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Review Article

Key Anti-nutrients of Millet and their Reduction Strategies: An Overview

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Abstract

Millet is the sixth economically important crop that has the potential to grow very quickly in dry environments. Different types of millets belong to the family Poaceae. Pearl millet (*Pennisetum glaucum*) is one of the commonly grown millet types in India and Africa. It is used as food as well as fodder worldwide. It is rich in nutrients and minerals (essential micronutrients), crucial in human growth and development. These nutrients and minerals also present anti-nutrients, such as tannins, phytates, trypsin, amylase inhibitors, etc. Anti-nutrients are natural constituents that limit the bioavailability of the essential nutrients and minerals in cereals and legumes. Usually, anti-nutrients don't have any significant harmful effect on an individual's health. However, their ability to inhibit the absorption of nutrients can cause malnutrition in rural people whose diet is based solely on cereals and grains. This is a major concern in a developing country, where millet is grown and consumed by a large population. Thus, there is a need to remove these anti-nutrients either entirely or partially. Several processing methods like decortication, heating, soaking, germination, and fermentation can reduce the content of anti-nutrients. This article reviews key anti-nutrients found in millet varieties, especially pearl millet, along with the methods used for their reduction.

Keywords: Millets; Anti-nutrients; Fermentation; Germination; Minerals

Abbreviations

PM: Pearl Millet; LAB: Lactic Acid Bacteria; LAF: Lactic Acid Fermentation; TPC: Total Polyphenolic Content; TFC: Total Flavonoids Content

Introduction

Plant-based foods/seeds such as cereals, nuts, legumes, millets, etc., are consumed due to their ample content of nutrients, including fats, proteins, essential amino acids, micronutrients, fibers, and vitamins [1,2]. Millet is an economically important crop that grows quickly in dry environments and is highly rich in minerals [3]. However, due to anti-nutrients such as phytate and polyphenol, the bioavailability of these minerals decreases [4]. Globally, millet production is assessed to be 27.8 million tons. India is the largest producer of millet at the global level, with a 41.04% share in the worldwide market [5]. Pearl millet (PM), a versatile grain for feed, food, and fodder, makes over 29 million hectares of all varieties of millet [6]. In India, PM is consumed by the rural people, especially

in Maharashtra, Gujarat, and Rajasthan, making almost 60% of the total grain consumption [7]. It contains a very high nutritional value for human consumption and livestock as well as has been further biofortified [8]. Biofortification is a process of enhancing the nutritional property through advanced plant breeding techniques [7]. Dhanashakti is the first successful biofortified variety of PM marketed in 2014 and is currently cultivated on 65,000 hectares across the country. Some other hybrids are HHB 299, HHB 311, RHB 233, and AHB 1200. These hybrids and biofortified varieties have 7.5- 8.0 mg of iron per 100 g and 3.5- 4.5 mg of zinc per 100 g of pearl millet. Consuming 200 grams of biofortified pearl millet-based products in a day covers almost 70% of an adult's daily zinc and iron requirement, and 130 grams fulfills 100% requirement for children. It will take approx. 5-7 years to commercialize biofortified and hybrid varieties of PM [9].

Despite all nutrients, some anti-nutrients too are found in pearl millet. The concentration of various anti-nutrients in different cultivars/varieties of pearl millets is shown in table 1. Tannins, phytic acid, and polyphenols are the most common anti-nutrients found in millets [10]. They combine with compounds or substances of natural or synthetic origin, thereby impairing nutrient absorption, digestion, and consumption. They may also produce other adverse effects, especially in people who survive only on cereals and grains. Even though it has been scientifically proven that anti-nutrients consumed in sufficient quantities can reduce the risk of certain diseases, such as breast cancer, coronary heart disease, and inflammation [11,12], yet the focus of research is on the adverse effects of these anti-nutrients and their management in cereals. Anti-nutrients can be reduced through various treatment methods such as soaking, germination/sprouting, cooking, malting, and fermentation of the grains [2,13]. Soaking is an easy and useful method and is also used before germination and fermentation [14]. Fermentation is considered the most convenient and popular process that lowers the anti-nutrients present in millets like tannins, trypsin inhibitors, phytic acid, etc., and enhances the overall nutritional quality of millets [15,16]. Mohamed., *et al.* observed that even time duration of treatment plays a vital role in lowering anti-nutrient content [17]. This article reviews the effects of various anti-nutrients present in PM and the strategies to reduce their content.

