REVIEW ARTICLE

Innovative genetic approaches to minimize anti-nutritional compounds in staple crops for future food security

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The increasing emphasis on consuming healthy food to boost the immune system is vital in addressing emerging pandemic concerns. Research in this domain encourages the diversification of human diets by incorporating highly nutritious, climate-resilient, yet underutilized crops. However, the bioavailability of nutrients and their absorption are critical in tackling malnutrition, especially in developing countries. Anti-nutritional factors such as phytic acid, gossypol, lectins and oxalic acid hinder nutrient digestion and absorption, presenting a significant barrier to effective nutrition. Traditional methods like soaking, roasting and fermentation reduce these factors but are limited to small-scale applications. Despite progress, there are substantial challenges in understanding and mitigating the accumulation of anti-nutrients in crops. Environmental factors like soil composition and fertilizer use significantly influence the expression of these compounds. Therefore, a balanced approach that incorporates advanced multi-omics and rapid estimation techniques is essential to optimize nutrient availability and develop non-toxic foods. Research indicates that while legumes are particularly high in anti-nutritional traits, crops like soybeans, *Brassicas* and cotton have seen significant advancements in reducing these factors. The integration of traditional processing methods with modern technologies, such as extrusion, offers scalable solutions to enhance food safety and nutritional quality. These combined approaches are significant in reducing hidden hunger and improving global nutritional outcomes. Future research should focus on crop-specific strategies and the stable expression of reduced anti-nutrient traits under diverse environmental conditions. This review highlights the need for continued innovation and education to promote the adoption of improved food processing methods, thereby ensuring better health and nutrition for populations worldwide.

Key Words: *Anti-nutritional factors; Nutrient bioavailability; Food processing techniques; Sustainable diets*

INTRODUCTION

The adoption of sustainable diets has substantial potential to alleviate hidden hunger in numerous countries by increasing the accessibility and absorption of essential nutrients. However, a significant obstacle to this goal is the presence of anti-nutrients in food. Anti-nutrients are substances that disrupt the digestion and absorption of nutrients, thereby decreasing their bioavailability [1]. Despite their significant impact on nutrition, these compounds have been largely neglected in research focused on addressing nutritional deficiencies and toxicities, especially with the rising global population. Anti-nutritional factors can impede digestion and nutrient absorption and in extreme cases, they can lead to serious health problems and even mortality. Consequently, it is vital to address the presence of anti-nutrients in key food crops to ensure effective mineral absorption from plant-based foods. Key anti-nutritional factors in foods include phytic acid, raffinose, saponins, tannins, enzyme inhibitors, lectins, gossypol, glucosinolates, goitrogens, oxalic acid, erucic acid, alkaloids, β-N-Oxalyl Amino Alanine (BOAA) and Hydrogen Cyanide (HCN). These substances have a significant impact on human health by hindering nutrient absorption through mechanisms like chelation and enzyme inhibition. Legumes are particularly problematic as they contain a higher proportion of anti-nutritional traits compared to other crops. This often leads to reduced consumer preference for legumes despite their potential nutritional advantages [2]. Traditional processing methods such as soaking, roasting, sprouting, fermentation, boiling and extrusion can effectively lower the levels of anti-nutritional components in grains. However, these techniques are generally used on a small scale, mainly in household cooking and some value-added agro-industrial products.

To address the limitations of traditional methods and improve nutrient bioavailability, advanced techniques like Ribonucleic Acid interference (RNAi) and gene editing are being explored. These modern agricultural technologies show potential in developing high-nutrition crops with reduced anti-nutritional factors [3]. The reduction of anti-nutritional traits has been an increasingly intense research area since the 1950s. However, there are considerable barriers to improving crop varieties by reducing these factors. The complete exploration of anti-nutrient accumulation in crops remains an ongoing challenge. While some anti-nutrients like phytic acid, raffinose, glucosinolates, enzyme inhibitors and erucic acid have been the main focus of breeding and transgenic approaches, others such as saponins, oxalic acid, alkaloids, HCN, goitrogens and BOAA need further investigation. Another critical factor in reducing anti-nutrients in crops is ensuring their stable expression across different environmental conditions. Anti-nutrients like phytic acid, glucosinolates and alkaloids are significantly influenced by soil composition, fertilizer use and other environmental factors. Therefore, alternative strategies involving advanced multi-omics, rapid estimation techniques and gene editing protocols are essential to optimize nutrient availability and develop non-toxic foods for human consumption. Additionally, it is significant to monitor the effects of reducing anti-nutrients on plant growth and metabolism. Anti-nutrients like saponins, raffinose, enzyme inhibitors, gossypol, glucosinolates and phytic acid play vital roles in regulating crop metabolism and growth. Studies indicate that these compounds influence essential aspects of crop metabolism and growth, necessitating a balanced approach to minimize negative pleiotropic effects on seed quality, yield and stability [4].

Among the primary crops, soybeans have been the most extensively researched for reducing anti-nutrient content, followed by *Brassicas* and cotton, which have also been examined to improve their acceptance for human and animal consumption [5]. This review highlights the progress made in breeding crops to reduce anti-nutritional traits and outlines future research directions in this critical area. By leveraging advanced biotechnological tools and understanding the environmental influences on anti-nutrient expression, we can develop high-value food crops with enhanced mineral availability, thereby contributing to global efforts to reduce hidden hunger and improve nutritional outcomes.

