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BEYOND ANTI-NUTRITION: THERAPEUTIC POTENTIAL OF LEGUME ANTI-NUTRITIONAL FACTORS IN HUMAN HEALTH

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ABSTRACT
Earlier, anti-nutritional factors (ANFs) from legumes (protease inhibitors, lectins, phytates, saponins, and polyphenols) were known to hinder the bioavailability of nutrients and were necessary to be inactivated by heat or chemical treatment. However, a series of molecular and clinical studies along with epidemiological evidence has radically changed our way of looking at these compounds as potential beneficial therapeutics for common and some non-infectious diseases. In this review, we discuss the transition from the traditional viewpoint of anti-nutrients to the modern bioactive compounds with an explanation of how ANFs from legumes exert their positive impact through modulation of bioactive pathways, the most important of which include Nrf 2-Keap1, NF-κB, and AMPK, providing positive effect in prevention of cancer and diabetes. Furthermore, one chapter of the review is devoted to the analysis of the concentration and distribution of ANFs from different legumes, enabling readers to understand the practical importance of this information. Finally, we analyze thoroughly the results of the trials on clinical use regarding Bowman-Birk inhibitors for treatment of leukoplaquia and the epidemiological data, revealing the prospective dependency between consumption of phytates and colorectal cancer incidence. The technology of processing legume seeds involving their germination and fermentation is treated in the review.This study also looks into the emerging concept of pharmafoods based on legumes and discusses the challenges involved in obtaining formulations. By considering peer-reviewed literature and findings, the examination provides a framework to consider legume ANFs not just as adverse dietary substances but also as promising nutraceutical substances for the future of prevention and personal nutrition.

KEYWORDS: Bioactive peptides, Hormesis, Nutraceuticals, Cancer chemoprevention

1. INTRODUCTION
Globally, the plant-based eating with sustainable reasons as well as health advice, has rendered legumes to be the main subjects of research in nutrition (Willett et al., 2019). The legumes (Fabaceae) has remained varied and have been a boon to humanity for thousands of years since it is a cheap and environmental-friendly source of protein, complex carbohydrates, as well as dietary fiber and micronutrients. However, due to the presence of the anti-nutritional factors (ANFs), an array of secondary metabolites that can hinder the digestion, absorption process, or the metabolic functionality of the nutrients, makes it hard for total incorporation as well as acceptance into the culinary regime (Samtiya et al., 2020). The traditional view includes the aspect of nutritional toxicology which was common during the early 20th century, where different compounds like protease inhibitors, as well as saponins, and tannins were perceived to have harmful effects only, making it important to limit the presence or completely remove them by processes like soaking, germination, fermentation, as well as cooking by application of heat (Samtiya et al., 2020; Petroski & Minich, 2020). The traditional view has been accepted in the laboratory setting like something that does not represent the reality of food consumption.
In the past decades, there has been an ideology shift that has occurred due to the combination of precision nutrition and advanced omics, as well as new knowledge related to hormesis. The term "anti-nutritional factors" is now being replaced by "bioactive compounds" or "dietary phytochemicals," which indicates the researchers are increasingly aware that the biological activity of these compounds depends on many factors, including the amount administered in a diet and the characteristics of an individual's digestion (López-Moreno et al., 2022; Mullins &Arjmandi, 2021).

Hormesis refers to a dual-phase dose-response phenomenon that causes positive responses in cells exposed to small doses of stresses, whereas high doses cause harmful reactions (Calabrese et al., 2019). At dietary concentrations, ANF acts as a regulator of the biological processes that can trigger cellular defense mechanisms (Martínez-Ballesta et al., 2018; López-Moreno et al., 2022).

Relevant epidemiological evidence from various studies and meta-analysis confirms the importance of legumes in relation to the occurrence of colorectal cancer, type 2 diabetes mellitus, cardiovascular diseases and overall mortality (Schwingshackl et al., 2017; Afshin et al., 2019). These results which cannot be explained by existing nutritional composition models based only on macronutrients and fiber are consistent with the animal bioactivity profile of the anti-nutritional factors present in legumes in regular consumption levels. This review aims to consolidate existing literatures including peer-reviewed studies in molecular pharmacology, clinical medicine, food science and nutritional epidemiology. The following activities are to be carried out in order to achieve the stated purpose: 1) the determination of the specific ANF profiles and concentration levels in key legume species; 2) the analysis of ANFs acting mechanisms; 3) Review of existing clinical studies; 4) Understanding of the application of different processing technologies in ANFs bioactivity control; 5) Highlighting the significance of gut microbiota for legume metabolism and ANF bioactivity.

2. The Evolution of a Concept: From Anti-Nutrients to Bioactive Compounds

The increased studies on ANFs from legumes characterizes an overall development of philosophical approach to nutrition science marked by a shift of reductionist view of toxicity to a holistic perspective for understanding of biological processes taking place in body. The establishment of 'anti-nutrition' concept in the 1950s and 1960s at the intersection of nutritional and agricultural science is attributed to ensure that legumes can be successfully incorporated as new food products in animal feed. Studies carried out on animal feed based on raw soybean meal and other leguminous seeds proved to inhibit growth and lead to pancreatic enlargement and lower feed efficiency in these animals under the influence of heat-labile trypsin inhibitors and lectins (Muzquiz et al., 2012). The studies resulted in the establishment of rigid biochemical framework based on the idea that the only criterion of a substance's nutritive value is its adverse impact on digestibility and utilization of macronutrients and minerals. One of the implications of this approach was the necessity of processing ANFs through thermal denaturation, hydrolytic degradation, or physical removal. The concept of ANFs became well-established for many years as it served as the basis for dietary recommendations and food processing practices.

