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Effect of germination on nutritional quality of soybean

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Abstract

Soybean are rich in functional nutrients such as protein, Essential amino acid, Polyunsaturated fatty acid, minerals, vitamins and dietary fibre which can help maintain optimal body weight, prevent Alzheimer and cardiovascular disease. At the same time, it also contains some anti-nutritional factors which have adverse effects on the digestion, absorption and utilization of nutrients. Cellular and metabolic events are induced by water absorption during seed germination. After absorbing water, soybean dry seeds begin to mobilize organelles and enzyme activities, repair and activate physiological and biochemical processes, and start basic metabolism. After that, by controlling the key factors of metabolism, the cells complete the process of material transformation, energy metabolism, ROS balance, cell wall acidification and looseness, hypocotyl elongation, etc. and finish germination. As a result, cell metabolism causes significant changes in proteins, lipids, sugars, vitamins and minerals, and increases in some beneficial functional nutritional factors for humans and animals, the other anti-nutritional factors decreased obviously, which greatly improved the taste quality and economic value of soybean. This paper introduced the germination physiology, functional nutrients and their metabolic pathways during soybean germination. This study has important reference value for the study of theory and practice technology of soybean germination.

Keywords: soybean; germination; functional nutrients; key control factors of metabolism.

Practical Application: Sprouted soybeans can be used to make bean products such as fermented soybeans, fermented tofu, soy sauce, soy milk, tofu, dried bean curd, etc., to cardiovascular system, constipation, diarrhea and other diseases have good preventive effect, can promote the absorption of calcium and phosphorus, improve female climacteric syndrome.

1 Introduction

Soybeans are native to China and the world's largest supplier is Brazil. In 2019, for example, global soyabean consumption was 351.9 m tonnes, of which China consumed 108.2 m tonnes, accounting for 30.75 per cent of global consumption, and the world produces 337m tonnes of soyabeans, compared with 18.1 m tonnes in China, which accounts for just 5.37 per cent of global production (Research Report Network, 2022). As a result, China relies on imports for more than 80 per cent of its soybean consumption. Rich in protein, essential amino acid, unsaturated fat, minerals, vitamins, and dietary fiber, soybean help maintain optimal weight and prevent Alzheimer's disease, improve human immunity and cardiovascular disease and other physiological functions (Yang et al., 2020; Sun, 2013) (Table 1). It also contains a number of anti nutritional factors such as trypsin inhibitor, lectins, lipoxygenase and phytic acid, which have adverse effects on the digestion, absorption and utilization of nutrients and make people and animals have adverse reactions, which greatly reduces the edible value and market value of soybean products. In domestic soybean industry, soybean is mainly used for oil extraction and consumption of vegetable protein food. With the development of soybean products industry, soybean products can be divided into fermented soybean products and non-fermented soybean products, such as fermented soybean, fermented bean curd, soy sauce, soybean milk, bean sprout and dried bean curd (Wang & Ying, 2007). According to its technological characteristics, soybean products can be also divided into traditional products, new products and functional health products (Yang et al., 2018). It can be seen from the above description that yield and anti-nutritional factors are the limiting factors of soybean and its product value, it is an effective way to improve the nutritional quality of soybean and its products by increasing the content of functional nutrient factors beneficial to human body and animals.

Many studies have shown that germination can awaken dormant soybean seeds, activate various key enzymes of endogenous metabolism, promote physiological changes of soybean, and increase the contents of isoflavones, soybean saponins, vitamin C and other nutrients, the content of anti-nutritional factors such as lipoxygenase and phytic acid was decreased, and promote protein decomposition and Lipid hydrolysis oxidation process (Gao et al., 2019; Teng, 2019; Bao, 2015). Therefore, germination is considered to be the most direct and effective means to enrich the nutrient content of soybean and reduce the harmful content (Guo et al., 2011a; Ikram et al., 2021).

This paper mainly introduced the physiology of soybean germination, the change rule of main functional nutrients in the process of soybean germination, and the effect of key factors on

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Table 1. Soybean bioactive substances and their effects (Sun, 2013).