LITERATURE REVIEW

Major anti-nutritional traits in food crops and their effects on consumption

Cereal and legume-based foods contain various anti-nutritional factors that can impact their nutritional value and digestibility. Some of the key anti-

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nutritional traits that hinder food digestion and nutrient absorption include phytic acid, gossypol, lectins, raffinose, enzyme inhibitors, goitrogens, saponins, tannins, oxalic acid, erucic acid, alkaloids, BOAA and HCN. This section details the impact of consuming these anti-nutrients and provides information on their typical levels in regular diets (Table 1).

TABLE 1

The major role of anti-nutrients in consumption and plant growth regulation

S.no.	Anti-nutrient	Effects on consumption	Role in plant growth
1	Raffinose	Raffinose not digested by humans and monogastric animals	Acts as a cryoprotectant
		Leads to flatulence in humans and animals	Acts as a storage metabolite and is absorbed in seeds and roots
		Prevents non-alcoholic fatty liver disease in humans	Acts as a source of energy for seed germination
		Reduces inflammation, diabetes, allergies and obesity	-
2	Saponins	Cause diarrhea and vomiting by damaging red blood cells	Act as phytoalexin during fruit and tuber development
		Affects the nutrient absorption by gut membranes	Resistance against diseases in vegetables
		Negative impact on chick development and feed efficiency	-
3	Erucic acid	Fat accumulation in heart muscles	–
		Cardiovascular diseases and myocardial lesions in the heart	-
4	Oxalic acid	Causes headache, coma and kidney stones	Precursors of oxalic acid play a major role in climate resilience
		Calcium oxalate has a severe impact on human nutrition and health	Growth regulation of crops during pollination
		Leads to death due to oxalate poisoning	-
5	Phytic acid	Nutritional inhibitor in monogastric animals	Phosphorus storage and chelation of micronutrients for growth and development
		Decreases the risk of colon cancer and inflammatory bowel disease	-
		Lowers blood glucose level	-
6	Gossypol	Acute poisoning on ingestion	Resistance to cotton bollworm
		Causes iron deficiency known as erythropoiesis	-
		Increases cytosolic Ca^{2+} activity	-
		Decreases antioxidant levels in tissues	-
7	Lectin	Agglutinates red blood cells	Regulation of cell signaling and plant response to biotic, abiotic and symbiotic stimuli
		Anti-tumor agent	-
		Antimicrobial, antifungal, antibacterial, antiviral	-
		Alters the integrity of intestinal mucosa	-
8	Goitrogen	Deficiency of thyroid hormone	-
		Reduces growth and reproductive performance	-
		Apoptotic and anti-proliferative effects in thyroid cancer cells	-

9	Enzyme inhibitors	Trypsin inhibitors trigger pancreatic hyperplasia	Confer biotic stress tolerance and act as biopesticides
		Prevention of type 2 diabetes and obesity	-
		Protease inhibitors reduce the activity of proteolytic enzymes during ingestion	-
		Alpha-amylase inhibitors affect post-meal plasma glucose levels	-
10	Glucosinolates	Cause rancidity	–
		Prevent cardiovascular and neurodegenerative diseases	-
11	Tannins	Inhibit digestive enzymes and cause intestinal damage	Antiparasitic properties of plant tannins
		Have been associated with reduced feed intake, growth rate, feed efficiency, and protein digestibility	Act against pathogenic bacteria, have antibacterial actions and are antioxidants
		Enhance the food product's oxidative stability	Prevent neurodegenerative diseases and have anti-tumor, anti-inflammatory and antibacterial properties
		Improve the quality of the meat and milk. Act as a natural preservative	-
12	Hydrogen cyanide	In animals stops cellular respiration process due to asphyxia	–
		Severe shortness of breath and frequent urination in animals	-
13	β-N-oxalyl amino alanine	Causes neurolathyrism, a neurologic condition that is irreversible in both humans and animals	Act as an Antioxidant

Raffinose: Pulses are rich in carbohydrates, proteins, dietary fiber, vitamins, minerals and other bioactive compounds beneficial to human health. However, their global consumption, particularly in industrialized countries, is limited by the high levels of Raffinose Family Oligosaccharides (RFOs) they contain. These oligosaccharides are present in beans, cabbage, brussels sprouts, broccoli, asparagus and whole grains. RFOs ($C_{18}H_{32}O_{16}$) are especially abundant in the seeds of legumes such as chickpeas (*Cicer arietinum*), lentils (*Lens culinaris*) and soybeans (*Glycine max*). They are also found in the leaves and tubers of vegetables and other storage organs like roots. Raffinose is present in Chinese artichoke tubers (*Stachys sieboldii*) and common bugle leaves (*Ajuga reptans*). Defatted soy flour contains raffinose levels ranging from 1.15% to 3.23%, lentils contain 4.5 to 5.5 mol per 100 g of flour and faba beans contain 0.12% to 0.29%. Humans and monogastric animals cannot digest RFOs, which are instead fermented by gut microflora, producing gases that cause flatulence and discomfort [6]. Despite this, RFOs offer health benefits, including antiallergic, anti-obesity, anti-diabetic effects and prevention of non-alcoholic fatty liver disease. They improve gut microbiota health and the overall health of the large intestine, making them potential therapeutic agents for reducing inflammation, diabetes and allergies. Thus, balancing the RFO content in crops is essential for promoting them as functional foods [7].

