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Research Article

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Effect of Various Pretreatments on Anti-nutrients of Mango Seed Kernel and its Utilization in Preparation of Pasta

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Abstract

The availability of mango (*Mangifera indica* Linn.) in India is abundant, leading to a significant amount of seed waste generated during processing. This research aims to eliminate anti-nutrients from the mango seed kernels (msk), particularly from the Safeda variety, which has larger fruits and a higher seed percentage. Various pretreatment techniques, including soaking in water, sodium chloride (NaCl) and potassium metabisulfite (KMS), followed by either boiling or autoclaving for varying time durations, have been explored to reduce the levels of tannin, phytate, saponin, and oxalate in the seed kernels. The initial anti-nutrient content in fresh MSKs was recorded as 0.294 ± 0.001 mg/100 g for tannins, 0.48 ± 0.002 mg/100 g for phytates, 0.28 ± 0.01 mg/100 g for saponins, and 0.054 ± 0.008 mg/100 g for oxalates. However, the most effective pretreatment, involving a 48-hour soaking period along with 60 minutes of extended boiling, significantly reduced all these anti-nutrient content to 0.135 ± 0.068 mg/100 g, 0.011 ± 0.0041 mg/100 g, 0.08 ± 0.001 mg/100 g, and 0.0014 ± 0006 mg/100 g, respectively. Subsequently, the pretreated MSKs were dried and ground into powder which was further utilized for pasta preparation. Further both MSK powder and the developed pasta were subjected to Fourier transform infrared (FTIR) spectroscopy analysis showing the presence of various functional components. Among the 11 treatments, after sensory analysis treatment T_6 (5% MSK powder) got the highest overall acceptability score (7.91 ± 0.45) based on color (8.91 ± 0.42), texture (8.86 ± 0.56), taste (7.62 ± 0.83) and aroma (5.86 ± 0.79) of developed product. Thus, the study concludes that soaking in water for 48 hours, followed by boiling for 60 minutes effectively helps in reduction of anti-nutrients from MSKs making them suitable for utilization in pasta.

Keywords

Mango, Anti-nutrients, Pretreatments, Soaking, Boiling, Autoclaving, Pasta

Introduction

India holds the distinction of being the world's largest mango producer. Mango production has seen a notable growth trend since 2010, with an increase in production up to 41 million tons in 2020 [1]. Revered as the "King of Fruits" mangoes occupy a paramount position within India. Cultivated over a vast expanse of 2,351 thousand hectares, the fruit production reaches approximately 20.77 million tons, contributing 40.48% to global mango production. The primary mango-producing states include Andhra Pradesh, Uttar Pradesh, Karnataka, Bihar, Gujarat, and Tamil Nadu [2]. Mango is consumed as fresh fruit and processed products like concentrated, dried, frozen, and canned products. Due to its rich flavor and nutritional value, various mango-based items like juices, canned slices, frozen chunks, pickles, and mango powder have been innovatively

developed [3]. The availability of mangoes in India is significantly high, and thus, their utilization and processing results in the production of large number of by-products, primarily mango peel and seed. The MSK contains significant amounts of starch, fiber, protein, and minerals, making it a potentially valuable source of nutrition for both human and animal feed. While the protein content in MSKs is relatively low, its protein quality is excellent due to its richness in all essential amino acids [4]. Several research studies have established that mango byproducts exhibit strong antioxidant properties attributed to the presence of bioactive compounds like carotenoids, tocopherols, and phenolic compounds [5, 6]. According to the research study conducted by Kuganesan and team, it is evident that the MSK demonstrates high levels of antioxidant and anti-inflammatory activity. This is due to the elevated phenolic content in the seed kernel, making them valuable as value-added products [7]. These compounds possess significant health-promoting potential owing to their ability to offer anticarcinogenic [8], antidiabetic [9], antiatherosclerotic [10] and, antioxidant and antimicrobial properties [11]. The MSK is also a rich source of starch and thus has versatile applications in the food industry, such as gelling agents, stabilizers, thickeners, and encapsulating agents [12].

While MSK can serve as a rich source of energy, containing soluble carbohydrates and proteins, it does contain certain limiting factors referred to as anti-nutritional components like alkaloidtannin, cyanogenic glucosides, oxalates, phytates, and saponinetc [13]. Anti-nutrients impact the utilization of nutrients in the human body, particularly proteins, vitamins, and minerals. This effect occurs as they bind to these compounds, decreasing their absorption within the digestive system [14]. Therefore, these anti-nutrients should be eliminated to the possible lower extents to make use of the nutrients present in the kernels without discarding them. Various pretreatment methods like soaking, alkali treatments, boiling, autoclaving, roasting, germination, and fermentation are being used in food processing industries to eliminate these anti-nutrients from the food materials to make them more nutritious [15]. These methods can reduce anti-nutrients in MSK, making them more suitable for consumption. Consequently, MSK with reduced anti-nutrient content can serve as a promising ingredient for value-added product development. Numerous value-enhancing items can be produced by blending mango seed powder flour with various other flours. Thus, study aimed to employ certain pretreatment techniques alone or in combination to reduce the anti-nutrients in MSKs easily without any degradation in nutrient quality, thus can be utilized in food products as a fiber source and helps in reduction of industrial waste. To narrow down the focus, the Safeda variety was chosen as the subject of research, based on its availability and fruit size. Furthermore, the MSK powder was subjected with various concentration along with *Salmonella* and water for optimization that can be utilized on industrial scales.

Materials and Methods

Procurement of raw material

Ripe, mature, and disease-free Safeda variety mangoes were purchased from the Phagwara fruit market in Punjab. The fruits were cleaned, then the peels, flesh and seeds were separated using a kitchen knife. The seeds were rinsed under tap water to remove any remaining pulp. Kernels were then carefully extracted from the seeds using a kitchen knife for analysis of major anti-nutritional content.

