quality and sensory acceptability of the developed products, thereby promoting the sorghum- based value-added products.

Keywords: Antinutritional factors, functional properties, product development, sensory evaluation, sorghum

Introduction
Millets are one of the most nutrient dense crops, having rich nutritional profile and several benefits in curing diseases. They are small sized cereal food crops that belongs to the *Poaceae* Family. They are known as 'Nutri-cereals' due to their rich nutritional profile and high resilience to climate change. While millets are abundant in both macro and micronutrients, their health-promoting properties are primarily attributed to their micronutrients content, which includes vitamins, minerals, antioxidants, flavonoids, and polyphenols.
Millets are also regarded as the suitable food crops for future human consumption due to their high tolerance to drought and hot temperature and required minimal input requirements during cultivation (Aljobair 2022) [5]. These ancient millet grains are used for the preparation of various recipes in the developing countries; however, they are still majorly used as animals feed in the developed countries (Ahmed *et al*.,2018) [4].
Sorghum is a significant crop cultivated globally for food, feed and fuel. It thrives mainly in arid to semiarid tropical and subtropical regions, showcasing a wide range of morphological and genetic diversity that allows it to adapt to various growing conditions. The crop's major use includes human consumption and livestock feed (Mehl, 2024) [20]. Due to water shortage, global warming, population growth, and health concerns about gluten-containing foods, the popularity and importance of sorghum and its products have greatly rise in recent decades.

Health Benefits of sorghum
Potential health benefits of sorghum include low glycemic index and anti-diabetics property that may be helpful in reducing the postprandial blood glucose level and glycosylated haemoglobin. If consumed daily, it helps in control of diabetes mellitus, obesity, and hyperlipidemia (Frankowski *et al*.,2025) [13]. Sorghum, being naturally gluten – free, serves as an excellent alternative for individuals with celiac diseases which is an immune mediated intestinal disorder tiggered by the ingestion of gluten found in rye ,wheat, and barley (Brites *et al*., 2018) [9]

Effect of processing on sorghum
Processing of sorghum involves various methods that significantly impact its bioactive compound, nutritional profile and anti-nutritional factors. These processes enhance its nutritional value and biological activities, making it more suitable for consumption and industrial applications.

Significance of the study
This study offers scientific evidence regarding the effects of soaking during on the nutritional composition, antinutritional factors, and functional properties of sorghum grains. Determining an optimal soaking condition that enhances mineral bioavailability and reduces antinutrient levels, can make the grains more suitable for consumption. The study supports utilization of processed sorghum as a functional and gluten - free ingredients for developing value- added food products and for promoting millet-based diets for sustainable nutrition and health.

