



## Nutritional and Anti-Nutritional Factors in Soybean

Riya Mishra<sup>1</sup>, MK Tripathi<sup>1,2\*</sup>, Niraj Tripathi<sup>3</sup>, Jagendra Singh<sup>2</sup> and Sharad Tiwari<sup>4</sup>

<sup>1</sup>Department of Genetics and Plant Breeding, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalyaya, Gwalior, India

<sup>2</sup>Zonal Agricultural Research Station, Morena, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalyaya, Gwalior, India

<sup>3</sup>Directorate of Research Services, Jawaharlal Nehru Krishi Vishwa Vidyalyaya, Jabalpur, India

<sup>4</sup>Biotechnology Centre, Jawaharlal Nehru Krishi Vishwa Vidyalyaya, Jabalpur, India

\*Corresponding Author: MK Tripathi, Zonal Agricultural Research Station, Morena, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalyaya, Gwalior, India.

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MK Tripathi., et al.

### Abstract

*Glycine max* is a vivacious crop famous for its high nutritional worth, mostly owing to its amusing protein content, important fatty acids, vitamins, minerals and bioactive compounds for instance isoflavones. Contempt these benefits, soybean also comprehends an array of antinutritional factors, like phytic acid, protease inhibitors, oligosaccharides, saponins and lectins, which can impede nutrient absorption and utilization. This review examines the different nutritional and antinutritional components found in soybean, accentuating their biochemical significance and health implications. Understanding the balance between nutritional benefits and antinutritional challenges is crucial for optimizing soybean's role in human and animal diets. Biochemical studies provide insights into the complex interactions between these components, facilitating the development of processing methods to reduce antinutritional factors while preserving or enhancing nutritional quality. Moreover, the detection and characterization of these factors through molecular markers represent a promising approach to soybean breeding and genetic improvement. Molecular markers enable the identification of specific genes and alleles associated with desirable traits, such as reduced antinutritional factors and enhanced nutritional content. This precision breeding approach can lead to the development of soybean varieties with improved health benefits and reduced negative impacts. The comprehensive study of soybean's nutritional and antinutritional factors, combined with advanced biochemical and molecular techniques, holds significant potential for improving the nutritional profile of soybean-based products. This, in turn, can contribute to better health outcomes and more efficient utilization of this essential crop in food and feed industries.

**Keywords:** Anti-Nutritional Factors; Carbohydrates; Isoflavones, Lectins; Lipids; Oligosaccharides; Nutritional Factors; Proteins; Protein Inhibitors; Phytic Acid; Saponins; Tannins

## Introduction

*Glycine max* (L.) Merrill, is a versatile crop, serves as both a leguminous and an oilseed crop. It is an annual, self-pollinated diploid legume of the subfamily Fabaceae [1]. This erect and productive crop was tamed for food from its viny wild predecessor, *Glycine soja* Sieb and Zucc., in eastern China over 3,000 years ago. Subsequently, it was introduced to Japan, Korea and Southeast Asia. Over the past century, it has spread to America and Brazil, where it now produces substantial yields [2]. It is grown in various soil types across temperate and subtropical regions worldwide [3]. It holds a taproot system, exhibit a short-day photoperiodic flowering response, and display either determinate or indeterminate growth habits. Each pod typically contains one to five seeds [4]. Soybeans have a large number of chromosomes ( $2n = 40$ ), and at metaphase, their mitotic chromosomes are short (1.42-2.82  $\mu\text{m}$ ) and have a comparable morphology.

Soybean production has seen amazing expansion over the past century. In this time, the United States has witnessed soybeans transform from a minor crop to becoming the second most valuable agricultural commodity [5]. In the year 2021-22, global soybean production was led by Brazil, which produced 144.000 million metric tons. The United States followed with a production of 119.884 million metric tons. Argentina contributed 52.000 million metric tons to the global output. China and India produced 19.000 million metric tons and 11.200 million metric tons, correspondingly [6-8]. In *Kharif* 2023, major soybean-producing states in India reported significant figures. Rajasthan saw a sowing area of 10.945 lakh hectares, with an expected yield of 925 kg per hectare, leading to an estimated production of 10.126 lakh metric tons. Madhya Pradesh had a sowing area of 52.050 lakh hectares, with an anticipated yield of 1008 kg per hectare, resulting in an estimated production of 52.470 lakh metric tons. Maharashtra reported a sowing area of 45.640 lakh hectares, with an expected yield of 1028 kg per hectare, culminating in an estimated production of 46.917 lakh metric tons [8].

The seeds of soybean are highly prized for their distinct composition and diverse applications, including use in food, animal feed and industrial products [9]. It is abundant in protein and vegetable oil and comprehends various phytochemicals, including isoflavones and phenolic compounds (Table 1). Owing to its superior

nutritional value, it is regarded as a traditional health-promoting food [10]. A wide array of soya products has been developed, viz., soymilk, roasted soybeans, soy mayonnaise, boiled soybeans, miso, soy yogurt, soy cheese, soy sauce, tempeh, tamari, textured vegetable protein (TVP), tofu and textured soy protein (TSP) [11]. It serves as a significant source of premium quality protein and oil and the quality of soybean seeds is often evaluated based on their nutritional and antinutritional properties (Table 2). It is also an excellent source of B-complex vitamins, as well as essential minerals such as potassium, phosphorus, sodium and magnesium [12]. It is a type of legume, which is characterized by absence of cholesterol and low saturated fat content. It is unique among vegetable foods that provide all eight essential amino acids. It is widely recognized for its nutritional value, attributed to its high levels of iso-flavonoids and folic acid, making it a vital component of a healthy diet. The consumption of dietary soy products has garnered significant scientific attention due to their potential positive effects on human health [13]. The global consumption of soy foods is rising, primarily due to their recognized health benefits. However, there are also some nutritional drawbacks associated with the consumption of soy foods [14]. The main purpose of this review is to provide a comprehensive overview of the nutritional and antinutritional components found in soybean, by biochemical analysis and using molecular markers for their detection, highlighting their implications for human and animal health.

## Nutritional content

### Proteins and Lipid content

Soybean is a crucial source of plant-based protein worldwide, offering a higher percentage of crude protein compared to many other commercially produced legumes or pulses [24]. Protein is a key component that determines both the economic and nutritional value of soybeans [25]. A European investigation discovered that individuals who consistently maintain a health-conscious lifestyle, such as pescatarians, vegetarians or vegans, exhibit a higher propensity to consume soy foods compared to the general population. Being a premium quality protein, soy protein boasts a protein digestibility-corrected amino acid score (PDCAAS) of 1.00, comparable to certain animal-derived proteins, such as those found in meat and dairy. It encompasses a well-balanced profile of essential amino acids, although it is relatively deficient in sulfur-containing amino acids like methionine [18].

