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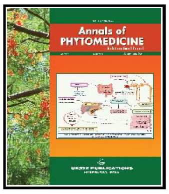

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Phytin in oilseeds: Physiology, breeding and implications for nutrition and health

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Abstract

Oilseed crops play a crucial role in providing vegetable oils for various purposes. Given the growing global demand for vegetable oils and their high agronomic value, oilseed crops are planted extensively worldwide. Several antinutritional factors are present in oilseeds. It is also worth noting that during the preparation of chemically purified protein from various seeds, a fraction containing organic phosphorus was isolated, which is predominantly present as phytin. This suggests that oil seeds could potentially contain phytin. Phytin is a crucial mineral storage compound found in seeds. It serves as the principal storage form of phosphorus in many plant tissues. Its significance lies in several aspects such as it is vital for seed and grain development as well as successful seedling growth, while often considered an antinutritional substance in human diets, phytin may also have positive nutritional roles. It acts as an antioxidant and an anticancer agent. Phytin is present in the parenchyma of ripe seeds in the form of globoids large basophilic concretions. Understanding phytin's role in seeds is essential for sustainable agriculture and human health. Researchers continue to explore its multifaceted effects on nutrition and well-being. Advances in breeding have led to the development of low phytate mutants in staple food crops like maize and barley, which is useful for oilseeds. These mutants offer potential benefits in areas such as enhancing the sustainability of lands used for crop cultivation. Reduced phytate levels positively impact mineral nutrition in human and animal nutrition. The quest for low phytic acid (LPA) crops has gained momentum due to their potential benefits for both human nutrition and agricultural sustainability. The LPA or "low-phytate" seed trait offers several advantages such as managing phosphorus (P) more efficiently. LPA crops enhance the global availability of essential minerals (iron, zinc, calcium, magnesium) for both humans and animals. Critics argue that breeding for the LPA trait may lead to reduced yields and field performance or diminish phytic acid's health benefits. Progress continues in breeding and genetically engineering high-yielding, stress-tolerant LPA crops. Alternative approaches, such as using the enzyme phytase as a feed additive or biofortification breeding, are also effective. Even with a moderate reduction in yield (*e.g.*, 5% to 10%), growing a nutritionally-enhanced crop variant seems justified. Prioritizing nutrition alongside yield can lead to crops that are both productive and highly nutritious. In summary, the present review aims to provide a critical understanding of phytin's role in seeds, physiology, genetics, crop improvement for low phytic acid contents, methods to reduce phytin in oilseeds using various methods and its impact on nutrition and health for sustainable agriculture and human well being.

1. Introduction

Oilseed crops play a crucial role in providing vegetable oils for various purposes. Major oilseed crops include rapeseed (*Brassica napus* L.), sunflower (*Helianthus annuus* L.), safflower (*Carthamus tinctorius* L.), soybeans (*Glycine max* L.), and peanuts (*Arachis hypogaea* L.). These plants are primarily cultivated for the oil present in their seeds. Given the growing global demand for vegetable oils and their high agronomic value, oilseed crops are planted extensively worldwide. Several antinutritional factors are present in oilseeds. These compounds can impact the nutritional quality and utilization of oilseed crops. Trypsin inhibitors are the compounds that interfere with protein digestion by inhibiting the enzyme trypsin. When ingested, they reduce the availability of essential amino acids. Lectins adversely affect carbohydrate utilization. They can bind to carbohydrates and interfere with their absorption and metabolism. Gossypol (found in cottonseed) is an antinutrient present in cottonseed. It can tie up iron, affecting its bioavailability. Glucosinolate is found in oilseeds like rapeseed, cottonseed, and soymeal. These compounds form insoluble complexes with phytic acid, further reducing nutrient bioavailability. The bioavailability of functional components and their levels are crucial in health maintenance (Nidhi Sharma and Maryam Sarwat, 2022). Phenolic

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compounds have antinutritional effects. Oxalic acid, chlorogenic acid, and protease inhibitors are also present in some oilseeds. As oilseeds are rich sources of nutrition, understanding and managing these antinutritional factors are essential for optimizing their use in human and animal diets. Phytic acid (also known as phytin) accumulates in seeds and acts as a phosphorus reservoir. However, it can reduce the digestibility of oilseeds and hinder mineral absorption. Hence, this review takes up a comprehensive look into phytin content in oilseeds, its metabolism, physiology, biochemistry, genetics, reduction strategies and implications of phytin for future research in oilseed crops.

Phytic acid is also known as inositol hexakisphosphate, inositol hexakisphosphate (IP6), or inositol polyphosphate. It is found within the hulls and kernels of seeds, including nuts, grains, and pulses (Vimal Singh *et al*., 2018). It is a calcium magnesium salt of phytic acid that occurs in plants as the main phosphorus storage reserve, especially in seeds or tubers. Phytin has a strong tendency to chelate metallic cations such as Ca, Fe, K, Mg, Mn, and Zn, forming an insoluble complex which renders them unavailable to animals or humans fed on a seed diet. This is because non-ruminant animals lack the enzyme phytase required to hydrolyze the inositol-phosphate linkages. In dry defatted flax seeds, phytin is present at concentrations ranging from 1.80% to 3%. Global estimate says that each year, up to 4.1 billion metric tonnes of crop seeds and fruits globally contain phytic acid. Approximately 35 million metric tonnes of phytic acid (containing 9.9 million metric tonnes of phosphorus) combine with potassium (K) and magnesium (Mg) to form over 51 million metric tonnes of phytate. Remarkably, the phosphorus content in this phytate is equivalent to nearly 65% of the elemental phosphorus sold worldwide for use in mineral fertilizers. Dry cereal grains contribute significantly, accounting for 69% of total crop seed/fruit production and 77% of the total phytic acid stored, annually.

