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REVIEW ARTICLE

# Sustainable strategies to combat anti-nutrition in legumes: Implications for global food security

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## Abstract

Vegetables are indispensable components of a balanced diet, providing essential nutrients for overall health and well-being. In Indian cuisine, where vegetables typically constitute approximately 20 % of dietary intake, their significance is further highlighted by leguminous vegetables, which play a critical role in addressing protein malnutrition. Despite their beneficial nutritional content, leguminous vegetables contain bioactive compounds known as anti-nutritional factors (ANFs), which can have a negative impact on human health. These ANFs include substances that either reduce nutrient availability or have toxic effects when consumed. To mitigate the adverse effects of ANFs, various traditional methods, including milling, soaking, boiling, thermal processing, sprouting, fermentation, and genetic modification, have been effectively employed. Additionally, modern techniques such as pulsed electric fields (PEF), ultrasonic treatment, and high hydrostatic pressure (HHP) are being increasingly applied to neutralize ANF activity. This review emphasises the essential role of leguminous vegetables in the Indian diet, the various challenges posed by anti-nutritional factors, and the range of both traditional and modern strategies developed to maximize nutrient retention while minimizing potential health risks.

## Keywords

anti-nutritional factors (ANFs); leguminous vegetable; thermal processing and non-thermal processing

## Introduction

Vegetables are integral to human nutrition, recognized for their rich array of bioactive, health-enhancing compounds (1). Despite India achieving self-sufficiency in carbohydrate-dense food production, the nation continues to grapple with a significant protein deficit, a prevalent nutritional shortfall in many developing countries, often manifested as protein-energy malnutrition. Legumes, as a notable source of secondary metabolites such as carotenoids (2), play a critical role in dietary diversification and contribute to environmental sustainability (3). With protein concentrations that are double those found in cereals, legumes represent a vital and expanding source of protein. Leguminous vegetables, including staples like peas and beans, are globally consumed for their high nutritional value, providing essential protein, fiber, carbohydrates, and vital minerals such as iron, calcium, potassium, magnesium and zinc, alongside a spectrum of antioxidants (4, 5) .

Legumes are rich in nutrients. Beyond their nutritional value, they offer various health benefits, potentially bypassing the chronic circumstances of cardiovascular disease, diabetes, and other cancers. Furthermore, they are crucial as a source of folate, which has been associated with reducing neural tube defects (6, 7). Within the Indian dietary framework, legumes are indispensable, particularly in vegetarian diets, where they act as the principal protein source. Additionally, they provide resistant starch, a prebiotic carbohydrate that undergoes fermentation into short-chain fatty acids, promoting metabolic health (8). Recognizing the extensive benefits of legumes, both the Food and Agriculture Organization (FAO) and the United Nations proclaimed 2016 as the International Year of Pulses, underscoring their significance in terms of nutrition, economy, environmental sustainability, and food security.

Leguminous vegetables encompass a wide range of species, including peas and beans such as field bean (*Pisum sativum spp. arvense*), garden pea (*Pisum sativum spp. hortense*), french bean (*Phaseolus vulgaris* L.), moth bean (*Vigna aconitifolia* (Jacq) *Maerchal*), scarlet runner bean (*Phaseolus coccineus*), cluster bean (*Cyamopsis tetragonoloba* L. Taub), hyacinth bean (*Dolichos lablab* L.), lima bean (*Phaseolus lunatus* L.), cowpea (*Vigna unguiculata* L. Walp), broad bean (*Vicia faba* L.), soybean (*Glycine max* L.), jack bean (*Canavalia ensiformis*), winged bean (*Psophocarpus tetragonolobus* L.), and sword bean (*Canavalia gladiata*). Of these, peas, beans. In contrast, others like cluster beans, winged beans, lima beans, broad beans, and cowpeas hold significant importance among leguminous vegetables. In contrast, others, such as cluster beans, winged beans, lima beans and broad beans, are of relatively lower economic relevance.

Recent studies have highlighted the correlation between legume consumption and a decreased risk of cardiovascular disease and overall mortality (1). Nonetheless, it is crucial to acknowledge that legumes contain anti-nutritional factors (ANFs) like enzyme inhibitors, phytic acid, polyphenols, and oligosaccharides, which can interfere with nutrient absorption and metabolism, thereby impacting protein digestibility and the bioavailability of trace elements (9). Fig. 1 highlights the positive health benefits of legumes on human health.

### *Anti-Nutritional Compounds in Leguminous Vegetables: Types and Significance*

Anti-nutritional compounds are biochemical substances synthesized by plants as part of their defense mechanisms against insects, fungi, and herbivores, thereby providing a protective function. These compounds are distributed throughout various parts of the plant and can disrupt its metabolic processes, which in turn affect nutrient absorption and stunt its growth (10). Anti-nutrients can be broadly classified into two categories: heat-stable (for example, phytic acid, alkaloids, condensed tannins, and saponins) and heat-labile (for example, lectins, protease inhibitors, cyanogenic glycosides and toxic amino acids) (11). Fig. 2 presents a detailed representation of the distribution of anti-nutritional factors found in various parts of plants. The inherent thermal sensitivity of certain anti-nutrients suggests that applying heat treatment can be an effective strategy for significantly improving the edibility and nutritional quality of leguminous vegetables. By employing such methods, we can potentially transform these vegetables into more wholesome food sources, enhancing their overall value in our diets. Anti-nutritional factors in legumes can thus be divided into proteinaceous factors that denature at standard cooking temperatures and non-proteinaceous factors that are thermostable and resistant to heat (12). Table 1 outlines the specific anti-nutritional factors associated with various leguminous crops.