In the research on tropical legume seeds, Betancur-Ancona et al. (2012) have noted this duality that anti-nutritional compounds have some bioactive properties that need to be studied further. The challenge to anti-nutritional ideas began with cancer epidemiology and molecular pharmacology such that the detection of low breast, colon, and prostate cancer cases in Asian countries with high soy dietary habits elicited mechanistic studies of the non-nutritional aspects of legumes. Discovery of the Bowman-Birk protease inhibitor (BBI) from soybeans in vitro and in vivo to be an inhibitor of carcinogenesis particularly pointed to the fact that it inhibits carcinogenesis differently from its activity as a trypsin inhibitor. Thus, the change from "anti-nutrient" to "non-nutritional bioactives" made way for extensive pharmacological studies. Since then, the debate has developed rapidly owing to the advancement of the concept of hormesis.Based on the theory of hormesis, the apparent contradiction between the toxicological data (indicating harmful effects at high doses) and the new data about the beneficial effects of low doses was explained. A proteinase inhibitor, like BBI, is not good or bad in nature; its impact on the human body depends upon different aspects, such as the concentration of the inhibitor, duration of the exposure, tissue environment, or physiological condition of the organism. In the study of Bosi et al. (2019), this idea was developed, and the researchers described the bioactive and anti-nutritional properties of grain legumes, emphasizing the complexity of the actual influence of legume consumption on body metabolism. Katoch et al. (2023) continued the discussion of this issue by providing a comprehensive review of rice bean *Vigna umbellata*, showing how the indigenous knowledge systems have found out the therapeutic properties of substances that were declared as anti-nutrients by the Western dietary science.In other words, the new study of the microbiome redefires the ANFs, and is no longer characterized by the rigid classification of substances into good or bad categories. Ozolina et al. (2023) observe that research on ANFs claims a lot of attention as knowledge about the various processing methods continues evolving. In this way, it can be argued that nowadays more and more people understand that it is impossible to universally exclude substances and instead, a number of factors should be taken into consideration in the health assessments. For this reason, recent developments changed the very understanding of the so-called ANFs as it is believed that scientists see the evolution of ANFs from the phytochemical network to the process of bioactives development based on numerous factors like dosage, interaction with matrix, host genetics, gut microbial metabolism, etc. Fig 1. Shows benefits of anti-nutritional factors present in legumes.

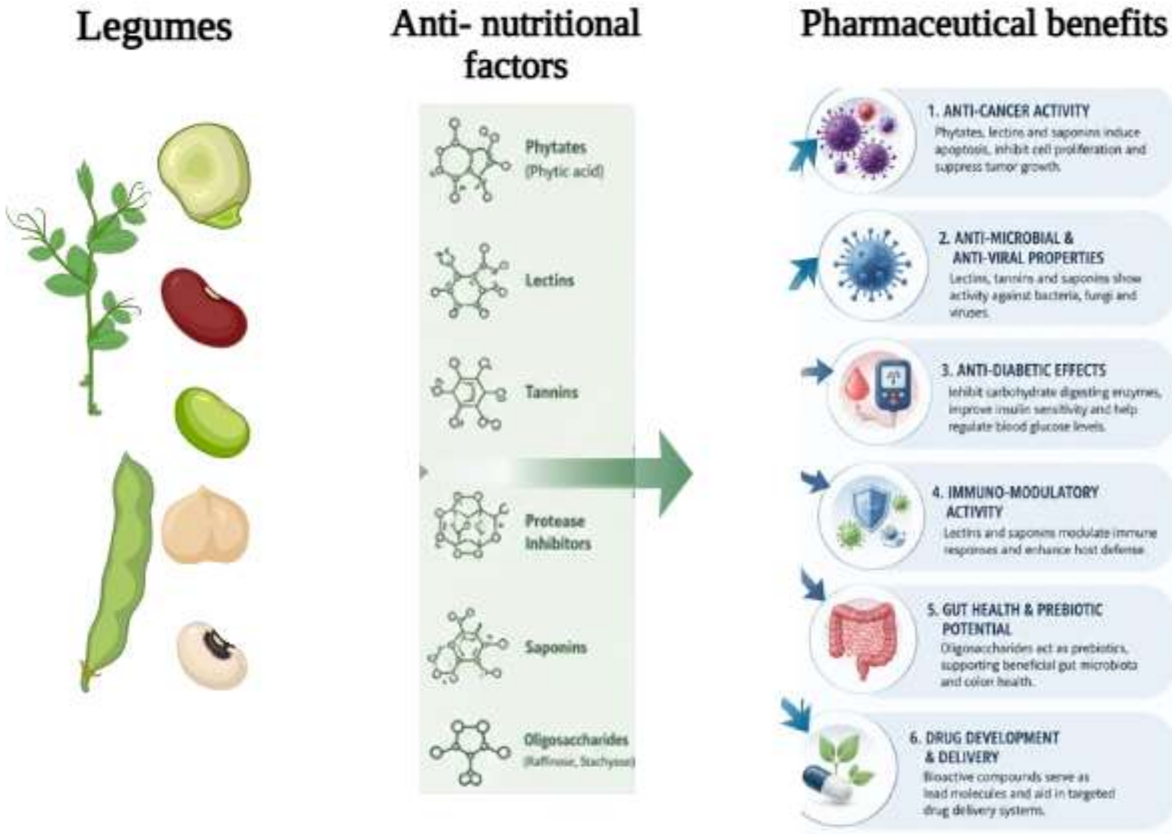

Fig 1. Benefits of Anti-nutrition factors of Legumes

3. Legume ANF Profiles: Species-Specific Distribution and Concentration Ranges

To grasp the therapeutic properties of ANFs in legumes in detail, one needs to familiarize oneself with their quantitative levels in different kinds of legumes, since both the type and amount of a particular ANF can vary incredibly with its genetic background, practices of cultivation, and afterward stages. In this part of work, the most important types of ANFs are described regarding their concentration levels in the most popular legumes with reference to several research studies. Table 1. gives a take on important ANFs.