2. Phytin in oilseeds

Phytin, or phytate, is a significant component in oil seeds. It serves as the primary form of phosphorus storage in the seeds. When seeds sprout, phytate breaks down, and the phosphorus is released for use by the young plant. In oilseeds, phytin has several important roles: Phytin acts as a storage form of phosphorus, which is crucial for the growth and development of the plant. Phytin is a natural antioxidant found in oil seeds. It has been linked to several health benefits, including preventing conditions like cancer. During the production of commercial protein products from defatted seed meals, phytin is often separated. This process allows for the isolation of a purer protein as well as a potentially valuable phytin concentrate. Oilseeds are rich in bioactive compounds, including phytosterols, phenolic acids, tocopherols, tocotrienols, and carotenoids. Many of these compounds, including phytin, have positive effects on health and can be used to design functional foods. Therefore, phytin plays a crucial role in the nutritional value (Bhuneshwari and Surya Pratap Singh, 2022), industrial applications, and health benefits of oilseeds.

2.1 Structure of phytin

Phytic acid, also known as phytin, is a six-fold dihydrogen phosphate ester of inositol, specifically of the myoisomer. It is also referred to as inositol hexaphosphate or inositol hexakisphosphate (IP6). At physiological pH, the phosphates are partially ionized, resulting in the phytate anion.The chemical formula of phytic acid is $C_6H_{18}O_{24}P_6$ (Figure 1). Its structure consists of a cyclohexane ring, where each carbon atom is attached to an oxygen atom, a phosphorus atom, and two hydrogen atoms. The phosphorus atoms are further bonded to four oxygen atoms, forming phosphate groups. In the structure of phytates, there is a pyrophosphate bond formed as the result of the substitution of a proton in the $PO_2(OH)_2$ group by the metal ion. This structure allows phytic acid to bind strongly to minerals, which can influence its bioavailability in the human digestive system.

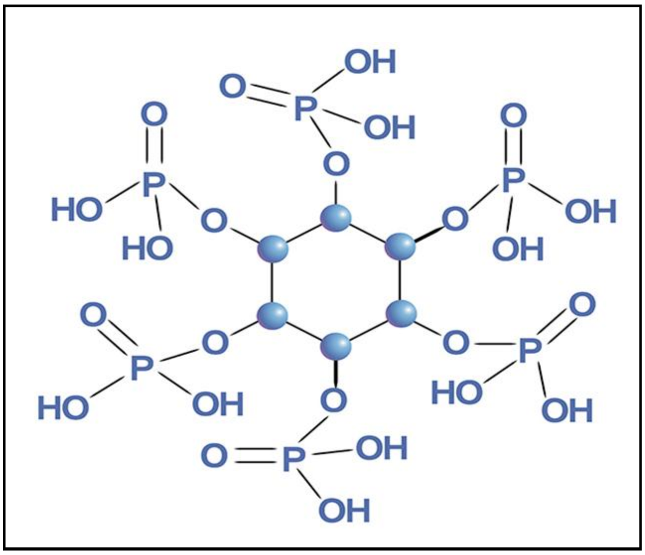

Figure 1: Phytic acid structure (Saburov and Kamilov, 1989).

2.2 Phytin role in plant physiology

Phytic acid, or phytin, plays a significant role in plant physiology. It serves as the principal storage form of phosphorus in many plant tissues, especially in bran and seeds. It is also present in many legumes, cereals, and grains. Phytin has a strong binding affinity to dietary minerals such as calcium, iron, and zinc, inhibiting their absorption in the small intestine (Pandey, 2018). This characteristic influences its bioavailability and nutritional value. In addition to its role in phosphorus storage, phytin also functions as an energy store, a source of cations, and a source of myo-inositol, which is a precursor for cell wall formation. These roles are particularly crucial for plants. However, it is important to note that the bioavailability of phytin's phosphorus and inositol is limited in non-ruminant animals, as they lack the enzyme phytase required to hydrolyze the inositol-phosphate linkages (Khan *et al*., 2014). This aspect has implications for agricultural practices and animal feed formulation.

2.3 Phytin role in the biochemical process

Phytin plays several important roles in biochemical processes. Phytin is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phytin has a strong tendency to chelate metallic cations such as calcium, iron, potassium, magnesium, manganese, and zinc, forming insoluble complexes. This property influences the bioavailability of these minerals in the diet (Robert and Elsie, 1935). Phytin plays several positive roles in metabolism, including protein and carbohydrate metabolism, responding to oxidative stress, drought tolerance by inducing stomatal closure, preventing senescence and apoptosis, and regulating phosphate homeostasis (Vimal Singh *et al*., 2018). Phytin acts as a shelter factor for maturing embryos in seeds. The lower inositol polyphosphates, such as inositol penta-(IP5), tetra-(IP4), and triphosphate (IP3), occur in nature as catabolites of phytic acid. It is important to note that while phytin has these significant roles, its phosphorus and inositol are not bioavailable to non-ruminant animals due to the lack of the enzyme phytase required to hydrolyze the inositol-phosphate linkages.

2.4 Phytin as a functional food in human nutrition

Phytin is a natural compound found in many plant foods. It has several potential uses and benefits: Phytin can bind minerals like calcium, iron, and zinc, reducing their bioavailability in the body. This property is often considered anti-nutritional, but, it can also be beneficial in certain contexts, such as reducing the risk of mineral overload. Phytin has been shown to have antioxidant properties, which means it can neutralize harmful free radicals in the body. Some research suggests that phytin may have anticancer properties. It has been found to inhibit the growth of various types of cancer cells in laboratory studies. By chelating calcium, phytin may help to prevent the formation of kidney stones. Some studies suggest that phytin may help manage conditions associated with metabolic syndrome, such as high blood pressure and high blood sugar (McCance and Widdowson, 1935). However, it is important to note that while phytin has potential health benefits, it can also interfere with the absorption of essential minerals, if consumed in large amounts. Therefore, it is important to maintain a balanced diet.