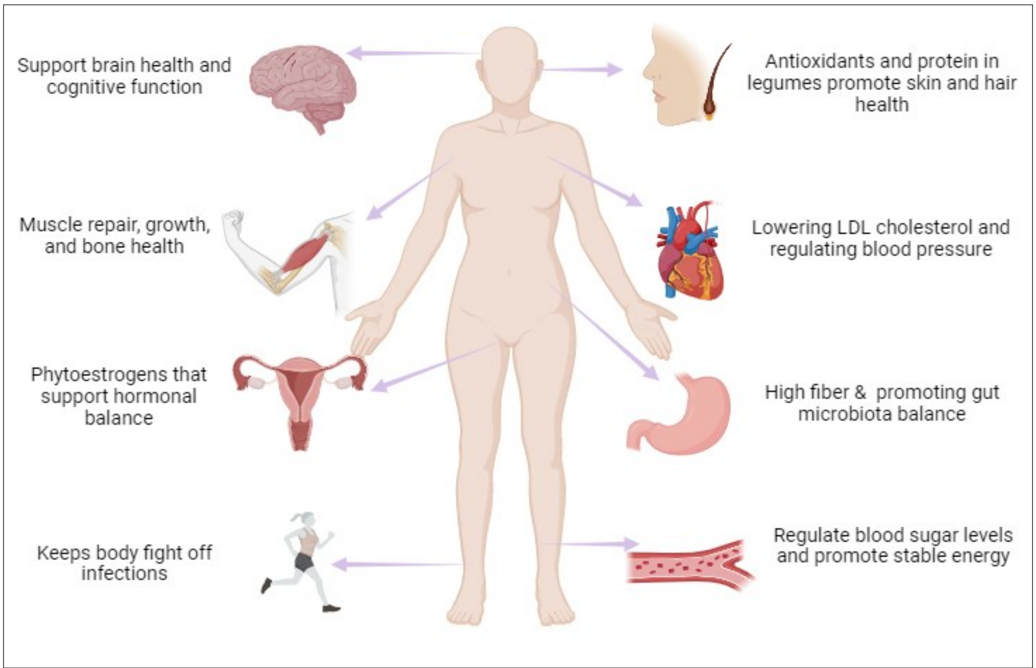


**Fig. 1.** Positive health effects of legumes on human being.

**Table 1.** Anti-nutrients and their corresponding leguminous vegetables (37)

| Vegetable legume source | Anti-nutrient factor |
|---|---|
| Broad bean | Phytic acid, phytohaemagglutinins, tannins |
| Cowpea | Phytic acid, phytohaemagglutinins. |
| Soya bean | Anti-vitamin A factor, anti-vitamin B12 factor, anti-vitamin D factor, anti-vitamin E factor, glucosinolates, phytic acid, phytohaemagglutinins, saponins |
| Pea | Lectins and tannins |
| Kidney bean | Amylase inhibitor, anti-vitamin E factors, cyanogens, phytic acid, phytohaemagglutinins, saponins |
| Hyacinth bean | Cyanogens, phytohaemagglutinins. |
| Field pea | Anti-vitamin E factors, cyanogens, phytic acid, phytohaemagglutinins, saponins. |

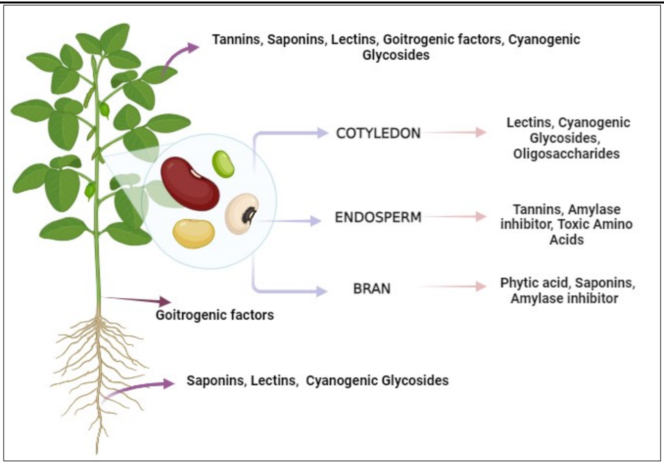


**Fig. 2**. Distribution of ANFs in different plant parts.

## Protenaceous factors

Legumes are often regarded as an excellent source of protein. However, it's worth noting that specific proteins found in legumes may not be beneficial for human consumption, collectively referred to as proteinaceous anti-nutritional factors. These factors are typically regarded as non-nutritional proteins.

### Lectins

Lectins are also known as hemagglutinins because they cause blood cells to clump together, a process called agglutination. They constitute about 2 to 10 % of the total proteins in seeds of legumes. These proteins, of great interest for clinical and immunological studies, have systemic physiological effects when ingested, including increased protein catabolism, mobilization of reserve fat and glycogen and alterations in mineral metabolism.

A major disadvantage of lectins is that they cause interference with digestion in the small intestine. The undigested and unabsorbed lectins move into the colon, where gut microbiota ferment the mixture of lectins producing short-chain fatty acids and gases, causing irritation in the gut. Lectins bind to the epithelial cells of the intestine at high enough concentration; they inhibit nutrient absorption and may well be damaging to the lining of the intestine that allows the translocation of bacteria into the blood. The natural concentration of lectins differs between legume species, with 0.6 % of total proteins containing garden peas, whereas kidney beans contain between 2.4 and 5 %, lima and soybeans contain about 0.8 % (13).

Ingestion at high concentrations of lectins about 5mg, may cause nutritional deficiency and allergic effects. The significant gastrointestinal symptoms associated with lectins are primarily due to their interaction with gut epithelial cells (14). More importantly, ingestion can lead to food-borne illness, hemolytic anemia and jaundice from inadequately cooked or raw kidney beans, leading to conditions like jaundice and other food-borne illness-associated conditions, particularly in G6PD deficient individuals primarily because of its toxic nature (15).

### Amylase inhibitors

Amylase inhibitors, or starch blockers, are substances that inhibit the enzymatic digestion of starch and hence, reduce the available energy in the forms of maltose and glucose. The inhibitors are heat-labile and have optimal activity at the pH of 4.5-9.5. Factors such as pH, temperature, ionic concentration and exposure time significantly impact functionalities related to their effectiveness. Pigeon pea contains relatively high amylase inhibitor activity (0.2-1.5 mg/g of dry seed weight), which is active over a pH range of 4.5 to 9.5 (16). In other field beans, the activity is comparatively lower (0.05-0.1 mg/g dry weight). On the other hand, winged beans, soybeans, lima beans and peas contain no detectible amylase inhibitor activity. The presence of these inhibitors leads to lower hydrolysis of polysaccharides, such as starch and glycogen, which may be associated with gastrointestinal disorders, diarrhea, nausea and vomiting caused by poor digestion (16). These inhibitors are produced in the later stages of seed development and subsequently break down during the latter germinative period of pigeon pea (17).

### Goitrogenic factors

Goitrogens are natural chemicals found in foods: soybeans a range of legumes, and probably affect the normal functioning of thyroid glands (18-20) They could interfere with the manufacture of thyroid hormones, which are essential for the regulation of metabolic processes and overall play a critical role in growth and reproduction health (18). The goitrogenic effects can be mitigated by an increase in iodine, as iodine is an essential substance for thyroid hormone synthesis (21). The investigation has observed that some legumes, such as peas, common beans, soybeans, and peanuts, could inhibit the uptake of the active radioactive iodine by the thyroid in human subjects (22). This observation indicates the possibility that these legumes could cause goitrogenic effects, especially by impairing the functions of the thyroid.

