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Role of lacto-fermentations in reduction of antinutrients in plant-based foods

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ABSTRACT

Plant-based food products are gaining more importance and they play an important role in maintaining sustainable, low-meat, and healthy diets. Plant-based food products, specifically legumes and cereals, are important staple foods in developing countries. However, it is important to know whether these plant-based systems are capable of delivering the minerals and is it beneficial to motivate consumption to decrease the manifestation of mineral deficiencies. Plant-based foods apart from containing a large number of macronutrients and micronutrients they also possess various anti-nutritional factors. Some of the major anti-nutritional components present in plants are saponins, tannins, phytic acid, lectins, protease inhibitors, and amylase inhibitors. Such kind interactions with minerals interfere in bioavailability from plant-based foods throughout the course of human digestion and lead to micronutrient malnutrition and mineral deficiencies. Lacto-fermentation is commonly used to disrupt such interactions and make nutrients and phytochemicals free and accessible to the consumers. The purpose of this review was to provide information about the different types of antinutrients present in plant sources, their possible effects on the human body, and the benefits of lacto-fermentation over other conventional food processing approaches such as soaking, germination, and heating in the reduction of antinutrients.

1. INTRODUCTION

The share of plant-based food materials is increasing promptly and is gaining popularity in the global market. Plant-based gaining interest because of several factors, such as changes in lifestyle, awareness toward healthy alternative foods, and increased understanding regarding the renewable production of food. Edible plants are a rich source of certain micronutrients such as minerals, vitamins, and phytochemicals [1,2]. Plant phytochemicals carry some important properties such as antioxidants, anticarcinogenic, and antimicrobial activities and have displayed possible protective benefits toward cardiovascular disorders, cancer, hormonal imbalance, and osteoporosis [3,4]. Various plant-based foods such as cereals and legumes contain an appropriate number of oligosaccharides and dietary fibers [5,6]. These oligosaccharides are non-digestible carbohydrates, therefore, possess the ability to improve certain physiological properties. Moreover, oligosaccharides act as prebiotics and help in promoting the growth of gut microbiota selectively. β-glucans add some health benefits by reducing the level of cholesterol and help in improving the sensory traits of the end product [7-9]. Despite all such functional characteristics of plant-based food, a careful assessment discloses that commercial plant-based foods are nutritionally imbalanced as compared to animal origin products. Plant proteins more often display low quality, less digestibility, and unwanted inhibitions in essential amino acids [10,11]. Furthermore, some vitamins such as Vitamin D and B_{12} are found in low amounts or absent in plant-based raw materials which are ultimately are responsible for vitamin deficiencies in a strict vegetarian diet [12,13].

Plant antinutrients directly interact with the minerals and micronutrients and lead to harmful effects on zinc and iron bioavailability as demonstrated *in vitro* [14,15] and *in vivo* conditions [16-18]. Moreover, an inadequate amount of micronutrient level is found in the diet of people who are vegetarian or vegan and they are at higher risk of micronutrient deficiency and associated disorders. Plant-based products, raw, or vegan diets often contain antinutrients associated with them as they are accumulated naturally within the plant tissues [18,19]. The most common antinutrient present in plant-based foods is phytates, tannins, lectins, saponins, enzyme inhibitors, and phenolics [Table 1 and Figure 1].

Moreover, mineral antinutrients also reduce the digestibility of certain macromolecules such as dietary proteins, starch, and carbohydrates by forming insoluble complexes with them. Another reason for decreased mineral bioavailability in the plant-based food system is because of the binding of antinutrients with each other like phytic acid (PA) with dietary fiber [20]. Anti-nutrients can have a toxic effect when consumed above a certain limit and causes various health disorders [Figure 1] [19]. Apart

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Table 1: Antinutrients associates with plant-based food sources