Hybrid/ Variety	Anti- nutrients	Concentration (per 100g)	References
Gazira	Tannin Phytic acid Polyphenols	415.38 mg 987.19 mg 220 mg	[91]
Gadarif	Tannin Phytic acid Polyphenols	420 mg 952.51 mg 170 mg	
CO7	Tannin Total phenol	152 mg 300 mg	[72]
Proagro- 9444	Tannin Phytic acid Total polyphenols	590 mg 580 mg 207 mg	[7,67]
ICMH- 1201	Tannin Phytic acid	420 mg 580 mg	
Dhanashakti	Tannin Phytic acid Polyphenols	232.71 mg 566.67 mg 445.38 mg	[104]
Shanti	Tannin Phytic acid Polyphenols	229.87 mg 596.30 mg 426.56 mg	
Pioneer 86M64	Tannin Phytic acid Polyphenols	225.68 mg 618.85 mg 403.59 mg	
Standard	Phytic acid Total polyphenols	943 mg 304 mg	[51]
Ugandi	Phytic acid Total polyphenols	1076 mg 444 mg	
IS 91333	Phytic acid	795 mg	[105]
IS 91666		530 mg	
IS 91777		618 mg	
Kalukombu	Tannin	230 mg	[65]
	Phytic acid	780 mg	
	Flavonoids	270 mg	
Maharashtra Rabi Bajra	Tannin	210 mg	
	Phytic acid	570 mg	
	Flavonoids	210 mg	

Table 1: Concentration (per 100g) of anti-nutrients in PM variety/cultivar/hybrid.

Anti-nutrients

The compounds that bind to the nutrients present in the food and make them less available for absorption by the human body are anti-nutrients. Some of the common anti-nutrients found in millets are described below.

Phytate

Phytic acid or phytate is myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate. It is the primary storage form of phosphorous comprising 1-5% by weight in cereals, legumes, nuts, oilseeds, etc. It is a secondary metabolic compound that is believed to be a nutrient reserve for the seed accumulated during ripening [4,18]. Humans neither absorb phytate nor have the ability to hydrolyze this molecule, resulting in its involvement in making minerals less bioavailable. Phytate being negatively charged ion works in pH-sensitive areas and negatively affects the bioavailability of positively divalent and trivalent mineral ions like Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+} [19]. Phytate levels are highly variable in grains, legumes, nuts, and seeds because they depend on growing conditions, processing, harvesting, and even testing methods. It is the most common substance known to inhibit mineral absorption [20]. Some biofortified varieties have been developed to overcome the effect of phytates, such as *Dhanashakti*, ICMH 1201, ICMH 1301, ICTP 8203, ICMV 221. ICTP-8203.

Polyphenols

Polyphenols are considered vital for life because they maintain body and health throughout life. They do not play a direct role in nutrition but have health benefits. They act as antimutagenic, anticarcinogenic, antiestrogenic, anti-inflammatory, antiviral, and platelet aggregation inhibitors, which can be useful in minimizing and preventing diseases [21]. Some epidemiologic studies on animals and humans have shown that various polyphenols have an antioxidant and anti-inflammatory function and can prevent and cure obesity, cancer, neurodegenerative diseases, and heart diseases [22,23].

Dietary polyphenols reduce the transport of thiamine and folic acid, alter drug activity through interactions that affect drug carriers or enzymes involved in the reaction, and, in some cases, increase inhibition and bioavailability. Polyphenols show an inhibitory effect on iron absorption, resulting in poor iron status [22,24]. Excessive amounts of polyphenols in the colon can prevent beneficial microbes from growing [25]. Some polyphenols may also have carcinogenic or genotoxic effects at high doses or concentrations.