S. No.	Content	Quantity per 100 g	Standard Unit
1	Water	8.54	g
2	Energy	446.00	kcal
3	Energy	1870.00	kJ
4	Protein	36.50	g
5	Total lipid (fat)	19.90	g
6	Ash	4.87	g
7	Carbohydrate, by difference	30.20	g
8	Fiber, total dietary	9.30	g
9	Total sugars	7.33	g
10	Calcium	277.00	mg
11	Iron	15.70	mg
12	Magnesium	280.00	mg
13	Phosphorus	704.00	mg
14	Potassium	1800.00	mg
15	Sodium	2.00	mg
16	Zinc	4.89	mg
17	Copper	1.66	mg
18	Manganese	2.52	mg
19	Selenium	17.8	µg
20	Vitamin C, total ascorbic acid	6.00	mg
21	Thiamine	0.874	mg
22	Riboflavin	0.87	mg
23	Niacin	1.62	mg
24	Pantothenic acid	0.793	mg
25	Vitamin B6	0.377	mg
26	Folate, total	375.00	µg
27	Folate, food	375.00	µg
28	Folate, DFE	375.00	µg
29	Choline, total	116.00	mg
30	Betaine	2.10	mg
31	Vitamin A, RAE	1.00	µg
32	Carotene, beta	13.0	µg
33	Vitamin A, IU	22.00	IU
34	Vitamin E (alpha-tocopherol)	0.85	mg
35	Vitamin K (phylloquinone)	47.00	µg
36	Fatty acids, total saturated	2.88	g
37	SFA 14:0	0.055	g
38	SFA 16:0	2.12	g
39	SFA 18:0	0.712	g
40	Fatty acids, total monosaturated	4.40	g
41	MUFA 16:1	0.055	g
42	MUFA 18:1	4.35	g

43	Fatty acids, total polyunsaturated	11.30	⊗
44	PUFA 18:2	9.92	⊗
45	PUFA 18:3	1.33	⊗
46	Tryptophan	0.591	⊗
47	Threonine	1.77	⊗
48	Isoleucine	1.97	⊗
49	Leucine	3.31	⊗
50	Lysine	2.71	⊗
51	Methionine	0.547	⊗
52	Cystine	0.655	⊗
53	Phenylalanine	2.12	⊗
54	Tyrosine	1.54	⊗
55	Valine	2.03	⊗
56	Arginine	3.15	⊗
57	Histidine	1.10	⊗
58	Alanine	1.92	⊗
59	Aspartic acid	5.11	⊗
60	Glutamic acid	7.87	⊗
61	Glycine	1.88	⊗
62	Proline	2.38	⊗
63	Serine	2.36	⊗

**Table 1:** Nutritional content of soybean seeds [15].

<b>A</b>			
<b>S. No.</b>	<b>Nutritional factor</b>	<b>Benefit</b>	<b>References</b>
1	Isoflavonoids	Antioxidant properties, may reduce risk of heart diseases and certain cancers, improve bone health	[16]
2	Folic acid	Supports DNA synthesis and repair, crucial for pregnant women to prevent neural tube defects in infants	[17]
3	Protein	High quality plant protein, supports muscle repair and growth, may help in weight management. Controls hyperglycemia, hyperlipidemia, hyperinsulinemia	[18]
4	Fiber	Enhances digestive health, supports satiety and maintaining optimum blood sugar levels	[19]
<b>B. Anti-nutritional factor</b>			
<b>S. No.</b>		<b>Side effects</b>	<b>References</b>
1	Phytic acid	Can reduce the absorption of essential minerals like zinc and iron	[20]
2	Saponins	High intake can cause gastrointestinal disturbances, such as nausea and diarrhea	[21]
3	Trypsin inhibitors	Can interfere with protein digestion and absorption, potentially leading to growth issues if consumed in excess	[22]
4	lipoxygenases	Produce off-flavors and odors	[23]

**Table 2:** Health benefits of nutritional and side effects of anti-nutritional factors found in soybean.

Roughly 29% of the world's oil and fat produced is soybean oil. The cotyledon, which makes up around 20% of the overall weight of the soybean, is where most of the soybean lipids are located. Fat content is determined using the Soxhlet extractor method [26]. The Soxhlet extraction method was used by Li., *et al.* [26] to calculate the oil content.

Protein and fat content in soybeans can be chemically analyzed using the Kjeldal method and Soxhlet extraction. Aulia., *et al.* (2023) [27] utilized hyperspectral imaging to assess the protein and lipid content in soybeans. Their findings indicated that the protein content ranged between 34.1 to 56.8 g/100g (34.1-56.8%) of the total seed weight, while the lipid content varied from 8.1% to 24% on a seed basis [27]. Mishra (2023) [28] estimated the protein and fat content using the traditional Mikrokjeldahl digestion and distillation and Soxhlet extraction procedure respectively, in RILs of soybean and reported the seed protein ranging between 34 to 42% and 16 to 20% fat content respectively. Prabakaran., *et al.* (2018) [29], assessed the stability of fatty acids in raw and roasted soybean flour under different storage conditions. Linoleic, oleic and linolenic acids were the major unsaturated fatty acids, with significant reductions observed after 48 weeks at high temperature, regardless of packaging material. Kholmurodova., *et al.* (2023) [30] carried out field research conducted in years between 2017-2021 to evaluate soybean varieties for protein and oil content. The CH3 variety had medium protein (38.16%) and oil (18.66%), while US-25 had higher protein (40.00%) and medium oil (19.05%). The total protein and oil content arrayed between 57.44% (Arletta) to 60.00% (Sparta) respectively. Jin., *et al.* (2023) [31] conducted a genome-wide association study (GWAS) on 320 soybean accessions to identify loci associated with protein (PC) and oil content (OC). PC ranged from 37.8% to 46.5%, and OC between 16.7% to 22.6%, identifying 23 loci for PC and 29 for OC, with 15 and 24 loci being newly discovered. Nine candidate genes linked to protein and oil biosynthesis were also identified, offering insights for marker-assisted selection in soybean breeding. Using cultivar Tai292 as a reference for comparison, Li., *et al.* (2012) [26] assessed the protein and oil content of soybean seeds at the R6-R7 stage. The percentage of oil and protein that varied was 39% and 26% respectively. It had been determined that the genotypes *viz.*, L-1, L66 and L-139/19 were excellent sources for creating vegetarian soybeans with reduced oil content without sacrificing protein content.

MAS using SSR markers has been effectively employed to identify genes that various nutritional and antinutritional components

of soybean (Table 3). In their 2023 study, Pokharel., *et al.* [32] screened soybean germplasm for specific markers linked to these genes, employing five SSR markers including Satt556, Satt006, Satt212, Satt144 and Satt449 across eighty-eight genotypes found in Nepal. The investigations results were auspicious, revealing the occurrence of protein and oil genes in the Nepalese genotypes. This indicates that SSR markers can be efficaciously used to detect genotypes of soybean with elevated protein and fat contents, thereby potentially improving their quality and nutritional value.