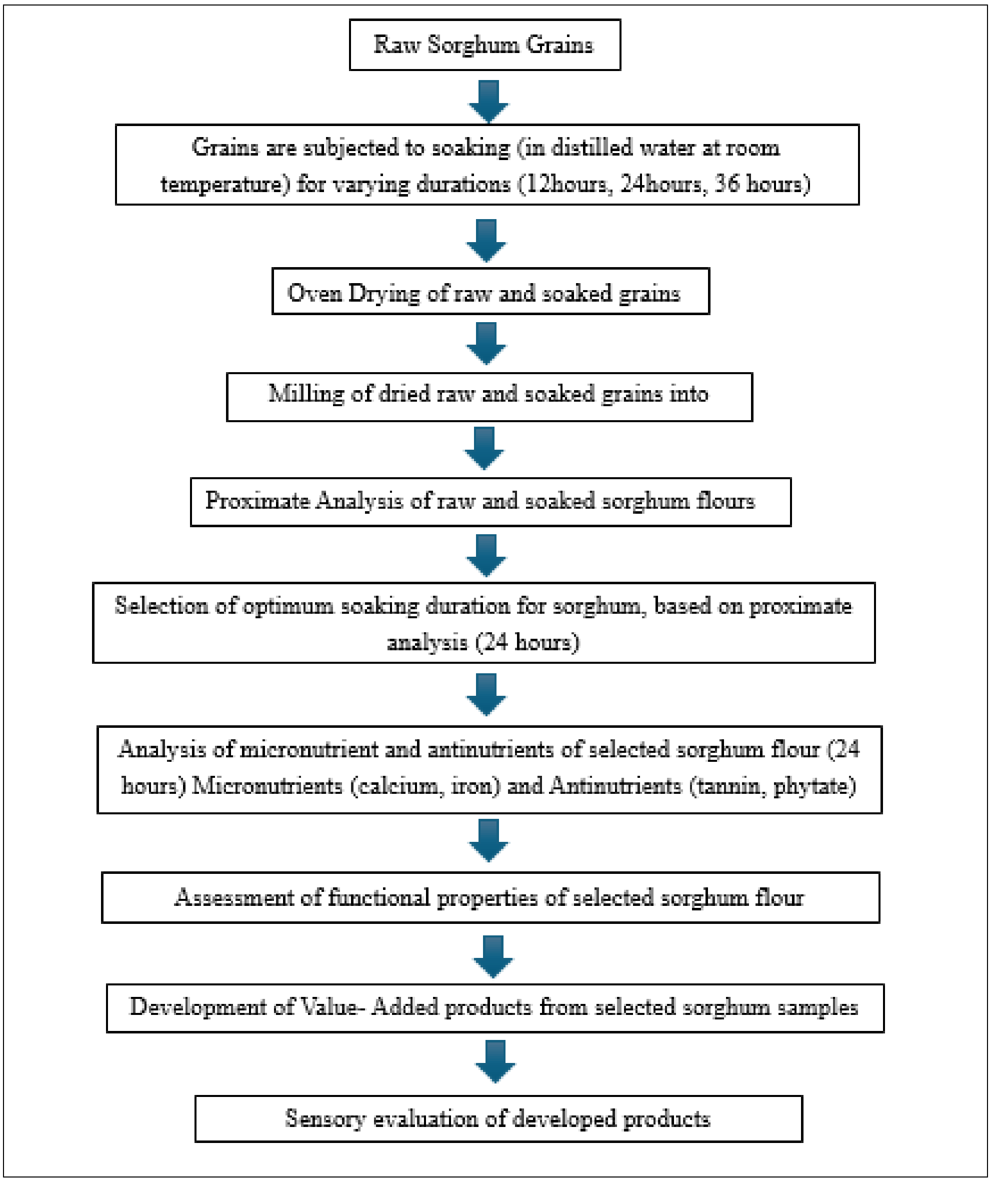
Objectives of the study

This study was carried out to achieve the following objectives:

- To analyze nutritional and antinutritional components of raw and processed sorghum grains.
- To evaluate the functional properties of nutritional superior processed sorghum grains.
- To develop and carry out sensory evaluation of sorghum-based food products.

Methods and Materials

Study Design



1. Sample Preparation

Sorghum grains were obtained from the retail market in Jaipur city and thoroughly cleaned to eliminate dust, hair, wilted grains, and other impurities. The grains were then subjected to soaking in distilled water for different durations-12 hours (as per Eltayeb *et al.*,2015 and Fadlalla *et al.*,2026), 24 hours (Baig *et al.*,2018[7] and Afify,2012), 36 hours (Baig *et al.*,2018) [7]. After soaking, grains were dried in a hot air oven for 90 minutes at 80°C. The processed and dried powered were then estimated for macronutrients to determine the best-performing processed stage.

2. Chemical analysis of samples

Raw and processed grains were ground into flour and then they were analyzed proximate composition (moisture, ash, crude protein, crude fat, crude fibre), minerals (iron, calcium) and antinutritional component (tannin, phytate) using standard analytical methods (AOAC,2023). The data obtained were statistically analyzed, and the differences were considered significant at 5% level of significance. Carbohydrate content was calculated by difference using the formula: Carbohydrate (%) =100- (moisture + ash + protein + fat + fibre). The estimations were carried out in the Research laboratory of the Department of Home Science, IIS (deemed to be University), Jaipur, Rajasthan.

3. Sensory evaluation

Organoleptic evaluation of the formulated products was performed by a panel of 10 semi-trained judges. The organoleptic evaluation with respect to flavour, colour, taste, texture, and overall acceptability was carried using nine-point hedonic scale.