3.1 Protease Inhibitors

In soybeans, 70-80% of the total trypsin inhibition is attributed to KTI while 20-30% is from BBI, with BBI amounting to approximately 2-5 mg/g of defatted soy flour (Gitlin-Domagalska et al., 2020). Chickpea (*Cicer arietinum*) has a distinct profile with low-molecular-weight trypsin-chymotrypsin inhibitors, having TIA values of around 8 to 18 mg/g, i.e., accounting for only about one-fifth of the trypsin inhibition in soybeans. Lentils (*Lens culinaris*) have moderate TIA level values, which range between 3 to 10 mg/g largely representing BBI inhibitors, with the activity being higher in red lentils than in green lentils. There is a large variation among common beans (*Phaseolus vulgaris*) regarding TIA level attributes, having TIA levels of about 5 to 25 mg/g while runner beans show higher amounts of inhibitors than white-seeded beans. Peas (*Pisum sativum*) and faba beans (*Vicia faba*) have low levels of protease inhibitors, having TIAs of around 2-7 and 1-5 mg/g respectively (Clemente et al., 2015; Samtiya et al., 2020). Cowpea (*Vigna unguiculata*) lies in the middle with TIA levels ranging from 10 to 20 mg/g (Gonçalves et al., 2016). In a review of the anti-nutritional components of pulses, Kumar et al. (2022) noted that the wide range of published data remains an indication of not only the genetic diversity but also the significant methodological changes in the assessment of the anti-nutritional constituents.

3.2 Lectins

Different species of legumes vary greatly in the levels of lectin found. However, the lectin content in common beans (*Phaseolus vulgaris*) is fairly high due to the prevalence of phytohemagglutinin (PHA) which is reported to have a high concentration of between 10 to 30 mg/g in the unprocessed seeds, red kidney beans containing a maximum level of as much as 40 mg/g of PHA (Muzquiz et al., 2012). This biochemical fact makes the consumption of kidney beans hazardous because eating them raw or untreated poses a high risk of having acute gastroenteritis where one may suffer from acute vomiting, severe nausea and diarrhea after eating the beans. Lectin from lentils (*Lens culinaris*) on the other hand, is present in low concentrations of between 2-5 mg/g. It has a very distinct preference in terms of binding to glucose and mannose residues unlike the lectin from soya beans which binds to N-acetylgalactosamine residues. The concentration of lectin from pea (*Pisum sativum*) is of low value (0.5-1.5 mg/g); whereas the lectin type from faba bean (*Vicia faba*) has a higher value of 1-2 mg/g. The lectin type from chickpea (*Cicer arietinum*) lies at the concentration of 0.8-2 mg/g, to bind with the complex form of N-linked glycans. In the context of cooking processes, it is worth noting the effect of various traditional methods of cooking that include boiling for 100°C for a time period of at least 10-30 minutes, with the resulting decrease in hemagglutinating activity of more than 99% in all types of legume strains studied.

3.3 Phytic Acid

Among all legumes, phytic acid (myo-inositol hexakisphosphate, InsP6) is one of the most common ANFs, being the main phosphorus storage compound in seeds that constitutes 60-80% of seed phosphorus (Gupta et al., 2015). Phytic acid in common beans (*Phaseolus vulgaris*) ranges from 6-18 mg/g; black beans and pinto beans are at the high end, while navy beans are at the low end of the range. Chickpeas (*Cicer arietinum*) have higher levels of phytic acid (5-12 mg/g), especially in desi (brown-seeded) varieties compared to kabuli (white-seeded) varieties. Lentils (*Lens culinaris*) have moderate levels of phytic acid, around 4-8 mg/g, mainly found in the protein bodies of the cotyledon rather than in the seed coat. Peas (*Pisum sativum*) have 4-9 mg/g of phytic acid, while faba beans (Vicia faba) have 6-14 mg/g (Gupta et al., 2015). Cowpea (*Vigna unguiculata*) rank in the average range at 7-12 mg/g (Gonçalves et al., 2016).According to Soni et al. (2022), due to its chelating effect on minerals in nutrition, phytic acid possesses many properties which can lead to various health benefits such as antioxidant activity and anti-cancer effect on cells. Phytic acid content is influenced through processing of the seeds for example, soaking in water for 12 to 24 hours can lead to 20-30% reduction in the phytic acid content. Germination can also lead to 40-60% reduction of phytic acid in seeds. Prolonged fermentation can even go beyond the 80% reduction of phytic acid contents, as the enzymes in the seeds/phytases and microbial phytases get activated (Samtiya et al., 2020; Gupta et al., 2015).

3.4 Saponins

The term "saponin" refers to an amphipathic glycoside (either triterpenoid or steroidal) with a significant structural variability from species to species within the legume family, which is responsible for their foaming property in cooking. It is reported that the chickpea saponin content is found to be in the range of 2-5 mg/g, composed mainly of β-amyrin triterpene (highest saponin concentration detected in the seed coat), while the concentration in the dark-seeded desi chickpeas is found to be twice that of kabuli chickpeas. Lentils have a special composition of saponins containing soyasapogenol (1-3 mg/g) and the red lentil type is usually richer in saponins than the green lentil type. Common beans (*Phaseolus vulgaris*) contain 1.5-4 mg/g saponins (ollow saponins) and totaled 1.9% and 0.5-2 mg/g of a saponin, respectively. It is further noted that the levels of saponin in peas are extremely low (0.5-2 mg/g) compared to the content of this glycoside in the common beans and lentils.

3.5 Polyphenols and Tannins

Legumes have a wide range of polyphenolic compounds with many chemical compositions from simple phenolic acids to complex structural types of tannins (proanthocyanidins). The compounds exhibit the highest amounts in the phenolic compound group (Mayo et al., 2021). The seeds of common beans (*Phaseolus vulgaris*) are characterized by wide variations of polyphenols which depend on the color of the seed coat; the content of tannins presents from 0.5 to 20 mg/g. A higher concentration of condensed tannins is recorded in dark forms, such as black and red beans, while varieties with white seeds have a low level of tannins (Ganesan & Xu, 2017). The amount of total phenolics (TPC) in common beans varies from a low of about 2 to 12 mg/g, though it is noted that the colored beans show a better antioxidant potential in comparison to white beans. In chickpea (*Cicer arietinum*), the phenolic compounds consist of phenolic acids and flavonols. The variety of TPC in chickpeas reaches 1-5 mg/g. In black-seeded varieties of desi chickpeas, the majority of polyphenols (over 80%) are found in the seed coat. Polyphenols are present in the lentils (*Lens culinaris*) in the form of flavonols and proanthocyanidins, particularly catechin, epicatechin, and kaempferol glycosides, with TPC being around 3-12 mg/g. Dark green lentils have the largest number of polyphenolics in their composition (Zhang et al., 2015).The main source of polyphenols in faba beans is the seed hull, although the tannin content will vary based on the flower color types (2-8mg/g in colored flower cultivars and <1mg/g in low tannin plant types produced through conventional breeding techniques) (Merghem et al., 2004). Tannin concentrations for *Vigna unguiculata* are in the range of approximately 2-7 mg/g in TPC. Lentils have some anti-nutritional factors and polyphenols are characterized with some strong inhibition of α-glucosidase and pancreatic lipase activity in the process (Rudra et al., 2023). This diversity has a great importance for the purpose of further targeted selection because polyphenolic composition of legumes is especially important due to the fact that it causes high inhibition of the activity of certain enzymes, making it possible to improve the glycemic index of dishes from lentils.