Antinutritional analysis

Tannins

A 5 g portion of the sample was boiled with 400 ml of distilled water. After allowing the mixture to cool, it was subsequently transferred to a 500 ml volumetric flask and subjected to filtration. A 10 ml aliquot of the resulting filtrate was carefully measured and introduced into a 100 ml volumetric flask containing 75 ml water. To this solution, 5 ml of Follin-Denis reagent and 10 ml of sodium carbonate were added. The total volume was adjusted to 100 ml using distilled water. The absorbance of the solution was measured at a wavelength of 760 nm. The determination of tannin content within the sample was achieved through comparison with the standard curve, established using tannic acid as the reference standard. The results were subsequently expressed as mg of tannin per 100 g of the sample.

Saponins

A 5 g aliquot of the sample was immersed in a solution consisting of 20% acetic acid in ethanol. This mixture was left to stand within a water bath set at a temperature of 50 °C for 24 hours. Following this period, the solution was subjected to filtration, and the resulting extract was concentrated using a water bath until its volume was reduced to one quarter of the original. To this extract, concentrated NH_4OH (ammonium hydroxide) was cautiously added drop by drop until the point of complete precipitation was reached. The entire solution was then permitted to settle, facilitating the precipitate collection by filtration. The collected precipitate was subsequently quantified by weighing. The saponin content in the sample was determined according to standard AOAC procedure.

$$\text{Saponin content} = \frac{\text{Weight of filter paper + Residue wt. of filter paper}}{\text{Weight of sample analyzed}} \times 100$$

Phytates

A 0.2 g portion of the sample was accurately weighed and introduced into a 250 ml conical flask. Subsequently, it was immersed in 100 ml of 20% concentrated HCl for 3 hours. After the soaking period, the sample underwent filtration. From the resulting filtrate, a 50 ml volume was transferred to a 250 ml beaker, to which an additional 100 ml of distilled water was added. Subsequently, 10 ml of a 0.3% ammonium thiocyanate solution was introduced as an indicator. The mixture was then titrated using a standard iron(III) chloride solution, with each milliliter containing 0.00195 g of iron. This titration process allowed for the determination of phytate content in the sample according to standard AOAC procedure.

$$\text{Phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{2}$$

Oxalates

This determination involved three major steps: digestion, oxalate precipitation, and permanganate titration.

Digestion

2 g of sample was suspended in 190 ml of distilled water in a 250 ml volumetric flask. 10 ml of 6 M HCl was added, and the suspension was digested at 100 °C for 1 hour. The solution was cooled and then made up to 250 ml mark before filtration.

Oxalate precipitation

Duplicate portions of the filtrate were measured into breakers, and four drops of methyl red indicator added. Then NH_4OH solution was added (drop wise) until the test solution changed from pink to faint yellow (pH 4.0-4.5). Each portion was then heated to 90 °C, cooled and filtered to remove precipitate containing ferrous ions. The filtrate was again heated to 90 °C and 10 ml of 5% $CaCl_2$ solution was added while being stirred constantly. The solution was then heated and left overnight at 25 °C. It was then centrifuged at 2500 rpm for 5 minutes. The supernatant was decanted, and the precipitate was completely dissolved in 10 ml of 20% (v/v) H_2S0_4 solution.

Permanganate titration

At this point, the total filtrate resulting from the digestion of 2 g of sample was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05 M standardized $KMnO_4$ solution to a faint pink color, which persisted for 30 s. The calcium oxalate content was calculated using the formula:

$$\text{Oxalate} = \frac{\text{T x (Vme) x Df}}{\text{(ME) x (Mf)}}(\text{Mg}/100)$$

Where T is the titer of $KMnO_4$ (ml), Vme is the volume-mass equivalent, Df is the Dilution factor = Vt/A. Where Vt is the total volume of filtrate (300 ml), and A is the aliquot used (250 ml), ME is the molar equivalent of $KMnO_4$ in oxalate and Mf is the mass of sample used.

Pre-treatment of kernels to reduce anti-nutrients

The seeds after washing with running tap water, were opened with the help of kitchen knife and the kernels were extracted. After separation, the kernels were treated with various pretreatments to reduce the amount of anti-nutrients present in the kernel. The kernels were first pretreated with various pretreatments such as soaking in plain water, soaking in NaCl (10%), and soaking in KMS (730 ppm) solution. After soaking, the best treatment from soaking was selected based on the results of the anti-nutrient analysis. The selected treatment was further proceeded for a combined pretreatments that are boiling and autoclaving. The combination of soaking for 48 hours and boiling and autoclaving for 10, 20, 30, and 40 minutes was performed. Various pretreatments given to MSKs are as mentioned in table 1.

The duration of best combination among the pretreatments from T_{13} to T_{20} were extended for 50 and 60 minutes (T_{21} and T_{22}) to minimize the anti-nutritional content. After these pretreatments, anti-nutritional analysis was performed, and the best pretreatment was selected which was effective in eliminating the anti-nutrients to the maximum possible extent. After the pretreatment with the best method, the kernels were dried in the tray dryer at 60 °C to remove the moisture content from the kernels.

Preparation of MSK powder for utilization in preparation of pasta

The pretreated kernels were dried in a mechanical dehydrator (Bio Techno Lab, Mumbai, Maharashtra) at 60 ± 2 °C

Table 1: Standardization of pretreatment for MSK.

Treatment	Description
T_0	Fresh kernels (non-pretreated)
T_1	Soaking in water for 12 hours
T_2	Soaking in water for 24 hours
T_3	Soaking in water for 36 hours
T_4	Soaking in water for 48 hours
T_5	Soaking in alkali solution like KMS (730 ppm) for 12 hours
T_6	Soaking in alkali solution like KMS (730 ppm) for 24 hours
T_7	Soaking in alkali solution like KMS (730 ppm) for 36 hours
T_8	Soaking in alkali solution like KMS (730 ppm) for 48 hours
T_9	Soaking in NaCl (10%) solution for 12 hours
T_{10}	Soaking in NaCl (10%) solution for 24 hours
T_{11}	Soaking in NaCl (10%) solution for 36 hours
T_{12}	Soaking in NaCl (10%) solution for 48 hours
T_{13}	Soaking (best of three soaking methods) and boiling for 10 minutes
T_{14}	Soaking (best of three soaking methods) and boiling for 20 minutes
T_{15}	Soaking (best of three soaking methods) and boiling for 30 minutes
T_{16}	Soaking (best of three soaking methods) and boiling for 40 minutes
T_{17}	Soaking (best of three soaking methods) and autoclaving for 10 minutes
T_{18}	Soaking (best of three soaking methods) and autoclaving for 20 minutes
T_{19}	Soaking (best of three soaking methods) and autoclaving for 30 minutes
T_{20}	Soaking (best of three soaking methods) and autoclaving for 40 minutes
T_{21}	Extended duration of boiling/autoclaving for 50 minutes
T_{22}	Extended duration of boiling/autoclaving for 60 minutes