Saponins: Saponins ($C_{58}H_{94}O_{27}$) are surface-active secondary metabolites that do not evaporate and are present in soybeans, sugar beets, peanuts, spinach, asparagus, broccoli, potatoes, apples, eggplants, alfalfa and ginseng root. These glycosidic triterpenoids are commonly found in the seed coats of various crops. Known for their structural diversity, saponins fall under triterpenes and steroid glycosides categories [8]. Their complex structures give them unique physical, chemical and biological properties, such as sweetness, bitterness and foaming and emulsifying abilities. Saponins exhibit pharmacological, medicinal, hemolytic, antimicrobial, insecticidal and molluscicidal properties. However, consuming saponins can cause diarrhea, vomiting and red blood cell breakdown. They can bind to intestinal cells, affecting nutrient absorption. In poultry, saponins negatively impact chick growth, feed efficiency and the absorption of lipids, cholesterol, bile acids and vitamins A and E.

Erucic acid: During digestion, triglycerides containing erucic acid release it into the bloodstream, where it's distributed to tissues for energy. However, cardiac muscle oxidizes erucic acid poorly, leading to fat accumulation in the heart, which can result in cardiovascular diseases and myocardial lesions.

Alkaloids: Alkaloids, particularly quinolizidine, are prevalent in commercially grown legumes. Recent advancements in detection techniques now facilitate the rapid identification of low HCN levels, significant for breeding sorghum varieties with reduced toxicity. BOAA is a neurotoxin found in both seeds and leaves. It arises as a by-product of nitrogen metabolism in plants, presenting a significant challenge in crops like *Lathyrus sativus* due to its association with lathyrism, a neurologic disorder that can lead to irreversible damage upon consumption. While extensive variation in germplasm has been observed, further research is essential to elucidate the genetic mechanisms responsible for BOAA biosynthesis. Molecular breeding techniques, including omic approaches, intron-based markers and gene editing, are currently under development to mitigate BOAA levels in *Lathyrus*, especially significant as it is a staple crop in rice-fallow systems across South Asian nations [9].

Several varieties such as Pusa-24, Pusa-305, Lysine-Specific Demethylase 1 (*LSD1*), *LSD-2* and *LSD-3* have been developed as low-BOAA cultivars, containing less than 0.2% BOAA [10]. These cultivars represent a potential avenue in agricultural research aimed at reducing the health risks associated with BOAA consumption. Efforts to standardize breeding methods emphasize the importance of genetic studies to enhance crop safety and sustainability in regions reliant on *Lathyrus* cultivation.

Oxalic acid: Oxalic acid ($C_2H_2O_4$) is a type of dicarboxylic acid found naturally as potassium and calcium salts within the cell sap of oxalis and rumex plant species. Once ingested, insoluble calcium oxalate compounds formed from oxalic acid cannot be effectively eliminated through the urinary tract. This inability to excrete calcium oxalate can lead to the development of kidney stones, posing a significant risk to human health and nutrition. Foods known to be high in oxalates include cruciferous vegetables such as kale, radishes, cauliflower and broccoli, as well as chard, spinach, parsley, beets, black pepper, chocolate, nuts, berries and beans [11]. To mitigate the potential negative effects of oxalates, it is advised to consume calcium supplements alongside foods rich in oxalic acid. This practice helps bind with oxalates in the gut, facilitating their removal from the body and thereby reducing oxalate levels in the bloodstream. Despite its rarity, excessive consumption of oxalates can lead to serious kidney conditions and in extreme cases, fatal oxalate poisoning. Efforts to manage oxalate intake are significant, especially for individuals prone to kidney stone formation or with pre-existing kidney conditions. Monitoring dietary oxalate levels and adopting strategies to enhance calcium binding and excretion of oxalates can significantly mitigate the health risks associated with high oxalate consumption. Understanding the sources and effects of oxalates in various foods remains vital for promoting optimal kidney health and overall well-being in populations exposed to oxalic acid-rich diets.

Phytic acid: Phytic acid ($C_6H_{18}O_{24}P_6$) is an antioxidant that naturally binds with minerals like phosphorus, iron and zinc. It is predominantly found in the grains, nuts and seeds of cereals, legumes and vegetables. Notably, it is present in the rice aleurone layer and abundant in the endosperm and embryo of maize. Seeds store phosphorus mainly as phytic acid after pollination and during germination, the enzyme phytase breaks it down to aid plant growth. Monogastric animals, lacking phytase in their digestive systems, experience nutritional inhibition from phytic acid as it binds essential micronutrients [12]. The insoluble mineral-bound form of phytic acid is problematic because its excretion in animal waste can lead to eutrophication and soil pollution. Therefore, decreasing phytic acid content in grains can enhance mineral availability in foods [4]. Despite its anti-nutritional effects, dietary phytic acid has health benefits, including reducing the risk of colon cancer and other inflammatory bowel diseases due to its antioxidant properties. It helps prevent lipid peroxidation, oxidative spoilage, discoloration, putrefaction and syneresis in foods. Consequently, while reducing phytic acid in foods is significant for improving nutrient absorption and supporting normal growth and metabolism, it should be balanced to retain its beneficial properties. The recommended safe consumption range for phytic acid is approximately 250-800 mg.