S. No.	Antinutrients	Plant source	Implications	References
1	Lectin	Wheat, beans, quinoa, peas	Acne, inflammation, migraines, or joint pains	[30,31]
2	Trypsin Inhibitors	Chickpeas, mung beans, red kidney beans	Protein digestion	[32]
3	Alpha-amylase Inhibitors	Seeds: Sesame, flax, poppy, sunflower, pumpkin	Inhibit the activity of salivary and pancreatic amylase	[33]
4	Protease Inhibitors	Cereals and legume	Inhibits growth, pancreatic hypertrophy, and poor digestibility	[34]
5	Tannins	Grapes and green tea	Inactivates digestive enzymes and reduces digestibility of proteins	[35]
6	Phytates	Vegetable products. seeds, grains, nuts	Decreased mineral bioavailability	[36]
7	Goitrogens	Broccoli, cabbage, cauliflower, Brussels sprouts, and kale	Hypothyroidism	[37]
8	Raffinose oligosaccharides	Beans, cabbage, broccoli, asparagus	Burping, flatulence, and stomach discomfort	[38]
9	Saponins	Legumes, pseudo-grains, potato, eggplant, tomato	Hypoglycemia or disturbs the protein digestion, vitamins, and mineral absorption in the gut, and also result in the development of leaky gut, hypo-cholesterolemic effect	[39,40]
10	Polyphenols	Vegetables, fruits, legumes, and cereals	Causes precipitation of macromolecules such as carbohydrates and proteins, therefore, decreasing their bioavailability and digestibility. Also, the bioavailability of vitamins such as vitamin B12 and minerals is reduced	[41]

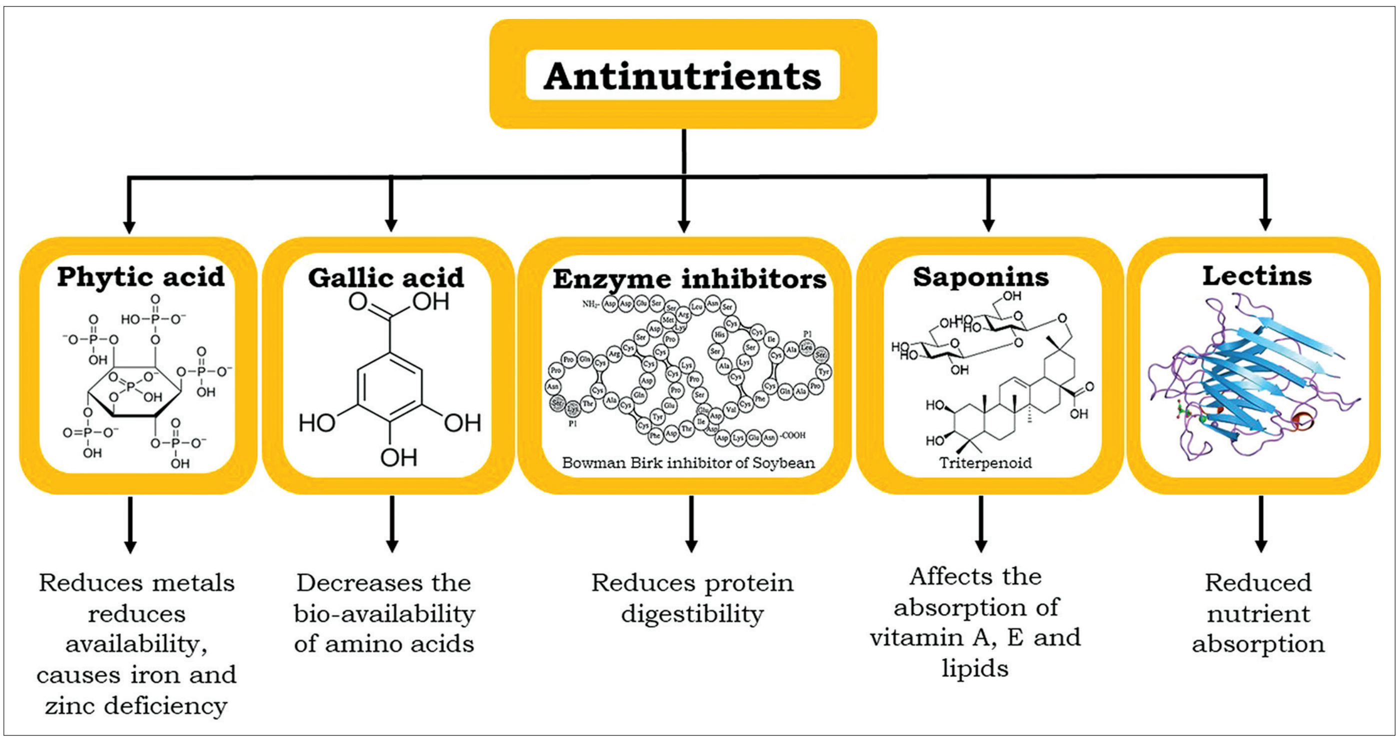

Figure 1: A brief overview of the types and adverse effects of key anti-nutrients.

from reduced bioavailability and biodigestibility the other common symptoms exhibited by many antinutrients are nausea, bloating, headaches, rashes, etc. [21]. Furthermore, quality improvement is necessary therefore removal of undesirable food components is desirable. Moreover, at low concentrations, these antinutrients have shown various positive health benefits such as reduced glucose levels, prevents cancer risks, antibacterial and antiviral properties, maintaining proper liver functioning, and highly antioxidants [22].