### Saponins

Saponins, which are foam-forming triterpene or steroidal glycosides, have been reported in several food commodities, including asparagus and cucumber. Their complex molecular structure confers a myriad of physical, chemical and biological properties, such as bitterness, foaming, sweetness, and emulsifying ability, in addition to pharmacological and medicinal properties. These properties also include hemolytic, antimicrobial, insecticidal and molluscicidal activities (23). Diets containing saponins have been reported to lower cholesterol levels; hence, potentially useful and invaluable for the prevention of preventing peptic ulcers and reducing the danger posed by cardiovascular diseases. Saponins have already been proven to function as hypocholesterolemic agents, immunostimulators and anticancer drugs, at least from evidence gathered in murine studies (24).

However, the bitterness and astringency of saponins are disadvantageous to food flavor; they also impair the digestion of proteins and enzymes by inhibiting chymotrypsin and trypsin digestive enzymes (25, 26). Some reports suggest that dietary consumption of saponin could be negatively correlated with renal stone formation (24). For instance, kidney beans contain approximately 106.02 mg of saponins per 100 g of fresh weight (27).

### Non - Protenaceous factors

Over 900 non-protein amino acids (NPAAs) have been documented, with notable prevalence in certain legume families, including *Mimosoideae, Vicieae, Phaseoleae and Caesalpinioideae.* These NPAAs exhibit structural similarities to proteinogenic amino acids and function as structural analogs (28).

When ingested by herbivores, microorganisms, or other plants, NPAAs can disrupt various biological processes. They can potentially substitute for standard amino acids in ribosomal protein synthesis, leading to the formation of defective proteins such as canavanine and azetidine-2-carboxylic acid. Furthermore, they can interfere with aminoacyl-tRNA synthetases or other stages of protein biosynthesis and competitively inhibit amino acid uptake systems. All these disturbances in vertebrates bring forth a variety of unfavourable effects, including fetal malformations, hepatic cirrhosis, hallucinogenic symptoms, neurotoxic disorders, gastrointestinal distress, alopecia, paralysis, hypoglycemia, and arrhythmias (29).

### Phytic acid

Dietary intake of phytate is highly undesirable because of its inhibitive effect on the absorption of minerals (30). It has been found to bind carbohydrates, thereby reducing its digestibility and bioavailability (31). Furthermore, phytic acid is an inhibitor for many gastrointestinal enzymes, such as tyrosinase, lipase, pepsin, amylase and trypsin. Although it has these inhibitory effects, phytate may have additional beneficial properties as an anticarcinogen antioxidant and is thought to lower blood clotting, cholesterol levels, and triglycerides with the possible reduction of cardiovascular diseases (32).

Phytic acid is a chelator with strong potential for binding proteins and minerals and obscures bioavailability. Its influence on zinc is severe, with diets of minimal animal protein being bound by it within the intestinal cavity, which reduces its bioavailability for absorption as well as reabsorption. Moreover, studies indicate that phytic acid causes inhibition of iron absorption in humans and diminishes calcium absorption. However, with the availability of phytic acid, it is still hard to attribute a negative effect on magnesium absorption based on whole food products.

Consumption of foods containing phytic acid should theoretically reduce the development of calcium oxalate crystals by maintaining an adequate urinary tract-free calcium concentration, contributing to the prevention of stone production. Estimates of mean daily intakes of phytate are considered to range from 2000 to 2600 mg for vegetarian diets and diets of rural populations in developing countries, while intake based upon mixed diets is supposed to range from 150 to 1400 mg (33). Phytate is very temperature sensitive; boiling can break its concentration by an amount of approximately 20 % (34).

In the pea, there is an increase from 0.16 % to 1.23 % during maturity, whereas in soybeans, the increase is from 0.87 % to 1.26 %. The phytic acid content in winged beans also increases proportionally with four stages of seed maturity. It is worth noting that phytate is a factor contributing to the low bioavailability of iron from soybeans. The average phytate content in beans ranges from 1 % to 2 % (35). Soybeans are reported to contain 1.16 mg/g of phytate on a fresh-weight basis (36). Whereas kidney beans have a phytate content of 627.33 mg/100 g of fresh weight (27).

### Tannins

Tannins are present in substantial quantities in legume plants and are recognized for their ability to form complex compounds with proteins, carbohydrates and polysaccharides (37). They can also bind with vitamin B (38). The precipitation of protein-tannin complexes also depends on factors such as pH, ionic strength, and the molecular mass of tannin, which is reportedly heat-stable. Astringent and bitter plant polyphenic compounds, very much known for chelating or precipitating proteins and other organic molecules, such as amino acids and alkaloids, are at play here (39) . Tannins are also present in coffee and tea. Excessive intake of tannins can lead to calcium and iron deficiencies, contributing to diseases such as osteoporosis and anemia. To mitigate the effects of tannins, consuming milk with plain tea or coffee is advisable. Additionally, vitamin C-rich foods can counteract tannins and promote gradual iron absorption (40). Apart from these, other noxious effects of tannins are satiety and poor food intake, enzyme digestive inhibition, enhanced excretion of endogenous proteins, gastrointestinal failures and possible toxicity from the absorbed tannins or their metabolites.

Fabaceae family is notable for its tannin content, with soybeans containing approximately 1.93 mg/g of tannin on a fresh weight basis (41). Diets high in tannin have been associated with weight loss. It is observed that condensed tannins are associated with the trapped trypsin inhibitor activity of faba beans testa (42).

### Cyanogenic Glycosides

Legumes are sometimes associated with toxicity due to the presence of hydrogen cyanide (HCN), a highly toxic compound. Among leguminous vegetables, lima beans are notable for containing cyanogenic glycosides. Cyanogens are generally present in minute quantities as glycosides, known as cyanide. If these glycosides remain within intact plant tissues, they are not poisonous. However, when tissue damage or the beginning of decay occurs, hydrolytic enzymes become activated and hydrogen cyanide is released. The activation of hydrolytic enzymes during tissue damage or the onset of decay is a well-documented phenomenon. These enzymes, which include proteases, amylases, and lipases, are typically inactive or present in an inactive form within cells. Upon injury or the initiation of decay, these enzymes become activated and begin to break down cellular components, which can lead to the release of various byproducts, including hydrogen cyanide. It has been calculated that this chemical constitutes approximately 90 % of the larger class of plant poisons (35). Cyanogenic glycosides fall into the category of phytoanticipins. In plants, their action is activated by β-glucosidases, which unbind toxic volatile HCN and ketones or aldehydes, wherein plants resist herbivores and pathogens (43). Lima beans contain 210-310 mg/100 g of HCN, peas and beans 2.3 and 2 mg/100 g, respectively (44).