Table 1. Antinutritional compounds and their activity

Anti-nutritional compound	Major legume source(s)	Reported pharmaceutical/health-related uses	Representative references
Phytic acid (myo-inositol hexakisphosphate; IP6)	Soybean, common bean, chickpea, lentil, pea, cowpea, mung bean	Anticancer activity, antioxidant, prevention of kidney stone formation, hypocholesterolemic effects, regulation of glucose metabolism	Urbano et al. (2000)
Saponins	Soybean, chickpea, lentil, pea, faba bean	Anticancer, cholesterol-lowering, immunomodulatory, anti-inflammatory, antimicrobial, vaccine adjuvant potential	Francis et al. (2002); Sparg et al. (2004)
Tannins (condensed tannins)	Cowpea, faba bean, lentil, common bean, pigeon pea	Antioxidant, antimicrobial, antiviral, anti-inflammatory, cardioprotective potential	Fraga-Corral et al. (2021); Samtiya et al. (2020)
Lectins (phytohemagglutinins)	Common bean, soybean, pea, lentil	Targeted cancer therapy research, antiviral activity, drug delivery, cell recognition and diagnostics	Lam & Ng (2011); Lagarda-Diaz et al. (2017)
Protease inhibitors (Kunitz and Bowman–Birk inhibitors)	Soybean, cowpea, mung bean, chickpea	Chemopreventive activity, anti-inflammatory effects, antiviral potential, suppression of tumor progression	Kennedy (1998)
α-Amylase inhibitors	Common bean, kidney bean, chickpea	Anti-obesity formulations, postprandial glucose control, adjunct therapy for type 2 diabetes	Barrett & Udani (2011)
Galacto-oligosaccharides (raffinose, stachyose, verbascose)	Soybean, chickpea, lentil, pea	Prebiotic activity, improvement of gut microbiota, enhancement of mineral absorption, immune modulation	Martínez-Villaluenga et al. (2008)
Oxalates	Winged bean, soybean, common bean, chickpea (lower amounts in many legumes)	Limited pharmaceutical use; mainly investigated for metal chelation and biochemical applications rather than as therapeutics	Holmes & Kennedy (2000)

4. Molecular Mechanisms of Therapeutic Action

The fact that ANFs from legumes can be reclassified as biologically active agents now seems guaranteed since a lot of evidence has been gathered in terms of molecular pharmacology. These agents cannot be said to work according to just one particular mechanism, since they exhibit polypharmacology and act upon different molecular targets at the same time in accordance with certain factors, such as concentration and types of cells.

4.1 Protease Inhibitors:

Bowman-Birk inhibitor (BBI) is considered to be the most studied ANF for its biological function. The biochemical possibility of chemoprevention has been established for the BBI that acts intracellularly and predominantly via a mechanism separated from its classical function of trypsin/chymotrypsin inhibition in the gut. Epithelial cells take the BBI through endocytosis and then inhibit the chymotrypsin-like function of the 20s proteasome effectively and directly (Gitlin-Domagalska et al., 2020). By means of blocking the ubiquitin-proteasome pathway, the action of BBI stabilizes important tumor suppressors, such as p53 and cdk inhibitors, leading to producing cell cycle arrest at the G1/S stage and apoptosis of the tumor cells (Clemente et al., 2015).

This is particularly relevant for epithelia since BBI become effective after ingestion. Along with the effects on the breakdown of proteins, BBI shows anti-inflammatory action through blocking the initial steps of the process by which NF-κB is formed, which happens through the inhibition of chymotrypsin activation (Gitlin-Domagalska et al., 2020). The principle of using these molecular actions has been proven in the important phase IIb randomized trial by Armstrong et al. (2013), where BBI was given to patients with oral leukoplakia. The results of the study showed a great clinical response accompanied by histological changes.

Gitlin-Domagalska et al. (2020) pointed out the possibility of the ability of BBI to be an epigenetic regulator due to its inhibition of specific HDACs, which leads to hyperacetylation of histones H3 and H4, loosening of chromatin, and any of the genes that are silenced epigenetically being turned back on in cancerous process. Therefore, BBI is seen not only as a protease inhibitor but also as a multi-target therapeutic agent that can function well in case of chemoprevention or combination therapeutic strategies.

4.2 Lectins: From Hemagglutinins to Autophagic and Immunomodulatory Agents

Lectins from legumes are composed of 4 components and have unique carbohydrate identification sites. They represent one of the most dangerous groups of anti-nutritional factors due to their capacity to bind to red blood cells and to bind in a highly efficient manner to the surface glycoproteins of the intestine that results in an increase in the permeability of the intestinal epithelium (Kumar et al., 2022). The strong toxicity of common bean (*Phaseolus vulgaris*) lectin at amounts from 10 mg up to 40 mg has generated attention concerning the importance of thermal treatment. However, despite the increased toxicity when raw bean seeds are ingested, lectins at much lower dosages are capable of identifying targeted carbohydrates. Panda et al. (2014) found that soybean lectin acts as a powerful agent against MCF-7 cancer cells in the sense that it induces processes of cell death such as autophagy and apoptosis through the involvement of the mechanisms leading to the formation of ROS, which are frequently connected with oxidative stress conditions.