Results and Discussion

1. Nutrient Analysis

Table 1: Mean proximate content of raw and processed sorghum grains

Nutrients (g/100g)	Raw grains	Processed grains		
		12 hours soaking	24 hours soaking	36 hours soaking
Moisture (%)	8.29±0.45	30.65±0.42	33.40±0.34	34.19±0.06
Ash	1.67±0.54	1.46±0.03	1.28±0.034	1.27±0.43
Crude Fat	2.08±0.28	4.40±0.34	8.44±0.75	6.04±0.87
Crude Protein	8.88±0.22	14.79±0.61	11.5±0.44	10.40±0.44
Crude Fibre	6.2±0.62	2.39±0.34	2.02±0.66	1.85±0.53
Carbohydrate	61.24±0.66	46.31±0.02	42.18±0.28	46.25±0.32

Moisture Content

The results revealed mean moisture content to increase substantially from 8.29g/100g in raw sorghum grain to 30.65, 33.40, and 34.19g/100g after 12, 24, and 36 hour of soaking, respectively (Table 1). This increase is due to water absorption by the grain during soaking, resulting in hydration of starch granules and softening of the endosperm. Similar increases in moisture content from 9-11% to 29-36% after soaking, depending on soaking duration and conditions have been reported in sorghum by Keyata *et al.*, (2021) [17] where pre-milling soaking treatments enhanced water uptake and altered grain structure (Javed *et al.*,2025) [16].

Ash Content

The estimation of ash content revealed its mean value to reduce from 1.67g/100g in raw grain to 1.27-1.46g/100g with increase in soaking duration (Table 1). Studies by Keyata *et al.*, (2021) [17] & Afify *et al.*, (2012) have shown that ash content in raw sorghum grains to range from 1.6 to 2.0g/100g, while on soaking this amount reduced to approximately 1.2-1.5g/100g, which is in line with the present study. This reduction is primarily due to the leaching of water-soluble minerals into the soaking water such as potassium, magnesium, and phosphorus. However, it has been reported that soaking may improve mineral bioavailability due to phytate degradation (Javed *et al.*, 2025 & Keyata *et al.*, 2021) [16, 17].

Crude Fat

The results showed mean crude fat to increase from 2.08g/100g in raw grains to 4.40g/100g after 12-hour soaking, followed by a decline with longer soaking (Table 1). The initial increase can be explained by a relative concentration effect resulting from reduction in carbohydrate and fibre content. The subsequent decrease may be due to lipid hydrolysis or leaching during prolonged soaking (Indrianingsih *et al.*, 2023) [15].Reported values indicate that crude fat content in raw sorghum grains generally ranges between 2.0 and 3.4g/100g, and short-duration soaking can increase it to about 3.8-4.8g/100g, which could be due to improved extractability, followed by a decline with longer soaking periods (Indrianingsih *et al.*, 2023[15] & Afify *et al.*, 2012).

Crude Protein

Crude protein content showed an increment from 8.88g/100g to 14.79g/100g after 12 hours of soaking, which however decreased with extended soaking (Table 1). The initial increase is associated with breakdown of protein-phytate and protein-tannin complexes which might have improved protein extractability. However, the decrease at 24 and 36 hours of soaking might be due to protein solubilization and loss into soaking water. Similar trends have been documented in soaked sorghum grains by Dey *et al.*, (2025) & Keyata *et al.*, (2021) [10, 17].

Crude Fiber

The estimation of mean crude fibre exhibited a substantial decrease in its value from 6.20g/100g in raw grain to 1.85-2.39g/100g after soaking (Table 1). This reduction is attributed to the softening of cell wall components and leaching of soluble fibre fraction during soaking. Sorghum studies consistently reported fibre reduction following wet processing (Huirem & Sahoo, 2024) [14]. Also, similar trends have been discussed in proximate studies on millets during traditional processing by Dey *et al.* (2025) [10].

Carbohydrates

The results of carbohydrate content showed a decrease from 61.24g/100g in raw grain to 42.18-46.31g/100g after soaking (Table 1). This decrease could be due to the loss of soluble sugars and partial starch solubilization into soaking water. Similar reductions in carbohydrate content after soaking (60-72g/100 to 43-50g/100g) have also been reported in various studies (Javed *et al.*,2025; Indrianingsih *et al.*,2023 & Keyata *et al.*,2021) [15, 16, 17].

2. Selection of processing duration (soaking) based on proximate analysis

Based on the proximate analysis, sorghum soaked for 24-hour exhibited a nutritionally improved profile, with higher protein and fat content, reduced carbohydrate level. This sample was subsequently analyzed for micronutrient composition and antinutritional factors, and the resulting flour was evaluated for its functional properties.