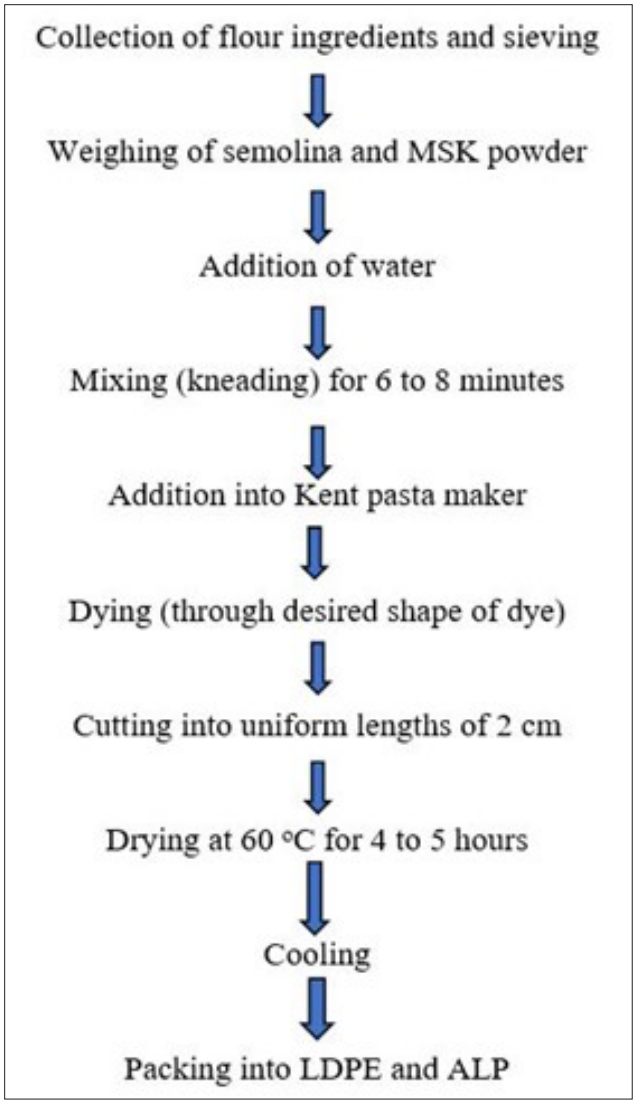


Figure 1: Flow chart for the preparation of MSK powder incorporated pasta.

for 8-10 hours. These were then ground using Sujata super mix mixer grinder (900 watt), followed by sieving through a 48 mesh (297 microns) metallic sieve to yield powder. The MSK powder was packed in polyethylene bags for subsequent use before the preparation of pasta.

Standardization of MSK powder for the preparation of pasta

The pasta was developed by using MSK powder, semolina powder, and water, as represented in figure 1. For the standardization of treatments, semolina powder was replaced by incorporation of MSK powder in different proportions, keeping water constant. Ten treatments of pasta were prepared by using different recipes comprising of various proportions of semolina powder, different concentrations of powder of MSK and water. All these ingredients were added into the pasta maker (Kent RO System Ltd, Noida, India) and was kneaded for 6 - 8 minutes and extruded through the desired dye. The pasta was then cut into uniform lengths of 2 cm and dried in a dehydrator for 4 hours. It was then cooked for 15 minutes with 0.5 g of salt and 400 ml water and subjected to organoleptic evaluation by a panel of ten judges to select the best ones to be used as base for further studies. Out of the ten treatments developed, the best treatment was selected (Table 2).

Table 2: Different treatments are used for the standardization of pretreated MSK powder for the preparation of pasta.

Treatments	Percentage replacement (%)	Semolina (g)	MSK powder (g)	Water (ml)
T_1	0	200	0	85
T_2	1	199	1	85
T_3	2	198	2	85
T_4	3	197	3	85
T_5	4	196	4	85
T_6	5	195	5	85
T_7	6	194	6	85
T_8	7	193	7	85
T_9	8	192	8	85
T_{10}	9	191	9	85
T_{11}	10	190	10	85

FTIR analysis

The collected samples of powder were analyzed qualitatively using FTIR analyzer (FTIR Spectrometer, equipped with Kbr beam splitter). Approximately 5 mg of each sample, 5 mg KBr was used for analysis and FTIR Spectrophotometer was operated at a spectral range 4,000 - 400 cm^{-1}.

Result and Discussion

Anti-nutritional analysis of fresh MSK

Data presented in table 3 provides four different anti-nutrients (tannins, phytates, saponins, and oxalates) found in MSKs of Safeda variety. Tannins are polyphenolic compounds and have a bitter or astringent taste. These are known to bind proteins and minerals, reducing their bioavailability [16]. In MSKs of Safeda variety, the tannin content was measured as 0.294 ± 0.001 mg/100 g. The tannin levels found in this study (0.294 ± 0.001) are less than what was documented by Akin-Idowu et al. [17] i.e. 0.54-0.63 mg/100 g and more than the value (0.142 ± 0.001) reported by Anta and Zomegni [18]. Phytates, or phytic acid, are naturally occurring compounds found in many plant seeds and grains. Phytates hinder the uptake of minerals such as iron, zinc, and calcium and the breakdown of protein and starch. The formation of phytate-to-calcium and phytate-to-iron complexes is likely to hinder the absorption of calcium and iron, respectively [19, 20]. The phytate content in MSKs was measured as 0.48 ± 0.002 mg/100 g, which is much lower than the value obtained by Umeobika [21]. The oxalate content in MSKs of Safeda variety was recorded as 0.054 ± 0.008 mg/100 g, less than the value (1.44 ± 0.05 mg/100 g) documented by Richa et al. [22]. Oxalates form insoluble crystals with calcium, leading to the formation of kidney stones in susceptible individuals [23]. Saponins exert their anti-nutritional effects by enhancing the permeability of small intestinal mucosal cells, which leads to the inhibition of nutrient transport. Additionally, saponins have been observed to decrease the protein digestibility [24]. The saponin content was measured as 0.28 ± 0.01 mg/100 g which is higher than the value (0.04 ± 0 mg/100 g) reported by Fowomola [13].