Gossypol: Gossypol ($C_{30}H_{30}O_8$) is a group of polyphenols known for its potential to cause acute poisoning when ingested [13]. Research indicates that cumulative toxic effects from gossypol can manifest after just 1-3 months of consumption. To avoid toxicity, it is recommended to limit gossypol intake to 20 mg per kg of feed. Cases of gossypol poisoning have been documented in broiler chicks, pigs, dogs, sheep and goats, with monogastric animals such as pigs, birds, fish and rodents being particularly susceptible compared to ruminants. Younger ruminants are more affected by gossypol than adults. A primary consequence of gossypol ingestion is anemia, commonly observed in animals fed with cottonseed. During digestion, gossypol binds to iron in hemoglobin, forming a gossypol-iron complex that inhibits iron absorption, leading to a condition known as erythropoiesis, characterized by the fragility and death of erythrocytes [14]. This process also increases cytosolic Ca^{2+} activity, causing cell membrane disruption and contraction. Moreover, gossypol poisoning is associated with decreased antioxidant levels in tissues. At high concentrations, gossypol interferes with energy production by disrupting enzymatic activity in the mitochondrial electron transport chain and oxidative phosphorylation. Additionally, gossypol affects both male and female gametogenesis and causes embryo lesions, contributing to male infertility. Due to these properties, gossypol is being considered for potential use as a male contraceptive in future pharmaceutical research [15].

Lectins: Lectins which are complex carbohydrate-binding proteins, are glycoproteins with non-catalytic carbohydrate-binding sites and are classified into various types, including animal, algal, bacterial, fungal and plant lectins. Also referred to as hemagglutinins, these "anti-nutrients" have collected significant attention due to their involvement in obesity, chronic inflammation and autoimmune diseases. They are primarily found in raw legumes such as kidney beans, lentils, peas, soybeans and peanuts, as well as in whole grains like wheat. In leguminous plants, lectin content is highest in seeds compared to bark, leaves, roots or stems and they are commonly present in nuts, cereals and leguminous seeds [16]. Consuming lectins in their active state can cause severe adverse reactions in humans; for example, even small amounts of raw or undercooked kidney beans, which contain phytohemagglutinin, can lead to red blood cell aggregation, causing symptoms such as nausea, vomiting, stomach upset and diarrhea. Milder side effects include bloating and flatulence. Studies on animal cells have shown that active lectins can interfere with mineral absorption, affecting the levels of calcium, iron, phosphorus and zinc in the digestive tract. A safe level for lectin consumption from leguminous foods is considered to be 200-400 hemagglutinin units (hau). Despite their negative effects, lectins have demonstrated potential benefits in cancer treatment due to their antiangiogenic, antimetastatic and antiproliferative properties [17].

Goitrogens: Goitrogens (C_5H_7NOS) derive their name from "goiter" indicating an abnormal growth condition. Goiter refers to the thyroid gland's enlargement caused by thyroid hormone deficiency. Rich in goitrogens, soybean and cassava are cruciferous vegetables belonging to the genus *Brassica*, though high levels of goitrogens are also found in other cruciferous vegetables. Goitrogens disrupt iodine utilization and thyroid hormone production, leading to impaired growth and reproductive performance due to hormone deficiency. Iodine supplementation is more effective than heat treatment in mitigating the effects of goitrogens. Despite their negative impact, foods containing goitrogens also have bioactive compounds that offer protection against thyroid cancer. For instance, sulforaphane, an isothiocyanate found in crucifers, has shown apoptotic and antiproliferative effects on thyroid cancer cells. Additionally, goitrogens have been utilized in COVID-19 treatment to activate the Nuclear Factor Erythroid 2-Related Factor 2-Kelch-like ECH-associated protein 1 (*NRF2-KEAP1*) pathway and mitigate the cytokine storm induced by the virus [18]. Therefore, ensuring the safe consumption of goitrogens is essential to avoid their adverse side effects while benefiting from their positive properties.

Enzyme inhibitors: Protease inhibitors, naturally occurring in plants, are a significant area of research due to their ability to limit enzyme activity through protein-protein interactions by blocking the enzymes active sites. Compared to legumes, cereals contain much lower amounts of these digestive inhibitors [19]. Protease inhibitors can significantly diminish the activity of proteolytic enzymes during digestion. Among the various enzyme inhibitors, trypsin inhibitors and alpha-amylase inhibitors are prominent in foods. Alpha-amylase inhibitors primarily affect the breakdown of polysaccharides into oligosaccharides. By inhibiting alpha-amylase activity, these enzyme inhibitors slow carbohydrate digestion, thereby affecting post-meal plasma glucose level. Additionally, trypsin inhibitors enhance the production of hormones like steatogenic hormone and Cholecystokinin (CCK), leading to reduced food intake and body weight. In humans, the intake of trypsin inhibitors can lead to reduced growth rates, slower protein digestion, decreased amino acid availability and pancreatic hyperplasia. Research has shown that inhibiting certain enzymes, such as alpha-amylase, alpha-glucosidase and lipase, can be beneficial, enhancing the digestibility of legume-based foods. While there are health benefits, such as the prevention of type 2 diabetes and obesity, potential digestive malfunctions should be considered in future studies [20].