3. Mineral content of the selected processed sorghum grains

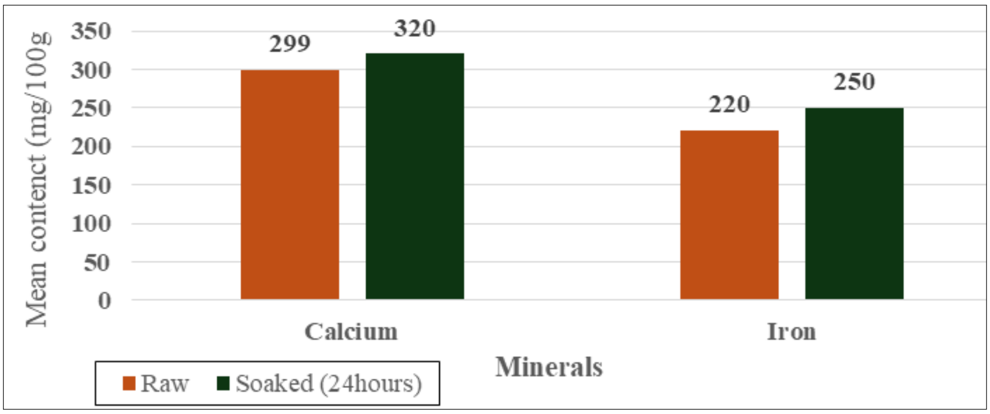


Fig 1: Mean mineral content of raw and processed (soaked) sorghum

Soaking for 24 hours resulted in a significant ($p<0.05$) increase in the mean content of extractable calcium (from 299 to 320 mg/100g) and iron (from 220 to 250mg/100g) in the sorghum sample (Figure 1). This increase reflects processing-induced alterations in the physicochemical structure of the grain rather than a true increase in total mineral content. Water uptake during soaking causes softening of cell walls and partial dissociation of protein-mineral and phytate-mineral complexes, which significantly($p<0.05$) enhances mineral solubilization and release during analytical extraction (Kruger *et al.*, 2014) [18]. Similar trends have been observed in a study by Kumar *et al.*, (2024) [19] where soaking for 24-48 hours significantly improved mineral availability ($p<0.05$). Iron availability increases from 29.1% in raw millet to 37.4% while phytic acid content decreased from 1.92 mg/100g to 1.12mg/100g on soaking.