Millet contains more than 50 phenolic compounds, like phenolic acids and their derivatives, flavonols, flavones, and flavanonols, and has a high antioxidant capacity. Soluble phenolic compounds are generally available in the pericarp of the kernel and cell wall consisting of the bound form [26]. Polyphenols can be categorized based on their carbon skeleton as simple phenols, phenolic acids, tannins, flavonoids, lignans, lignins, curcuminoids, coumarins, and stilbenes [27]. Polyphenolic content ranging between 502.78- 767.54 mg/100g has been found in pearl millet by Sangwan [28], Sharma and Kapoor [29], and Archana., *et al.* [30]. Similarly, Kaushik and Grewal studied thirteen different PM varieties for anti-nutrient content (polyphenol and phytic acid). These varieties were grown in Haryana. These varieties' polyphenolic and phytic acid content ranged from 403.7 to 521 mg/100g, and 577.7 to 620.7 mg/100g, respectively [31]. The phenolic, phytic acid and flavonoid content of different varieties of pearl millet are presented in tables 2 and 3.

Variety	Total phenolic content (mg GAE g^{-1})	Total flavonoid content (mg QE g^{-1})
RHB-177	152.54	82.56
RHB-173	156.73	85.71
HHB-67	162.32	87.39
GHB-538	175.60	142.08
GHB-558	198.96	107.04
MPMH-17	202.81	147.20

Table 2: Total phenolic and flavonoid content in different varieties of PM grown in Rajasthan.

Source: Meena *et al.* [106].

Varieties	Polyphenolic content	Phytic acid content
HC- 10	418.3	604.0
HC- 20	448.1	593.0
HHB- 67	505.4	620.7
HHB- 146	521.0	589.3
HHB- 197	501.0	605.0
HHB- 223	439.0	618.3
HHB- 226	510.0	597.7
HHB- 234	445.7	577.7
WHC- 901- 445	403.7	612.3
HMP- 802	461.0	590.3
HHB- 256	416.0	583.3
HHB- 272	411.7	599.7
HHB- 265	451.9	603.0

Table 3: Polyphenolic and phytic acid content (mg/100g) in different varieties of pearl millet.

Source: Kaushik and Grewal [31].

Tannins

Tannins are considered an important group of anti-oxidant polyphenols, usually present in food and beverages. After cellulose, hemicellulose, and lignin, tannins are considered the fourth most abundant constituent of the plant. Tannin is a polyphenolic biomolecule, having an astringent and bitter taste and a high molecular weight of 500-3000Da. They are organic, nitrogenous, and inorganic substances and serve the plants' protection [32]. In the human body, tannins show multifunctional properties that attract the researcher's interest [10,33]. However, they negatively affect the nutritional value of food. Tannin combines with protein and makes a complex, lowering food efficiency, growth, and iron absorption. Other severe side effects that can appear from excessive tannin consumption are damage to the gastrointestinal tract and the elimination of specific tissues, proteins, and essential amino acids. Therefore, they should be reduced to a safe level before being consumed by humans [34,35].

According to dietary analysis, the daily intake of tannin in India is 1500-2500 mg. Daily consumption of less than 1.5 to 2.5g tannin does not show any side effects. However, consumption beyond this range leads to diseases like anemia and osteoporosis and worsens cancer [36-38]. Decreased serotonin levels increase the severity of migraines. This is because starch gets bound by tannin and is a serotonin precursor [39]. They can inhibit the growth of cancer cells. However, some carcinogenic molecules can induce cancer and cause hepatic cell necrosis [40]. When tannins precipitate protein, it gives a sensation of astringency, reducing the palatability of food products. Therefore, tannins are a disadvantage for the beverage industry [36].

Tannins have various industrial applications as they have antioxidant and anti-bacterial properties. Therefore, they are used in the food industry, preventive medicine, and the leather and chemical industry. Hydrolyzable tannin is an ester of sugar and phenolic acids or their derivatives. It consists of gallic acid, ellagic acid, hexahydroxy diphenic acid, and penta galloyl glucose with a central glucose molecule attached to many gallic acid units [33]. Gallotannins contain a polyphenolic and a polyol residue, so they are the simplest hydrolyzable tannins. Oxidative coupling of at least two galloyl units forms ellagitannins [41]. Gallotannins are found in grapes, berries, pomegranate, and persimmons, whereas ellagitannins are present in tea, coffee, fruits, nuts, and wine from fermented oak barrels. Condensed and complex tannins are usually found in fruits (e.g., apples, cranberries, and pears), chocolate, coffee, cocoa, legumes (e.g., red kidney beans, chickpeas, black-eyed peas, and lentils), green grapes, nuts, red wine and tea [42].