2.5 Phytin metabolism

Phytate is instead considered a nutraceutical (Perez-Gregorio and Simal-Gandara, 2017). Its properties and vivid health benefits are presented in the scientific literature. The FDA classified phytin as generally recognized as safe (Buades *et al*., 2017). The comprehensive overview of the current understanding of phytin metabolism in oilseed seeds, including its biosynthesis, degradation, factors influencing phytin levels (Valluru and van den Ende, 2011; Gillaspy, 2011) and strategies for phytin reduction is as follows:

2.5.1 Biosynthesis of phytin

Phytin biosynthesis in soybean seeds involves the condensation of inositol with phosphorus-containing molecules, primarily in the form of myo-inositol hexakisphosphate (IP6). Several enzymes, including inositol phosphate synthase and inositol phosphate kinases, catalyze sequential phosphorylation reactions leading to the formation of IP6. The regulation of phytin biosynthesis in soybean seeds is influenced by genetic factors and environmental cues such as phosphorus availability and plant developmental stage.

2.5.2 Degradation of phytin

Phytin degradation in soybean seeds primarily occurs through the activity of phytase enzymes, which hydrolyze the phosphate groups of IP6, leading to the formation of lower inositol phosphates (IP5, IP4, *etc*.) and free phosphate. Soybean seeds contain both endogenous and exogenous phytases, with different isoforms exhibiting varying substrate specificities and pH optima. The expression and activity of phytase enzymes in soybean seeds are regulated by genetic factors, environmental conditions, and post-harvest processing methods.

Factors influencing phytin levels

Several factors influence phytin levels in soybean seeds, including genetic variation, phosphorus availability, plant genotype, agronomic practices, and post-harvest processing techniques. Breeding efforts to develop low-phytic soybean varieties and optimization of agricultural practices can help mitigate phytin accumulation in soybean seeds.

Strategies for phytin reduction

Various strategies have been proposed to reduce phytin levels in soybean seeds, including genetic engineering, conventional breeding, processing techniques (*e.g*., soaking, germination, fermentation), and enzymatic treatments. These approaches aim to enhance the nutritional quality and mineral bioavailability of soy-based products while preserving their sensory and functional properties.

Phytin metabolism in soybean seeds is a complex process influenced by genetic, environmental, and processing factors. Understanding the biosynthesis, degradation, and regulation of phytin in soybean seeds is essential for developing strategies to reduce phytin levels and improve the nutritional quality of soy-based products.

3. Phytin in oilseed crops

The oilseeds reported phytin in varied levels based on area, soil types, cultivars and inputs used in cultivation.

3.1 Sesame

Phytin is found in sesame seeds (Sharma *et al*., 2021). As a plant matures, phytate rapidly accumulates in its seeds. Sesame seeds are reported to be a rich source of phytates compared to soybeans. Toma *et al*. (1979) reported 4.7, 5.2, 4.7, and 5.1% phytin content in whole, dehusked, roasted dehusked, and roasted sesame seeds, respectively. Makinde and Akinoso (2013) reported 30 mg/100 g phytate in the dehusked white cultivar and 25.07 mg/100 g in the black cultivar of sesame seeds.

3.2 Sunflower

Phytic acid, also known as phytin, is found in sunflower seeds. As a plant matures, phytate rapidly accumulates in its seeds. This substance is the primary way phosphorus is stored in many plants, including seeds. When consumed, it binds to other minerals to create phytates. Because humans do not have any enzymes that can break phytates down, their nutrients cannot be absorbed into the body. However, the effect of phytic acid on mineral absorption only occurs when large amounts of phytates are consumed within a diet that is already lacking nutrition. It also only affects the absorption of nutrients eaten at the same meal.

3.3 Safflower

Phytinis a compound found in various plant seeds, including safflower. In safflower, antinutritional factors (ANFs) include phytin, tannins, oxalates, luteolin, acacetin, glycosides, and serotonin derivatives. Phytin, specifically, is a salt of calcium, magnesium, and phosphorus.These ANFs can reduce the availability of nutrients and may cause growth inhibition if consumed by humans. Interestingly, the presence of phytin (inositol hexaphosphate) in safflower seeds may have contributed to increased seed weight. Safflower seeds are valued for their oil content, which ranges from 20% to 40% (Singhal *et al*., 2019; Bhite *et al*., 2021). The oil extracted from safflower seeds contains various fatty acids, including palmitic acid, linoleic acid, and oleic acid. Application of NPK (nitrogen, phosphorus, potassium) fertilizers, along with organic manure (FYM), positively impacts yield, oil content, and nutrient uptake. For optimal results, consider applying 50:25:00 kg/ha NPK along with 1 t/ha FYM and 40 kg/ha sulfur. This combination leads to higher plant height, capsule production, grain weight, and overall yield, including oil yield. In summary, phytin plays a role in safflower seeds, and understanding its effects can enhance safflower cultivation and oil production.