4. Anti nutrient content of the selected processed sorghum grains

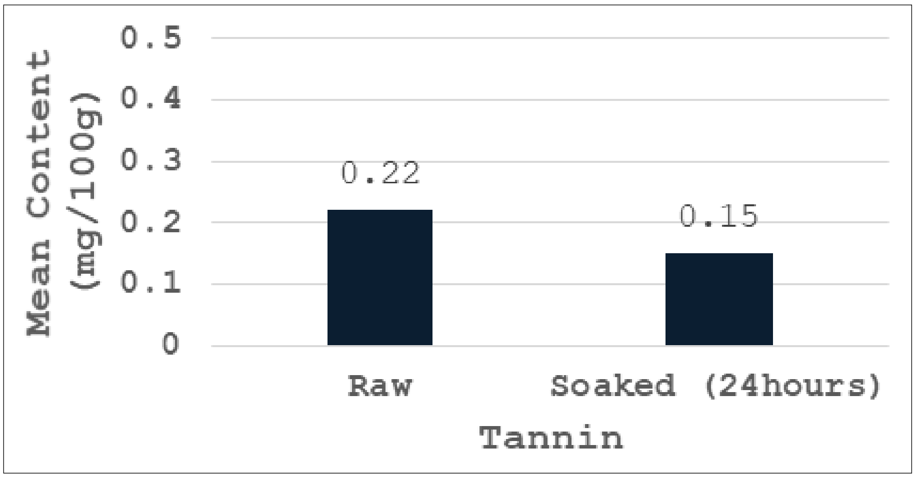


Fig 2: Mean tannin content of raw and processed sorghum

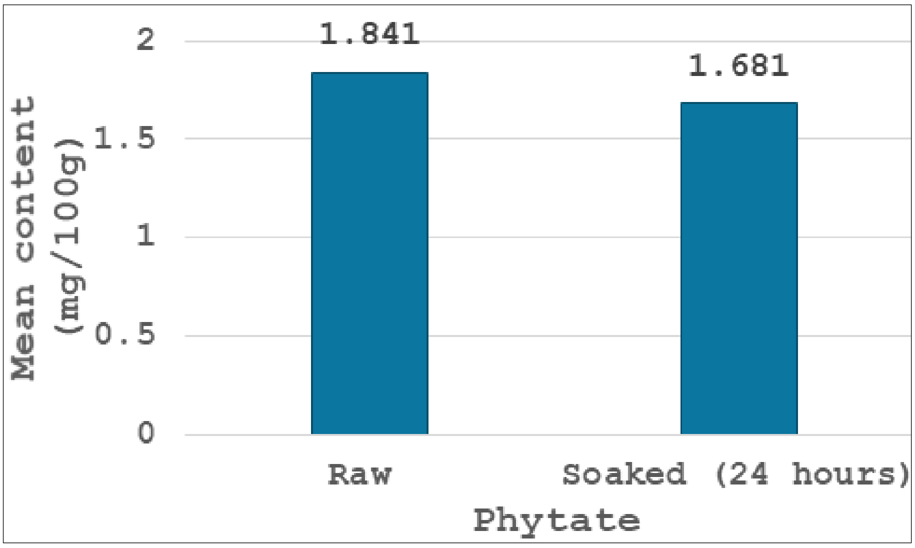


Fig 3: Mean phytate content of raw and processed sorghum

The significant reduction (p<0.05) in antinutritional factors further explain the enhance mineral extractability, as tannin content decreases from 0.22% to 0.15% (Figure 2) and phytate content from 1.84 to 1.68mg/100g following soaking (Figure 3). These results are consistent with study those reported significant reduction (p<0.05) in tannins and phytic acid due to leaching into the soaking medium and activation of endogenous phytase enzyme responsible for phytic acid hydrolysis (Adeyanju *et al.*, 2025 & Singh *et al.*, 2025) [10, 23]. In pearl millet and sorghum millet, soaking for 24 hours resulted in significant decrease (p<0.05) in phytic acid (up to 38%) and tannins (up to 42%), accompanied by corresponding significant improvements (p<0.05) in in-vitro iron and zinc bioavailability (Singh *et al.*, 2025) [23]. Since phytic acid and tannins strongly chelate divalent minerals such as iron and calcium forming insoluble complexes that limit absorption, their reduction significantly increases (p<0.05) the pool of unbound, extractable minerals (Adebo & Oyeyinka, 2023) [15].

5. Functional properties of the selected processed sorghum flour

Table 3: Mean functional properties of the processed sorghum flour

Parameters	Raw sorghum flour	Soaked sorghum flour (24 hours)
Bulk Density(g/ml)	0.70±0.02	0.82±0.012
Water Absorption capacity (g/g)	149.712±0.02	132.114±0.11
Oil Absorption capacity (g/g)	109.3±0.21	82.977±0.32
Swelling Power (g/g)	5.5±0.27	5.32±0.31

Bulk density

Table 3 shows that soaking for 24 hours significantly (p<0.05) altered the functional properties of sorghum flour. Bulk density increased from 0.70±0.02g/ml in raw flour to 0.82±0.012g/ml after soaking, likely due to hydration-induced structural rearrangement and improved particle packing. Similar increases in bulk density after soaking have been reported in sorghum and proso millet flours, where values increased from approximately 0.62-0.68g/ml in raw samples to 0.75-0.85g/ml following hydration treatments (Adebowale *et al*., 2010; Sharma *et al.*, 2025) [1].

Water absorption capacity

Water absorption capacity reduced significantly (p<0.05) from 149.7±0.02%in raw flour to 132. ±11%after soaking (Table 3). Comparable reduction have been reported in millet flours, where water absorption reduced from about 155-165% to 125-140% following soaking mainly due to leaching out of soluble components and modification of starch- protein interactions (Bhosle *et al., 2024;* Kumar *et al.*, 2024) [8, 19].

Oil absorption capacity

Oil absorption capacity was also reduced significantly (p<0.05) from 109.30±0.2%in raw flour to 82.98±0.32% after soaking (Table 3). Similar treads have been observed in millet – sorghum composite flours, where oil absorption declined from approximately 105-120% to 80-95% after soaking, which has been attributed to protein conformational changes and reduced hydrophobic binding sites (Adeyanju *et al*., 2025; Sharma *et al*., 2025) [10].