### *Oligosaccharides*

Oligosaccharides such as raffinose, stachyose, ciceritol, and verbascose are present in legumes and are known to cause flatulence in human subjects (35). The antinutritional activity of these oligosaccharides is attributed to their accumulation in the large intestine. In this context, gut flora containing α-galactosidase enzymes break down these oligosaccharides through anaerobic fermentation, producing $H_2$, $CO_2$ and trace $CH_4$ in the gut lumen, which causes gas distension leading to flatulence. Soybeans and peas contain significant levels of raffinose and stachyose. These oligosaccharides act as a carbon source for sprouting seeds; thus, germination can reduce their levels to zero.

### *Toxic Non-protein Amino Acids*

Two of the non-protein amino acids in grain legumes are highly toxic to animals and humans: mimosine and 3-N-oxalyl -L-2, 3-diaminopropanoic acid (ODPA). These compounds are known to cause extreme adverse effects in both animals and humans. Mimosine, produced in grass peas (*Lathyrus sativus*) and broad beans, is linked to lathyrism, a chronic motor neuron disease. Symptoms of mimosine toxicity are stunted growth, hair and wool loss, lameness, mouth and esophageal lesions, reduced serum thyroxine levels and goiter (21).

Another non-protein toxic amino acid is canavanine, which is found in several legume plants at concentrations as high as 63 g/kg dry weight in some seeds. Jack bean (*Canavalia ensiformis*) is rich in canavanine. Due to its structural resemblance to the amino acid arginine, the toxicity of canavanine results in interference with arginine metabolism and RNA synthesis. Even though the adverse effects of canavanine on feed intake have been mainly documented at dietary levels equivalent to 300 g/kg of raw faba beans, its presence has led to metabolic favism, an illness characterized by hemolytic anemia resulting from the ingestion of broad beans. This illness affects individuals with a genetic deficiency of glucose-6-phosphate dehydrogenase, causing an inability to metabolize glutathione in their red blood cells.

### *Anti vitamins factors*

Antivitamins are substances that inhibit the action of vitamins, thereby depressing their biological activity. These are commonly found in many edible plant foods, particularly legumes. Thus, it has been reported that soybeans contain an anti-vitamin A, which can destroy carotene, the precursor of vitamin A, though it may be destroyed by simple heat treatments, such as steaming for five minutes, which is widely used in daily households.

Similarly, anti-vitamin D compounds found in soybeans have been known to interfere with calcium and phosphorus absorption in animals, particularly in chicks, leading to a condition known as rechitogenic activity (45). Such anti-vitamin E compounds have been identified in field peas, soybeans, and kidney beans as contributing factors to liver necrosis and muscular dystrophy in experimental animals. Raw soybeans also contain anti-vitamin B12, which lowers the biological availability of this vitamin. Anti-vitamins D and K are inactivated with heat treatment; thus, their potency is destroyed during cooking or through other forms of thermal processing of the foods (46).

### *Approaches to Minimize Anti-Nutritional Factors in Vegetable Legumes*

Previous reports have suggested that the frequent may lead to zinc and mineral deficiencies among Egyptians (5). It was observed that phytate administration harmed the metabolism of calcium, zinc, and phosphorus in female rats. In addition to diminishing vitamins and minerals, a high intake of these antinutritious-rich foods can result in toxicity.

To minimize the negative impacts of these anti-nutritional factors in food products, various traditional methods and technological processing techniques have been adapted. These include soaking, milling, debranning, roasting, cooking, germination, and fermentation (Table 2, Fig. 3). Each of these techniques targets and diminishes the levels of anti-nutritional factors, thereby improving the nutritional value and hygiene safety of processed foods.

### *Post-harvest processing*

In post-harvesting processing, milling is the common practice to reduce phytic acid lectins and tannins, especially those confined in the outer layers of the grain brans. This process enhances the digestibility and bioavailability of nutrients by removing these anti-nutrients, which otherwise interfere with mineral absorption and protein digestion (47). However, the most significant disadvantage of milling is the loss of essential minerals, such as iron, zinc and magnesium, which are also found in the bran. This mineral loss can compromise the nutritional value of the grains, making it challenging to balance the reduction of anti-nutrients with the retention of essential nutrients (45). Thus, while milling enhances some aspects of grain quality, it may simultaneously reduce the overall mineral content and nutritional benefits of the final product (48).

### *Soaking and Germination*

Soaking plays an essential role in post-harvest techniques as it offers required moisture, which supports germination and reduces the content of enzyme inhibitors and other anti-nutrients that increase digestibility and nutrient value (49). Some of the endogenous enzymes during soaking, such as phytase, are released during this process, especially when legumes are soaked for more than an hour before cooking; thus, these compounds and other non-nutritional substances

**Table 2.** Effectiveness of different processing methods in lowering antinutrient levels across various legume varieties

| Eradication process | Produce | Lectins (%) | Phytic acid (%) | Saponins (%) | Tannins (%) | Raffinose (%) | Stachyose (%) | Verbascose (%) |
|---|---|---|---|---|---|---|---|---|
| Soaking | Broad bean, common bean, cowpea, green pea, jack bean | 0.1-60 | 0.2-35 | 3-23 | 0-79 | 22-97 | 7-99 | 18-66 |
| Soaking and cooking | Broad bean, common bean, cowpea, green pea, jack bean | 0.9-100 | 1.8-81 | 13-100 | 29-100 | 55-100 | 49-100 | - |
| Soaking and autoclaving | Broad bean, common bean, cowpea and lima bean | 93-100 | 0.3-100 | 21-100 | 36-75 | 79-88 | 75-80 | - |
| Extrusion cooking | Broad bean, common bean, green pea, jack beanVand African yam bean (*Sphenostylis stenocarpa*). | 100 | 1-99 | 9-70 | 73-100 | 20-61 | 15-66 | 26-90 |
| Germination | Lablab bean, jack bean, cowpea, common bean, and broad bean. | 2-80 | 17-96 | 6-64 | 13-80 | 100 | 100 | - |
| Gamma irradiation | Velvet bean and broad bean | - | - | - | - | 78-100 | 75-100 | 74-100 |
| Sprouting + autoclaving | Common bean | - | - | - | - | 100 | 100 | - |