3.4 Soybean

Phytin has a strong tendency to chelate metallic cations such as Ca, Fe, K, Mg, Mn, and Zn, forming insoluble complexes. This makes these nutrients unavailable to animals or humans fed on a seed diet.In most oilseeds and grain legumes, phytin is associated with protein and concentrated within sub-cellular inclusions called globoids that are distributed throughout the kernel. However, in soybean seeds, there appears to be no specific location for phytin (Greenwood, 1989). While, phytin reduces the dietary nutritional value by making certain nutrients unavailable, it also plays several positive roles in metabolism (Thunyarat *et al.*, 2008). These include protein and carbohydrate metabolism, responding to oxidative stress, drought tolerance by inducing stomatal closure, preventing senescence and apoptosis, regulating phosphate homeostasis, and acting as a shelter factor for maturing embryos in the seeds.

3.5 Mustard

The seeds of mustard species such as *S. alba* (white mustard or yellow mustard), *B. juncea* (brown mustard), and *B. nigra* (black mustard) are increasingly used in the food and beverage industry due to their nutritional and functional properties. However, the presence of phytin in these seeds can reduce their dietary nutritional value (Mieth *et al*., 1983).

3.6 Groundnut

Groundnut seeds, also known as peanuts or *A. hypogaea*, are rich in carbohydrates, crude lipids, and crude protein. However, the presence of phytin in these seeds can reduce their dietary nutritional value. Despite its anti-nutritional properties, phytin plays several positive roles in metabolism. These include protein and carbohydrate metabolism, responding to oxidative stress, drought tolerance by inducing stomatal closure, preventing senescence and apoptosis, regulating phosphate homeostasis, and acting as a shelter factor for maturing embryos in the seeds.

3.7 Cotton

Cottonseed oil is extracted from the seeds of cotton plants, mainly *G. hirsutum* and *G. herbaceum*. The oil is used for various purposes, including cooking, due to its flavour stability. However, unrefined cottonseed oil contains a toxin called gossypol, which gives the unprocessed oil its yellow colour and protects the plant from insects. This toxin is removed during the refining process. While the presence of phytin in cottonseed oil is not explicitly mentioned in the sources, it is important to note that phytin is generally found in seeds of cereals and legumes. Therefore, cottonseed oil may contain some amount of phytin before refining. Castor oil is a vegetable oil pressed from castor beans. It is a colourless or pale yellow liquid with a distinct taste and odour. The main active component of castor oil is ricinoleic acid, a type of fatty acid.

4. Phytin content in various crops

The phytin values are approximate and can vary depending on factors such as genetic variation, environmental conditions, processing methods, and analytical techniques used for measurement. Additionally, newer research findings may provide updated information on phytin content in various crops (Table 1). For specific and accurate data, it is recommended to consult scientific literature or databases focusing on food composition and nutritional analysis.

Table 1: Phytin content in various crops

Crop	Phytin content (g/100 g)	Notes
Soybeans	1.5 - 2.5	Phytin content varies based on variety and processing.
Corn	0.6 - 1.5	Phytin content may vary depending on maturity.
Wheat	0.4 - 0.8	Phytin content is primarily found in bran and germ.
Rice	0.1 - 0.6	Phytin content varies depending on the rice variety.
Barley	0.3 - 0.8	Phytin content varies based on barley type.
Oats	0.4 - 0.8	Phytin content is primarily found in the outer layers.
Sorghum	0.3 - 0.6	Phytin content varies based on sorghum variety.
Millet	0.3 - 0.6	Phytin content varies depending on millet species.
Legumes (*e.g*., peas, beans)	0.5 - 1.0	Phytin content varies among legume varieties.
Sunflower seeds	0.5 - 1.2	Phytin content may vary based on seed type.
Canola (Rapeseed)	0.4 - 0.8	Phytin content depends on rapeseed variety.

5. Genetics and improved phytin resistance and nutritional quality

Continuous research and collaboration among plant breeders, geneticists, physiologists, and biotechnologists are essential for advancing the development of oilseed varieties with improved phytin resistance and nutritional quality.

5.1 Phytin genes

While the presence of phytin in castor oil is not explicitly mentioned in the sources, it is important to note that phytin is generally found in seeds of cereals and legumes. Therefore, castor oil may contain some amount of phytin before refining. It is also worth noting that during the preparation of chemically purified protein from various seeds; a fraction containing organic phosphorus was isolated, which is predominantly present as phytin (Bolley and McCormack, 1952). This suggests that oil seeds, including castor, could potentially contain phytin. However, the exact amount in castor oil specifically would require further investigation.

The genetic mechanisms regulating phytic acid biosynthesis and accumulation have been studied in crops like maize, rice, and wheat (Sharma *et al*., 2022). However, only a few leguminous crops have been targeted for such studies. Understanding these genetic mechanisms can help in developing strategies to regulate phytic acid content in crops, which is important for improving the bioavailability of minerals in food and feed (Sahu *et al*., 2024). The genetic engineering of oilseed crops, such as soybean and maize, has seen significant advancements over the past two decades (Porokhovinova *et al*., 2022). Several quantitative trait loci (QTLs) controlling seed oils have been identified in different oilseed crops. However, there are no reports of their successful pyramiding in a suitable genetic background leading to significant improvement of oil content (Porokhovinova *et al*., 2022).

In soybean, a major oilseed crop, a high-density genetic linkage map was constructed using the specific-locus amplified fragment sequencing (SLAF-seq) method. The study identified 24 stable QTLs for seed oil content and compositions, including a novel locus for palmitic acid on Chr. 10 (Savadi *et al*., 2017). Some interesting genes involved in lipid metabolism were detected in the QTL regions (Yao *et al*., 2020). These advancements in genetic engineering and the identification of gene targets for enhancing seed oil content will aid the successful development of new-generation high-yielding oil crops (Savadi *et al*., 2017). Ongoing research efforts to understand lipid biosynthesis and the possibilities of genetic modifications of key regulators of oil accumulation will set a platform to produce transgenic oilseed crops with enhanced oil content.