Tannins: Tannins ($C_{76}H_{52}O_{46}$) are plant-derived polyphenolic compounds that interact with proteins and other organic substances such as amino acids and alkaloids, forming complexes with vitamin B12 during digestion. Tannins are categorized into two types: hydrolyzable tannins and Proanthocyanidins (PAs), also known as condensed tannins. Hydrolyzable tannins are more resistant to both enzymatic and non-enzymatic hydrolysis compared to PAs, which are generally more soluble in water. Condensed tannins are particularly prevalent in leguminous forages and seeds. Tannins form complexes with dietary proteins, creating indigestible compounds that inhibit endogenous proteins, including digestive enzymes. Furthermore, tannins exhibit anti-nutritional properties, potentially causing intestinal damage, hindering iron absorption and posing carcinogenic risks. Beyond their nutritional impacts, tannic acid, a form of tannin, is utilized in the production of rubber, inks and dye fixatives. Reducing tannin content in foods can promote a healthier digestive system by minimizing these adverse effects.

Improving food safety and nutrition: Traditional and innovative approaches to combat anti-nutrients

Various traditional processing methods are utilized to improve the bioavailability of micronutrients in plant-based diets. Currently, a range of techniques is employed to mitigate the effects of food anti-nutrients, including milling, soaking, germination, autoclaving, microwave treatment and fermentation [21]. This section highlights the processing methods used to diminish anti-nutritional properties in crops (Table 2). It also details the specific processing techniques effective in reducing individual anti-nutritional factors. Recently, the market has seen the introduction of value-added products created using these methods. Traditional methods, such as milling, can physically remove parts of the grain where anti-nutrients are concentrated. Soaking can leach out soluble anti-nutrients, while germination activates enzymes that break down these compounds.

Autoclaving and microwave treatments can denature anti-nutrients through heat, making them less active. Fermentation, through microbial action, can degrade anti-nutrients and enhance the nutritional profile of foods. These processing techniques not only reduce the anti-nutritional content but also improve the overall digestibility and nutritional quality of plant-based foods.

Boiling: Boiling is an effective method to mitigate the presence of anti-nutrients like lectins, tannins and protease inhibitors in various foods. Research indicates that subjecting pigeon peas to boiling for an extended period, specifically 80 minutes, led to substantial reductions in these anti-nutrients: protease inhibitors decreased by 70%, lectins by 79% and tannins by 69%. Furthermore, studies highlight the effectiveness of boiling in reducing calcium oxalate in cooked green leafy vegetables by 19% to 87%, surpassing the efficiency of baking and steaming methods. Another investigation by Maphosa et al., observed significant improvements in the nutritional quality of beans following boiling, primarily due to decreased concentrations of lectins and saponins [22]. Boiling has been recognized for its ability to alter the chemical composition of foods, particularly by degrading or leaching out anti-nutritional compounds. The prolonged exposure to high temperatures during boiling breaks down complex structures such as lectins, which are known for their adverse effects on nutrient absorption and digestive health. Similarly, tannins, which bind to proteins and other nutrients are significantly reduced through this process, enhancing the bioavailability of essential minerals in foods. Protease inhibitors, another class of anti-nutrients that interfere with protein digestion, also undergo substantial degradation during boiling, thereby facilitating better protein utilization by the body. Comparative studies emphasize that boiling is particularly effective compared to other cooking methods like baking or steaming when it comes to reducing anti-nutrients. This method not only enhances food safety by neutralizing harmful substances but also contributes to improving the overall nutritional profile of boiled foods. By employing traditional cooking practices like boiling, food processors and consumers alike can mitigate the detrimental effects of anti-nutrients, ensuring that cooked foods retain higher levels of essential nutrients and are safer for consumption.

Autoclaving and roasting: Autoclaving and roasting are two effective cooking methods known for their ability to enhance the nutritional quality of foods by reducing anti-nutrients. Autoclaving, a process that involves high-pressure steam cooking, has been widely recognized for its efficacy in decreasing the levels of various anti-nutrients present in foods. The duration of cooking plays a significant role in determining the extent of reduction in anti-nutrient content, with longer cooking times generally resulting in greater reductions. Previous studies have highlighted that autoclaving can significantly enhance the nutritional value of foods by eliminating anti-nutrients, particularly tannins and trypsin inhibitors [23]. Roasting another heat-based cooking method, has also been investigated for its impact on reducing anti-nutrients. The study focusing on soybean meal demonstrated a significant reduction in trypsin inhibitor activity following roasting, underscoring the effectiveness of this method in improving protein digestibility. Similarly, studies involving beans have shown that heating, soaking and autoclaving can substantially decrease the levels of enzyme inhibitors and tannins, thereby enhancing the overall nutritional profile of grains. Both autoclaving and roasting work by applying high temperatures to food substances, which helps break down complex chemical structures responsible for anti-nutrient activities. Tannins known for their ability to bind proteins and inhibit nutrient absorption are particularly susceptible to degradation under these conditions. Likewise, trypsin inhibitors, which interfere with protein digestion are effectively neutralized through heat treatment, ensuring better utilization of dietary proteins by the body. These cooking techniques not only improve the safety and palatability of foods but also contribute to enhancing their nutritional value. By reducing anti-nutrient content, autoclaving and roasting play a critical role in promoting the bioavailability of essential nutrients in foods, making them more beneficial for overall health and well-being. Incorporating these traditional cooking methods into food preparation practices can help optimize the nutritional benefits derived from various food sources.