### Carbohydrate content

Carbohydrates make up a significant amount of soybean seed, but their composition and content have not been investigated as much as those of their protein and fat components. Soybeans typically have a carbohydrate content of 30-35%, however soybean flour can have much as 40% of carbs [33]. Non-structural types of carbohydrates, such as low molecular weight sugars, oligosaccharides and trace amounts of starch, make up around half of the dry matter of soybeans. Structural polysaccharides make up the remaining fraction, with a notable proportion of pectic polysaccharides among them. Raffinose, stachyose and verbascose are examples of galacto-oligosaccharides that make up approximately 5% of the dry matter in soybeans, whereas starch makes up less than 1% [34]. The majority of the carbohydrates found in soybeans are oligosaccharides, especially stachyose. These oligosaccharides enter the colon and support the growth of good bacteria like bifidobacteria because they are resistant to breakdown by intestinal enzymes. Nevertheless, flatulence may arise from the colon's fermentation of these oligosaccharides producing more gas [35].

Saldivar., *et al.* (2010) [36] investigated how the composition of two commercial cultivars and five specialized genotypes of soybeans changed as the seeds developed. When seeds reached maturity, their starch content declined from 6–15% in developing seeds to 0.2–1%, but sucrose also decreased and non-digestible oligosaccharides stayed low. There were notable variations in oil, protein, soluble saccharides and starch across the seven genotypes of soybeans. Agume., *et al.* (2016) [37] with the goal to evaluate how various procedures impact the flour's characteristics, investigated the combined effects of roasting and soaking (0–72 hours) on the physicochemical and pasting characteristics of soybean flour. Following soaking, dehulling, drying and partial roasting, the total carbohydrates in the beans ranged from 22.8 to 27.9 g/100 g. The study by Murai., *et al.* (2024) [38] analyzed carbohydrate variability in soybeans, focusing on sucrose and stachyose as the

main sugars in 2020 samples. In 2021 samples, heat treatment (autoclaving) caused significant reductions in sucrose (20.88%), glucose (11.18%) and fructose (1.46%). Nobile, *et al.* (2023) [39] evaluated air temperature, solar radiation, precipitation and evapotranspiration during seed-filling to assess the impact of climate conditions on the carbohydrate composition of two soybean varieties ( $R_5$ – $R_7$ ). The results indicated that lower temperatures were linked to lower quantities of sucrose and total sugar, suggesting that cooler weather is best for growing soybeans with better nutritional profiles. The study conducted by Dhungana, *et al.* (2017) [40] assessed the seed starch content (SSC) of 17 soybean genotypes across a range of planting periods and settings. Late-planted soybeans had greater SSC, which interacted adversely with crude protein but positively with crude fat. Temperature and cloudiness were major environmental factors that affected SSC, with IT189276 being the most stable high-starch genotype.

### Tocopherol content

Essential lipid-soluble antioxidants called tocopherols are found in food and in both human and animal tissues. They are mostly found in regions of cells that are high in lipids, such as the mitochondrial membranes, fat reserves, and lipoproteins like cholesterol and low-density lipoprotein. In addition to acting as antioxidants, tocopherols also affect signal transmission, control gene expression, and modify a number of cellular processes, all of which are beneficial to health [41].

The study performed by Ghosh, *et al.* (2022) [42] measured the tocopherol content of 493 Chinese-grown soybean accessions from different age groups and nations. Russian accessions had more  $\alpha$ -tocopherol than U.S. accessions, which had the highest amounts of both total and  $\gamma$ -tocopherol. The tocopherol content of soybeans can be improved by considering maturation groups and geographic considerations, as evidenced by the greater levels of  $\alpha$ - and  $\gamma$ -tocopherol found in early maturing accessions and higher levels of  $\delta$ -tocopherol in late-maturing ones. Rani, *et al.* (2007) [43] measured the tocopherol content of 66 genotypes of Indian soybeans and discovered considerable variation, with 'Co Soya2' having the highest total tocopherol. The samples 'Ankur' and 'VLS1' contained the highest  $\alpha$ -tocopherol and 'PK327' the highest  $\gamma$ -tocopherol, respectively. High correlation was detected between tocopherol isomers and  $\gamma$ -tocopherol had a significant relationship with the amount of linoleic acid. In a soybean RIL population, Zhang, *et al.* (2023) [44] found 38 QTLs for tocopherol content, with stable QTLs located on chromosomes 5 and 12.

Missense variations in 47 SNPs, including GmVTE4 and GmHSFA8, were found during analysis. These variants may have an effect on tocopherol accumulation. These results provide information for high-tocopherol soybean breeding. The Northern Region accessions had the highest levels of  $\alpha$ - and  $\gamma$ -tocopherols, according to the study by Ghosh, *et al.* (2021) [45] that assessed the tocopherol content of 1151 Chinese soybean accessions. Compared to landraces, cultivars often had higher levels of tocopherol, and there was a substantial correlation between tocopherols and characteristics of seed quality as well as geographic location. Chu, *et al.* (2023) [46] discovered five important loci in the tocopherol pathway and established a significant association between tocopherol and oil content in over 800 soybean accessions. Tocopherol composition and content were influenced by genetic differences in three tocopherol pathway genes, but levels of fatty acids and tocopherol were elevated by the transcription factor GmZF351.

Another study in a soybean RIL population conducted by Knizia, *et al.* (2022) [47] mapped 32 QTLs for different tocopherol levels and found a significant QTL on Chromosome 6 linked to  $\delta$ -tocopherol,  $\alpha$ -tocopherol and total tocopherol. These QTLs were close to candidate genes for tocopherol biosynthesis, such as  $\gamma$ -tocopherol methyltransferase and tocopherol cyclase. Kim, *et al.* (2024) [48] examined isoflavones, B vitamins and tocopherols in 13 Korean soybean cultivars that were produced over a three-year period in three different sites. The  $\beta$ - and  $\delta$ -tocopherols were strongly controlled by genotype, but the  $\alpha$ - and  $\gamma$ -tocopherols, B vitamins and isoflavones were regulated by environmental factors and their interactions. Lower isoflavone levels were found in early maturing cultivars with black seed coats; these findings may be used to evaluate compositional equivalency in genetically engineered soybeans.

### Amino Acids profiling

There are eighteen amino acids in soybean protein, all of which are necessary. These amino acid compositions are usually examined using high-performance liquid chromatography (HPLC) or wet chemical techniques [49]. Soybeans are rich in protein, but they lack the sulfur-containing amino acids methionine and cysteine, which are essential for diets that are monogastric. Since neither monogastric animals nor humans are able to produce methionine, it is categorized as an essential amino acid. Since the body may produce cysteine from methionine under some circumstances, it is regarded as conditionally necessary [50].