Swelling power

Swelling power decreased slightly but significantly (p<0.05) from 5.50±0.27% to 5.32±0.31% after soaking (Table 3). Adebowale *et al*. (2010) reported comparable reductions in sorghum starch swelling power (from~6.1 to 5.2–5.5), and similar decreases have been noted in soaking finger millet flours (Kumar *et al.*, 2024) [19].

Overall, the significant changes (p<0.05) in functional properties indicate that 24 hours soaking effectively modifies sorghum millet flour for food applications. The soaked flour, with higher bulk density and lower water and oil absorption capacities, is suitable for bakery products, porridges, complementary foods, and low-fat formulations, while raw flour may be preferred where higher hydration is required.

6. Sensory evaluation of value-added food products developed using processed sorghum flour

Sensory evaluation is a critical aspect of products development, as it helps determine consumer acceptability based on organoleptic properties. A semi-trained panel of judges assessed the sample, using a 9-point hedonic scale, focusing on attributes such as appearance, texture, taste, colour, and overall acceptability.

Two products are formulated, using processed sorghum flour, namely tomato khakra *(*roasted) and beetroot *muthiya* (steamed) to which pulses and dried vegetables powder were added to enhance their nutritional quality.

Tomato khakra

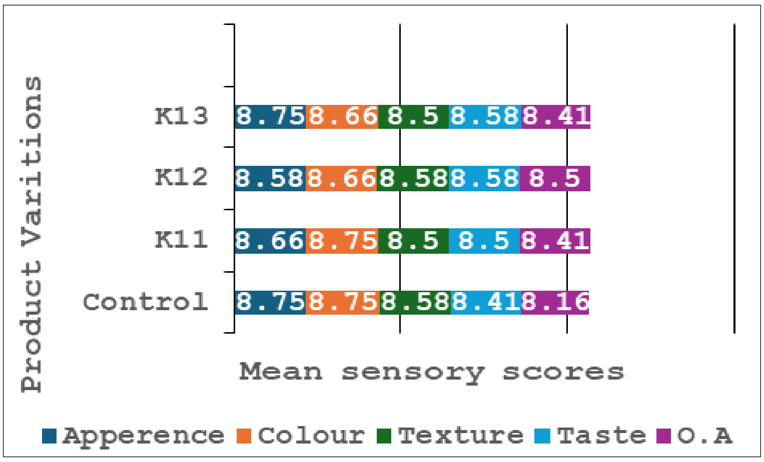


Fig 4: Mean Sensory Scores of Tomato khakra

Fig 5: Different variations of Tomato khakra

To the basic recipe of khakra *prepared* using standard wheat-chickpea flour formulation sorghum flour was incorporated in varied proportions, i.e. 25%, 50%, and 75 %(Figure 5). The various having 50% of sorghum flour was found to be the most acceptable one and was selected as the control. To this, tomato powder was added in three proportions- 5% (K11), 10% (K12), and 15% (K13). The sensory evaluation results revealed mean scores for overall acceptability to be highest (8.5) for sample K12 (50%). For texture and taste, the highest scores were obtained by K12, with mean value of 8.58 for both the attributes. The highest mean scores for appearance and colour were observed in sample K13 (8.75), containing 75% sorghum flour, and K11 (8.75), containing 25% sorghum flour, which were comparable to the control sample (8.75) (Figure 4). Rehal *et al.*, (2022) [22] reported that 10% tomato pomace incorporation in gluten-free snacks yielded the highest overall acceptability, while higher levels reduced texture and flavour scores due to increased fibre content. Similarly, Yagcie *et al.*, (2022) observed that extruded snacks containing around 10% tomato powder achieved better sensory scores compared to higher inclusion levels.