Oligosaccharides from plants, including raffinose, stachyose, and verbascose, are consumed by gut bacteria through fermentation, resulting in flatulence, diarrhea, etc., [20,23-25]. Unfortunately, the natural flavor of plant-based products displays less acceptance [26]. Plant-based food is to date commonly regarded as products having a displeasing taste, probably due to past experiences with less desirable products available in the market [27]. Plant phenols, terpenes, glucosinolates, and flavonoids based on their molecular weight they produce undesirable changes in organoleptic properties such as bitter taste and pungent smell [28,29].

2. STRATEGIES TO REDUCE ANTINUTRIENTS

Various methods that have been employed to reduce antinutritional factors present in foods are discussed below:

2.1. Soaking

Soaking is a physical process and it is considered one of the easiest ways through which soluble antinutritional factors can be removed and also helps in reducing the cooking time. Soaking also enhances the release of enzymes (e.g., endogenous phytases), which are present in plant foods such as almonds, nuts, grains, and other edible seeds, soaking also reduces the number of enzyme inhibitors to improve both digestibility as well as nutritional value [30-42]. Many of the anti-nutrients are water-soluble in nature, which enhances their removal from foods through leaching. One of the studies reported that the concentration of PA was decreased in chickpea from 47.45% to 55.71% on increasing the time of soaking from 2 to 12 h [43]. Soaking with distilled water, 1% sodium bicarbonate and mixed salt solutions decreased total phenols, ortho-dihydroxy phenols, tannins, and phytates by 33%, 41%, 35%, and 21%, respectively [44]. Soaking of soybean flour has also reduced the total protein, soluble sugar, and tannins [45]. Some drawbacks are also associated with soaking is that it causes leaching of some important water-soluble proteins and minerals [41].

2.2. Heating

The autoclave is an instrument, which is widely used for the application of heat treatments. Autoclaving of plant-based food stimulates the production of endogenous enzymes such as phytase, tannase, and also increases acidity [41]. On consumption of autoclaved plant-based food enhanced health benefits have been observed. Another research showed a significant reduction in anti-nutritional factors in legumes after autoclaving, soaking, and cooking [46]. Most of the previous studies have concluded that autoclaving is the best method for the reduction of various anti-nutritional components relative to other processing methods [47-49].

2.3. Cooking

Cooking helps in reducing various antinutrients such as PA, tannins, and oxalic acid from whole grains, beans, and vegetables. Protease inhibitors are proteinaceous in nature and they get readily denatured by heat treatment [50]. Various studies have shown that reduced antinutrient amounts can be obtained by heating under controlled conditions, that is, when the temperature is needed to be maintained lower than that of boiling point for about 15 min [51]. Boiling of Bambara groundnut seeds for about 1 h greatly decreased the amount of raffinose and also enhanced protein digestibility of seeds was obtained [52]. The cooking of a mixture of sweet potato leaves and lemon together decreased the amount of both phenolic (56%) as well as oxalate components [53].

2.4. Germination

Germination is another process used for the reduction of anti-nutritional factors present in plant-based foods [54]. Germination of seeds typically activates endogenous enzymes such as phytase which hydrolysis the phytate and decreases the amount of PA found in samples. Germination usually brings a change in the nutritional level, biochemical property, and physical characteristics of the food. Germination is the most preferred process and is frequently used for reducing the antinutritional content in cereals [55-57]. Latest studies stated that germination helps in changing the isoflavone profile usually found in soybean due to the activation of an enzyme called β-glucosidases; this helps in improving the nutritional value of soybean as isoflavones display chelating properties [58,59]. Singh *et al*. [60] stated that on the processing of millet with germination, the polyphenol contents have shown a maximum reduction of about 75% as compared to other processes such as soaking and microwave.