5.2 The reduction of phytin gene expression

The reduction of expression of phytin genes in oilseed crops can be achieved through various genetic engineering approaches. One such method is the use of RNA interference (RNAi) technology. For instance, seed specific RNAi silencing of cytosolic pyrophosphatases in Arabidopsis resulted in a 1-4% increase in oil content at the expense of protein content in transgenic seeds. Another promising approach is the use of gene editing technologies such as CRISPR/Cas9 (Savadi et al., 2017). This technique allows for site-directed mutation, enabling the knocking down of genes involved in undesirable seed components such as starch and polysaccharides (Savadi *et al*., 2017). By suppressing these genes, the oil content can be enhanced, and anti-nutritional components can be reduced. These methods provide a pathway for the modification of fatty acid profiles and the enhancement of oil content in oilseed crops. However, it is important to note that these are complex processes that require careful implementation and a thorough understanding of the genetic makeup of the crops.

5.3 Phytin inhibitors in oilseeds

In recent years, researchers have investigated various compounds that inhibit phytin activity in oilseeds, aiming to enhance nutrient absorption and mitigate mineral deficiencies. Oilseeds, such as soybeans, sunflower seeds, and rapeseed (canola), are integral components of human and animal diets, providing essential nutrients and bioactive compounds. However, the presence of phytin in oilseeds can limit the absorption of minerals such as iron, zinc, and calcium, posing challenges to optimal nutrient utilization. Phytin inhibitors offer a promising approach to mitigate this issue by reducing phytin activity and improving mineral bioavailability. Examples include (Ingale *et al*., 2011):

5.3.1 Phytase enzymes

Oilseeds naturally contain varying levels of phytase enzymes, which catalyze the hydrolysis of phytin into inositol and inorganic phosphate. Higher phytase activity in certain oilseed varieties can reduce phytin levels and improve mineral bioavailability.

5.3.2 Polyphenols

Polyphenolic compounds found in oilseeds, such as flavonoids and tannins, have been shown to bind to phytin and inhibit its activity. By forming complexes with phytin, polyphenols may reduce their ability to chelate minerals, thereby enhancing their absorption.

5.3.3 Fermentation products

Fermentation of oilseeds can lead to the production of organic acids and other fermentation byproducts that inhibit phytin activity. Fermented oilseed products, such as tempeh and natto, may exhibit reduced phytin content and improved mineral bioavailability compared to their non-fermented counterparts.

5.3.4 Genetic modifications

Advances in biotechnology have enabled the development of genetically modified oilseed varieties with reduced phytin content or enhanced phytase activity. By targeting genes involved in phytin metabolism, researchers aim to produce oilseeds with improved nutritional profiles and reduced antinutritional effects.

5.3.5 Dietary Fibers

Fiber-rich components in oilseeds can bind to phytin, limiting its interaction with minerals and promoting their bioavailability.

5.3.6 Metal chelators

Compounds like citric acid and EDTA can chelate metal ions, disrupting phytic-mineral complexes and enhancing mineral solubility.

Phytin inhibitors found in oilseeds offer promising opportunities to improve nutrient absorption and mitigate mineral deficiencies. Overall, the exploration of phytin inhibitors in oilseed crops represents a promising avenue for enhancing their nutritional quality and addressing global nutrition and health challenges. Further research is needed to elucidate the specific mechanisms of action of phytin inhibitors and their potential applications in agricultural and food systems.

6. Methods to reduce phytin in oilseeds

Phytin, a form of phytic acid, is often found in oilseed crops and can be reduced through various methods (Nout and Rambouts, 1993).

6.1 Mechanical expression

This process involves the compression of oil seeds in various types of equipment, such as hydraulic presses.

6.2 De-gumming

This method is used for seeds that have high phosphatide content. The phosphatide, which forms a gummy residue, is removed by mixing the oil with 2 to 3% water. This hydrated phosphatide can then be removed by settling, filtering, or centrifuging.

6.3 Solvent extraction

This method is employed for the manufacture of oil from the oil seeds.

6.4 Genetic engineering

Genetic engineering approaches have been explored to enhance oil content in oilseed crops, which could indirectly reduce the phytin content (Savadi *et al*., 2017).

These methods can help in reducing the phytin content in oilseed crops, thereby improving their nutritional value and digestibility. However, it is important to note that these processes require careful implementation and a thorough understanding of the oilseed crop's characteristics (Ijaz *et al.,* 2019).

7. Crop improvement methods for phytin reduction

Several breeding methods such as germplasm screening, transgenic plants and biofortification (Divya, 2022) are much investigated to reduce the phytin content in oilseeds. They are detailed below:

7.1 Gemplasm screening for phytin resistance in oilseeds

Screening for phytin resistance in oilseeds involves identifying varieties or genotypes that exhibit reduced phytin content or enhanced tolerance to high phytin levels (Till *et al*., 2007). Phytin, also known as phytic acid, is the primary storage form of phosphorus in plant seeds and can limit the bioavailability of important nutrients like minerals to humans and animals. Here is a general overview of the process:

7.1.1 Selection of plant material

Begin by selecting a diverse range of oilseed varieties or genotypes for screening. These may include different cultivars, wild relatives, or breeding lines.

7.1.2 Growth conditions

Grow the selected plant material under controlled conditions in a greenhouse, growth chamber, or field. Ensure uniformity in environmental conditions such as light, temperature, water, and nutrient availability.

7.1.3 Phytin content analysis

Harvest mature seeds from each genotype and analyze their phytin content. This can be done using various analytical techniques such as spectrophotometry, high-performance liquid chromatography (HPLC), or enzymatic assays.

7.1.4 Phytin extraction

Extract phytin from the seeds using appropriate extraction methods. This may involve techniques such as acid extraction followed by purification.