The importance of this cooperative death mechanism being investigated here is so promising in cancer because this offers us an alternative to get rid of cancerous cells that have been resistant to drug treatment because of modifications in their survival mechanisms. The property of lectins to bind to carbohydrate makes it a potential tool to selectively destroy cancer cells due to the fact that these cells are marked by certain modifications at the level of glycoprotein and glycosphingolipid structure. According to Muzquiz et al. (2012), general results of various studies of legumes' influence on health and nutrition bring to light the ambivalent effects of lectins whose mode of action is said to enhance the processes of lymphocyte proliferation and cytokine production at small doses, which is, at the same time, both encouraging and dangerous depending on both dose and conditions under which lectins are used. When legumes are cooked properly, the amount of lectin that one gets in terms of diet reduces by over 99%. The residual interactions between lectins and glycans in the intestines could play a part in GALT functions in the body.

4.3 Phytic Acid (InsP6): Chelation and Intracellular Signaling

Phytic acid (myo-inositol hexakisphosphate, InsP6) is a compound that is present in all types of leguminous seeds at a concentration ranging between 4 mg/g to 18 mg/g. Due to its chelation properties in the food system, phytic acid has been considered as the archetypal anti-nutrient in the preface of food. The external anti-nutritional effects which occur when the phytic acid produces insoluble mineral-phytate complexes under neutral conditions in the intestine remains as a nutritional concern among people who consume repetitive diets which are based on cereals and legumes lacking minerals (Schlemmer et al., 2009). Despite all this, it has been increasingly found that this ability of phytic acid to chelate minerals might have therapeutic value. The impact of the phytic acid in the digestive tract is that it may bind to surplus ionic iron and thus inhibits iron-mediated production of damaging hydroxyl radicals, thereby reducing chances of developing colorectal cancer (Schlemmer et al., 2009).

However, it is the pharmacological importance of this process that takes place in the inside of cells. The phytic acid enters into mammalian cells where it is accumulated in high concentrations sufficient for the effects. In addition to its role as an inhibitor, InsP6 alters important cellular functions through its involvement in the modulation of important signaling pathways like PI3K/Akt and DNA-dependent protein kinase (DNA-PK) (Schlemmer et al., 2009). Gupta et al. (2015) provide evidence that low levels of InsP5, InsP4, and InsP3 due to microbial phytase-mediated partial dephosphorylation are important as second messengers regulating calcium signaling, transport, and signaling as a result of the enzymatic processes occurring in the colon, emphasizing the interconnected relationship between food intake of phytic acid and the effects of host physiology and microbial activities in the mutual concurrence of various physiological influences. Soni et al. (2022) pointed out the need to take into consideration the positive effects of phytic acid as an antioxidant, anticancer, and antidiabetic agent since it can serve as a mineral chelator.

4.4 Saponins and Polyphenols: Membrane Interactions and Metabolic Signaling

Saponins of legumes act as modulators of the biological functions through their interaction with the cholesterol-rich lipid domains, called 'lipid rafts' in the membranes of cells. This interaction leads to the alteration of membrane biophysical properties, such as fluidity, permeability, and arrangement of signaling molecules (Guang et al., 2014). One of the mechanisms of action of saponins mimics that of artificial bile acids sequesters by disturbing the enterohepatic cycle of bile acids and by stimulating an expression of hepatic LDL receptor as a result of activation of cholesterol 7α-hydroxylase (CYP7A1), which results in the reduction of plasma LDL cholesterol levels (Guang et al., 2014). Other than that, the activities of saponins concerning membrane disruption can also be associated with their selective cytotoxicity towards cancer cells due to the distinct composition of cholesterol and lipid rafts in the membranes of cancer cells, which makes them more sensitive to membrane damage caused by saponins as compared to their non-cancerous counterparts. Among other legume constituents, polyphenols shows a different mode of action and most of the intracellular activities of polyphenols are performed by microbial metabolites of polyphenols with lower molecular mass.

High-molecular-weight condensed tannins are able to cause the precipitation of salivary proline-rich proteins as well as the cleavage of digestive enzymes in the mouth and upper gastrointestinal tract and affect the astringent perception associated with certain pulses. The overall health consequences are mostly determined by the molecules derived from the gut microbiome of low-molecular-weight phenolic compounds (Tomas-Barberan et al. 2017).

The authors Zhang et al. 2015 undertook the research into the phenolic profile of lentils grown in Canada, analyzing 20 diverse varieties. It was shown that lentil polyphenols possess very strong in vitro inhibitory activity towards α-glucosidase and pancreatic lipase, essential for the enzymatic breakdown of starch and triacylglycerides respectively. The inhibition of these enzymes clearly explains the very low GI values in lentils and the effect of regular consumption of the pulses in the metabolism involving glucose and fat. The molecular pharmacology of ANFs of legumes reveals a complicated multi-level system that comprises different bioactive components that can interact with cell systems at many levels from the gut lumen till the regulatory cellular mechanisms.

5. Technological Approaches for Harnessing ANF Bioactivity: From Processing to Formulation

There has been a consistent and important link between food processing techniques and legume ANFs, which has always focused on the removal of ANFs. Various methods that have been used traditionally in treatments like soaking, dehulling, boiling, autoclaving, roasting, and fermentation have been developed on the principles of the anti-nutrition model, whereby effectiveness of treatments measured only by the percentage of decrease in the amount of ANFs present (Samtiya et al., 2020). The progress in understanding the role of ANFs in human health as bioactive agents with therapeutic effects requires a broader understanding of food processing technologies and processes used. Today, food processing technologies should not be seen as an effective way of complete removal of potentially harmful substances, but as a way in which various characteristics can be modified, including the kind, amount, structure, bio-availability, and eventually the action of these elements. Ozolina et al. (2023) provided a detailed discussion of the available options for treatment of legume ANFs that stresses the idea of the need to align safety standards and bioactive potential of the treatment products.

5.1 Germination: Controlled Metabolic Activation

Germination is considered to be one of the most biochemically traditional processes for legumes as it activates the internal enzymatic machinery of a dormant seed which, in turn, leads to the transformation of its chemical structure. In particular, during germination there occurs a partial breakdown of phytic acid resulting in approximately 40%-60% decrease of InsP6 levels within 48 to 72 hours depending on the temperature and a type of seed (Gupta et al., 2015). This process should be regarded not just as some kind of a method for improving mineral bioavailability. Rather, this process should be interpreted as a process of bioprocessing of seeds after germination which is important for the germinated seeds due to the presence of lower inositol in InsP3, InsP4, and InsP5 transforming into various bioactive substances. The correct manipulation of germination parameters will allow changing the content of certain metabolites in the seeds. Other than phytates, the composition of polyphenols in legumes is also modified during germination.