Bioactive substances	Concept	Effect
Soybean polypeptides	A mixture of low molecular weight peptides obtained by hydrolysis of soybean protein with protease and special treatment.	Lower cholesterol, prevent cancer, lower blood pressure, lose weight, and prevent aging.
Soybean lecithins	A group of phosphorous-containing lipids in soybeans, which are products of glycerol, fatty acids, and phosphates, including lecithin, phosphatidylcholine, and Phosphatidylinositol.	Treatment and protection of the nervous system, cardiovascular system and immune system, physical defense, anti-aging, brain health, beauty, blood nutrition, to promote reproductive fetal pregnancy and sexual function, prevention of Alzheimer's disease.
Soybean oligosaccharides	Any of a group of soluble carbohydrates found in soybeans, mainly stachyose, raffinose, and sucrose.	Promote the proliferation of Bifidobacterium, enhance intestinal function and detoxification, to prevent dental caries.
Soybean Isoflavones	Isoflavone glycosides and their corresponding aglycones form the secondary metabolite formed during soybean growth.	Anti-cancer effects, especially to prevent breast cancer, prostate cancer, colon cancer, prevent osteoporosis, inhibit or reduce menopausal syndrome in women, anti-oxidation, anti-hemolysis, anti-hyperlipidemia, anti-fungal effects.
Soybean saponin	A pentacyclic triterpenoid saponin derived from oligosaccharides and oleanolic triterpenes.	Lower cholesterol, inhibit cancer, protect X-rays, prevent lipid peroxide production, delay aging, anti-thrombotic, weight loss, anti-cancer, anti-fatigue effect.
Soybean dietary fiber	An insoluble carbohydrate in soybeans.	Lower cholesterol, improve lipid metabolism, prevent colorectal cancer, arteriosclerosis and coronary heart disease, improve diabetes, and lose weight.

the metabolism of functional nutrients in the process of soybean germination. This study has important reference value for the study of theory and practice technology of soybean germination.

2 Physiology of soybean germination

Cellular and metabolic events are induced by water absorption during seed germination. According to the degree of water absorption and utilization of seeds, the process of seed germination can be divided into three stages: the beginning of rapid self-absorption of water stage, slow stage, a large amount of water absorption stage (Bove et al., 2001). As shown in Figure 1, the rapid self-priming stage (Stage 1) begins, and the seed immediately after water uptake initiates the recovery of basic metabolism, initiating repair and activation of physiological and biochemical processes, such as mobilization of organelles, enzyme activity, and so on. In this phase, called"Physical" absorption, the result of step-by-step activation of metabolic pathways is a gradual increase in hydration (arrow). When the hydration of the seed exceeds 60%, the water absorption of the seed enters the slow stage (Stage 2), the metabolic activity inside the seed begins to be active, and the seed embryo cells grow rapidly, the accumulation of osmotic active substances such as sugars, amino acids, potassium ions, cell wall acidification promotes cell wall polymer loosening. At the same time, with the increase of H^+-ATPase activity, it further promotes the seed water absorption, weakens the restriction of the surrounding tissues (such as endosperm), and finally causes the hypocotyl elongation, the embryo breaks through the seed coat, and the germination is completed. After that, the seeds re-enter the stage of mass water absorption. During seed germination, the rich nutrients (lipid, protein, starch) in the seeds are broken down and utilized to maintain the early growth of the seedlings until the seedlings are self-sufficient (Figure 1).

The physiological mechanism of seed germination has been studied in oat, tomato, tobacco and Arabidopsis thaliana (Bove et al., 2001). The mechanism of crop seed germination is mainly focused on model crops such as rice, rape and a few other crops, and relatively little research has been done on other crops and vegetables (Zheng et al., 2017).

Germination is influenced by environmental factors (availability of water, oxygen and light, and temperature), it is also influenced by internal factors (dormancy, permeability of Testa to water and oxygen, and resistance of endosperm to the appearance of radicle) (Bove et al., 2001). Tian et al. (2011) considered that in addition to the influence of seed vigor on seed germination, some physical factors such as temperature, water content, seed soaking, time and frequency, electric field and some chemical factors such as chemical gas, chemical agent, acid-base, metal ion, biological seed coating agent, sewage irrigation, plant tissue extract, soil organic compounds and soil fungi may also have some promoting or inhibiting effects on soybean seed germination.