7.1.5 Phytin resistance evaluation

Subject the extracted phytin to screening assays aimed at identifying resistance or tolerance traits. This could involve exposing the phytin to conditions that mimic those encountered during seed digestion in the gastrointestinal tract, such as acidic pH and enzymatic degradation. Measure the rate of degradation or release of phosphorus from phytin as an indicator of resistance.

7.1.6 Genetic analysis (optional)

If working with a genetically diverse population, conduct genetic analysis to identify molecular markers associated with phytin resistance. This can aid in marker-assisted selection or breeding efforts to develop cultivars with improved phytin resistance (Diaz *et al*., 2007).

7.1.7 Field trials

Validate the phytin resistance traits identified in controlled environments through field trials under natural growing conditions. This step helps to assess the performance of selected genotypes in real-world agricultural settings.

7.1.8 Selection and breeding

Based on the screening results, select promising genotypes with reduced phytin content or enhanced phytin resistance for further breeding efforts. Utilize traditional breeding methods or modern biotechnological approaches such as genetic engineering or genome editing to develop improved oilseed varieties with desirable phytin traits.

7.1.9 Characterization and release

Characterize the selected varieties in terms of agronomic performance, nutritional quality, and other relevant traits. Once proven stable and superior, release these varieties for commercial cultivation, making them available to farmers.

7.2 Transgenic plants for phytin content reduction

Developing transgenic plants for phytin reduction involves genetic engineering techniques to modify the expression of genes involved in phytin metabolism. Here is a general outline of the process:

7.2.1 Identification of target genes

Identify genes involved in phytin metabolism, such as those encoding enzymes responsible for phytate synthesis or degradation. Genes involved in phosphorus storage and metabolisms are also potential targets.

7.2.2 Gene silencing or overexpression

Modify the expression of target genes using techniques such as gene silencing (RNA interference) or gene overexpression. For example, downregulating the expression of genes involved in phytate synthesis or upregulating the expression of genes encoding phytase enzymes, which hydrolyze phytate, can lead to reduced phytin levels (Shi *et al*., 2007; Gupta *et al*., 2011).

7.2.3 Gene cloning and vector construction

Clone the target genes along with regulatory sequences (promoters, terminators) into plant transformation vectors. These vectors often contain selectable marker genes for identifying transformed plants.

7.2.4 Plant transformation

Introduce the constructed vectors into plant cells using Agrobacterium-mediated transformation or biolistic methods. This results in the integration of the transgenes into the plant genome.

7.2.5 Selection and regeneration of transgenic plants

Select transformed plant cells using selectable markers (*e.g*., antibiotic resistance) and regenerate whole plants from these cells using tissue culture techniques.

7.2.6 Molecular characterization

Confirm the presence and expression of the transgenes in regenerated plants using molecular biology techniques such as PCR, RT-PCR, and gene expression analysis.

7.2.7 Phenotypic analysis

Evaluate the phytin content and composition in transgenic plants compared to non-transgenic controls. This may involve biochemical assays, such as HPLC or spectrophotometry, to quantify phytin levels.

7.2.8 Field trials

Conduct field trials to assess the agronomic performance, yield, and phytin traits of transgenic plants under real-world growing conditions. Evaluate the stability of the transgenic phenotype across different environments and growing seasons.

7.2.9 Regulatory approval and release

Obtain regulatory approval for the cultivation and commercialization of transgenic plants with reduced phytin levels. Ensure compliance with biosafety regulations and address any environmental or health concerns.

7.2.10 Deployment and adoption

Once approved, release transgenic plants with reduced phytin levels to farmers for cultivation is taken care. Promote their adoption by highlighting their potential benefits, such as improved nutrient availability and reduced environmental phosphorus pollution.

Continuous monitoring and further research may be necessary to assess the long-term impacts of transgenic plants on the environment, human health, and agricultural sustainability.

7.3 Biofortification for phytin reduction in oil seeds

Biofortification for phytin reduction in oilseeds involves conventional breeding or genetic engineering techniques to develop varieties with naturally reduced phytin levels or enhanced phytase activity. The biological functions of phytic acid and the identification of germplasm and pathways for breeding high-yielding, stress-tolerant low-phytate genetic resources have been reviewed by many academicians and researchers (Raboy, 2001, 2003, 2009; Rose *et al*., 2013). Here is how biofortification can be implemented:

7.3.1 Genetic variation screening

Identify natural genetic variation within oilseed crops or related species for traits associated with phytin reduction or enhanced phytase activity. This can involve screening germplasm collections, landraces, and wild relatives for favorable traits.

7.3.2 Marker-assisted breeding

Utilize marker-assisted selection (MAS) to breed oilseed varieties with reduced phytin levels. Molecular markers linked to genes controlling phytin metabolism or phytase activity can facilitate the selection of desired traits in breeding populations.

7.3.3 Conventional breeding

Employ traditional breeding techniques such as recurrent selection, hybridization, and backcrossing to develop oilseed varieties with improved phytin traits. Select and cross parental lines with desirable phytin characteristics to generate progeny with enhanced phytin reduction potential. From the mutant studies, it was established that LPA mutants have seed phytic acid reductions from 10% to 90% (Sparvoli and Cominelli, 2015).

7.3.4 Trait introgression

Transfer genes or genomic regions associated with phytin reduction from wild relatives or exotic germplasm into elite oilseed cultivars through introgression breeding. This broadens the genetic diversity available for improving phytin traits in commercial varieties.

7.3.5 Marker development

Develop molecular markers linked to phytic-related traits using genomics and bioinformatics approaches. These markers can aid in the selection of breeding lines with reduced phytin content or enhanced phytase activity, accelerating the breeding process.