Enzyme inhibitors

Various types of enzyme inhibitors are protease inhibitor, alpha-amylase inhibitor, and trypsin inhibitor. These inhibitors protect plants against pests and microbes [43]. Plants have a variety of protein inhibitors to regulate proteins and self-protect from herbivores. These are mainly stored in seeds and tubers and are, therefore, called storage proteins. The contents of such proteins range from 6 to 10%. Trypsin inhibitors have an inhibitory effect exclusively against trypsin proteins that bind to peptides after lysine and arginine. They contribute significantly to the trypsin and chymotrypsin loss, preventing proteins' digestion [10,44]. Protein enzymes are known as proteases that catalyze the hydrolytic cleavage of peptide bonds in the target proteins.

Also, proteases are involved in various proteolytic processes that regulate and prevent extreme and unwanted protein damage. The function of these proteases is sometimes affected by some inhibitors called protease inhibitors. These are smaller proteins usually present in high concentrations in storage tissues and represent up to 10% of the total protein content [45,46]. Protease inhibitors play an essential role in various physiological processes in plants. Plant functions include storage proteins, regulators of endogenous proteolytic activity, and protective components associated with plant resistance to insects and pathogens. The binding of protease inhibitors to proteins slows down their digestion in the small intestine and facilitates the release of proteins from the body. As a result, the bioavailability of sulfur-containing amino acids (such as cysteine and methionine) is reduced [2]. Also, by modulating protease activity, protease inhibitors interfere with various biological processes such as apoptosis, blood clotting, fibrinolysis, inflammation, and hormonal pathways in mammals. Hence, there is a need to remove or considerably reduce the content of protease inhibitors from foods. However, protease inhibitors have biotechnological potential as insecticides, anticancer agents, and anti-bacterial [47-49].

Amylase inhibitors can behave as endogenous, mammalian, and insect alpha-amylases, which bind to alpha-amylase to make

them non-functional. Proteinaceous and non-proteinaceous inhibitors are the two classes of alpha-amylase inhibitors. In industries, α-amylase inhibitors are used in brewing and baking to maintain starch degradation rate and improve the properties of flour. The stability of beer foam could be better with α-amylase inhibitors as they interact with iso- α-acid from the hop. Researchers found that it also has an active role in diabetes [10,43].

Reduction strategies

Several processing methods include decortication, heating, soaking, germination, and fermentation, which have been used for many years to reduce the concentration of anti-nutrients in plant-based foods. Recently, low phytic acid lpa mutants have been developed, which lower the phytic acid content naturally in legumes and cereals.

Decortication

Decortication or dehulling involves the removal of the pericarp (outer covering) of the grains. Various millets, including PM, have a different fraction of husk ranging from 1.5 to 29.3%. Traditionally, decortication was done manually with the help of mortar and pestle, but nowadays, a rice huller/rice milling machine is used [50]. El Hag., *et al*. found that decortication reduced phytic acid up to 53% and total polyphenolic content (TPC) up to 9% in PM [51]. In contrast, Pal., *et al.* found a 52.63-56.00% reduction in the phytic acid content of lentil [52]. The effect of dehulling on the anti-nutritional and nutritional profile of Gampella and IKMP-5, cultivars of PM (cultivated in Burkina Faso), was investigated. It was found that dehulling significantly reduced some of the anti-nutrient components present in the bran [53]. The concentration of condensed tannins was also found to be lowered by dehulling. Pal., *et al.* reported that the combination of decortication and germination resulted in a considerable reduction in anti-nutritional factors like tannins, phytate, trypsin inhibitors, TPC, etc in horsegram [54]. Ghavidel and Prakash, found the reduction to be 47-52% and 43-52% for phytic acid and tannin, respectively [55]. In a recent investigation, this method decreased phytic phosphorus content by 39% in little millet, 23% in barnyard millet, 25% in kodo millet, and 12% in common millet [56]. It is pretty clear from these studies that the removal of the pericarp and aleurone layer (peripheral parts) of grains reduces the content of anti-nutritional factors. Therefore, decortication is a suitable method for eliminating phytates and tannins [57].