TABLE 2

The major role of anti-nutrients in consumption and plant growth regulation

S. no	Anti-nutrient traits	Traditional methods	Effective method
1	Phytic acid	Milling, soaking, germination, fermentation, blanching	Soaking, germination, fermentation
2	Lectins	Milling, boiling, soaking, fermentation	Soaking, boiling, heating, fermentation
3	Tannins	Milling, soaking, autoclave, germination, fermentation, blanching, boiling	Boiling, soaking
4	Saponins	Boiling, washing, fermentation, roasting	Fermentation
5	Oxalic acid	Milling, blanching, boiling, soaking	Boiling, soaking
6	Enzyme inhibitors	Soaking, autoclave, roasting, fermentation, boiling	Fermentation, boiling
7	Polyphenols	Germination, soaking, fermentation	Germination
8	Gossypols	Extrusion, fermentation	Extrusion
9	Raffinose	De-hulling, germination, alcoholic extraction, microbial treatment	Cooking
10	Goitrogens	Steaming, cooking, fermenting, milling, soaking, washing	Soaking
11	β-N-oxalyl amino alanine	Soaking, boiling, fermentation, cooking, autoclaving	Soaking and cooking
12	Alkaloids	Soaking, washing, germination, fermentation, aqueous thermal treatment alkaline treatment	Soaking, cooking fermentation and alkaline treatment

Fermentation: Fermentation is a metabolic process known for its ability to enhance the nutritional quality and digestibility of grains. During fermentation, carbohydrates undergo oxidation to produce energy, which is essential for metabolic activities. Studies have demonstrated that fermentation significantly improves the nutritional profile of grains by increasing the bioavailability of essential nutrients such as lysine, methionine and tryptophan [24]. These critical amino acids are significant for various physiological functions in the human body, including protein synthesis and immune function. One of the notable benefits of fermentation is its ability to reduce the levels of anti-nutrients present in cereals. Anti-nutrients like phytic acid, tannins and polyphenols can interfere with the absorption of minerals and proteins in the digestive system. Fermentation processes, particularly those involving Lactic Acid Bacteria (LAB), have been shown to significantly decrease the concentrations of these anti-nutrients in grains. For example, lactic acid fermentation has been effective in reducing tannin levels, thereby enhancing the absorption of iron from cereals [25]. Recent research has explored various fermentation techniques to optimize the reduction of anti-nutritional components in maize flour. Studies have demonstrated that prolonged fermentation periods with LAB mixture result in greater reductions of anti-nutrients such as tannins, polyphenols, phytate and trypsin inhibitors compared to spontaneous fermentation methods. This highlights the effectiveness of controlled fermentation processes in enhancing the nutritional quality of fermented foods. Overall, fermentation not only improves the digestibility and bioavailability of nutrients in grains but also contributes to reducing the adverse effects of anti-nutrients. By breaking down complex compounds and enhancing nutrient absorption, fermentation plays a significant role in promoting the nutritional value and health benefits of fermented grain products. Incorporating fermentation into food processing practices offers a sustainable approach to improving the nutritional quality of staple foods and enhancing overall dietary health.

Milling: Milling is a widely employed technique in grain processing, primarily aimed at separating the outer bran layer from the grains. This process is instrumental in reducing the concentration and distribution of anti-nutritional factors, which are predominantly located in the bran layers of grains. By removing the bran, milling effectively eliminates various anti-nutrients such as phytic acid, lectins, tannins and enzyme inhibitors from the grain matrix [12]. These anti-nutrients can impair the absorption of essential nutrients and minerals in the human digestive system, thus affecting overall nutritional quality. Study focusing on pearl millet has highlighted the transformative impact of milling on the chemical composition and distribution of oxalic acid within the grain structure. Oxalic acid, known for its potential to bind with minerals and form insoluble complexes, is notably affected by the milling process, which alters its presence in the aleurone layer and bran of grains. Consequently, milling not only enhances the nutritional value of grains by reducing anti-nutrients but also contributes to improving their digestibility and bioavailability. The effectiveness of milling lies in its ability to physically separate the outer layers of grains, where anti-nutrients tend to accumulate, from the inner endosperm, which is richer in starch and protein content. By removing the bran layers, milling significantly diminishes the concentration of substances that can hinder nutrient absorption and utilization in the body. This process is significant for enhancing the nutritional quality of grains intended for human consumption, particularly in regions where cereals and millets form staple foods. Milling represents a pivotal step in grain processing that not only refines the texture and appearance of grains but also plays a vital role in improving their nutritional profile by reducing anti-nutritional factors. The widespread adoption of milling in food processing industries underscores its importance in ensuring that grains provide optimal nutritional benefits without compromising dietary health.