2.5. Fermentation

Fermentation is another alluring option to achieve the goal of reduction of antinutrients from plant-based foods. It is considered a natural solution to food processing since the earlier times of mankind, and nowadays, fermented foods are gaining more popularity than ever before [61]. Fermentation is an ancient and simple technique which is often employed to prevent and improves the safety, nutritional, rheological, and sensorial characteristic of plant-based materials while being natural and economical [62]. Plants can support the growth of a large number of microorganisms [63]. Plant-based fermentation most commonly employs lactic acid bacteria (LAB), bacilli, and yeasts (e.g., *Saccharomyces*) [64,65]. They have been studied mostly as monocultures; these microbes possess certain proven properties through which they increase necessary nutritional and sensory characteristics. Fermentation has shown considerable improvement in the nutritional quality of cereals. It may also serve to enrich the reservoir of available amino acids, vitamins, and minerals; as a result, fermentation enhances the total digestibility and sensory characteristics of food [65]. Furthermore, fermentation is of immense importance in the degradation of oligosaccharides which are usually found in beans and vegetables and are accountable for digestive difficulties such as flatulence. Fermentation can increase the concentration or bioaccessibility of functional (bioactive) compounds. In a recent report, fermentation of maize flour has been done with a mixture of LAB through the standard method with 12 h intervals to verify the impact of fermentation on the reduction of anti-nutritional components [66]. Significant reduction of antinutrient components (tannin, polyphenol, phytate, etc.) in fermented maize was observed with the increase in fermentation time. Furthermore, it was concluded that fermentation with LAB-consortium reduced more antinutrients than that of spontaneous fermentation [66]. Etsuyankpa *et al*. [67] performed the microbial fermentation on local cassava products to assess the reduction in anti-nutritional composition present in them. Results showed a significant decrease in the level of cyanide, tannins, phytate, oxalate, and saponins from cassava products due to the microbial fermentation. Fermentation is one of the food processing strategies, which is adopted in Africa to made cereals crops edible and also increases the nutritional properties as well as safety concerns of these foods because cereals are not easily consumed in natural/raw forms. Fermentation of cereals by LAB has been reported to increase free amino acids and their derivatives by proteolysis and by metabolic synthesis. Fermentation has been shown to improve the nutritional value of grains by increasing the content of essential amino acids such as lysine, methionine, and tryptophan.

3. ROLE OF FERMENTATION IN MINERAL AND MICRONUTRIENT BIOAVAILABILITY

Lactic acid fermentation increases the nutritional profile and aids in enhancing the bioavailability of vitamins, minerals, and other nutrients. Therefore, it is considered the most sustainable way for the biological fortification of vitamins, minerals, and essential amino acids in certain food products. This can be achieved in different ways; first, microorganisms apart from containing catabolic property also possess anabolic property in them and can produce certain vitamins and some growth factors. Second, fermentation by LAB, yeast, and mold can hydrolyze coatings and cell walls which are indigestible in nature both chemically as well as physically, and resulting in the release of nutrients trapped within the plants complex structure. Cereals-based foods suffer from low nutritional availability mainly because of the presence of a large amount of anti-nutritional components that impose negative consequences on mineral bioavailability, also in developing countries cereals are the main staple food; therefore, they greatly influence the nutritional status of such area.

However, microbial fermentation can enhance the nutritional value as well as the digestibility of plant foods suffering from low bioavailability [Figure 2]. Microbial enzymes display maximum activity under acidic conditions produced during fermentation and under temperature ranges varying between 22°C and 25°C [68].

Microbial enzymes under an acidic environment including amylases alter the main food components by the degradation of polysaccharides, proteins, phytates, and lipids, respectively. In addition to improving the function of enzymes, fermentation often decreases the number of antinutrients including PA, polyphenols, tannin, trypsin inhibitors, and dietary fibers [19,69] in food that contributes toward enhanced bioavailability of minerals including iron, B vitamin, thiamine, riboflavin, niacin, protein, and simple sugars. Cassava on fermentation has revealed a reduction in the cyanogenic toxin content [70-81] [Table 2].