Murai, *et al.* (2024) [38] examined amino acid variability in soybeans, with L-arginine (9.82 g/100 g), leucine (5.29 g/100 g) and glutamate (4.90 g/100 g) being the most prevalent in 2021 samples. Heat treatment (autoclaving) caused an 8.47% median loss of free lysine. In order to quickly determine the amino acid content of edamame (Vegetable soybean) among 20 genotypes of soybeans, Jiang, *et al.* (2023) [51] assessed several drying techniques. Outcomes revealed that low-heat drying (65°C) and freeze drying generated similar and dependable outcomes, making low-heat drying a viable alternative for large-scale use. It was determined that there are six genotypes with increased amino acid content that can enhance the nutritional value of edamame. Wang, *et al.* (2022) [52] increased the A2B1a subunit and decreased 7S globulin in soybeans by RNA interference, which increased the amount of sulfur-containing amino acids by 79.194 nmol/mg. The protein ratio of 11S/7S increased 1.89 times. Confirmed structural alterations in 11S globulin provide a way to improve the soy proteins' quality for food production. Kudelka, *et al.* (2021) [53] examined critical amino acids, protein, and moisture content in traditional and genetically modified soybeans and their derivatives. While there were variances in the magnitude of amino acids in each product, there were no notable differences between the varieties of soybeans. There was no tryptophan because of acid hydrolysis. Ban, *et al.* (2021) [54] examined how ethylene treatment affected the amount of free amino acids in soybean leaves and found that both essential and non-essential amino acids increased five to six times. Twenty-five times more asparagine was added, accounting for 41 percent of the total; arginine and branched-chain amino acids also increased dramatically. The study performed by Assefa, *et al.* (2018) [55] demonstrated the composition and quality of amino acid (AA) found in soybeans by analyzing 35,101 data points from 2012 to 2016 in 14 US states. Spatial autocorrelation was seen in essential AA concentrations, and there was a negative connection with both protein and oil content across latitudes. There were significant connections between the AAs, especially between isoleucine and valine ( $r = 0.93$ ). Based on the essential amino acids (EAAs) needed for human nutrition, Tessari, *et al.* (2016) [56] reassessed the environmental impact of diets derived from plants and animals. With the exception of soybeans, which have the least impact, it is shown that the synthesis of animal proteins has a similar footprint to that of plant proteins questioning the notion that diets high in plants are invariably more environmentally friendly. The impact of increased CO<sub>2</sub> and phosphorus deficit on soybean seed yield and composition was investigated in the study of Singh, *et al.* (2016) [57]. P deficit resulted in lower levels of oil, minerals and total seed production, but higher concentrations of car-

bohydrates and amino acids. Carrera, *et al.* (2011) [58] examined amino acid composition in soybean seeds from 31 field trials in Argentina, finding total amino acids ranged from 31.69% to 49.14%. Environmental factors, including temperature, solar radiation and precipitation, significantly influenced amino acid content during seed filling. The response of every amino acid differed in different environmental conditions, showing compensatory effects.

### Isoflavones

Isoflavones are chemically defined as flavonoid compounds with a skeleton made up of three phenyl chromones. Soybeans are the main source of them, and the hypocotyl is where they are most prevalent [59]. Key soybean chemicals called isoflavones have been the subject of much research because to their physiological and nutritional effects. These chemicals are rare in nature; among commonly consumed goods, they are only found in considerable concentrations in soybeans and foods derived from soybeans. Traditional soy meals typically have quantities of about 25 mg per serving of the three principal isoflavones *viz.*, glycitein (7,4'-dihydroxy-6-methoxyisoflavone), daidzein (4',7-dihydroxyisoflavone) and genistein (4',5,7-trihydroxyisoflavone) [60]. Twelve different isoflavones, divided into four families *i.e.*, aglycones, glycosides, acetylglycosides and malonylglycosides, are found in soybean seeds. Malonylglycosides are the most common among them, although aglycones are only found in trace amounts [61]. Eating foods high in isoflavones or soy has been associated with a decreased risk of bladder and endometrial cancer. During pregnancy, isoflavones assist lessen depressed symptoms, hypertension, bone loss and vasomotor symptoms. Their effects on urogenital symptoms and cognition, however, are yet unclear [62]. The FDA considers 50 mg of isoflavones to be a safe daily dose. The average daily intake of soy isoflavones in Asian nations including China, Japan and South Korea is between 25 and 50 mg. In comparison, less than 2 mg is typically consumed daily in the US and other European nations [63]. Although isoflavones have many benefits, some researchers view them as anti-nutritious compounds instead of nutritious due to their negative consequences, which include an increased risk of menopause and hormone-dependent cancer [64].

Azam, *et al.* (2024) [65] measured the amounts of isoflavones in several Chinese-grown soybean germplasm and discovered an 8-fold variance in the total isoflavone (TIF) content. The highest TIF levels were found in US accessions, followed by late-maturity groups and black seed coat soybeans. Individual isoflavones and TIF content were shown to be significantly correlated. Kuligowski, *et al.* (2022) [66] evaluated the effects of various processing tech-

niques on isoflavones in soybeans. Cooking lowered numerous isoflavones while raising genistein and daidzein; sprouting resulted in the largest reduction in total isoflavones; and tempe fermentation reduced several isoflavones while increasing glycitin. By reducing different isoflavones during the frying and cooking process, soy pasta's nutritional profile was also affected. The isoflavone content of 1,168 soybean accessions obtained from China was examined by Azam, *et al.* (2020) [67], and the results showed a seven-fold variance in total isoflavone (TIF) levels as well as notable regional differences. With 35 elite accessions found, the Huang Huai Hai Valley Region's accessions had the highest TIF. Geographic origin had a significant impact on the composition of isoflavones, with glycosides and malonylglycosides being important constituents. Choi, *et al.* (2020) [68] evaluated the effects of seed weight on 24 black soybean cultivars' anthocyanin, isoflavone, phenolic contents and antioxidant activities. While small seeds exhibited higher total phenolics and some antioxidant activities, large seeds had higher total anthocyanins and isoflavones. Yuk, *et al.* (2016) [69] created a technique to treat soybean leaves with ethylene to boost the amount of isoflavones, which resulted in levels of daidzin, genistin and its derivatives up to 13,854 µg/g. Important genes in the isoflavonoid biosynthesis pathway were activated by ethylene, greatly increasing the production of isoflavones. According to the research, ethylene has the potential to increase the nutraceutical value of soybean leaves. The isoflavone concentrations in 40 soybean cultivars were investigated by Zhang, *et al.* (2014) [70], and the results indicated considerable genetic, environmental, and maturity group heterogeneity. Cultivars that matured later showed increased levels of isoflavones, ranging from 551.15 to 7584.07 µg/g. Plant height and seed characteristics were positively connected with isoflavone levels, however oil content was negatively associated. After analyzing 204 different soybean germplasms for useful components, Kim, *et al.* (2012) [71] discovered that tiny seeds had the highest concentration of both phenolics and isoflavones, and that Korean soybeans had the highest average isoflavone content. Chinese soybeans displayed the highest levels of tocopherol and soyaapogenol, while American soybeans had the highest overall phenolic compounds. Overall, the amounts of these beneficial chemicals were greatly impacted by seed size and origin.