The emergence of phenolic compounds as the germination proceeds indicates the physiological adjustment of the germinating seed to the oxidative stress associated with the transition from dormancy to active metabolism. The investigation of Zhang et al. (2015) presents an evidence that lentil germination process can contribute to the increase in total phenolic content by 50-200% and increase the concentration of different bioactive compounds such as flavonoids and phenolic acids, directing to the improvement of the antioxidant and enzyme-inhibiting characteristics of the obtained sprouts. Germination can be considered as a practical, eco-friendly and scalable biotechnological process for synthesizing the nutritionally and pharmacologically enriched food components from legumes, without necessity of any solvents or chemical substances for the production.

5.2 Fermentation: Microbial Metabolic Engineering

Fermentation, executed either through spontaneous, backslopping, or defined culture methods is considered an advanced technique of microbial metabolic engineering with the intention of promoting safety as well as bioactivity. Studies show that the lactic acid fermentation of legumes using specific strains of Lactobacillus, Pediococcus and others significantly reduces the content of phytic acid as much as 80% due to a synergistic effect played by the endogenous phytases activated in the acidic environment of fermentation along with the newly-generated phytases produced during fermentation (Gupta et al., 2015). Simultaneously, the proteolytic activity of fermentative bacteria results in the production of numerous types of proteases and peptidases capable of hydrolyzing trypsin inhibitors and lectins, hence reducing their activity by 70-95% and providing safety as well as digestibility without clamping up on any heating processes which might adversely affect heat-sensitive bioactive compounds (Samtiya et al., 2020). The conversion of polyphenols of legume plants during fermentation involves the activity of various glycosidases which liberate aglycones from glycosylated forms thus increasing their bioavailability and consequently their biological activity (Tomás-Barberán et al., 2017).

The fermentation of soybeans, in the production of traditional foods from East Asia such as miso, tempeh, and natto, leads to an increase in levels of free genistein and daidzein aglycones that are readily absorbed in the intestine and are more effective as Nrf2 activators compared to glycosylated forms. In addition, the proteolytic activity of fermenting microorganisms can help recover hidden bioactive peptide sequences from legume storage proteins, some of which exhibit substantial activity in ACE-inhibiting, antioxidation, opioid and immunomodulatory activity. Kumaretal. (2022) noted that fermentation is one of the most viable strategies to overcome the negative effects of ANFs and enhance the nutraceutical value of legume foods.

5.3 Controlled Thermal Processing: The Goldilocks Principle

The method of thermal processing is still the most common technique used and required by laws for the assurance of safety for legume food products. Particularly true when it comes to the destruction of heat-sensitive antinutritional factors like lectins and protease inhibitors. The method of moist heat treatment which means boiling at temperature of 100 °C for 10 to 30 minutes shows how the lectin agglutination activity decreases by 99 % and the trypsin inhibitor activity decreases by 80 to 95 % in almost all legumes of commercial importance (Samtiya et al., 2020; Ozolina et al., 2023). The application of the right amount of thermal processing presents the Goldilocks Principle, whereas the lack of thermal processing leads to the presence of toxic active lectins which can be responsible for acute gastrointestinal toxicity. On the other hand, too much or prolonged thermal processing usually applied at high temperatures and in low moisture conditions destroys the useful bioactive substances, leads to oxidation of polyunsaturated fatty acids (PUFAs), loss of thermolabile vitamins, and decline of protein quality due to the process of Maillard reaction involving the reducing sugars and the ε-amino group of lysine.

The development of new regulated thermal processes, such as microwave baking, radio frequency heating, and ohmic heating, offers the discovery of innovative technologies that allow the use of fast and uniform heating profiles with the utmost control of the heating process. These technologies help achieve a significant reduction of lectin and protease inhibitors compared to the traditional process, while the level of total phenolics and antioxidant activity remains higher. The thermal processing of the bioactive products is carried out with the idea to create the optimal range for the presence of harmful and bioactive compounds, as it is not the process of complete elimination of ANFs.

5.4 Emerging Technologies for Pharmafood Development

To produce isolated legume ANFs from conventional sources into standardized, clinically proven pharmafoods and nutraceuticals requires sophisticated processing technologies. These must be used to isolate the specific bioactive components from the plant source while at the same time keeping their chemical and biological activity intact. Martínez-Ballesta et al. (2018) have published literature on the different systems that are used in producing nanoparticles and their applications in the medical field. Encapsulation technologies can be applied to encapsulate BBI, phytic acid, saponin fractions or polyphenolic extracts into different grades of edible biodegradable wall materials. This encapsulation system performs several significant functions: it protects the bioactive component from the acidic pH and proteolytic action of the stomach during the digestive process; it provides for controlled and targeted release in the intestine through the selection of the appropriate wall material with pH-sensitivity or susceptibility to some types of bacterial enzymes; it hides the unpleasant bitter, astringent, or beany flavor of certain purified legume-derived bioactives; and it improves considerably the stability and processability of the bioactive material. All of these technologies represent a crucial link between the large body of mechanistic and epidemiological quantified evidence gathered and available for identifying the bioactive properties of legume ANFs and the possibility of actual production of legume-based pharmafoods, which are ready for clinical assessment and practice in preventive and therapeutic nutrition.

6. Clinical and Epidemiological Evidence

The connection between the molecular pharmacology of legume ANFs and the various human health benefits supported by a growing body of clinical and epidemiological data which has progressively moved from correlation only evidence to mechanistic explanation.

6.1 Cancer Chemoprevention

A well-established conclusion in nutritional epidemiology is that the more legumes we eat, the less likely we are to suffer several types of cancer, especially colon cancer. Grosso et al. (2017) conducted a broad systematic review and meta-analysis that looked at the relationship between consumption of flavonoids and lignans, and death rates from cancer given that many of the plant bioactive substances present in legumes lower the rates of death from cancer. Among the inhibitors, Bowman-Birk has made the most progress in terms of going from the laboratory to practical clinical trials. Armstrong et al. (2013) conducted a major phase IIb clinical trial in which they assessed the efficacy of this inhibitor using a placebo control group. The trial was successful and proved that 31% of patients receiving treatment showed a clinical and histological response compared to the effectiveness of 9% in the control group.