Beetroot *Muthiya*

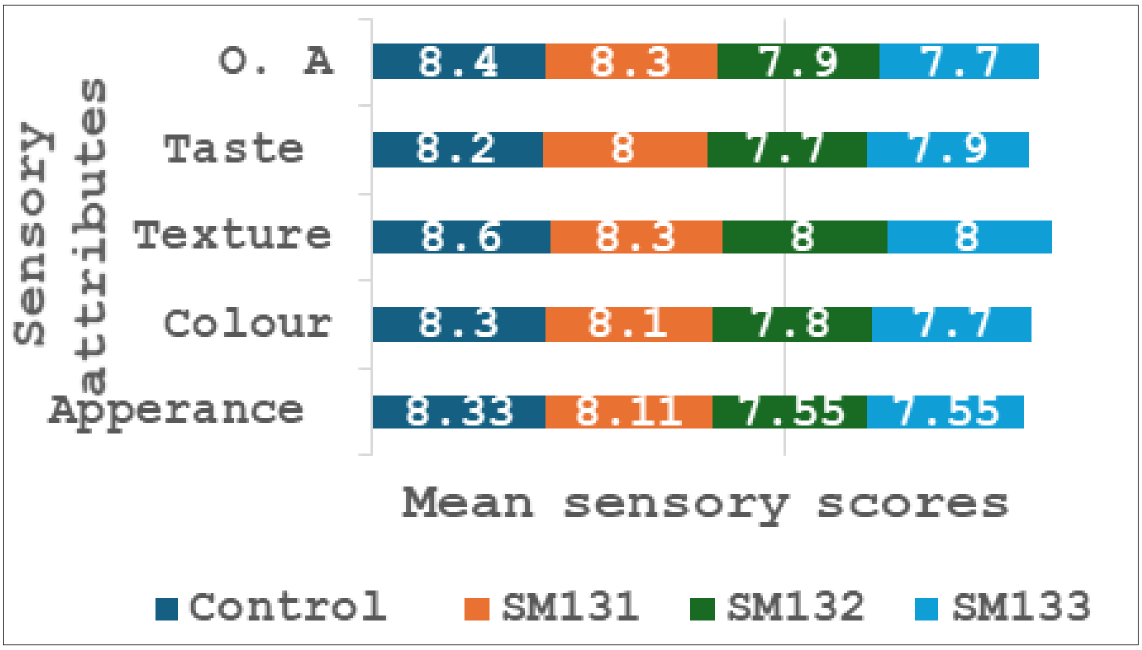


Fig 6: Mean sensory scores of Beetroot muthiya

Fig 7: Different variations of Beetroot muthiya

The control and three beetroot-incorporated variations of sorghum *muthiyas* (SM131, SM132, SM133) were assessed using a 9-point hedonic scale (Figure 7). The results demonstrated that beetroot incorporation influenced all the sensory attributes, with moderate inclusion levels maintaining high acceptability.

The control *muthiya* showed the highest overall acceptability (8.40), followed by SM131 (8.30), while higher beetroot levels SM132 (7.90) and SM133 (7.70) showed a decline in acceptability scores (Figure 7). Similar trends were reported by Tangariya *et al*., (2023) [24] where beetroot-enriched snacks bars with moderate incorporation achieved overall acceptability scores around 8.0, while higher levels reduced scores to approximately 7.5-7.8. Likewise, sorghum – based vegetable snacks reported overall score 8.0 at optimized formulation (Fatima *et al*., 2025) [12], supporting the importance of moderate incorporation for sensory quality.

Conclusion

The present study clearly demonstrates that soaking duration significantly influences the nutritional composition, antinutritional factors, functional properties, and sensory quality of processed sorghum. Soaking resulted in improved hydration, reduced crude fibre and carbohydrate content, and modified fat and protein levels due to leaching and biochemical transformations. Among the treatments, 24-hours soaking emerged as the optimal processing condition, offering a balanced nutritional profile with significantly enhanced extractable calcium and iron, along with notable reductions in phytate and tannin contents. These improvements are attributed to the dissociation of minerals-phytate complexes and activation of endogenous phytase enzymes, thereby improving minerals bioavailability.

Functional property analysis revealed that 24 hours soaking significantly increased bulk density while reducing water and oil absorption capacities, making the flour suitable for diversified food applications.

The successful development and high sensory acceptability of sorghum- based value-added products-particularly tomato khakra with 10% tomato incorporation and moderately fortified beetroot *Muthiya* with 5% further validate the technological and consumer relevance of the optimizes flour.

Overall, the study confirms that controlled soaking is a simple, cost-effective, and scalable processing method to enhance the nutritional characteristics and utilization potential of sorghum. The finding strongly supports the promotion of sorghum as a functional, gluten-free, and sustainable millet for improving dietary diversity and public health.

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