7.3.6 Phytase enhancement

Identify and characterize natural variants or alleles of phytase genes with superior enzymatic activity. Introduce these variants into oilseed crops through breeding or genetic engineering to enhance phytase activity and reduce phytin levels in seeds.

7.3.7 Transgenic approaches (optional)

Employ genetic engineering techniques to modify the expression of genes involved in phytin metabolism or phytase activity. Develop transgenic oilseed crops with reduced phytin content or enhanced phytase activity for improved nutritional quality.

7.3.8 Field trials and evaluation

Conduct field trials to assess the agronomic performance, yield potential, and nutritional quality of biofortified oilseed varieties under diverse environmental conditions. Evaluate the stability and effectiveness of phytin reduction traits across different growing regions and management practices.

7.3.9 Regulatory approval and release

Obtain regulatory approval for the cultivation and commercialization of biofortified oilseed varieties with reduced phytin levels. Ensure compliance with biosafety regulations and address any concerns related to genetically modified organisms (GMOs) if using transgenic approaches.

7.3.10 Deployment and adoption

Release biofortified oilseed varieties to farmers for cultivation and promote their adoption through extension services, farmer training programs, and market incentives. Highlight the nutritional benefits of reduced phytin content for human and animal consumption.

Biofortification efforts for phytin reduction in oilseeds require collaboration among plant breeders, geneticists, agronomists, food scientists, policymakers, and other stakeholders to address global health and nutrition challenges effectively.

7.4 Molecular breeding for phytin reduction in oilseeds

Molecular breeding for phytin reduction in oilseeds involves leveraging genetic information and molecular markers to select and develop varieties with lower phytin content. Here is how the process typically works:

7.4.1 Identification of genetic variation

Conduct genome-wide association studies (GWAS), quantitative trait loci (QTL) mapping, or other genetic analyses to identify genomic regions associated with phytin content or phytase activity in oilseed crops.

7.4.2 Marker development

Develop molecular markers linked to phytic-related traits identified. These markers could be single nucleotide polymorphisms (SNPs), insertion/deletion (indel) markers, or simple sequence repeats (SSRs) located near genes controlling phytin metabolism or phytase expression.

7.4.3 Marker-assisted selection

Use molecular markers associated with low phytin content or enhanced phytase activity to select breeding lines with desirable traits. MAS allows for more efficient and precise selection compared to traditional breeding methods, enabling breeders to directly target specific genomic regions of interest.

7.4.4 Genomic selection

Implement genomic selection approaches that utilize genome-wide marker data to predict breeding values for phytic-related traits. This data-driven approach allows breeders to indirectly select for complex traits such as phytin content, even in the absence of specific markers.

7.4.5 Population improvement

Utilize marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), or other breeding strategies to introgress favourable alleles associated with phytin reduction from diverse genetic sources into elite oilseed cultivars. This involves crossing elite lines with donors containing the desired traits, followed by marker-assisted selection to recover the recurrent parent genome while retaining the target alleles.

7.4.6 Phenotypic evaluation

Conduct field trials and laboratory analyses to evaluate the phytin content and agronomic performance of the selected breeding lines and populations. Phenotypic data is used to validate the effectiveness of marker-assisted selection and guide further breeding decisions.

7.4.7 Genomic editing

Employ advanced biotechnological tools such as genome editing (*e.g.*, CRISPR-Cas9) to directly modify genes involved in phytin metabolism or phytase activity. Targeted gene editing allows for precise modification of specific genomic sequences, potentially leading to the development of oilseed varieties with tailored phytin traits.

7.4.8 Regulatory approval and release

Ensure compliance with regulatory requirements for genetically modified organisms (GMOs), if employing genome editing or transgenic approaches. Obtain regulatory approval for the commercial release of phytin-reduced oilseed varieties, following thorough safety assessments and public consultation.

7.4.9 Deployment and adoption

Release phytin reduced oilseed varieties to farmers and promote their adoption through extension services, seed distribution programs, and market incentives. Demonstrate the agronomic and nutritional benefits of these varieties to facilitate their uptake in agricultural production systems.

Molecular breeding approaches offer powerful tools for accelerating the development of oilseed varieties with reduced phytin content, contributing to improved nutritional quality and sustainable agriculture.

8. Oilseed varieties with less phytin content in India

As of my last update in January 2022, specific oilseed varieties in India with inherently lower phytin content may not have been widely identified or commercialized. However, research institutions and agricultural organizations in India are actively engaged in breeding programs and genetic studies aimed at developing oilseed varieties with improved nutritional profiles, including reduced phytin content.

9. Processing techniques used in oilseeds to reduce phytin

Processing techniques can be employed to reduce phytin levels in oilseeds, including soybeans. While phytin serves as a storage form of phosphorus in seeds, it can also bind to minerals and reduce their bioavailability (Grases *et al*., 2017; Bora, 2014). Here are several processing techniques commonly used to reduce phytin levels in oilseeds:

9.1 Soaking

Soaking oilseeds in water for a certain period before further processing can help reduce phytin levels. Phytin is water-soluble, and soaking allows some of it to leach out into the water. Discarding the soaking water before further processing can help reduce the phytin content of the oilseeds.

9.2 Germination

Germination involves soaking the oilseeds in water and allowing them to sprout for a specified period. During germination, enzymes are activated, including phytase, which breaks down phytin into its parts, reducing overall phytin levels in the seeds (Kaukovirta-Norja *et al.*, 2004).

9.3 Fermentation

Fermentation is another method that can help reduce phytin levels in oilseeds. During fermentation, microorganisms produce phytase enzymes, which break down phytin. Fermented products such as tempeh, miso, and natto are examples of fermented soybean products where phytin levels may be reduced compared to unfermented soybeans.