3.1. PA

PAs also commonly known as myo-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate and are usually found in plant-based food between the range of 0.1 and 6.0% [82]. PA is a secondary metabolite and is mainly found in legumes, peanuts, cereals, and oilseeds, and other plant sources [83]. The increasing trend of veganism results in the ingestion of a large amount of PA which occurs naturally in plant food [84,85]. According to earlier reports, PA affects the activity of enzymes, required for the hydrolysis of protein within the small intestine and stomach [86]. In general, PA affects the bioavailability of minerals and has a strong impact on infants, pregnant, and lactating women on the consumption of a large amount of cereal-based foods [87,88]. PA form insoluble salts with alkali earth metal and transition metals such as zinc, calcium, manganese, and iron [89]. Strong complexation is obtained between two minerals and phytate. This is commonly documented in the case of zinc and calcium where a strong complex is developed between the calcium-zinc phytate than for any of the minerals [89,90]. Phytases originating from microbial sources can degrade PA. The ideal conditions on which phytase activity depends is on its source: For example, the optimum pH of 4.5–5 is required for the activity of phytases which are found endogenously present in cereals [91,92]. Anastasio *et al*. [91] demonstrated that the addition of phytase-positive starter culture in sourdough increased the solubility of iron and zinc by 98% and 89.8% in comparison to phytase-negative starter culture where the solubility of both iron and zinc was only about 41% and 60%, respectively. Magala *et al*. [93] also observed a somewhat similar trend and recorded 89% of degradation of PA in samples of Tarhana and about 100% degradation of PA in beverages made up of rice and oats after fermenting them with *Lactobacillus sanfranciscensis* CCM 7699 culture.

3.2. Phenolic Compounds (PCs)

Phenolic substances can be defined as the most diverse group of plant components present in plants which range from simple phenolic acid to complex tannic acid. They are abundantly present in vegetables, fruits, legumes, and cereals. PCs bind with macromolecules such as carbohydrates and protein thus causing their precipitation. Their antinutrient property also causes chelation of some minerals with their hydroxyl groups and form insoluble compounds that are not consumed by the human body [20]. To chelate iron PCs should possess either

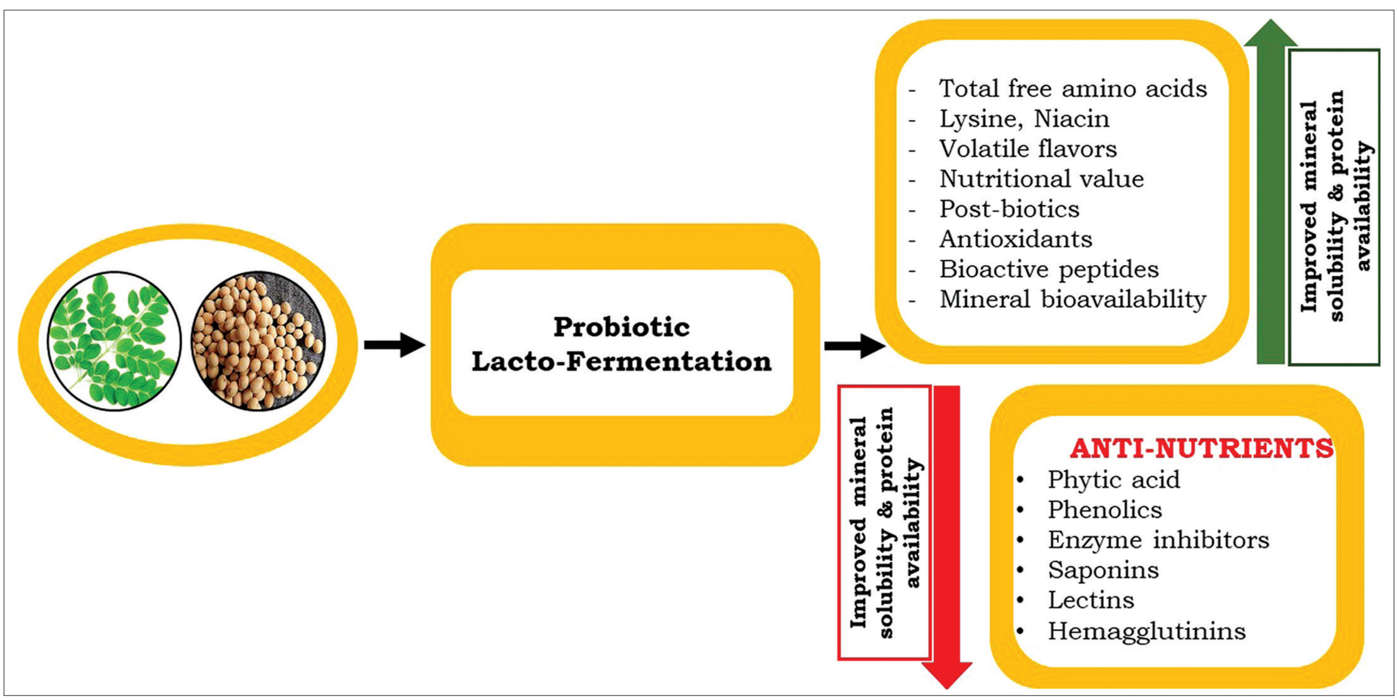

Figure 2: Graphical overview of the lacto-fermentation of plant based.