### Anti-nutritional content

Grain legumes are nutritious, but their use as food sources is limited because of a number of antinutritional factors (ANFs) that have been linked to negative health effects in humans. ANFs occur naturally as secondary metabolic products that act as plant defense mechanisms against biological stressors like insect infestations

[72]. Anti-nutrients can be harmful and decrease the digestibility of protein and the bio-availability of minerals [22]. Phytic acid, Raffinose family oligosaccharides (RFOs), Kunitz trypsin Inhibitor (KTI) and soybean agglutinins are important antinutrients among the many anti-nutritional factors (ANFs) found in soybean [73].

### Trypsin inhibitor

One of the greatest significant ANFs is trypsin inhibitors (TIs), which diminish the digestion and fascination of dietary proteins. Legume trypsin inhibitors (TIs) are categorized into two families according to their molecular weight: Bowman-Birk inhibitors (BBTIs), which have a molecular weight of around 8 kDa, and Kunitz inhibitors (KTIs), which have a molecular weight of about 20 kDa. Certain legumes, like lentils and common beans, only have one kind, but others, like soybeans, have both [74]. While Bowman-Birk inhibitors (BBIs) inhibit both trypsin and chymotrypsin, Kunitz trypsin inhibitors (KTIs) mainly inhibit the digestive enzyme trypsin. Three main trypsin inhibitors are found in soybeans *viz.*, KTI-1, KTI-2 and KTI-3. KTI-3 is the most prevalent, closely followed by KTI-1 and KTI-2, which is believed to be inoperative. Traditionally, urease activity (UA) is utilized as a subsidiary pointer of trypsin inhibitor activity (TIA) [75].

Using overexpression of six TI genes in soybean and *Arabidopsis*, Sultana, *et al.* (2023) [76] investigated the function of soybean trypsin inhibitors (TIs) in plant defense. Less leaf defoliation and larval weight of maize earworms (*Helicoverpa zea*) were observed in transgenic lines, particularly KTI7 and BBI5. But there was no change in soybean cyst nematode (SCN) resistance and transgenic plants did not exhibit any different growth patterns from herbivore-free controls. Khan, *et al.* (2022) [77] focused on isolating, purifying and characterizing the trypsin inhibitor protein from soybean seeds. The protein, with a molecular weight of 21.5 kDa, was purified through ammonium sulfate precipitation and analyzed using SDS-PAGE. GC-MS identified steroid derivatives like stigmaterol, campesterol, beta-sitosterol and gamma-tocopherol [77]. Trypsin inhibitor activity (TIA) was shown to be equivalent between KTI/BBI-containing and KTI-lacking soybean cultivars in the study comparing Kunitz (KTI) and Bowman-Birk (BBI) trypsin inhibitors by Takacs, *et al.* (2022) [78]. Although some BBI activity persisted in the stomach, radiofrequency heat treatment greatly reduced TIA, with 110°C being the most effective temperature. In order to deactivate trypsin inhibitors and preserve the quality of soybean products, effective heat treatment is required. Using isoelectric precipitation and chromatography, soybean trypsin inhibitor (STI) was effectively recovered from waste water made from soybean by



Zhang, *et al.* (2022) [79], exhibiting an 89.47% recovery rate and high purity. Additionally, the purified forms of the KTI and the BBI have specific activity of 1733.5 TIU/mg and 2588.3 TIU/mg, respectively. Good stability and resistance to pepsin hydrolysis were shown by STI, offering important information for STI recycling and active chemical usage. By employing a single extract level, adding the enzyme last sequence and adding  $\text{Ca}^{2+}$  for better control, the trypsin inhibitor (TI) assay procedure for soy products was enhanced by Liu (2019) [80]. While enhancing sensitivity and using less reagent, a half-volume technique produced findings identical to the standard method. A total of 1.0–79.8% of the total trypsin inhibitor activity (TIA) was contributed by genetic diversity in KTI levels, which were assessed in 102 genotypes of soybeans by Kumar, *et al.* (2019) [81]. KTI was completely inactivated by boiling and autoclaving, although it was greatly diminished by microwave radiation and sprouting. Processing techniques and genotype both affected KTI inactivation. In 180 soybean genotypes, Kumar, *et al.* (2018) [82] optimized the extraction conditions for KTI. Three KTI alleles *i.e.*, Tia, Tib and Tic were identified, with Tia predominating in Indian genotypes. The trypsin-KTI complex experiment revealed a 2.51-fold binding of Tia to trypsin. The densitometry and ELISA measurements of KTI and BBI activities revealed a considerable difference from the usual spectrophotometric method's estimate of total trypsin inhibitor activity (TIA).

In 2019, Bulatova, *et al.* [83] examined a soybean germplasm assemblage to detect accessions with abridged trypsin inhibitor contents in seeds. A total 29 accessions, along with parental lines and 2 hybrid populations, were selected and analyzed using genetic markers to detect alleles at the Ti3 locus, which encodes the KTI. Among the three molecular markers used including Satt228, Satt409 and Ti/ti gene-specific markers, the SSR marker *namely* Satt228 proved to be the most effective diagnostic marker [83].

### Lipoxygenase

Lipoxygenase is regarded as an anti-nutritional factor due to its negative impact on unsaturated fatty acids and flavor profile. This enzyme catalyzes the oxidation of linoleic and linolenic acids, ensuing in the formation of off-flavor [84].