9.4 Roasting

Roasting oilseeds at high temperatures can help reduce phytin levels by partially breaking down the phytin molecule (Sinha and Khare, 2017). However, excessive heat may also reduce the nutritional quality of the oilseeds, so careful control of roasting conditions is essential.

9.5 Enzymatic treatment

Enzymatic treatment involves using exogenous phytase enzymes to degrade phytin in oilseeds. Phytase enzymes can be added during processing to hydrolyze phytin into its parts, reducing overall phytin levels and increasing mineral bioavailability.

9.6 Sprouting

Sprouting involves germinating seeds under controlled conditions. Similar to germination, sprouting activates phytase enzymes, leading to the breakdown of phytin and reduction in phytin levels in the oilseeds.

9.7 Acidification

Acid treatment, such as soaking oilseeds in acidic solutions or fermenting them with lactic acid bacteria, can help reduce phytin levels. Acidic conditions promote the activity of phytase enzymes, leading to the degradation of phytin.

9.8 Combination methods

Combining different processing methods, such as soaking, germination, and fermentation, may have synergistic effects on reducing phytin levels in safflower seeds. Experimentation with different combinations of processing techniques can help optimize phytin reduction while preserving the sensory and nutritional qualities of safflower products.

It is important to note that while these processing techniques can help reduce phytin levels in oilseeds, they may also affect other nutritional and sensory properties of the seeds. Therefore, it is essential to carefully consider the impact of processing on overall product quality and nutritional value. Additionally, the specific processing method chosen may depend on factors such as the type of oilseed, the intended use of the final product, and consumer preferences.

10. Future scope of phytin research in oilseeds

The study of phytin in oilseeds presents several avenues for future research and practical applications. Here are some potential future scopes:

10.1 Development of low phytic varieties

Continued breeding efforts aimed at developing oilseed varieties with naturally low phytin content or enhanced phytase activity. This can involve traditional breeding methods as well as biotechnological approaches such as genetic engineering (Rose *et al.*, 2013).

10.2 Phytin reduction technologies

Exploration of novel technologies for reducing phytin content in oilseeds during processing is required. This may include enzymatic treatments, fermentation, and other bioprocessing techniques to hydrolyze phytin and improve mineral bioavailability.

10.3 Understanding phytic acid-mineral interactions

Further, elucidation of the mechanisms underlying phytic-mineral interactions and their impact on nutrient bioavailability need to be studied. This can inform the development of strategies to mitigate the adverse effects of phytin on mineral absorption.

10.4 Phytin as a functional ingredient

Investigation of the potential functional properties of phytin in food and feed formulations happens. Phytin's ability to chelate metal ions and form complexes with proteins may have applications in food processing, texture modification, and encapsulation of bioactive compounds.

10.5 Nutritional implications

Conducting clinical studies to assess the nutritional implications of phytin in oilseed-based diets for humans and animals should be attempted. This includes evaluating mineral bioavailability, nutrient absorption, and overall nutritional status.

10.6 Phytin as a bioactive compound

Exploration of the potential health benefits of phytin beyond its role as an antinutrient. Phytin's antioxidant properties, ability to modulate gut microbiota, and potential anti inflammatory effects warrant further investigation.

10.7 Sustainable agriculture

Integration of phytin research into sustainable agricultural practices is required. Understanding the dynamics of phytin in soil-plant systems can inform nutrient management strategies, reduce environmental phosphorus pollution, and improve the sustainability of oilseed production.

10.8 Biofortification

Utilization of phytin research to enhance the nutritional quality of oilseed crops through biofortification strategies (Gupta *et al.*, 2015). Developing oilseed varieties with improved mineral bioavailability can contribute to addressing global malnutrition and food security challenges.

10.9 Phytin in non-food applications

Exploration of potential non-food applications of phytin-rich oilseed byproducts are plenty. This includes utilization in animal feed formulations, biofuel production, and industrial processes.

10.10 Consumer education and awareness

Increasing consumer awareness about the nutritional implications of phytin in oilseeds and promoting balanced diets that optimize mineral bioavailability. Educating consumers about food processing techniques that can reduce phytin levels and enhance nutrient absorption are essential.

11. Conclusion

Oilseed crops play a crucial role in providing vegetable oils for various purposes. Major oilseed crops include rapeseed, sunflower, safflower, soybeans, cotton, and peanuts. These plants are primarily cultivated for the oil present in their seeds. Several antinutritional factors including phytin or phytic acid present in oilseeds, which can impact the nutritional quality and utilization of oilseed crops. Phytin inhibitors found in oilseeds offer promising opportunities to improve nutrient absorption and mitigate mineral deficiencies. Overall, the exploration of phytin inhibitors in oilseed crops represents a promising avenue for enhancing their nutritional quality and addressing global nutrition and health challenges. Various strategies have been proposed to reduce phytin levels in soybean seeds, including genetic engineering, conventional breeding, processing techniques (*e.g.*, soaking, germination, fermentation), and enzymatic treatments. These approaches aim to enhance the nutritional quality and mineral bioavailability of soy-based products while preserving their sensory and functional properties. Specific oilseed varieties in India with inherently lower phytin content may not have been widely identified or commercialized. Several breeding methods such as germplasm screening, transgenic plants and biofortification are much investigated to reduce the phytin content in oilseeds. Overall, phytin research in oilseeds encompasses a wide range of scientific, technological, and practical applications aimed at improving human nutrition, agricultural sustainability, and food system resilience. Continued interdisciplinary collaboration and investment in research and innovation are essential for realizing the full potential of phytin and best reduction methods in oilseed crops.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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