Table 3: Anti-nutritional analysis of MSKs.

Anti-nutrient (mg/100 g)	Amount (mean value + SE)
Tannins	0.294 ± 0.001[a]
Phytates	0.48 ± 0.002[bc]
Saponins	0.28 ± 0.01[a]
Oxalates	0.054 ± 0.008[b]

Effect of various pretreatments on the anti-nutrient content of MSK powder

The study assessed the impact of different pretreatment methods on the anti-nutrient levels found in MSKs. These methods included soaking the kernels in water, soaking in NaCl, and soaking in KMS for varying durations of 12, 24, 36, and 48 hours. Table 4 shows that, as the soaking time increases, the tannin content gradually decreases. After 12 hours of soaking in water, the tannin content was reduced to 0.256 ± 0.08 mg/100 g, and it further decreased to 0.207 ± 0.10 mg/100 g after 48 hours. Likewise, the decline in tannin content when soaked in NaCl follows the pattern observed during water soaking, showing a consistent decrease in tannin levels over time. The reduction is less pronounced compared to water soaking. After 12 hours of soaking in NaCl, the tannin content was 0.323 ± 0.004, and it decreased to 0.208 ± 0.01 after 48 hours. Like the previous treatments, soaking in KMS led to a reduction in tannin content over time. After 12 hours of soaking in KMS, the tannin content was 0.351 ± 0.02, and it decreased to 0.274 ± 0.004 after 48 hours. Similarly, as the time interval increased, there was a gradual reduction in the amount of phytates. After 12 hours of soaking in water, the phytate content was approximately 0.26 ± 0.07 mg/100 g, which decreased to 0.24 ± 0.01 mg/100 g in 24 hours, 0.22 ± 0.6 mg/100 g in 36 hours, and 0.2 ± 0.002 mg/100 g in 48 hours. The results for soaking in NaCl show that the phytate content decreased with longer soaking times. At 12 hours, the phytate content is around 0.36 ± 0.01 mg/100 g, which decreased to 0.33 ± 02 mg/100 g at 24 hours, 0.29 ± 0.04 mg/100 g at 36 hours, and 0.24 ± 0.04 mg/100 g at 48 hours. Again, the phytate content decreased over time. At 12 hours, the phytate content was approximately 0.37 ± 0.03 mg/100 g, which decreased to 0.33 ± 0.08 mg/100 g at 24 hours, 0.32 ± 0.06 mg/100 g at 36 hours, and 0.27 ± 0.09 mg/100 g at 48 hours, when they are soaked in KMS. The results show that all the pretreatment methods and longer time duration contribute to the reduction of phytate content in MSKs. Soaking in water results in more reduction in phytates over 48 hours. The reduction in phytate content observed during the soaking of the samples may be attributed to the leaching of phytate ions into the soaking water. This leaching process is driven by a concentration gradient, which controls the rate of diffusion [25].

Similarly, after 12 hours, the saponin content was 0.24 ± 0.06 mg/100 g, and it decreased to 0.12 ± 0.021 mg/100 g at 48 hours. Similarly, soaking in NaCl and KMS solutions also resulted in a reduction in saponin content. However, the reduction was not as significant as in the water soaking. The saponin content after 48 hours of soaking in NaCl and KMS was recorded as 0.13 ± 0.01 mg/100 g and 0.12 ± 0.011 mg/100 g, respectively. So, the results demonstrate that soaking in water for longer durations, i.e., 48 hours, appeared to be more effective in reducing saponin content. After soaking MSKs in water for 12 hours, the reduction in oxalates was 0.0241 ± 0.006 mg/100 g. As the soaking time increased to 24, 36, and 48 hours, the values decreased slightly as 0.0198 ± 0.0012, 0.0192 ± 0.0023 mg/100 g, 0.0186 ± 0.0032 mg/100 g, respectively, indicating a diminishing effect on oxalate reduction. Similarly, after soaking in NaCl for 12 hours, the reduction was 0.0397 ± 0.001 mg/100 g, and it decreased slightly over longer soaking times of 24, 36 and 48 hours to 0.038 ± 0.0047, 0.0298 ± 0.0026, 0.281 ± 0.0067 mg/100 g respectively. It indicates that longer soaking times in water for 48 hours can effectively reduce oxalate levels in MSKs. The decrease in oxalate levels indicated that the duration of soaking played a significant role in reducing their concentration. Consequently, this reduction in oxalates diminished their capacity to form complexes with food nutrients, as mentioned earlier, thereby enhancing the bioavailability of nutrients in the samples [26]. These findings indicate that with longer soaking periods, there was a corresponding increase in the reduction of anti-nutrients. This increase in reduction occurred because anti-nutrients were leached into the water during extended soaking.