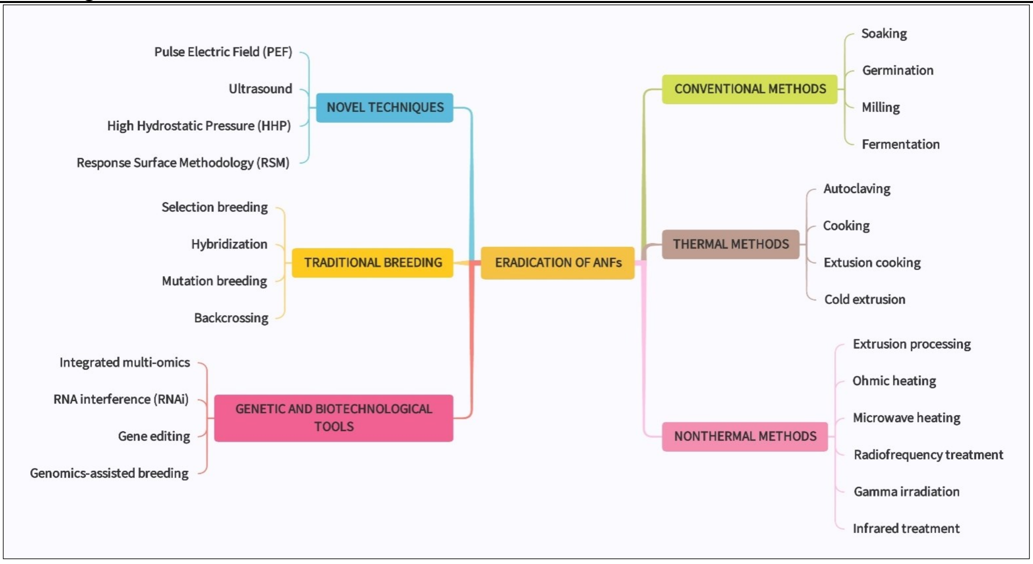


**Fig. 3**. Overview of strategies for reducing antinutritional factors.

leach into the water of soaking (50).

Adding sodium bicarbonate to the soaking water further improves the efficiency of soaking because it increases the permeability gained by partial solubilization of cell walls. Several water-soluble anti-nutrients are leached off during prolonged soaking, as shown previously (30), who demonstrated a significant reduction in phytate content with soaking at 45 °C and 65 °C. The success was achieved by soaking grains and beans to enhance mineral concentrations, protein availability, and the lowering of phytic acid levels (51).

For example, soaking cowpea seeds in alkaline water for 16 hours removed raffinose and decreased stachyose by more than 80 %. It is also reported that the release of endogenous phytase and tannins from seeds, when kept for 24 hr due to the modified ionic environment, resulted in increased seed coat permeability. Studies have shown that soaking seeds in a salt solution results in a higher reduction of RFO compared to soaking in distilled water (52). Moreover, the researcher observed a reduction in lectins when broad beans and common beans were soaked in distilled water (25).

Germination, a process adopted mainly in Mediterranean areas, involves seed hydration up to radical emergence. In germination, galactosidase in the mature seed becomes activated; it catalyzes the hydrolysis of RFOs for energy generation. Germination is also a highly effective means of reducing anti-nutrient content in plant-based foods (53). Recent studies have demonstrated that germination indeed affects the isoflavone content in soybeans by the activation of β-glucosidases thereby enhancing the nutritional value since isoflavones possess chelating ability (54, 55).

## *Autoclaving and Cooking*

The principle behind cooking and autoclaving for eliminating anti-nutritional compounds involves osmosis, wherein pressure forces water into seeds, nullifying or reducing the effects of heat-sensitive anti-nutritional factors. For instance, when velvet beans were boiled for 3 hr, the contents of raffinose, stachyose, and verbascose were reduced by 29-47 %. However, autoclaving at 121 °C for 90 min brought out a more significant loss, wherein the compounds were reduced by 46-69 %. Boiling also resulted in decreases in vicine (18.9 %) and convicine (22.5 %) (56).

Coupling soaking and autoclaving significantly reduced stachyose (76-78 %) and raffinose (65-72 %) in common beans and the combined effect was more significant than either method alone. Furthermore, sprouting for over 48 hr followed by autoclaving eliminated RFOs completely (9). In cooking, if the temperature does not exceed 100 °C, it is known as extrusion cooking. Among all the thermal processing methods, extrusion cooking has been shown to retain a higher percentage of major anti-nutrients, such as tannins and RFOs. The technique is widely used in pulse flour-making industries. When the produce is cooked below 50 °C, thermolabile compounds like vitamins, functional proteins, and flavors are conserved in cold extrusion. It is the most common method when making fortified pasta with legume products like common beans and green peas (57). Still, it should be borne in mind that anti-vitamins E and D are autoclave-resistant (58).

### *Fermentation*

Fermentation is an ancient food preservation process that has been recognized as a safe and effective means to enhance food safety, sensory characteristics, and nutritional value. One of the most prevalent forms of fermentation is lactic acid fermentation, which involves LAB. LAB can break down α-galactosides, including stachyose, verbascose and raffinose, which cause digestive discomfort and flatulence when fermented by gut bacteria in the large intestine. Studies have shown that lactic acid fermentation can significantly reduce the concentration of toxins, such as ODAP, in plants like *Lathyrus sativus*. Fermentation is the most effective detoxification method, eliminating up to 95 % of toxins.

Lactic acid fermentation is observed to reduce the activities of trypsin inhibitors, saponins, and phytic acid content, as well as biogenic amines, in legumes. This also leads to tannin degradation; this results in an improvement of the nutritional quality of legumes, enhancing the iron absorption capacity. Furthermore, it raises the isoflavones content, which acts as antioxidants. Beneficial bacteria such as *Pediococcus pentosaceus Pediococcus acidilactici, Lactobacillus brevis, Lactobacillus plantarum,* and *Lactobacillus sakei,* are known for their ability to reduce anti-nutritional factors (59).

For instance, research has shown that faba beans and field peas fermented with *Lactobacillus reuteri* and faba beans fermented with *Pediococcus pentosaceus* contained reduced raffinose levels in the legumes (60, 61). Generally, fermentation is a versatile and efficient approach to enhancing legumes nutritional value and safety.