Heating

Heating includes roasting, boiling, cooking, or autoclaving. Roasting reduced 74.6, 28.4, 98.3, and 97.5% of tannin, phytate, trypsin inhibitor, and protease inhibitor, respectively. However, cooking reduced these by 42, 75.8, 95.8, and 95.8%, respectively [35]. Sade, also found that roasting eliminates tannins (from 0.51 to 0.29 mg/100g) and phytate (from 0.21 to 0.11 mg/100g) [58]. According to Jambrec., *et al.* TPC increases after cooking [59]. However, Abdelrahman., *et al.* found that cooking caused a 6-10% and 5-8% reduction in phytic acid and polyphenols [60]. A study on different types of beans found that boiling decreased 5- 51% phytate and 42- 48% TPC [61]. In a study, autoclaving kidney beans reduced tannins by 50-72%. Earlier studies reported autoclaving as one of the best methods for reducing the levels of various anti-nutritional factors [15,62]. Boiling and autoclaving help improve the quality of protein in winged beans by reducing the levels of anti-nutrients. Pressure cooking improved protein digestibility in black grams due to tannins' removal [63]. Vijayakumari., *et al.* evaluated the effects of soaking, cooking, and autoclaving on reducing phytic acid content in pulses. Results showed that 3 hours of cooking significantly reduced phytic acids level [64]. They also found that autoclaving was more effective in reducing phytic acid content in the Indian tribal pulse germplasms, *Mucuna monosperma*. Around 20-25% decrease in tannin and phytic acid and a 17% decrease in tannins after pressure cooking was reported by Pushparaj and Urooj [65] and Mohapatra., *et al.* [66], respectively. Sihag., *et al.* observed maximum removal of phytic acid by malting (38.23%) and of polyphenols by pressure cooking (49.28%) [67]. Different heating treatments (boiling, autoclaving, and microwave cooking) were used by Hefnawy, and found to decrease anti-nutrients significantly. They also used microwave cooking and found that trypsin inhibitors, tannin, and phytic acid were reduced by 81.5, 34.3, and 39.1%, respectively [68]. Pal., *et al.* [54] and Pal., *et al.* [52] observed a reduction in trypsin inhibitor by 26.79% in germinated samples of horse gram and 80.51-85.41% reduction trypsin inhibitor of lentil after cooking, respectively.

Soaking

Soaking is done before treatments like germination, cooking, and fermentation. Soaking is the easiest way to reduce anti-nutrients [14]. Some studies reported that soaking in water for 12 to 18 hrs is quite efficient in reducing levels of partially or entirely

soluble phytic acid and proteolytic enzyme inhibitors [69]. Roy., *et al*. studied five varieties of chickpea- Virat, Annigeri, IC268966, BGM 408, and CUML4 and reported that soaking decreased tannin, phytic acid content in the range of 16.90 - 23.28% and 15.19 -17.78%, respectively [70]. According to Fernandes., *et al.* [71] and Nithya., *et al.* [72], soaking and discarding water can remove a significant percentage of the anti-nutritional factors as it reduces anti-nutrients by leaching out polyphenols. Some studies have found a remarkable decrement in the levels of oligosaccharides, tannins, and phytates in soaked and cooked beans without using soaking water. It is recommended to discard the water used for soaking as it has been proved to be an efficient method of eliminating phytates and phytic acid. Hithamani and Srinivasan, studied a reduction in tannins content from 2.72 to 0.70 mg/g after soaking. During soaking, the enzyme polyphenol oxidase gets activated, which reduces the polyphenolic content [73]. Another study conducted by Sharma and Kapoor [29], found a 36.8% reduction in phytic acid and 39.57% reduction in polyphenol content. Ibrahim., *et al.* concluded that tannins reduced from 210.17 to 210.03 mg/100g, phytic acid from 4.54 to 4.18 g/100g, and trypsin inhibitors from 29.65 to 24.91 TIU/mg after 16 hrs of soaking [74]. Soaking reduced phytate content in sorghum by 4% and in maize by 21%. Tannins were decreased by 22% in chickpeas. However, others have reported 17 to 30% reduction in various bean varieties after soaking [11]. Singh., *et al.* concluded a reduction of up to 70% of polyphenol when millet was treated with soaking, germination, microwave treatment, and fermentation [75]. Soaking of soybean for 96 hrs reduced 35% of trypsin inhibitors. It proved a very simple and economical processing treatment for inactivating trypsin inhibitors [76].