Effect of combined pretreatments on the anti-nutrient content of MSK powder

For the effective reduction, additional pretreatment methods, involving soaking in water followed by boiling or autoclaving were given to the MSKs. The combination of soaking and heat-treatment was found to be highly effective in reducing anti-nutrients in jack beans, indicating that this method is suitable for efficiently eliminating anti-nutrients [27]. Tannins and phytic acid content in mung beans [28] and cowpeas [29] underwent significant reduction following the boiling process. During soaking in water and boiling for 10 minutes the tannin content was reduced to 0.159 ± 0.006 mg/100 g and it further reduced to 0.138 ± 0.022 mg/100 g. In case of soaking for 48 hours and autoclaving for 10 minutes, tannin content was reduced to 0.162 ± 0.02 mg/100 g and at 40 minutes it got reduced to 0.146 ± 0.04 mg/100 g. As the soaking and boiling combination reduced the tannin content more significantly than autoclaving, it was further heated for 60 minutes to reduce more anti-nutrients. When the time of boiling increased furthermore, tannin content was reduced to the minimum level of 0.135 ± 0.014 mg/100 g. The results suggest that longer durations of soaking for 48 hours followed by boiling for 60 minutes generally lead to higher tannin reduction. The processing outcomes demonstrated that the most significant reduction in tannin levels was achieved through the combined effects of soaking and autoclaving, like the results concluded by Legesse and Emire [30]. The phytate content decreased significantly with increasing time duration of soaking in water and boiling. After 10 minutes, the phytate content was approximately 0.046 mg/100 g, which further decreased to 0.023 mg/100 g after 20 minutes, 0.026 mg/100 g after 30 minutes, and 0.011 mg/100 g after 40 minutes. Similarly, for soaking in water and autoclaving, the phytate content decreased as the time interval increased. After 10 minutes, the phytate content was around 0.047 mg/100 g, which decreased to 0.034 mg/100 g after 20 minutes, 0.034 mg/100 g after 30 minutes, and 0.011 mg/100 g after 40 minutes.

Similarly, the results of soaking the MSKs in water for 48 minutes followed by boiling shows that, as the time interval increased, there was a gradual reduction in saponin content. After 10 minutes, the saponin content was 0.13 ± 0.008 mg/100 g, and it decreased to 0.08 ± 0.001 mg/100 g after 40 to 60 minutes. For the soaking and autoclaving pretreatment, the saponin content also decreased with increasing time duration. The saponin content after 10 minutes was 0.16 ± 0.002 mg/100 g, and it decreased to 0.09 ± 0.06 mg/100 g after 40 minutes. The results of soaking the MSKs in water for 48 minutes followed by boiling shows the reduction in oxalates. Moreover, after soaking in water and boiling for 60 minutes reduced the number of oxalates to significant level of 0.0014 ± 0.0006 mg/100 g as the reduction in soaking for 48 hours followed by boiling for 10 minutes was 0.0057 ± 0.001 mg/100 g. The decrease in phytates, oxalates, and tannins became more pronounced as the duration of boiling and soaking increased. This trend of reduced anti-nutrient levels with longer boiling and soaking times aligns with findings from a study conducted by Sotelo et al. [31]. Similarly, in soaking and autoclaving, at 10 minutes it was reduced to 0.0087 ± 0.0002 mg/100 g, and it decreased further to 0.0028 ± 0.0006 mg/100 g as the autoclaving time increased to 40 minutes.

From the results presented in table 4 it was observed that the anti-nutrients that are present in the MSKs were reduced significantly when they were subjected to the pretreatment of soaking in water for 48 hours and boiling these soaked seeds for 60 minutes. The combination of soaking and boiling shows the maximum reduction of anti-nutrients. As the boiling time increased, the anti-nutrient content reduced significantly due to the heat treatment, which is like the observation for Bambara groundnut *(Vigna subterranea* L.) seeds [32]. Because of the longer soaking and longer boiling period, the anti-nutrients present in the MSKs leached out of the kernels and made them edible. In the case of short durations of pretreatment, the anti-nutrients present in MSKs were bound within the cells. So, the seeds that are treated with the best-selected pretreatment were selected for the preparation of MSK powder to utilize it in the development of pasta.

Comparison of anti-nutrient content of fresh kernels with pretreated kernels

The most substantial decrease in anti-nutrient levels was observed in the pretreatment, involving soaking for 48 hours in water followed by 60 minutes of boiling. Consequently, these pretreated kernels were chosen for the drying process to produce MSK powder. The tannin content in the fresh MSK was observed as 0.294 ± 0.001 mg/100 g. After the soaking and boiling pretreatment, the tannin content was significantly reduced to 0.138 ± 0.022 mg/100 g. The phytate content in the fresh MSK was recorded as 0.487 ± 0.06 mg/100 g. After the soaking and boiling pretreatment, the phytate content decreased significantly to 0.011 ± 0.0016 mg/100 g. This indicates that the combined pretreatment effectively reduced the phytate content. This observation was relatable to the results concluded by Mazahib et al. [33]. The saponin and oxalate content in the fresh MSK was recorded as 0.28 ± 0.01 mg/100 g and 0.054 ± 0.01 mg/100 g. However, after the soaking and boiling pretreatment, the content decreased to 0.08 ± 0.002

Table 4: Effect of various pretreatments on the anti-nutrient content of MSK.