Soaking: Soaking is a widely employed method for mitigating the presence of anti-nutrients in foods, particularly in nuts, grains and edible seeds. This process involves immersing the food in water for a specified period, which facilitates several beneficial changes. Firstly, soaking enhances the moisture content essential for the activation of endogenous phytases present in plant foods, which are enzymes responsible for breaking down phytic acid [26]. Phytic acid is known for its ability to chelate minerals, thereby reducing their bioavailability in the body. By activating phytases through soaking, the breakdown of phytic acid is promoted, consequently improving the digestibility and nutrient absorption of grains. Studies have demonstrated that soaking effectively reduces various other anti-nutrients as well. For instance, soaking legumes overnight has been found to decrease the levels of phytate, protease inhibitors, lectins and tannins. Research indicates that soaking seeds for 24 hours can significantly lower hydrogen cyanide content, an important consideration for enhancing food safety and nutritional quality. Additionally, soaking treatments have been shown to reduce stachyose and raffinose content, which are oligosaccharides known for causing flatulence and digestive discomfort. The duration of soaking plays a significant role in its effectiveness. For instance, soaking peas for 12 hours can reduce phytate content by up to 9%, while soaking pigeon peas for 6-18 hours can lead to substantial reductions in lectins, tannins and protease inhibitors. Moreover, soaking grains like wheat and barley for 12-24 hours has been recommended to enhance their nutritional value by increasing protein and mineral content.

Sprouting: Sprouting is a highly effective method utilized to diminish the levels of anti-nutrients present in plant-based foods. This process involves the germination of seeds, during which various anti-nutrients such as phytate and protease inhibitors undergo degradation. Studies have demonstrated significant reductions in phytate levels ranging from 37% to 81% following sprouting, attributed to the activation of phytase enzymes that break down the phytate-mineral complexes in grains. This enzymatic activity is particularly significant in enhancing the bioavailability of minerals like calcium, iron and zinc in sprouted grains. Additionally, sprouting has been observed to reduce lectin and protease inhibitor content, although the decreases may vary depending on the specific type of grain or legume. Research documented substantial reductions in phytate levels in cereal grains after 10 days of sprouting, underscoring the effectiveness of this method in enhancing nutritional quality. Moreover, sprouting has been linked to alterations in the profile of bioactive compounds such as isoflavones in soybeans, which can contribute to their nutritional benefits [27]. Furthermore, sprouting appears to be particularly advantageous in reducing polyphenol concentrations in millets, with studies reporting reductions of up to 75%. This surpasses the decreases achieved by other processing methods such as soaking, microwave treatment and fermentation, highlighting sprouting as a superior technique for enhancing the overall nutritional value of grains.

Extrusion: Extrusion is a highly valued processing technique in the food industry due to its wide application and numerous advantages. This method involves pushing food ingredients through a small opening using either a single screw or a series of screws. Research has consistently shown that extrusion is remarkably effective in reducing the levels of anti-nutrients present in foods, including phytic acid, tannins, phenols, alpha-amylase inhibitors and trypsin inhibitors. Specifically, extrusion has been noted for its ability to decrease the ratio of phytic acid phosphorus to total phosphorus in processed foods. Studies have highlighted the significant benefits of extrusion in enhancing the nutritional quality of legumes, particularly when they are pre-soaked in water for 16 hours prior to extrusion. This process not only improves the digestibility of legumes but also enhances their utilization by both humans and animals. For instance, the extrusion of sesame oilseed meal using a single-screw frying extruder has been shown to effectively reduce the content of tannins, thereby improving its nutritional suitability. Moreover, extrusion has been instrumental in reducing free gossypol levels in various food products. According to test results conducted under the standards of the American Oil Chemists' Society, extrusion has achieved significant reductions ranging from 71% to 78% in free gossypol levels, underscoring its effectiveness in improving the safety and nutritional value of gossypol-containing foods [28].

DISCUSSION

Breeding strategies to alter anti-nutritional components for enhanced nutrient bioavailability in foods

The reduction of anti-nutrients in crops is a pivotal breeding strategy that significantly enhances the quality of produce. Various breeding techniques, including selection, mutation, backcrossing, hybridization and population improvement, have been employed using natural and induced genetic resources. The breeding for reducing anti-nutrients in crops began in the early 1960s with the development of glandless cotton. More recently, gene silencing and editing techniques have been used to create low anti-nutrient lines of major crops. Conventional breeding for reducing anti-nutrient content started with identifying germplasm accessions that naturally accumulated fewer anti-nutrients. For instance, in 1960, reduced gossypol content in cotton was selected, revealing that glandlessness is controlled by two genes. This discovery eventually led to the development of genotypes with glandless seeds but glanded plants, effectively reducing gossypol in seeds while maintaining it in vegetative parts. In soybean, lines with zero Kunitz inhibitors were identified, controlled by a recessive gene which was later introduced into elite cultivars. Similarly, low vicine and covicine lines were selected from faba bean germplasm, with these traits also governed by recessive genes. Recurrent selection in maize, involving synthetic populations, successfully developed high-iron and high-zinc lines with low phytic acid. In quinoa, pedigree breeding reduced saponin accumulation, although dominance of the trait required alternate strategies. Induced mutations, such as those in soybean using gamma rays, produced mutants with lower lectin content and normal germination rates, suggesting potential donors for improving meal quality. Backcrossing and mutation breeding are predominant strategies to reduce anti-nutritional traits in crops. These traits often play significant roles in plant defense and stress tolerance, so their reduction can negatively impact yield. For example, reducing phytic acid in crops through identified mutants resulted in improved nutrient bioavailability. Marker-assisted backcrossing has been employed to introduce low-phytic acid traits into elite cultivars, with near-isogenic lines demonstrating reduced phytate content without other morphological changes [29-33].