More importantly, the correlative biomarker analysis in the matched pre and post treatment mucosal biopsies provided direct evidence of target engagement and confirmed suppression of chymotrypsin-like proteasome activity and suppressed nuclear translocation of p65 subunit of NF-kB. The rationale for considering phytate as a treatment for the prevention of colorectal cancer is both mechanical and epidemiologically established. Schlemmer et al (2009) provided a definitive statement on phytate in food and their implication in health, reviewing available research data in vitro, animal, and human models that supports the role of dietary phytate to inhibit colon cancer. Soni et al (2022) explored the health benefits of anti nutritional factors and stated that the anti carcinogenic role of phytate occurs through different mechanisms that complement one another including antioxidant effects via chelation of iron, augmented Natural Killer cells function, and inhibition of tumor cell growth through intervention with intracellular signaling.

6.2 Metabolic Syndrome and Type 2 Diabetes

Strong and broad clinical evidence indicates a beneficial influence of the regular consumption of legumes on obesity, insulin resistance, dyslipidemia, and hypertension associated with metabolic syndrome. (Reynolds et al. 2020) In a meta-analysis of RCTs on the effect of dietary fibre and whole grains, including legumes, on diabetes management, (Reynolds et al. 2020) concluded that dietary fibre from whole grains, including legumes, improves both HbA1c and reduces risk factors for cardiovascular disease.

In a randomized crossover study using multi-omics analysis, (Chambers et al. 2019) showed that oral supplementation of the novel inulin-propionate ester-designed to mimic the propionate-rich colonic environment resulting from high-fibre diets rich in legumes-resulted in substantial improvements in insulin sensitivity in overweight and obese individuals.

Interestingly, the study provided mechanistic evidence that links a specific microbial fermentation product to a significant metabolic outcome and that the intervention induced changes in both the gut microbiome and the plasma metabolome towards increased concentrations of short-chain fatty acids. In a thorough review of the health effects of dietary fibre and individual dietary fibre components on metabolic health, (Lattimer & Haub 2010) concluded that the combination of soluble fibre, resistant starch, and the associated phytochemicals in whole legumes produces positive effects on glucose regulation, lipid metabolism, and satiety by synergistically influencing a complex web of factors. Focusing on the health effects of beans in cardiometabolic diseases, (Mullins &Arjmandi 2021) reviewed clinical evidence demonstrating that regular intake of common beans and other legumes is associated with significant reductions in LDL-cholesterol, improvements in postprandial glucose regulation, blood pressure reduction and beneficial effects on body composition. (Bosi et al. 2019) discussed the health effects of grain legumes focusing on bioactive and anti-nutritional components in the prevention and treatment of diabesity, highlighting how the phytochemistry of legumes supports the observed effects.

6.3 Gut and Systemic Inflammation

This inflammation is now recognized as the common thread running through all major chronic non-communicable diseases and diet plays an essential role in modifying inflammation and influencing health outcomes throughout life. In a controlled dietary intervention with a colitis mouse model, Monk et al. (2017) showed that navy and black bean flours effectively reduced colitis-related inflammation and injury to the colonic epithelium. They found that the beans prepared the colonic environment to promote a healthy gut and the anti-inflammatory effects were associated with increased concentrations of short-chain fatty acids (specifically butyrate) in the cecum and colon and the induction of anti-inflammatory signaling pathways in the colonic epithelium.

An informative and equitable review that addressed and debated the classification of different plant components (e.g. Lectins, phytates, and oxalates found in legumes) as anti-nutrients, was delivered by Petroski and Minich (2020), who argued that a number of these substances can have positive impacts on the gut and inflammation when consuming a healthy varied diet. Lpez-Moreno et al. (2022) directly investigated this topic, seeking answers to the question of whether anti-nutrients are friends or foes, and concluded the answer is complex, depending on dose, food preparation, individual nutrient status, and the gut microbiota.

7. The Gut Microbiome: A Central Mediator of Bioactivity

The gut microbiota now represents an indispensable metabolic organ which significantly determines the in vivo pharmacology of consumed phytonutrients, for example legume ANFs. This microbial metabolic ecosystem offers an enormous variety and breadth of enzymes, far in excess of what the human genome encodes, which profoundly affects both bioavailability and biological impact of these extremely complex compounds from legumes. Toms-Barbern et al. (2017) provided the important development and extension of the concept of metabotypes – distinct stable metabolic phenotype, characterized by composition of host's gut microbiota and the ability of the microbiome to convert certain substances derived from food. This concept have extensive implications for understanding the observed differences between people on legumes' intake in epidemiology and isolated legumes' bioactive substances in clinical trials.

For an "equol-producer" (the host have genes coding necessary bacterial species for reductive metabolism of soy isoflavone daidzein toequol) the positive effects on cardiovascular metabolism and hormones by consumption of soy could be substantially stronger then in "non-producer". Beyond isoflavones, the transformation of many legume polyphenols by the gut microbiota also includes the entire set of legume polyphenols from seeds. High molecular-mass complex tannins and condensed proanthocyanidins present in the seed coat of darker colored common beans, lentils and faba beans do not readily absorb in the small intestine due to their enormous molecular mass and numerous hydrogen-binding sites. However, they are favorite substrates for some clostridia and Bacteroidetes species and among Eubacterium, Clostridium, Gordonibacter, and Eggerthella species.

These specialists utilize the enzymes of the 'polyphenol breakdown toolbox', including tannases, esterases and dehydroxylases, which hydrolyze complex polyphenols to form a unique series of smaller phenolic acids capable of absorption in the bloodstream and leading to the observed systemic effects.

Analogous to polyphenols is the transformation of phytic acid. Humans and other monogastric animals show minimal endogenous intestinal phytase, but their colon is enriched with a diverse and metabolically active microbiome that contains bacteria endowed with enzymes that progressively remove phosphate groups from the fully phosphorylated molecule InsP6. The intermediate products of hydrolysis are different lower inositol phosphates (InsP5, InsP4, InsP3, and finally myo-inositol) that could be absorbed across the colonic epithelium (Schlemmer et al., 2009).