Anti-nutrient treatment	Tannins (mg/100 g)	Phytates (mg/100 g)	Saponins (mg/100 g)	Oxalates (mg/100 g)
T_0	0.294 ± 0.001^{bc}	0.48 ± 0.002^{ab}	0.28 ± 0.01^{b}	0.054 ± 0.008^{cd}
T_1	0.256 ± 0.08^{ab}	0.26 ± 0.07^{c}	0.24 ± 0.06^{da}	0.0241 ± 0.006^{ac}
T_2	0.267 ± 0.03^{c}	0.24 ± 0.01^{bcd}	0.21 ± 0.03^{bc}	0.0198 ± 0.0012^{de}
T_3	0.242 ± 0.01^{a}	0.22 ± 0.60^{de}	0.16 ± 0.014^{d}	0.0192 ± 0.0023^{d}
T_4	0.207 ± 0.10^{abc}	0.20 ± 0.02^{ae}	0.12 ± 0.021^{de}	0.0186 ± 0.0032^{c}
T_5	0.323 ± 0.004^{de}	0.36 ± 0.01^{cde}	0.27 ± 0.002^{a}	0.0397 ± 0.001^{bc}
T_6	0.303 ± 0.001^{c}	0.33 ± 0.02^{a}	0.16 ± 0.05^{ac}	0.0380 ± 0.0047^{a}
T_7	0.234 ± 0.002^{a}	0.29 ± 0.04^{bc}	0.15 ± 0.007^{bd}	0.0298 ± 0.0026^{ad}
T_8	0.208 ± 0.01^{c}	0.24 ± 0.04^{bd}	0.13 ± 0.01^{cd}	0.0281 ± 0.0067^{bcd}
T_9	0.351 ± 0.02^{d}	0.37 ± 0.03^{ac}	0.22 ± 0.01^{ab}	0.0546 ± 0.004^{ac}
T_{10}	0.288 ± 0.06^{bc}	0.33 ± 0.08^{d}	0.18 ± 0.04^{a}	0.0488 ± 0.002^{de}
T_{11}	0.282 ± 0.02^{cd}	0.32 ± 0.06^{e}	0.16 ± 0.006^{cd}	0.0281 ± 0.001^{a}
T_{12}	0.274 ± 0.004^{a}	0.27 ± 0.09^{de}	0.12 ± 0.011^{ae}	0.0273 ± 0.011^{c}
T_{13}	0.159 ± 0.006^{c}	0.046 ± 0.003^{bc}	0.13 ± 0.008^{b}	0.0057 ± 0.0001^{ab}
T_{14}	0.146 ± 0.01^{b}	0.034 ± 0.07^{cd}	0.11 ± 0.009^{e}	0.0043 ± 0.0002^{ad}
T_{15}	0.142 ± 0.04^{a}	0.026 ± 0.008^{d}	0.09 ± 0.01^{c}	0.0026 ± 0.0014^{c}
T_{16}	0.138 ± 0.022^{ab}	0.011 ± 0.0016^{a}	0.08 ± 0.002^{cd}	0.0014 ± 0.001^{d}
T_{17}	0.162 ± 0.02^{cd}	0.047 ± 003^{c}	0.16 ± 0.002^{ab}	0.0087 ± 0.0002^{a}
T_{18}	0.157 ± 0.06^{e}	0.038 ± 0.001^{bcd}	0.14 ± 0.004^{ac}	0.0071 ± 0.001^{e}
T_{19}	0.151 ± 0.03^{cd}	0.023 ± 0.004^{a}	0.12 ± 0.001^{c}	0.0043 ± 0.0002^{c}
T_{20}	0.146 ± 0.04^{a}	0.016 ± 0.009^{a}	0.09 ± 0.06^{b}	0.0028 ± 0.0006^{b}
T_{21}	0.135 ± 0.014^{a}	0.011 ± 0.003^{c}	0.08 ± 0.003^{bc}	0.0014 ± 0.0002^{ac}
T_{22}	0.135 ± 0.014^{b}	0.011 ± 0.003^{de}	0.08 ± 0.003^{cd}	0.0014 ± 0.0002^{d}

mg/100 g and 0.0014 ± 0.001 mg/100 g, respectively. The pretreatment resulted in a significant reduction in oxalate content. Table 5 illustrates that the preliminary treatment involving a 48-hour water soaking followed by a 60-minute boiling period effectively decreased the concentrations of anti-nutrients in the MSKs. All these anti-nutrients experienced significant reductions after this pretreatment, rendering the kernels nutritionally more advantageous and potentially safer for consumption.

As represented in figure 2, the anti-nutrient content present in the MSK was affected by the pretreatments, and the amount of these anti-nutrients was reduced significantly to the possible minimal levels. The duration of the soaking period can differ based on the processing conditions. Several crucial factors, such as the type of seeds and soaking medium, soaking temperature, and the duration of soaking, play a significant role in influencing the elimination of anti-nutrients [34, 35]. Due to the water-soluble nature of the anti-nutrients, longer soaking periods in water resulted in greater reductions in anti-nutrient content. This suggests that more anti-nutrients leached out when the kernels were soaked in water for extended durations. Conversely, shorter soaking durations led to less anti-nutrient leaching, resulting in smaller reductions in anti-nutrient levels. Similarly, in the case of soaking in NaCl and KMS, the reduction was more when the duration of soaking was longer. But the reduction of anti-nutrients was more in kernels soaked in water than the kernels soaked in NaCl and KMS. This clearly indicated that the anti-nutrient content present in the MSKs could be effectively reduced by soaking in water for 48 hours. Soaking the seeds of soybean, chickpea, green pea, lentil, broad bean, and common bean in distilled water led to a significant reduction in the levels of lectins, as well as total and soluble oxalates [36]. More reduction was observed when the water-soaked kernels were boiled for 60 minutes. The application of heat treatment led to a more significant disruption of seed membranes, which, in turn, allowed for a greater release of soluble sugars, fibers, and anti-nutrient components into the medium. From these observations, it was recorded that, the combined pretreatments, like soaking MSKls in water for 48 hours followed by boiling for 60 minutes, resulted in a significant reduction of anti-nutrients in the MSKs. The percentage reduction of these anti-nutrients is mentioned in table 5.

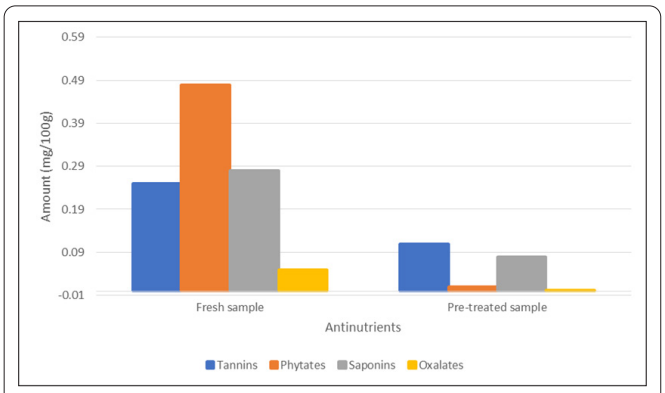


Figure 2: Graphical representation of the effect of pretreatment method on anti-nutrient levels of MSK.

Table 5: Comparison of anti-nutrients present in fresh and pretreated kernels.

Anti-nutrient (mg/100 g)	Fresh kernels	Pretreated kernels
Tannins	0.254 ± 0.03	0.135 ± 0.014
Phytates	0.48 ± 0.06	0.011 ± 0.0041
Saponins	0.28 ± 0.01	0.08 ± 0.001
Oxalates	0.054 ± 0.01	0.0014 ± 0006

FTIR observations of pretreated MSK powder

In FTIR spectroscopy, infrared radiation is passed through a sample, and the resulting spectrum is obtained by measuring the transmitted light intensity at different wavenumbers. Each peak in the spectrum corresponds to a specific vibrational mode of the molecules present in the sample (Figure 3).