Gonzalez-Gordo, *et al.* (2023) [23] discovered that by S-nitrosation and persulfidation, hydrogen sulfide ( $\text{H}_2\text{S}$ ) and nitric oxide (NO) block soybean lipoxygenase type 1 (LOX 1). The sole nitrated tyrosine residue that has been found to impair enzyme function is Y214, according to mass spectrometry. According to structural study, these signaling molecules can effectively reach LOX 1's in-

ternal tunnels to change cysteine residues, which in turn inhibits the enzyme. Using a variety of fatty acid substrates, Smirnova, *et al.* (2023) [85] examined the catalytic characteristics of recombinant soybean LOX2 (GmLOX2). Mostly oxidizing linoleic acid, GmLOX2 produced 94% optically pure (13S)-hydroperoxide with strong substrate-specificity in both regio- and stereospecificity. Similar to GmLOX1, the enzyme has optimal activity at a certain pH. To lessen beany flavor, Wang, *et al.* (2020) [86] employed CRISPR-Cas9 to produce soybean mutants *viz.*, GmLox1, GmLox2 and GmLox3 devoid of the lipoxygenase gene. It was established that key mutants, such as GmLox-28 and GmLox-60, were triple mutants with hereditary mutations. As a result, lipoxygenase activity was effectively eliminated in the transgene-free mutants, providing an unrestricted method of enhancing soybean flavor. The nutritional value and lipoxygenase activity of soybean lines with various tegument colors were examined in the investigation of Ciabotti, *et al.* (2019) [87]. The yellow-tegument lineages *viz.*, MGBR10-16601 and MGBR10-16201 were devoid of lipoxygenase and possessed low fat and high protein content. While all of the lineages under examination fulfilled commercial quality criteria, the black-tegument lineage *namely* BRN07-50543 exhibited substantial quantities of isoflavones. In order to achieve a 94.30% LOX inactivation rate, radio frequency (RF) treatment of soybeans was applied for 210 seconds by Jiang, *et al.* (2018) [88]. When the functional characteristics of soybean protein isolate (SPI) were assessed following radiofrequency (RF) treatment and contrasted with traditional thermal treatment (110°C), the results showed that SPI quality had been better preserved. Hexanal and 1-hexanol, two volatile substances found in soy milk, were examined to determine whether the sensory qualities had improved. By examining mutations in the Lox1, Lox2, and Lox3 genes, Lee, *et al.* (2014) [89] described a soybean mutant line H70 produced by gamma radiation that exhibited lipoxygenase-free characteristics. H70 showed three mutations that affected the activity of the enzyme: a histidine-to-arginine change in Lox3, a 74 bp deletion in Lox1 and a histidine-to-glutamine change in Lox2. These genetic changes point to the possibility of creating more flavorful soybean varieties. The soybean cultivar Jinpumkong 2, which lacks the LOX2 isozyme responsible for beany flavor, had sequence changes in its LOX2 gene, according to the study by Shin, *et al.* (2012) [90]. SNPs, missense mutations, and indels were among the variations. To create soybean cultivars devoid of LOX2, a SNAP marker derived from the missense mutation can be employed in breeding. Using the soybean mutant line OX948 that is devoid of lipoxygenase proteins, Reinprecht, *et al.* (2011) [91] examined the molecular basis of lipoxygenase null characteristics. A 74 bp deletion in Lx1, a histidine-to-glutamine change in Lx2 and changes

to the promoter region in Lx3 were among the important mutations that resulted in the loss of enzyme activity. These results can help with breeding soybean varieties for better flavor. In order to pinpoint certain genetic reasons for the inactivity of the soybean lipoxygenase genes Lox1 and Lox3, Lenis, *et al.* (2010) [92] examined mutations in these genes. To distinguish between mutant and wild-type alleles of Lox1, Lox2, and Lox3, molecular markers were developed. The lack of lipoxygenase activity was linked to homozygous mutant alleles, according to analysis, and the connection between the Lox1 and Lox2 loci was determined to be broken by an induced recombination event.

### Phytate content

Excessive levels of phytate in seeds are considered as a negative aspect of soybean as it binds to micronutrients like Zinc and reduce their availability for humans and animals leading to malnutrition, particularly deficiencies of Zn and Fe [93].

A total 7,945 genes with differential expression were found in the study conducted by Jin, *et al.* (2021) [94] using RNA-Seq to compare soybean seeds of low-phytic acid mutant 2mlpa with normal 2MWT lines. Important discoveries in 2mlpa seeds include decreased photosynthesis, increased sucrose metabolism and changed gene expression in the metabolism of phytic acid. A study on 256 soybean genotypes from China were conducted by Kuerban *et al* (2020) [95] revealed significant variation in seed phytate-phosphorus (P) concentration, ranging from 0.69 to 5.49 mg P g<sup>-1</sup> dry weight. Genotypes from Hainan Province had the highest phytate-P, while those from Inner Mongolia had the lowest. Two genotypes Siliyuan and Diliuhuangdou-2, were identified with low seed phytate concentrations and desirable root traits for efficient P acquisition, offering potential for breeding soybeans with low phytate-P [95]. Eight genotypic classifications were formed by the analysis of 173 recombinant inbred soybean lines carrying mutations in two MRP loci and MIPS in a study performed by Redekar, *et al.* (2020) [96]. The mips mutation did not further lower phytate, while the double mutants (mrp-l/mrp-n) decreased seed phytate by about 55%. In the presence of mrp-l/mrp-n mutations, however, the mips mutation reversed the expected high-sucrose, low-raffinose phenotype. By comparing the proteome profiles of the low-phytate soybean mutants *viz.*, TW-1 and TW-1-M, Yu, *et al.* (2019) [97] was able to discover 282 proteins that had differential accumulation and were associated with energy metabolism and stress response. Because TW-1-M had improved seed field emergence, it is most likely because of variations in proteins linked to plant hormones and reactive oxygen species. These findings add to

our knowledge of the mechanisms behind seed germination in low-phytate crops. In the study of Gobner, *et al.* (2019a) [98] low-phytic acid soybean mutants of MIPS1 and IPK1 were crossed with cultivars of the wild type as well as with each other. While IPK1 progenies displayed greater InsP3-InsP5 levels and a 43–71% InsP6 reduction, MIPS1 progenies reduced phytic acid by 44%. The highest reduction in phytic acid (up to 87%) was seen in double mutants with moderate accumulation of InsP3-InsP5, which improved the nutritional quality. In a study of Redekar, *et al.* (2017) [99] examined the gene regulatory networks in low-phytic acid soybeans and found that seed emergence and development are also impacted by mutations that lower phytic acid levels. Important transcription factors were found to be involved in hormone signaling and myo-inositol production. Two low-phytate soybean mutants with different seed field emergence patterns TW-1 and TW-1-M were compared by Yuan, *et al.* (2017) [100]. A total 3,900–9,200 differentially expressed genes were found using RNA-Seq; these genes are important for hormone signaling, antioxidant defense, and stress response. These pathways most likely play a role in TW-1-M's greater germination rate. Redekar, *et al.* (2015) [101] compared gene expression across five phases of seed development in low-phytic acid (lpa) and normal soybean lines using RNA-Seq. It found 4,235 differently expressed genes, with early lpa seeds exhibiting higher expression of genes linked to defense and cell walls and later stages exhibiting lower expression of genes associated with photosynthetic processes. These modifications demonstrate how lpa mutations affect seed development. Scaboo, *et al.* (2009) [102] confirmed that SSR markers Satt237 and Satt561 are linked to quantitative trait loci (QTLs) controlling seed phytate concentration in soybean, named cqPha-001 (major QTL) and cqPha-002 (minor QTL), respectively. The research showed significant correlations between seed phytate and traits like plant height, seed protein, and seed oil.