Gupta et al. (2015) illustrated that the metabolic impact of the host's gut microbiota dictates the majority of human health impacts from diet phytate. By far these transformations eliminate any rigid practical and theoretical division between "anti-nutrient" and "bioactive", and rather view the host-microbe supraorganism as determinant of the dietary effect and impact. In this context the gut microbiome plays significant role in individual responses to legumes and can be a target for improving therapeutic benefits from legumes consumption.

8. Future Perspectives: Towards Legume-Derived Pharmafoods and Precision Nutrition

The scientific journey of legume ANFs – from the dogma and the wholesale eradication of these compounds to their validation and therapeutic application – serves to illustrate that rather than a health risk to be purged from our food and diets, ANFs are in fact a highly complex, evolved pharmaceutical aspect, engineered by plants. The future of ANFs lies in harnessing the incredible ANF diversity offered by legumes within a framework of personalized nutrition and precision therapy. Pharmafoods- foods or food-derived products designed specifically to deliver doses of a bioactive substance in a standardized, bioavailable, and clinically active format to elicit a desired health benefit – represent the next frontier of translational research for legume ANFs. This approach has been successfully modeled by Armstrong et al. (2013), who evaluated the use of the soybean Bowman-Birk inhibitor concentrate (BBIC) as an oral topical treatment for oral leukoplakia.

Future work in this field will focus on employing innovative encapsulation and targeting systems that protect purified legume bioactives from degradation in the upper GI tract, control their release in targeted intestinal sites where action/absorption are required, and enhance their bioavailability and therapeutic effectiveness (Martnez-Ballesta et al., 2018). The continued ascent and validation of the metabotype concept – individuals classified by their capacity to metabolically convert dietary precursors into bioactives via the gut microbiota – will be crucial for the success of personalized ANF interventions. Future clinical trials that explore the use of bioactive compounds extracted from legumes will likely recruit participants based on their initial metabotype, measured non-invasively via fecal metagenomic/metabolomic assessments, in an attempt to ensure targeted intervention with optimal outcome potential, and minimized potential adverse effects. This approach, known as precision nutrition, will allow for significant improvements in the risk-benefit profile of dietary interventions and facilitate the translation of population-wide dietary recommendations into specific, data-driven therapeutic advice.

Likewise, sophisticated plant breeding technologies and biotechnological tools, such as marker-assisted selection (MAS), genomic selection (GS) and genome editing using systems such as CRISPR/Cas9 will also be key in finely tuning the legume crop's ANF profile (Katoch et al., 2023). Rather than seeking to systematically reduce overall levels of ANFs, a more targeted breeding approach could lead to the development of "designer legumes" tailored with unique phytochemical profiles to meet the demands of particular health challenges and consumer populations; examples could include developing lentil lines with an optimal saponins-phytate ratio for combined cholesterol and glycemic control, or soybeans with elevated and consistent Bowman-Birk inhibitors for cancer prevention. Rudra et al. (2023) pointed to a need to balance minimizing detrimental anti-nutritional effects with promoting the many important health benefits conferred by plant-derived phytochemicals within a processing and usage strategy, which will shape the future of how we process and utilize lentils.

Therefore, the future of legume ANFs does not rest in their removal from our diets, but in their intelligent, evidenced-based, personalized application based on their complex biological properties. The small, legume seed offers a powerful, biologically rich illustration of complexity and evolutionary design, packed with vast and previously unrealized medicinal potential that is just now beginning to be explored and intelligently exploited.

9. CONCLUSION

The story of anti-nutritional factors (ANFs) found in legume seeds has been transformed in the last 20 years, and perhaps irretrievably, by the advancements made in molecular nutrition, pharmacology, and the study of the microbiome. Those previously dismissible evolutionary features, metabolic flaws or unequivocally detrimental attributes are now coming into sharper focus as part of an extremely refined and complex, hormetic phytochemical system that contains a vast but context-dependent therapeutic potential. The concept has shifted away from the reductionist approach of traditional nutritional toxicology that judged the efficacy of a compound purely on its toxicity at isolated high-dose concentrations, toward a far more sophisticated systems-level molecular pharmacology where efficacy is dose- and context- dependent on host physiology, and critically, on the metabolic actions of microbes.

There is growing evidence that when ingested at their native dietary doses within whole leguminous foods, compounds such as the Bowman-Birk inhibitor, phytic acid, lectins, saponins and condensed tannins, can modulate many of the same oncogenic, metabolic, and inflammatory pathways Targeted by our pharmaceutical arsenal, but in the more subtle, chronic and multi-targeting way of nutrition. Supported by the increasingly convincing evidence of rigorous clinical randomized controlled trials such as the phase II b trial of the Bowman-Birk inhibitor concentrate for oral leukoplakia, and complemented by robust and consistent epidemiological evidence that is biologically sound, there is now sufficient weight to prompt a re-regulation of our advice to the consumer, and the design and processing of our foods, so as to move from a simplistic maximisation of nutrient supply and avoidance of ANFs toward one that includes the proactive optimisation of chronic disease prevention through the intentional inclusion of dose-appropriate, hermetic bioactives. The techniques that have for so long been used for the removal of ANFs-including soaks, germinations, fermentations and thermal treatments-can now be reinterpreted, redesigned, and deployed as sophisticated tools of bioprocessing designed to tune not only the level, but importantly the chemical type, configuration and bioavailability of specific leguminous ANFs, and of course to be rigorously compliant with safety standards. The challenge now is not the technically uncomplicated yet intellectually limiting task of getting ANFs out of the food. Instead it involves the far more nuanced, complex and indeed far more exciting endeavor of characterizing precisely, the conditions under which they exert their beneficial effects –dose, duration, formulation, interactions with other foods, microflora composition, host genetics and health status– using exactly the same level of pharmacological precision that is now being developed in the formulation of novel pharmaceutical drugs.

In an era where we have been talking about precision nutrition and tailored medicine and developing new pharmafoods the seed of the legume with its unparalleled genetic and phytochemical diversity stands out as a marvelously intricate object that offers enormous potential, when handled thoughtfully with respect and a dose-specific approach.

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