Table 2: Different lactic acid bacteria strains used for the plant-based fermentation which improved the availability of micronutrients

S. No.	Plant sources	Microorganisms	Bioavailability	References
1	Carrot juice	*L. acidophilus* NCDO1748	Minerals (Ca, P, Fe) and b-carotene	[72]
2	Carrot juice	*L. pentosus* FSC1 and *L. mesenteroides* FSC2	Increase in iron solubility, decrease in mineral inhibitor phytate	[73]
3	Wheat flour	*L. mesenteroides*	Increase in Ca and Mg solubility, decrease in phytate content	[74]
4	Maize	*Lactobacillus* sp.	Increases Iron bioavailability decreases phytate content	[75]
6	*Moringa oleifera* and beetroot (*Beta vulgaris* L.)	*L. plantarum* and *Enterococcus hirae*	Increase in calcium, iron, high radical scavenging activity	[76]
7	Tef flour	*Lactobacillus buchneri* and *Pediococcus pentosaceus*	Zinc absorption PA degradation	[77]
8	Kale juices	*L. acidophilus*	Increase in calcium, phosphorus, and magnesium	[78]
9	Chinese paocai	*L. pentosus* and *L. mesenteroides*	Decreases nitrite formation	[79]
10	Melon juice	*Lactobacillus reuteri* JCM1112	Increases folate production	[80]
11	Fruit drink (containing an oat base included grape, mango, passion fruit, banana)	*L. plantarum* 299v	Increase iron absorption	[81]

L. acidophilus: *Lactobacillus acidophilus*, *L. mesenteroides*: *Leuconostoc mesenteroides*, *L. plantarum*: *Lactobacillus plantarum*, *L. pentosus*: *Lactobacillus pentosus*

ortho dihydroxy (catechol) or trihydroxy (galloyl) groups in their structure [94,95]. Mladenka *et al*. [96] and Macakova *et al*. [97] revealed that complex formation depends upon pH by demonstrating the pH-dependent formation of iron-flavonoid complexes. Iron formed a stable complex with flavonoids at neutral physiological pH but under acidic pH less iron chelation was observed. At acidic pH more $fe3^{+}$ was reduced to $fe2^{+}$ due to flavonoids and less at neutral pH [97]. Tannins bind with iron with more affinity than that of PA. Studies making use of the Caco-2-cell model system showed that PC produced maximum inhibition of iron (90%) at a molar ratio of 1:1 iron: Tannin acid as compared to the phytates where 90% iron inhibition took place at a molar ratio of 1:10 iron:PA [98]. The biggest problem associated with PC's is their well-established therapeutic benefits in the human body. It is therefore important that equilibrium be achieved in such a manner so that PCs are not present at sufficiently high amounts to prevent the absorption of minerals, but at the same time, it should not be present in too low concentrations as a result of which human body would not be benefitted from their antioxidant activity. Metabolism of tannins or other polyphenols by LAB has been characterized only in a few plant fermentations including tempeh [99] and sorghum [92,100]. Among LAB, *Lactobacillus plantarum, Lactobacillus paraplantarum,* and *Lactobacillus pentosus* seem to be the only species capable of degrading hydrolyzable tannins through a tannase activity (tannin acyl hydrolase, EC 3.1.1.20) which breaks ester bounds of tannic acid, thus releasing glucose and gallic acid [92,101-103]. Most of the tannase producers were found in fermented vegetables but also human feces. In *L. plantarum*, tannase is very well characterized. Its activity was demonstrated and characterized [104] and genetic analysis showed it constitutes a novel family of tannases [105]. LAB tannases are intracellular. *L. plantarum* strain has been reported to produce a very efficient tannase during anaerobic fermentation [106]. Genes involved in tannin degradation are regulated in a coordinated way and are inducible by tannin and other PCs [107]. Characterization of fermented cassava LAB allowed identifying uncommon tannase producers such as *Weissella cibaria* and *Leuconostoc mesenteroides* ssp. [108].