### Saponins

High molecular weight secondary metabolites called saponins are present within the bark, stems, roots, leaves, and flowers of many plant species. They usually taste bitter and are composed of water-soluble sugar residues combined with a lipid-soluble aglycone, which can be a triterpenoid or sterol. Saponins are very surface-active because of their amphiphilic qualities, and their chemical structures and biological activities are intimately related [103]. Saponins can be divided into four subgroups according to the structures of their aglycones, or sapogenins. Groups A through E of soyasaponins are assigned to the glycosides of soyasapogenols A through E, correspondingly. Furthermore, group DDMP soyasa-

ponins, which have C-22 chains linked to 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) residues, are glycosides of soyasapogenol B [104]. The presence of group A saponins in particular from soybeans is known to contribute to astringency and bitterness, which can cause problems with foaming and an unwanted taste when tofu is produced. Studies show that saponins from groups B and E, on the other hand, are less astringent and bitter than those from group A [105].

Sun., *et al.* (2024) [106] quickly and non-destructively measured the amount of soybean saponin by using preprocessing and hyperspectral analysis techniques. By using ensemble learning models and a two-step variable selection process, good prediction accuracy ( $R^2 = 0.9216$ ) was attained. The accuracy of the model was increased by combining spectral and imaging data, providing a useful method for saponin detection. Using LC-PDA-MS/MS, Chitisanukul., *et al.* (2018) [107] detected and measured 105 soyasaponins in the seed hypocotyls and cotyledons of nine soybean varieties, including fully, partly and deacetylated soyasapogenol A glucosides (SAGs) and DDMPs. The seed structure and variety had an impact on the saponin composition, with fully-acetylated SAGs contributing to unfavorable traits in soy-based meals. After being identified by Sundaramoorthy., *et al.* (2019) [108], two recessive soybean mutants with no DDMP saponins were connected to the Sg-9 gene on chromosome 16. A single-nucleotide polymor-

phism in Glyma.16G033700, encoding UDP-glycosyltransferase UGT73B4, which is implicated in the production of DDMP saponin, was discovered by nucleotide sequence analysis. This is the first report of a gene directly implicated in the manufacture of DDMP saponins, as functional experiments verified that Sg-9 encodes a DDMP transferase essential for DDMP saponin formation. The Sg-5 gene, which is in charge of soyasapogenol A (SA) production in soybeans, was found and described in the work conducted by Yano., *et al.* (2016) [109]. The sg-5 mutant lacks group A saponins and accumulates more DDMP saponins due to a loss-of-function mutation in Glyma15g39090. By turning down this gene, soybeans can metabolically transition from producing harmful to helpful saponins, indicating this gene's potential to enhance the quality of soy products. Krishnamurthy., *et al.* (2014) [110] found that the saponin content varied throughout the eight wild soybean accessions studied in terms of saponin composition. The average saponin content of mature seeds was 16.08  $\mu\text{mol/g}$ , whereas two-week-old seedlings had 27.94  $\mu\text{mol/g}$ . Group A and Sg-6 saponins reduced during germination, whereas newly produced DDMP saponins increased. The primary objective of the work conducted by Tantry and Khan (2013) [111-122] was to authenticate dietary supplements using soybean chemical profiling. Liquid chromatography was used to isolate seven known soysaponins and three novel soysaponins *viz.*, M1, M2 and M3. All of the substances were described using spectroscopic techniques, such as two-dimensional NMR spectroscopy.

Trait	SSR Marker	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')	References
Protein Content	Satt556	GCGATAAAACCCGATAAATAA	GCGTTGTGCACCTTGTTTTCT	[37]
	Sat_135	GCGCCTCGCCTATATTTAAATTACAAAA	GCCTCCGCCTCCGAAATACACTTA	[112]
	Satt 263	CACCCAATCATGATAGCATTTTAT	CTCATGGAATTGTCTTTCAGTTTC	
	Satt 151	ATTGCCTAATTTCTGTTTGTGTGTA	CCAAAATCAAGGCAGTGAC	
	Satt 173	TGCGCCATTTATTCTTCA	AAGCGAAATCACCTCTCT	
	Satt431	GCGTGGCACCCTTGATAAATAA	GCGCACGAAAGTTTTTCTGTAACA	
	Satt510	GCGAGTTTCGCCGTTACCACCTCAGCTT	CCCTCTTATTTACCCTAAGACCTACAA	[113]
	Satt584	GCGCCAAACCTATTAAGGTATGAACA	GCGGGTCAGAAGATGCTACCAAACCTCT	
	Satt534	CTCCTCCTGCGCAACAACAATA	GGGGATCTAGGCCATGAC	
	Satt294	GCGGGTCAAATGCAAATTTATTTT	GCGCTCAGTGTGAAAGTTGTTTCTAT	
	Satt100	ACCTCATTTTGGCATAAA	TTGAAAAACAAGTAATAATAACA	
	Satt005	TATCCTAGAGAAGAACTAAAAAA	GTGATTAGGCTTGAAATA	
	Satt373	TCCGCGAGATAAATTCGTAATAAT	GGCCAGATACCCAAGTTGTACTTGT	
	Satt463	TTGGATCTCATATTCAAACTTCAAG	CTGCAAATTTGATGCACATGTGTCTA	
	Satt012	GCAATTAGTTTTAAAATGTTTC	AGAATAGAGCTACATATAATCATA	
	Satt449	GCGTGCTTCTATATTAGGTGTTAGT	GCGCATTGGAGTTTTTGCTTTT	
Satt185	GCGCATATGAATAGGTAAGTGCCTAA	GCGTTTTCTACAATAATTTTCAT		