Gene silencing using RNAi technology offers an efficient way to optimize anti-nutrient expression in crops. For instance, ultra-low-gossypol cotton was developed by silencing the d-cadinene synthase gene, reducing gossypol accumulation in seeds and vegetative parts. This approach has been patented and shown to improve yield and fiber quality. Similarly, metabolite engineering reduced raffinose levels in soybean, enhancing its nutritional value for poultry. Advanced gene editing techniques, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9), have been employed to reduce anti-nutrients. For example, knocking out specific genes in soybean reduced raffinose content without affecting plant growth. RNAi-mediated silencing of multiple genes in wheat revealed increased trypsin inhibition, beneficial for non-celiac wheat-allergic patients [34]. Manipulating anti-nutritional traits involves careful consideration of their roles in plants. For instance, trypsin inhibitors provide defense against pests but hinder digestion in humans. Advanced gene editing offers potential solutions, focusing on seed-specific reduction to maintain plant defense mechanisms while enhancing food quality. Traditional breeding has also addressed glucosinolate content in *Brassicas*. High-density genetic mapping and comparative genomics identified significant Quantitative Trait Loci (QTLs) for glucosinolate biosynthesis, leading to the development of crops with altered glucosinolate profiles. For instance, high-glucoraphanin broccoli was developed through marker-assisted selection. Similar strategies have been applied to radish and other crops, identifying candidate genes for glucosinolate synthesis. Breeding efforts have also focused on reducing erucic acid in *Brassicas* due to its health risks [35-38]. Mutations in the Fatty Acid Elongase 1 (*FAE1*) gene were induced to produce low-erucic acid lines, which were then introgressed into elite cultivars. Advanced progenies showed improved morphological traits, suggesting effective recombination and reduction of negative pleiotropic effects. Marker-assisted breeding and QTL detection have played significant roles in reducing anti-nutrients in crops. Genetic manipulation of these traits requires monitoring to avoid negative impacts on plant defense and stress tolerance. Integrative omics and advanced bioinformatics tools are essential for further reducing anti-nutrients without compromising yield. Gene silencing using RNAi technology has been effective in reducing anti-nutrients in major crops. For example, RNAi-mediated silencing of genes involved in phytic acid biosynthesis in rice and *Brassicas* has successfully reduced phytic acid content. CRISPR-Cas9 has been used to edit similar genes in soybean, resulting in stable transgenic lines with altered phytate concentrations. Future research should focus on integrating traditional processing methods with modern technologies to enhance food safety and nutritional quality [39]. Traditional methods such as soaking, germination, boiling and fermentation have been effective in reducing anti-nutritional factors like phytic acid, lectins, tannins and enzyme inhibitors. These methods improve nutrient bioavailability and preserve the sensory qualities of foods. Innovative food processing technologies, such as extrusion, complement traditional methods by offering efficient and scalable solutions. Extrusion reduces levels of anti-nutrients through the application of heat and pressure, enhancing the safety and nutritional suitability of food products. The combination of traditional wisdom and modern scientific advancements is significant for addressing global nutrition challenges. Education and awareness initiatives are essential to inform consumers, food producers and policymakers about the benefits of processed foods that retain nutritional integrity. Supporting research and development in food processing technologies can further optimize these methods for maximum efficacy and sustainability. Efforts should also focus on promoting the adoption of improved food processing methods across different food systems and regions [40-42]. By integrating traditional and innovative approaches, stakeholders can ensure that processed foods meet safety standards and consumer expectations while retaining high nutritional value. Overall, the reduction of anti-nutrients in crops through breeding and advanced processing techniques is essential for improving nutrient bioavailability and addressing global malnutrition. Continued innovation and education are necessary to promote these methods and ensure better health and nutrition for populations worldwide [43].

CONCLUSION

The presence of anti-nutritional factors in plant-based foods poses health risks, but effective processing methods offer solutions to mitigate these issues. Traditional techniques like soaking, germination, boiling and fermentation, practiced for centuries, significantly reduce anti-nutrient levels, enhancing nutrient absorption and preserving cultural diets. Innovative technologies like extrusion complement these methods by providing scalable solutions to further lower anti-nutrient content, improving the safety and nutritional quality of foods. Research highlights that integrating traditional wisdom with modern advancements is essential for addressing global nutrition challenges, particularly in vulnerable populations. Promoting the adoption of these processing methods, supported by education and research, can optimize food safety and nutritional integrity across diverse food systems. This approach ensures that processed foods meet both safety standards and consumer expectations while contributing to improved global dietary health.

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