3.3. Dietary Fibers

Dietary fibers mainly include non-digestible carbohydrate and non-carbohydrate components like lignin in them. Dietary fibers are not usually broken down by the human digestive enzymes and therefore these components are not absorbed within the upper alimentary tract [109]. A diet containing a large number of dietary fibers is associated with mineral deficiencies because dietary fibers and other components related to it such as PA and tannins are known to interact with minerals (such as zinc, iron, and calcium) and thus prevent their absorption. The dietary fibers interacts with minerals through electrostatic interaction and adsorption. Dietary fibers possess electrostatic interaction because of the presence of various free carboxylic as well as sulfonated groups that contain a negative charge and therefore they interact with positively charged metal ions resulting in their chelation. pH plays an important role in mineral binding and has been extensively studied. Mineral binding at low pH 4–4.5 has been observed low and maximum at high pH range 5.8–6.5 similar pH range is found in human intestine [110]. The iron-binding was found low at pH 4.0 and at pH 6.5 iron-binding was found high. The decrease in iron-binding could be attributed to the fact that fermentation may have resulted in a decrease in pH due to which mineral binding by fibers will be lowered. *In vitro* studies have not been able to demonstrate the fermentation of some of the dietary fibers caused by bacterial enzymes that take place within the human intestines. Mineral ions are released on the degradation of various fermentable dietary fibers such as pectin and the released mineral is absorbed eventually [111-113]. Fermentation of kale juice with *Lactobacillus acidophilus* IFO 3025 showed an increase in calcium, phosphorus, and magnesium [78]. Whole wheat flour contains a large amount of PA naturally. Fermentation of whole wheat flour in presence of *L. mesenteroides* strain 38, for 9 h established the decrease in PA and the generation of lactic acid leading toward greater calcium and magnesium solubility in comparison to the control medium [114]. The impact of natural fermentation (NF) and controlled fermentation (CF) in the reduction of antinutrient components, α-galactosides, and increase *in vitro* protein digestibility was examined. The dry bean (*Phaseolus vulgaris*) flour was used as a raw material for this analysis. Results showed that a reduction in raffinose oligosaccharide, antinutritional constituents, and pH was found in both cases of fermentation. The natural lactic fermentation of ground beans produced a substantial improvement in protein digestibility. Both forms of fermentation decreased the antinutrients and enhanced the nutritional value of the bean flour, and specified the possibility of bean flour to be used as an ingredient for processed foods [115]. Different selected LAB strains in tef fermentations were able to decrease PA content, among different LAB strains *Lactobacillus buchneri* MF58 displayed the maximum amount of degradation in PA (68%) and is supposed to enhance zinc absorption in humans from tef-injera, and more degradation of PA is possibly required to improve iron absorption [77].

3.4. Saponins

Saponins can be defined as non-volatile phytochemicals, ubiquitous in nature but are mainly present in plants. Saponins are a category of plant composed of water-soluble glycosides are connected to either a lipophilic steroid or triterpenoid. Triterpenoids are generally present in most cultivated crops such as legumes, sunflower seeds, and allium species. However, steroids are usually found in food plants such as oats, yucca, tomato seed, and yam [116,117]. Saponins are distinguished by their bitter taste, a surfactant activity, they can hemolyze red blood cells, they also influence the functioning of the intestinal epithelium, aids in the movement of allergens, and interrupting cell regeneration [118]. They form insoluble complexes with nutrients and inhibiting their absorption along the small intestine [119]. The fermentation with LAB decreases the SC content through the process of glycosylation in which an enzyme glycosyltransferase provides both water solubility as well as chemical stability to the aglycone. Fermentation of quinoa with *L. plantarum* decreases the saponin content in them up to the permissible level. The pasting, as well as functional properties in quinoa, was also improved. It then brings added benefits to this raw material and offers the potential to obtain a foundation for gluten-free cream soups with somewhat comparable properties to wheat soup [119]. To establish safe dietary supplements, soymilk was fermented for 24 h together with *Streptococcus thermophilus* 14085 and *Bifidobacterium infantis* 14603 at 37°C. Results showed that fermentation with LAB reduced the saponin levels with antinutritional activity and increased overall phenolic content and antitumor cell proliferation activity of soymilk against HT-29 and Caco-2 cells [120].