The peak 3291.65 cm^{-1}, 92.89% T corresponds to a stretching vibration of the hydroxyl (OH) group, typically found in alcohols and phenols. The high transmittance percentage suggests the presence of a significant amount of hydroxyl groups (mostly water) in the MSK powder. The peak 2918.80 cm^{-1}, 85.97% T corresponds to the stretching vibration of C-H bonds in alkanes or methyl groups [37]. The transmittance percentage indicates that there is a moderate amount of lipids and proteins in the sample. The peak 1740.56 cm^{-1}, 94.11% T corresponds to the stretching vibration of carbonyl (C=O) groups. In the context of MSK powder, this could indicate the presence of compounds such as proteins. The high transmittance percentage suggests a relatively high concentration of carbonyl-containing compounds. The peak 1379.07 cm^{-1}, 92.80% T corresponds to the bending vibration of the methyl (CH_3) group. The transmittance percentage indicates a moderate concentration of lipids in the sample. The peak 1045.22 cm^{-1}, 84.08% T corresponds to the stretching vibration of C-O bonds. It suggests the presence of compounds containing carbohydrates [38]. The transmittance percentage indicates a moderate concentration of C-O bonds in the sample.

In general, the FTIR analysis findings displayed in table 6 for the MSK powder indicate the existence of various functional groups typically encountered in organic compounds. These include hydroxyl, carbonyl, methyl, and C-O bonds. These outcomes offer valuable insights into the sample's composition, offering a basis for comprehending its chemical characteristics and potential uses.

FTIR observations of MSK powder incorporated pasta

The T_6 treatment, identified as the top choice based on

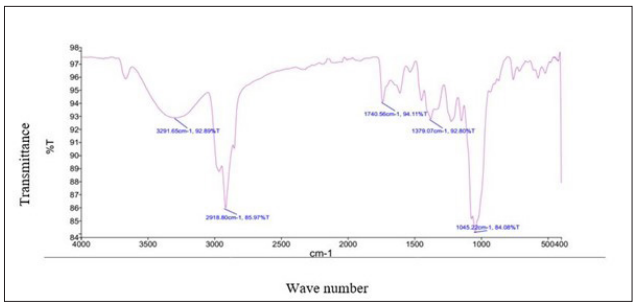


Figure 3: FTIR spectrum for pretreated MSK powder.

Table 6: FTIR observation of the MSK powder.

Absorption range (cm^{-1})	Peak observed (cm^{-1})	Peak type	Bond type	Type of vibration	Presence of nutritional elements
3000 - 3500	3291.65	Strong	O-H (Water)	Bending	Water
2500 - 3000	2918.8	Medium	C-H (Methyl)	Stretching	Lipids and proteins
1500 - 2000	1740.56	Strong	C=O (Carbonyl)	Stretching	Proteins
1000 - 1500	1379.07	Medium	CH_3 (Methyl)	Bending	Lipids
1000 - 1500	1045.22	Strong	C-O (Esters)	Stretching	Carbohydrates

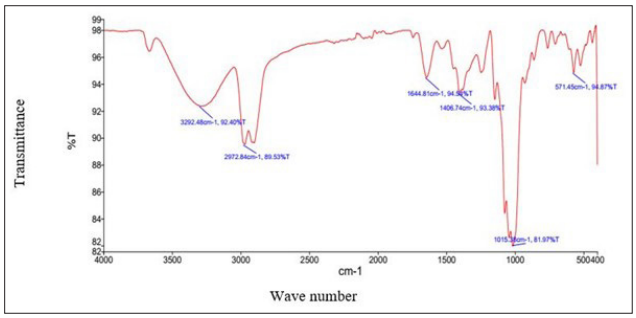


Figure 4: FTIR spectrum of MSK powder incorporated pasta.

sensory analysis, was subjected to FTIR evaluation. The outcomes revealed the presence of water, aliphatic hydrocarbons, proteins, and lipids in relatively elevated concentrations within this treatment (Figure 4).

The peak 3292.48 cm^{-1}, 92.40% T is attributed to the presence of the hydroxyl group (OH) stretching vibration, which indicates the presence of water molecules in the sample [39]. The relatively high transmittance percentage suggests that water is abundant in pasta. The peak 2972.84 cm^{-1}, 89.53% T corresponds to the C-H stretching vibration in the aliphatic hydrocarbon chains. The presence of this peak indicates the presence of lipids in the pasta. The peak 1644.81 cm^{-1}, 94.54% T is associated with the C=O (carbonyl) stretching vibration, which is typically found in proteins [40]. It suggests the presence of proteinaceous components, possibly from pasta ingredients or MSK powder. The peak 1406.74 cm^{-1}, 93.38% T corresponds to the bending vibrations of the methyl (CH_3) group. It is commonly observed in lipids, indicating the presence of lipids in the pasta sample. The peak 1015.38 cm^{-1}, 81.97% T corresponds to the stretching vibrations of C-O (ether or alcohol) bonds, indicating the presence of compounds such as polysaccharides or other organic components. The lower transmittance percentage of 81.97% suggests a lower concentration of these compounds compared to the other peaks. The peak 571.45 cm^{-1}, 94.87%T is related to the bending vibrations of the C-H bonds in an aliphatic chain. It further confirms the presence of carbohydrates in the pasta. Based on the information provided in table 7, the FTIR analysis of the pasta containing MSK powder reveals the existence of water, aliphatic hydrocarbons, proteins, and lipids. The substantial transmittance percentages indicate that these constituents are present in significant amounts within the pasta sample.