Germination

Germination is an active phase of metabolism in which anti-nutrients are reduced [14]. It could improve legumes and cereals' nutritional content by modifying the composition of chemicals and reducing the anti-nutrient factors [15]. Sokrab., *et al.* stated that germination reduces phytic acid but enhances polyphenol content [13]. Another study by Ying., *et al.* found that germination increased TPC by 5.57% in peanuts and decreased by 25.96% in soybean [77]. Masud., *et al.* stated that germination is one the best way to reduce phytic acid by up to 40% [78]. The phytic acid content in different varieties of PM varies from 588 to 1382 mg/100 g. When PM grains are germinated for 24 hrs at 30˚C, it leads to a reduction of phytates by more than 50%. This is due to the activity of an endogenous enzyme called phytase. It hydrolyzes phytic acid during the process of germination and eventually reduces phytic acid content [63,79]. In another study, the elimination of 40.00-59.38% of phytic acid was recorded [52]. The content of phytic acid in the cereal grains was analyzed, and it was discovered that germination for more than ten days led to an effective reduction ($P < 0.05$) in phytate content of all the selected cereals [18]. Kumari., *et al.* stated that the duration of germination affects the content of anti-nutrients like TPC and tannin content [14]. Handa., *et al.* found that 48 hrs germination reduces TPC from 134.71 to 65.19 mg GAE/100g and tannin content from 199.85 to 100.30 mg/100g [69]. In another study, Mohamed., *et al.* also analyzed the effect of germination duration and found a linear reduction of phytic acid in kidney beans and mung bean [17]. Owheruo., *et al*. reported a 60% reduction in phytate after germination [80]. In another study, change in total flavonoid content (TFC) was analyzed up to 7 days of germination. It was found that TFC increased till the 3rd day and then started decreasing. This also indicated that germination time plays an essential role in removing anti-nutrients [81]. The germination process activates the phytic enzyme and is responsible for the hydrolysis of phytic acid [82]. The concentration of tannin, phytate, TPC, and TFC in the control sample was 3.12, 28.90, 8.12, and 1.81 mg/g, respectively, which changed gradually till 72 hrs of germination [14]. Germination reduced phytic acid by 44.18% and polyphenol content by 46.41% [29]. According to Avilés-Gaxiola., *et al.* there was 88.2, 52.5, 34.0, 25.0, and 19.2% reduction in trypsin inhibitor activity in black gram, white bean, chickpea, black gram, and pigeon beans, respectively [76]. Lakshmanaswamy and Narayanan, worked on three biofortified PM varieties Dhanshakti, ICMH 1201, and Proagro 9444, having 0.56, 0.58, and 0.58 mg/100g phytate content and 0.54, 0.42, and 0.59 mg/100g tannin content, respectively. After germination and drying, they reported 28.57, 31.03, and 60.3% reduction in phytate and 59.2, 19.04, and 52.54 in tannin levels in the three varieties [7]. Using traditional methods, Roy., *et al.* studied five varieties of chickpea-Virat, Annigeri, IC268966, BGM 408, CUML4 [70]. These varieties were soaked for 9 hrs and germinated for 24 hrs. It was reported that soaking decreased tannins and phytic acid in the range of 16.90-23.28% and 15.19-17.78%. Germination also reduced tannin content from 22.66-35.22% and phytic content from 25.87-32.84%. Azeke., *et al.* concluded that germination reduces phytate content in millet and sorghum from 5.7 to 0.85mg/g and 6.2 to 1.2 mg/g, respectively [83]. Singh., *et al.* concluded that germination and fermentation

give good results by decreasing the anti-nutrient factors due to the loss of polyphenol when soaked in water [75].