### *Combined and advanced thermal techniques*

The combination of germination and autoclaving techniques was found to reduce anti-nutrients in kidney beans significantly. Lectins. Germination primarily targets stable anti-nutrients, such as phytates, tannins and compounds that cause flatulence. Subsequent cooking methods, including using water or saline solutions followed by autoclaving, effectively reduce heat-sensitive anti-nutrients like saponins and lectins (9).

Extrusion processing is a combined thermo-mechanical treatment involving accurate control over feed composition, cooking temperature along the extruder, moisture content, and screw speed, which can be an effective method to induce chemical and thermal breakdown of anti-nutritional factors simultaneously with enhancing the chemical, physical and nutritional characteristics of nutrients in a positive manner (62).

A study comparing extrusion processing with traditional non-thermal methods like dehulling, soaking (12 hr & 30 min in double deionized water), and germination (72 hr at 25 °C) in faba and kidney beans found that germination for 72 hours significantly lowered the phytate content by 60.8 % in faba beans and 30.2 % in kidney beans. In contrast, extrusion alone resulted in smaller reductions of around 26.7 % in faba beans and 21.4 % in kidney beans. When it came to tannins, which are primarily found in the outer layers of the beans, dehulling proved to be the most effective method, resulting in a reduction of approximately 92.8 % in both bean types. Conversely, extrusion led to reductions of 54.4 % in faba beans and 83.8 % in kidney beans.

Given the different distribution of some ANFs in edible seeds among species, for instance, tannins present mainly in the testa, phytates primarily in the bran and aleurone layer, results indicate that dehulling (physical removal) and milling (size reduction) may be required to be able to sufficiently decrease certain ANFs before prior utilising them as raw materials for extrusion (63).

Ohmic heating is an advanced thermal process that is described by (64) It deals with passing an electric current through the food. The electrochemical effects of ohmic heating deactivated trypsin inhibitors considerably. With the shortest period of heat application, as short as 3 min, trypsin decreased significantly. Ohmic heating resulted in a 13 % loss of trypsin activity, while electric cookers exhibited a loss of up to 19 %.

Microwave heating has proven to be highly effective in reducing protease inhibitors in certain legume seeds, all while maintaining the quality of their nutrients and proteins. Soaking legumes before microwave treatment further enhanced this effect, significantly lowering trypsin inhibitor activity compared to using dry microwave methods. For instance, a ten-minute microwave treatment at 2450 MHz reduced trypsin inhibitor activity (TIA) to just 8 % in soaked velvet beans (soaked for 8-12 hr), while a similar treatment lasting 12 min on unsoaked velvet beans showed less reduction (66). Furthermore, microwave heating for 3-5 min at 2450 MHz eliminated trypsin inhibitors in winged bean seeds.

Radiofrequency treatment, a form of dielectric heating, provides a more efficient alternative to traditional thermal processing methods (65). Gamma irradiation, another technique used to reduce anti-nutritional factors, has been explored for inactivating or removing specific anti-nutrients in legumes. For example, exposure to γ-rays at doses of 5 and 10 kGy significantly lowered the tannin content in raw and processed soya flour. Additionally, gamma irradiation has been applied to eliminate anti-nutritional compounds in broad and velvet beans, with studies reporting reductions of 74 % to 100 % in substances like raffinose, stachyose, and verbascose (66, 67).

Infrared treatment is gaining popularity as a promising new processing technique in the food industry. This approach employs electromagnetic radiation to convey energy at reduced temperatures, enhancing water uptake, decreasing cooking durations, and minimizing anti-nutritional elements in cowpeas (68). Also referred to as micronization, infrared heating operates within a frequency range of 1.8-3.4 μm, offering several advantages. These include efficient heating at temperatures between 750-930 °C, faster cooking times, more even heat distribution, lower energy consumption, and minimal impact on food quality.

### *Emerging Non-Thermal Processing Techniques: Recent Advances and Applications*

Traditional thermal processing methods are cost-effective particularly in heat-sensitive compounds such as vitamins, proteins, and amino acids. To address this, earlier chemical detoxification techniques were introduced. One example is the detoxification of *Canavalia ensiformis* seeds, where canavanine is broken down into deaminocanavanine under alkaline conditions. While chemical detoxification can be effective, it is not always ideal, as it may alter the food's sensory qualities or nutritional value in undesirable ways.

Innovative technologies, such as pulsed electric field (PEF), ultrasound, and high hydrostatic pressure (HHP), are gaining attention as effective methods for ensuring food safety while preserving nutritional quality. PEF, for example, applies short bursts of electric pulses ranging from 10 to 80 kV/cm over nanoseconds. This innovative technique breaks down cell membranes, making nutrients more accessible and effective while maintaining a cool temperature. It's a game changer for enhancing bioavailability without the risk of overheating. Ultrasound and HHP, on the other hand, work by influencing macromolecules through non-covalent interactions. These methods have demonstrated significant potential in reducing anti-nutritional compounds while maintaining the integrity of nutrients. A study highlighted the use of ultrasound treatment on common beans, where exposing them to 40 kHz frequency and 130 W power for 10 to 30 min effectively reduced α-galactosides more than soaking alone. This process also improved water diffusivity and reduced cooking time, showcasing its practical benefits (69).

These innovative approaches mark a significant step forward in post-harvest processing, particularly for legumes. By safeguarding essential nutrients and improving digestibility, they address the shortcomings of traditional methods. They enhance the nutritional value of the food and improve the overall eating experience, making legumes more enjoyable and healthful for consumers.

### *Tackling Anti-Nutrients in Leguminous Vegetables: Response Surface Optimisation*

Response Surface Methodology (RSM) is a promising approach for reducing antinutrients in traditional legume-based fermented foods, offering a systematic approach for optimizing processing conditions to achieve the desired reduction in antinutrient content. However, the application of RSM includes several key steps. Firstly, researchers identify independent variables such as processing time, temperature, pH, moisture content, and other relevant parameters that may affect anti-nutrient content. Secondly, an experimental design is developed to systematically study the effects of these variables on antinutrient content, the resulting responses. Thirdly, mathematical models are created to represent the relationship between independent variables and the response variable (antinutrient content), aiding in understanding interactions and predicting optimal conditions. Fourthly, an interaction study is conducted to assess how each variable influences antinutrient content and understand combined effects. Later, the response surface model is used to determine optimal conditions, which are then verified experimentally. Lastly, statistical analysis is employed to analyze data, validate models and determine the significance of variables. RSM has successfully been applied in various legume-based traditional fermented foods, including idli, kinema, soya-derived probiotic food, and dhokla. For example, under preconditioning and infrared heating parameter optimization for precooked cowpea, RSM identified the optimal processing conditions that led to substantial oxalate reduction. Therefore, RSM can be an efficient means of optimizing the processing conditions to reduce antinutrient content and enhance the nutritional quality of legume-based products.