Sensory scores of MSK powder incorporated pasta

Sensory scores were recorded by the panel of judges and were presented in table 8. The sensory score of the pasta incorporated with the MSK powder was obtained as follows. The scores of color analysis range from approximately 5.92 to 8.91. Higher scores indicate that the pasta samples were perceived to have a more appealing color. For instance, T_6 received the highest score (8.91 ± 0.42), indicating that it had the most visually appealing color, while T_{11} scored the lowest (5.92 ± 0.44), suggesting that it had a less visually attractive color. The scores for texture range from 6.72 to 8.86. Higher scores imply a more desirable texture. T_6 had the highest texture score (8.86 ± 0.56), indicating that it had the most preferred consistency, whereas T_{11} received the lowest score (6.72 ± 0.63), suggesting it had less texture. Similarly, the scores for taste range from approximately 5.68 to 7.62. Higher scores indicate a more appealing taste. T_6 obtained the highest taste score (7.62 ± 0.83), implying it had the most favorable flavor, while T_{10} had the lowest taste score (5.68 ± 0.92), suggesting it had a less preferred taste. The scores for aroma range from 5.34 to 5.94. Higher scores show that it has more appealing aroma. T_3 received the highest aroma score (5.94 ± 0.63), suggesting it had the most pleasant aroma, while T_5 scored the lowest (5.34 ± 0.83), indicating it had a less desirable aroma. The scores for overall acceptability range from approximately 6.18 to 7.91. T_6 had the highest overall acceptability score (7.91 ± 0.45), suggesting it was the most liked sample, while T_{11} received the lowest score (6.18 ± 0.54), indicating it had the least overall acceptance. The treatment T_6 was awarded highest, and it was selected as the best treatment. These results show that 5 percent replacement of semolina with MSK powder is preferred for incorporation in pasta.

Table 7: FTIR observations of the MSK powder incorporated pasta.

Absorption range (cm^{-1})	Peak observed (cm^{-1})	Peak type	Bond type	Type of vibration	Nutritional elements found in pasta
3000 - 3500	3292.48	Strong	OH (Hydroxyl)	Bending	Water
2500 - 3000	2972.84	Strong	C-H (Aliphatic chains)	Stretching	Lipids
1500 - 2000	1644.81	Strong	C=O (Carbonyl)	Stretching	Proteins
1000 - 1500	1406.74	Medium	CH_3 (Methyl group)	Bending	Lipids
1000 - 1500	1015.38	Strong	C-O (ether or alcohol)	Stretching	Carbohydrates
500 - 1000	571.45	Strong	C-H (Aliphatic chains)	Bending	Lipids

Table 8: Sensory scores of MSK powder incorporated pasta.

Sample	Color	Texture	Taste	Aroma	Overall acceptability
T_1	8.55 ± 0.74	7.66 ± 0.51	5.84 ± 0.92	5.46 ± 0.33	7.68 ± 0.56
T_2	7.56 ± 0.79	6.77 ± 0.46	5.80 ± 0.92	5.14 ± 0.72	7.31 ± 0.43
T_3	7.72 ± 0.44	7.27 ± 0.51	6.72 ± 0.99	5.64 ± 0.63	7.34 ± 0.51
T_4	8.23 ± 0.41	7.02 ± 0.07	6.87 ± 0.99	5.67 ± 0.44	7.59 ± 0.53
T_5	8.50 ± 0.67	8.15 ± 0.59	6.79 ± 0.99	5.34 ± 0.83	7.21 ± 0.83
T_6	8.91 ± 0.42	8.86 ± 0.56	7.62 ± 0.83	5.86 ± 0.79	7.91 ± 0.45
T_7	7.98 ± 0.72	7.16 ± 0.37	7.01 ± 0.83	5.55 ± 0.51	7.62 ± 0.56
T_8	7.53 ± 0.67	7.44 ± 0.62	6.00 ± 0.92	5.66 ± 0.55	7.22 ± 0.45
T_9	6.83 ± 0.75	7.68 ± 0.76	5.88 ± 0.92	5.68 ± 0.23	6.43 ± 0.60
T_{10}	6.61 ± 0.49	6.94 ± 0.56	5.68 ± 0.92	5.66 ± 0.61	6.24 ± 0.67
T_{11}	5.92 ± 0.44	6.72 ± 0.63	5.72 ± 0.83	5.50 ± 0.53	6.18 ± 0.54

Note: T_1 - Control (0% MSK powder), T_2 - 1% MSK powder, T_3 - 2% MSK powder, T_4 - 3% MSK powder, T_5 - 4% MSK powder, T_6 - 5% MSK powder, T_7 - 6% MSK powder, T_8 - 7% MSK powder, T_9 - 8% MSK powder, T_{10} - 9% MSK powder and T_{11} - 10% MSK powder.

Based on the data presented in table 8, the highest color score was recorded for the treatment T_6 while the lowest was recorded for T_{11}, because of the presence of higher tannins than the other treatments, resulting in darker coloration. The highest texture score was observed in the same treatment T_6, while the lowest was for T_{11}. Similarly, the taste and aroma were also recorded high for T_6. The overall acceptability score was recorded as high as 7.91 ± 0.45 for the treatment T_6. This suggests that the panelists favored T_6 as the most preferred treatment in relation to sensory characteristics like color, texture, flavor, and scent. It was noted that as the concentration of MSK powder increased, the sensory rating declined. This decline can be attributed to the rise in anti-nutrient levels with higher powder concentrations. Consequently, the treatment with the ideal powder amount, T_6, received greater approval compared to the other options assessed in the sensory evaluation of pasta infused with MSK powder.

Conclusion

This study offered valuable insights into various pretreatment techniques for reducing anti-nutrient levels in MSK. Since anti-nutrients hinder the effective utilization of minerals and nutrients, it is essential to eliminate them from food materials to mitigate their toxic effects and enhance nutrient availability for better absorption. Pretreatment methods such as soaking, boiling, autoclaving, and combinations thereof were found to significantly reduce anti-nutrient levels in MSKs. Among these methods, the most effective combination was soaking for 48 hours followed by boiling for 60 minutes, resulting in a percentage reduction of 55.51% for tannins, 97.71% for phytates, 71.43% for saponins, and 97.41% for oxalates in the MSKs. Therefore, based on these research findings, soaking the kernels for 48 hours, followed by a 60-minute boiling was considered the optimal pretreatment method for reducing anti-nutrient content in MSKs. Additionally, the study standardized the processing method for preparing pasta by incorporating MSK powder. It involved replacing 5% semolina with MSK powder to enhance the nutritional content of the pasta without compromising its textural properties. This research also demonstrated how factors such as soaking duration and temperature can influence the nutritional and anti-nutritional composition of mango kernel flour used in food applications.

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Conflict of Interest

None.

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