Lipid Content	Satt006	CAATGTGATTAGTTTTGGAAA	GGGTTAATGTTGTTTTTATA	[37]
	Satt212	CCAATCCAAACAAATCCACT	CAGCAATGATGATAATGAATGA	
	Satt144	CGTCGCCATCACTATGAGAA	CCATCTTGAGCAGAGTTGAAGTT	
	Satt449	GCGTGCTTCTTATATTAGGTGTAGT	GCGCATTGGAGTTTTTGCTTTT	
	Sat_217	GCGAAAAATTGTCAATGATATGATCAGTAAG	GCGGTCCTAGATGAAAAATGCTTTGTAA	[114]
	Satt200	GCGATAAATGGTTAATGTAGATAA	GCGAAAGGACAGATAGAAAAGAGA	
	Satt236	GCGTGCTTCAAACCAACAACTTA	GCGGTTTGCAGTACGTACCTAAAATAGA	
	Satt258	CGAGGCGATTTGG	CCGAAAAGTGAAACAAGT	
	Satt094	CCAAGTGCCAAATGAAG	ATCCATGGTTTTTTGATG	[112]
	Sat_001	GCGGATACGACCAAAAAATTGTT	GCGAACTGCGAAGATACTACCC	
	Satt257	GCGACTTTCCTTTCAATTTCACTCC	GCGCAATTGTCACCAACACAT	
	Sat_135	GCGCCTCGCCTATATTAATTACAAA	GCCTCCGCCTCCGAAATACACTTA	
Carbohydrate Content	Satt598 (Glucose, Fructose)	CGATTTGAATATACTTACCGTCTATA	CACAATACCTGTGGCTGTTACTAT	[115]
	Satt372 (Fructose)	CAGAAAAGGAATAATAACAACATCAC	GCGAAAACATAATTCACACAAAAGACAG	
	Satt324 (Fructose)	GTTCCCAGGTCCCACCATCTATG	GCGTTTCTTTTATACCTTCAAG	
	Satt359 (Sucrose)	GCGAGAAAATAATCCTGCTCAAG	GCGTTTAAGTCCAATAACAAAGATAAC	
	Satt680 (Sucrose)	GCGGGATATCGTGAGCATAGTTTAC	GCGGCTGAATATTTTAGGTTTAGAGTT	
	Satt270 (Sucrose)	TGTGATGCCCTTTTCT	GCGCAGTGCATGGTTTTCTCA	
	Satt359 (Raffinose)	GCGAGAAAATAATCCTGCTCAAG	GCGTTTAAGTCCAATAACAAAGATAAC	
	Satt150 (Raffinose)	AAGCTTGAGGTTATTCGAAAATGAC	TGCCATCAGGTTGTGTAAGTGT	
	Satt359 (Stachyose)	GCGAGAAAATAATCCTGCTCAAG	GCGTTTAAGTCCAATAACAAAGATAAC	
Satt635 (Stachyose)	GCGGTGTATTAATAATGTCCATGT	GCGCTGTCCTAATTTAAATGAGAAAAC		
Isoflavonoids	BARC_Satt129	TTCAGTACAAGTCGGGTGAATAATAATA	TCACATGTTCCGGACTTAAGGTAT	[116]
	BARC_Satt063	AAATGATTAACAATGTTTATGAT	ACTTGCATCAGTTAATAACAA	
	Satt144	CGTCGCCATCACTATGAGAA	CCATCTTGAGCAGAGTTGAAGTT	[117]
	Satt587	GCGAATGGTTGCTCAAATAATC	GCGCAAACCGCACAGTTTATGT	
Tocopherol	Sat_243	GCGATGTCGAATGATTATTAATCAAAATC	GCGGCAACCGCTTAAAAATAATTTAAGAT	[118]
	Sat_167	AAGGCACTCTTCCATCAATACAA	TTGAGCCGAAAGTTCAATTCTA	
Phytate Content	Satt237	GCGTGATTTCAATCCTTTTTC	GCGGTTGTCTGTTAGAACCT	[119,102]
	Satt561	GCGGACGAATTTCCAGA	GCGGGCAACAATATTTGAATCTA	
	Satt339	TAATATGCTTTAAGTGGTGTGTTATG	GTTAAGCAGTTTCTCTCATCAG	
	Satt 527	GCGGTTACATCTTGCAAATAAATAAC	GCGGAATTTTGCACATAAATAAATAACT	
Trypsin Inhibitor	Satt409	CCTTAGACCATGAATGTCTCGAAGAA	CCTAAGGACACGTGGAAGATGACTAC	[89]
	Satt228	TCATAACGTAAGAGATGGTAAAACCT	CATTATAAGAAAACGTGCTAAAGAG	
	Ti/ti, gene specific marker	CTTTTGTGCCTTACCACCT	GAATTCATCATCAGAACTCTA	
	Satt429	GCGACCATCATCTAATCACAATCTACTA	TCCCCATCATTTATCGAAAATAATAATT	[120]
Lipoxygenase	Satt656	GCGTACTAAAAATGGCAATTATTTGTTG	GCGTGTTCAGTATTTGGATAATAGAAT	[121]

Oleate content	Satt200	GCGATAAATGGTTAATGTAGATAA	GCGAAAGGACAGATAGAAAGAGA	[122]
	Satt156	CGCACCCCTCATCCTATGTA	CCAACCTAATCCCAGGGACTTACTT	
	Satt418	GCGAAAGCACATATGGGTTTGAAT	GCGAGGGCATATATATGATGAGGTA	
	Satt153	GGGTTATATCAGTTTTTCTTTTGT	CCATCCTCGTTAGCATCTAT	
	Satt243	GCGCATTGCACATTAGGTTTTCTGTT	GCGGTAAGATCACGCCATTATTTAAGA	
Linolenic acid	Satt135	CACGGATTTTAAATCATTATTACAT	TTCCAATACCTCCCAACTAAC	
	Satt581	CCAAAGCTGAGCAGCTGATAACT	CCCTCACTCCTAGATTATTTGTTGT	

**Table 3:** SSR Markers employed for Nutritional and Anti-nutritional Contents in Soybean with Corresponding Primer Sequences.

## Conclusion

The biochemical analysis of nutritional and antinutritional compounds in soybean, combined with SSR marker studies, provides a comprehensive understanding of the genetic diversity and breeding potential of soybean varieties. The identification of key nutritional compounds such as proteins, oils and essential amino acids, alongside antinutritional factors like phytic acid and trypsin inhibitors, enables the selection of genotypes with desirable traits for crop improvement. SSR markers, being highly polymorphic, allow for precise genetic mapping and the detection of quantitative trait loci (QTLs) associated with both nutritional and antinutritional compounds. Incorporating this molecular and biochemical data into breeding programmes accelerates the development of superior soybean cultivars with enhanced nutritional value, reduced antinutritional factors and improved resistance to diseases and environmental stresses. This integrated approach contributes significantly to achieving sustainable crop improvement, ensuring better food security and promoting soybean's role as a major protein and oil crop globally. Ultimately, this research lays the foundation for breeding soybean varieties that meet the growing demands of both consumers and industries for high-quality, nutritionally balanced crops.

## Author's Contribution

All the authors contributed to the development of this manuscript. Mishra R is responsible for conceptualization, literature review and writing of this manuscript. Tripathi N, Singh J and Tiwari S assisted with manuscript revision and literature organization. Tripathi MK provided overall supervision, critical revision and final shape to this manuscript. All the authors have approved this final manuscript.

## Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this review paper.

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