Fermentation

A few studies have shown that the fermentation of legumes improves their nutritional value and antioxidant properties. It eliminates certain endogenous anti-nutritional factors, for example, phytic acid, and has a positive effect on protein digestion and bioavailability. During food processing, fermentation helps in (Karovičová and Kohajdova [84]):

- Enrichment of human nutrition through the development of various aromas, flavors, and food textures.
- Preservation of large quantities of food via lactic acid, acetic acid, alcohol, and alkaline fermentation.
- Biological enhancement of food substrates with essential fatty acids, protein, essential amino acids, and vitamins.
- Detoxification
- Reduction in cooking time and fuel consumption.

In a study, Rasane., *et al.* observed that non-fermented roasted and germinated PM samples have high phytic acid content than fermented, roasted, and germinated samples [85]. Various microorganisms are used for fermentation, such as *Saccharomyces, Lactobacillus*, and different types of fungi, yeast, and mold, all of which show different results. In their study on soybean, Mohamed., *et al.* used four different strains of *Lactobacillus* - *L. plantarum, L. acidophilus, L. casei,* and *L. bulgaricus.* Of these, *L. bulgaricus* was found to be the best in reducing phytic acid content from soybean [17]. In a study by Salar., *et al*. samples of "Pearl millet koji" were fermented for ten days using *Aspergillus awamori* (MTCC-548). Fermentation resulted in a significant increase in the TPC up to 8 days but later started decreasing. TPC content increased from 18.00 mg GAE/g dw to 85.12 mg GAE/g dw. After ten days of fermentation, it decreased to approximately 52 mg GAE/g dw [86].

Lactic acid bacteria (LAB) play an essential role in maintaining and producing healthy foods. Lactic acid fermentation (LAF) is generally cheap and often requires little or no heat. Hence, they are fuel-efficient [87]. Svanberg., *et al.* found a significant reduction in anti-nutrients levels through the LAF process while improving the bioavailability of micronutrients [88]. *L. plantarum* plays a vital role in increasing food's shelf life and flavors [89]. *L. plantarum* proved efficient for reducing anti-nutrients in soybean, which can be used to produce soy-based weaning food [35]. In a study, mung bean, soybean, and kidney bean were fermented by *L. bulgaricus.* Maximum reduction of phytic acid was observed in kidney beans that is 85.4%, followed by 77.0% for soybean and 69.3% for mung bean [17]. 24 hrs of LAB fermentation resulted in a 40% reduction in phytate in chickpea flour [90]. PM cultivars, Ashana and Dembi, were investigated by Abdelrahman., *et al.*, a significant reduction in phytate and polyphenols content was found after fermentation for 14 hrs [60]. Mohamed., *et al.* studied two PM cultivars- Gazira and Gadarif and concluded that phytic acid and polyphenolic contents were significantly decreased when fermented for 24 hrs [91]. However, tannin content got reduced during processing but increased during germination. The data collected is shown in table 4. Tannin content increased with the increase in fermentation duration.

Treatment	Anti-nutrients	Gazira		Gadarif	
		0 hr	24 hr	0 hr	24 hr
Finely ground	Phytic Acid	987.19	341.92	952.51	456.10
	Tannin	415.38	348.15	420.25	391.02
	Polyphenols	220	90	170	110
Soaked	Phytic Acid	597.50	138.31	722.20	421.07
	Tannin	392.16	357.55	376.25	322.50
	Polyphenols	170	120	150	120
Autoclaved	Phytic Acid	363.27	102.35	338.21	105.25
	Tannin	390.20	345.88	397.27	339.06
	Polyphenols	100	80	80	40
Germinated	Phytic Acid	327.50	111.10	329.20	108.79
	Tannin	407.91	415.45	369.47	410.22
	Polyphenols	250	240	210	270

Table 4: Phytic acid, tannin, and polyphenolic contents (mg/100g) of Gazira and Gadarif cultivars after fermentation of the processed samples for 24 hrs.

Source: Mohamed., *et al.* [91].