In a specific study (70) conditions, the influence of optimizing preconditioning and infrared (IR) heating parameters on the cooking properties of precooked cowpeas was studied using RSM. The researchers identified optimal processing conditions as follows: 45 % moisture content, a heating temperature of 185 °C, and a heating time of 5 min. Under these optimized conditions, the oxalate levels were significantly reduced by 42 %.

### *Maximizing Antinutrient Reduction: Genetic and Biotechnological tools*

Advanced genetic tools, such as genetic modification, can create more palatable and less toxic cultivars by selecting genotypes with reduced levels of anti-nutritional factors (ANFs). This process relies on isolating specific genes related to ANF production, like lectins or non-protein amino acids and employing genetic engineering techniques to downregulate or knock out these genes. Recent advances in gene silencing and editing have enabled the development of crops with lower anti -nutrient content.

A seed-specific deletion strategy could focus on legume strains that do not accumulate ANFs in their seeds while still producing them in other parts of the plant. This targeted approach preserves seeds and seedlings without affecting the overall plant. This is particularly useful in cases where ANFs are transported to seeds from parts of the plant, such as leaves, or when they are produced in all areas except the seeds.

Conventional breeding techniques to reduce ANFs include selection, backcrossing, and mutation breeding. For example, gamma irradiation of soybean variety MAC450 reduced lectin content without affecting normal germination up to the $M_3$ generation (71). Additionally, phytic acid reduction has been successfully achieved in various major crops with identified donor plants from spontaneous and induced mutants (72).

Genetic engineering offers vast choices to effect seed-specific deletion of ANFs. If the gene encoding a toxin protein or a critical enzyme in the biosynthetic pathway of ANF is determined, then various methodologies of genetic engineering would be helpful in down-regulating or knocking out their activity. Strategies such as the expression of antisense mRNA, gene targeting, synthetic oligonucleotides, and ribozymes could be adapted. Another approach could be molecular, where new traits are introduced into crop plants, such as new lectins or proteins with a higher level of methionine or cysteine. While the general approach may seem relatively simple on paper, its application in practice and during a short period is a different matter. Recent research (73) identification a QTL associated with the Vicine-convincing trait on Chromosome 1 in Faba bean linked to hemolytic anemia.

Metabolite engineering activities effectively targeted the regulation of raffinose levels in soybeans (74). By silencing the *RS2* gene through an RNAi construct, we significantly downregulated raffinose synthase 2, demonstrating a successful approach in controlling this metabolite. This allowed for an increase in the total metabolizable energy content of soybean meal for poultry. More recent technological advancements in gene editing facilitated the lowering of raffinose levels in soybean soybeans using the knockout of *GmGoLS1A* and *GmGoLS1B* genes. This advanced technique demonstrated a reduction in raffinose content, accompanied by simultaneous increases in verbascose, protein, and fat content, without affecting plant growth. This finding underlines the potential of this technique to alter raffinose levels in soybean genotypes.

### *Anti-Nutritional Factors: Biological Implications and Functions*

Indeed, anti-nutritional factors (ANFs) cannot be summarily dismissed as hazardous to human health, as certain levels of ANFs present in legumes may offer health benefits. The intake of grain legumes in a diet can reduce dietary damage caused by affluence. Legumes possess properties such as cholesterol-lowering effects, dietary fiber, and trypsin inhibitors, which positively impact human health.

The dual nature of ANFs in legumes, particularly *Vicia faba*, is well documented. While this legume has been linked to favism in individuals with G6PD deficiency, it also contains L-Dopa, a precursor to dopamine that can be utilized in the costly treatment of Parkinson's disease. This sets the example of how some compounds that were wrongly assumed to be harmful could be therapeutically helpful.

The accumulation of these ANFs in plants is often an adaptive response to environmental stress, enhancing plant resilience and growth. For example, tannins and lectins provide natural defense mechanisms against pests and pathogens. However, in human diets, they can interfere with nutrient absorption, digestion, and overall health. Table 3 provides an overview of various anti-nutritional factors, highlighting their harmful effects, potential health benefits in humans, and their roles in plant growth. The entire review has been mind-mapped systematically and attached in Fig. 4.

## Conclusion

In recent years, there has been a growing emphasis on nutrition security, particularly in combating malnutrition. Leguminous crops are crucial as they offer nearly double the protein content of cereals, aiding undernourished and overnourished populations while reducing chronic disease risks, such as cardiovascular disorders and diabetes. Some legumes are nutrient-rich, though anti-nutritional factors can hinder their utilization by affecting mineral bioavailability. Understanding these compounds is essential for addressing nutritional disorders, especially in India. Different processing techniques impact the nutrients and anti-nutrients in legumes. Soaking and cooking are the most effective methods for reducing anti-nutritional factors, with wet heat treatments superior to dry heat. These processes enhance mineral bioavailability and improve digestibility. Advances in biotechnology and genetic engineering hold promise for developing food products with improved nutritional outcomes. When applied in the food processing sector, these