3.5. Lectins and Hemagglutinins

Lectins and hemagglutinins are proteins or glycoproteins that contain at least one non-catalytic domain thus exhibiting reversible binding toward particularly monosaccharides or oligosaccharides. Lectins and hemagglutinins can bind carbohydrate moieties present on the surface of the erythrocyte and agglutinate the erythrocytes, without bringing any change in the characteristics of the carbohydrates [121]. These anti-nutrients are primarily present in foods that are taken in raw forms [122]. Lectins which are glycoprotein are found in seeds such as cereals and beans and in tubers such as potatoes. Lectin hinders absorption of nutrients by getting adhered to cells of the epithelial lining, resulting in damaging the intestinal tract, therefore allowing the bacterial population to come in close contact with the blood stream [123]. The impact of NF on the lectin contents in the seeds of *Lens culinaris* cultivar Magda 20 was examined. The results were confirmed by ELISA which showed that the lectin component after 72 h of NF was nearly disappeared under the ideal concentration of flour and temperature conditions [124]. Dual solid-state fermentation of soya bean meal (SBM) was performed using the inoculum mixture of two strains of *Aspergillus* spp. and *Bacillus* spp. Fermentation resulted in a substantial decrease in lectin levels and enhanced organic acid production particularly lactic acid from non-detectable in SBM to 6.16 ± 0.22% in fermented SBM (FSBM) [125].

3.6. Enzyme Inhibitors

Protease inhibitors are generally found in plants and they commonly act as antinutritional factors. They are capable of suppressing the function of proteolytic enzymes in the gastrointestinal tract of animals. They are gaining importance in the field of research because of their potent way of reducing the activity of enzymes through the formation of protein-protein interactions. They prevent the activity of an enzyme by their catalytic mode of action by blocking the active site of enzymes. Trypsin inhibitor and chymotrypsin inhibitor are two types of protease inhibitors that mainly occur in raw grain legume plants. Trypsin inhibitors hinder the function of the enzymes trypsin and chymotrypsin within the gut, thereby resulting in the inhibition of protein digestion [18]. Cereal seeds primarily possess plant serpins, known as the major protease inhibitor family. They are also known as "suicide inhibitors" and are generally found in other species of plants [126,127]. Serpins act as potent inhibitors, which in particular suppress the activities of trypsin and chymotrypsin by acting on their overlapping reactive sites [128]. Dual solid-state fermentation of soybean meal was done using an inoculum mixture of *Aspergillus* spp. and *Bacillus* spp. and a significant reduction in trypsin inhibitors was observed from 2.56 ± 0.42 mg/g in soybean meal to 0.97 ± 0.14 mg/g in FSBM [125]. A significant decrease in trypsin inhibitors was observed from 7.33 to 6.65 when fermentation was done for 24 h using traditionally fermented pearl millet flour for the preparation of lahoh bread [129]. Ejigui *et al*. [130] recorded about 41.7% decrease in trypsin inhibitory activity found in yellow maize when it was kept under fermentation for about 4 days under a controlled environmental chamber. Likewise, Osman (2011) [131] obtained a 37–58% reduction in TIA after 24 h of fermentation in three sorghum cultivars. The influence of fermentation on amylase inhibitor activity found in pearl millet suggested a substantial reduction in AIA as the fermentation time increased. After 24 h, the amount of AIA decreased from 80.16 to 39.45 (50.8%) [129].

4. CONCLUSION AND FUTURE ASPECTS

Plant-based food products are becoming more popular as they play an important role in sustainable, low-meat, and balanced diets. Antinutritional factors are well-known plant components and pose difficulty for those who principally choose plant-based food as a diet. Antinutrients may produce unwanted impact when taken in a large amount. Therefore, the occurrence of antinutrients (e.g. lectins, PA, saponins, and enzyme inhibitors) in foods can trigger different reactions when the consumer has little awareness about the environmental effect on the detoxification ability of the human organism. Microbial fermentation by bacteria or fungi has the potential to increase the nutritional value. Fermented plant food materials are superior in nutrients to their unfermented counterpart because of the activation of endogenous enzymes in them and they are having the potential of reducing the antinutritional factors. Fermented foods have displayed great antioxidant potential as compared to unfermented ones due to an increase in Vitamin C content and also due to the release of various health-promoting bioactive components due to the weakening of plant matrix during fermentation. Disruption of plant-based food matrices containing different minerals embedded in them helps in improving the bioavailability of minerals.

At present, the bioaccessibility of minerals is primarily assessed by various *in vitro* methods which provide a standardized, high-throughput screening method and help in forecasting the mineral bioaccessibility. An idea of the bioavailability of nutrients can be provided by extending these methods to relevant cell lines. However, *in vivo* studies should be taken under consideration while estimating the nutrient bioavailability from food along with the whole range of nutritional, physiological, and ecological factors that may have an impact on absorption. No doubt, *in vitro* methods may provide better screening methods; however, confirmation by *in vivo* methods is important. Furthermore, researchers should not rely only on pulses and cereals but they should also focus on fruit and vegetables, which may be good sources of minerals along with some relevant antinutrients.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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