In a study, PM was fermented for 24 hrs, and the phytic acid content was analyzed, which decreased by 51.9% [92]. The fermentation of germinated PM buds at 30°C for 72 hrs with pure mixed culture *S. cerevisiae, S. diasticus, L. brevis*, and *L. fermentum* reduced the phytate content by reducing the phytate content 88.3%

[93]. Mohapatra., *et al.* concluded that the total phenol content was decreased by 28% during fermentation, and tannin content decreased by 30-39% in whole grain sorghum [66].

During fermentation, the metabolic activities of microorganisms modify the concentration of bioactive compounds. The cell wall of cereal grain ruptures due to fermentation, which leads to the production of several bioactive compounds. Some enzymes like amylases, proteases, and xylanases present in the cereals and microorganisms modify the grain's nutritional content [94]. Adeyemo and Onilude, worked on soybean for reducing anti-nutritional factors enzymatically by fermenting it with *L. plantarum.* Some pretreatments included were cooking, roasting, and fermentation for five days. It was reported that raw soybean contains 1.93 mg/g tannin, 1.16 mg/g phytate, 1.20 mg/g trypsin inhibitor, 1.20 mg/g protease inhibitor. After fermentation, tannin content was reduced to 0.120 mg/g, and phytate content became 0. 047 mg/g. Protease inhibitor and trypsin inhibitor also decreased from 1.2 to 0.020 mg/g and 1.20 to 0.010 mg/g, respectively [35]. In a study, soaked and germinated PM samples were fermented naturally and selectively.

L. acidophilus and *L. plantarum* were used for selective fermentation. It was concluded that both strains showed good results, but *L. acidophilus* showed comparatively more effective results. No phytic acid was detected in the germinated sample after fermentation with *L. acidophilus*. After natural fermentation, no phytic acid was found in both the samples [29]. Kanekar., *et al.* [95] found a 6% reduction after 18 hrs LAF, and Sharma and Kapoor reported an 80% reduction in amylase inhibitor [29]. Traditional processes can improve nutritional quality by increasing the palatability and assimilation of food or increasing the availability of nutrients by eliminating anti-nutrients or minimizing their effects [96]. Literature evidence has concluded that fermentation is one of the best approaches, which could be used to reduce the anti-nutritional content in plant-based food and improve the overall nutritional value of food.

Biotechnological interventions

Genetic engineering techniques have been used to eliminate the genes involved in the metabolic pathways for reducing the production and/or inactivation of anti-nutrients. Low phytate soybean and rice cultivars (approx. 40-50% less) have been developed [97-99]. It was found that lpa mutants reduce phytic acid content, affecting growth, stress response, seed development, and germination in plants [100]. A zinc-finger nuclease was designed to mutate the IPK1 gene in corn, one of the phytic acid biosynthesis genes, resulting in both herbicide tolerance and the expected alteration of the inositol phosphate profile in developing seeds [101]. Perera., *et al.* analyzed that lpa mutants of rice have 45-95% less phytic acid content than wild-type seeds [102]. Genomic resources can be used as pathways to RNA interference and removing anti-nutrient factors, but this technology has yet to be tried out *in vivo* [103].

Conclusion

Anti-nutrients are present in a wide range of foods, which is a big challenge for those opting for a vegetarian (plant-based) diet. These can have beneficial effects on health if consumed in a limited amount or else cause malnutrition. PM contains some anti-nutritional factors such as phytate, tannins, polyphenols, saponin, protease inhibitor, and trypsin inhibitor, which can inhibit mineral utilization, cause toxicity, and some other diseases. Therefore, to get proper nutrients from pearl millet, some common treatments like decortication, roasting, boiling, soaking, germination, autoclaving, and fermentation have proven useful. From all the processing treatments, germination and fermentation have been found to be the most effective methods. In addition to treatment methods, some biotechnological techniques are being used on cereal plants to prepare mutant crops, e.g., lpa crops having low phytate content.

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Consent to Participate

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Code Availability

Not applicable.

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Authors' Contribution

The search of the studies and manuscript preparation were performed by M.S., K.S., S.C., A.P., and M.S., S.S., S.C and T.D design and analyzed the study. The manuscript was edited by S.C., A.P., S.S., and T.D. All authors read and approved the final manuscript.

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