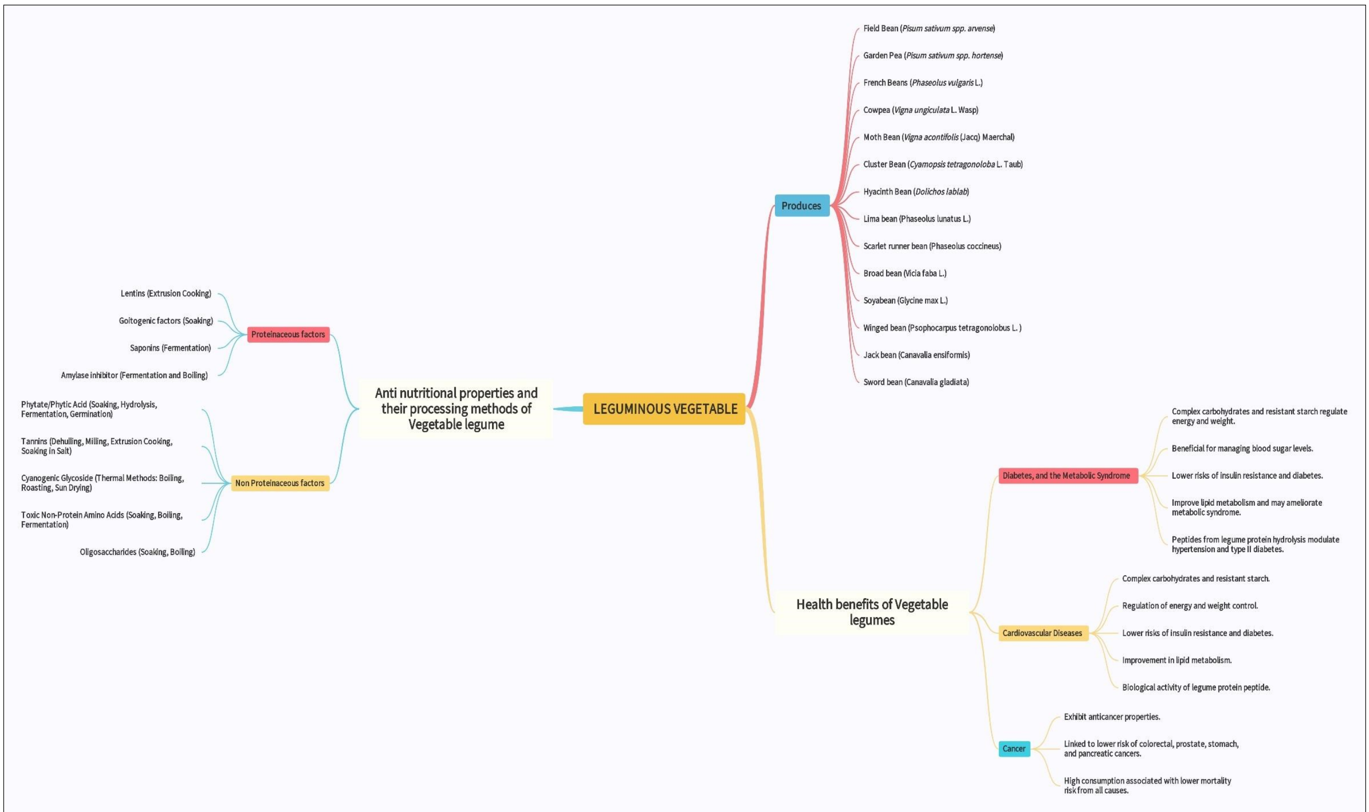


**Fig. 4**. Overview of anti-nutritional factors present in leguminous vegetables and strategies to control them.

**Table 3.** Pros and cons of anti-nutritional factors on humans and plants

| ANFs | Product source | Description | Health benefits | Harmful effect | Role in plant growth |
|---|---|---|---|---|---|
| Cyanogenic glycosides | Common bean and lima bean and | Glycosides of α-hydroxynitriles | - | Spontaneously degrade, causing potentially fatal HCN release; interfere with iodine organification, causing tropical ataxic neuropathy, goiter and cretinism; cause growth retardation and symptoms of cyanide poisoning including diarrhea, dizziness, headache, nausea, stomach pain, vomiting, and weakness | Releases hydrogen cyanide when its tissues are damaged, deterring herbivores |
| Phytic acid | *Phaseolus* sp., Faba beans, Jack bean, lablab bean, sword bean, lima bean, common bean, velvet bean, and soyabean | Often called myo-inositol hexakis dihydrogen phosphate, it serves as the primary phosphorus storage compound in seeds. | Possesses anticarcinogenic, antidiabetic, antioxidative, litholytic properties. | Interferes with the absorption of calcium, iron, magnesium and zinc, leading to deficiencies in these essential minerals. | Phosphorus storage and micronutrient chelation are crucial for proper growth and development.. |
| Saponins | Soyabean and French bean | Possess at least one glycosidic bond at the C-3 position that links the aglycone to a sugar chain, enabling them to create stable, soap-like foams in water-based solutions. | Lower the absorption of bile acids, cholesterol, and dietary fats, while also demonstrating antibacterial, antiprotozoal, insecticidal, and molluscicidal properties. | Able to attach to cells in the small intestine, reducing the absorption and utilization of nutrients. They also bind to vital dietary minerals, proteins, and starch. | Act as phytoalexins during the development of tubers and fruits, providing vegetables with resistance to diseases. |
| Lectins | Peas, Kidney bean and soyabean | Part of a group of carbohydrate-binding proteins, they remain stable even in acidic conditions.. | Exhibiting antimicrobial, insecticidal, antitumor, and antioxidant properties, they also help regulate blood sugar levels and prevent bacterial biofilm formation. | Can contribute to obesity, chronic inflammation, autoimmune disorders, and cause symptoms such as vomiting, nausea, and irritable bowel syndrome. | Control of cell signaling and plant reactions to abiotic, biotic, and symbiotic factors. |
| Tannins | Lima bean, Kidney bean, cowpea, and garden pea | Intricate, astringent, and water-soluble polyphenolic compounds. | Have antioxidant, antidiabetic, anti-inflammatory, anti-allergic, antimicrobial, anthelmintic, and anticancer properties. | Block digestive enzymes, thereby lowering the digestibility of nutrients, especially proteins and carbohydrates. They exhibit antinutritional effects, increased indigestibility, and have been associated with mutagenic, carcinogenic and hepatotoxic activities. | Plant tannins have antiparasitic effects and combat harmful bacteria, showing antibacterial activity and acting as antioxidants. Additionally, they help prevent neurodegenerative diseases and possess anti-tumor, anti-inflammatory, and antibacterial properties. |
| Vicine and convicine | Broad bean, Faba bean | Pyrimidine glycosides are present in broad beans. | They help prevent cardiac arrhythmias, inhibit the growth of malaria parasites, and have anti-inflammatory and antitumor properties. | Affect individuals with genetic G6PD deficiency, causing hemolytic anemia, a condition referred to as favism. | - |
| α-Galactosides | Soyabean and common bean | Ciceritol and Oligosaccharides (raffinose, stachyose, verbascose and ajugose) | Exhibit prebiotic effects, decrease the levels of N-nitroso compounds (potential carcinogens) in the gastrointestinal tract, and help alleviate constipation. | Lead to the production of gas in the digestive system | - |
| Gamma (γ)-Aminobutyric acid | soyabean | A non-protein amino acid containing four carbon atoms is formed by the decarboxylation of L-glutamic acid. | Lowers blood pressure, inhibits the growth of cancer cells, and helps regulate blood cholesterol levels | Increased levels can result in hypersomnia.. | - |

approaches not only promote economic growth but also enhance nutritional security.


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## Authors' contributions

RM did the resource collection and wrote the original draft. CI, BK, AS, MS and KG contributed the resources and also supervised and validated the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

**Ethical issues:** None

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