3 Functional nutrients and their metabolic pathways during soybean germination

Soybean is a kind of high quality protein resource. 100 grams of soy contains about 40-50 grams of protein, followed by soy fat, accounting for about 20% of the total weight of soy. Soy is also rich in a variety of minerals and vitamins (Gallego et al., 2021). In addition, soybean also contains a variety of functional nutrients, such as isoflavones, nattokinase, saponins and other

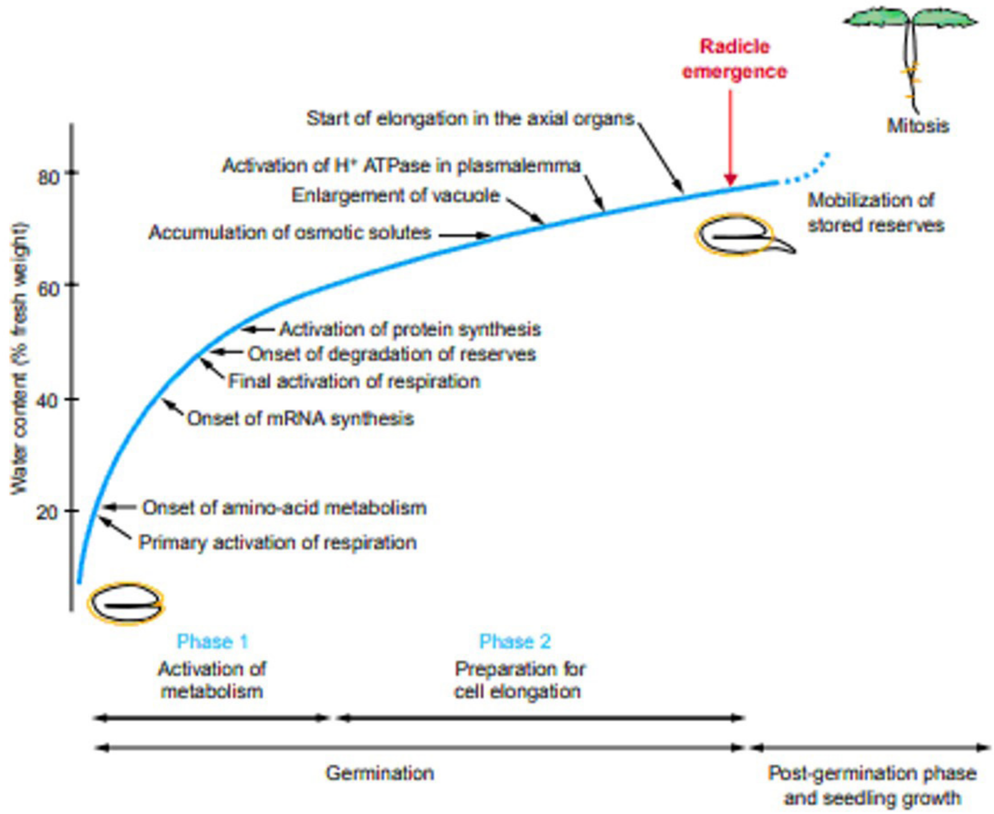

Figure 1. Germination of seed and physiological process (Bove et al., 2001).

special components (Sagara et al., 2019). During the germination of soybean seeds, the contents of polypeptides, isoflavones, saponins, Gaba and vitamin C increased, but the contents of trypsin inhibitor, agglutinin, lipoxygenase and phytic acid decreased. Generally speaking, these changes of functional nutrition factors which reflect the effect of soybean germination cannot be summarized without the changes of enzyme during soybean germination, so it is very important to study the change rule of soybean functional nutrient factors and the effect of key enzymes of endogenous metabolism.

In this paper, the metabolic pathways of soybean functional nutrient factors were divided into four types, which were protein metabolism, lipid metabolism, glycometabolism, vitamin and mineral metabolism.

3.1 Protein metabolism

Protein, polypeptide, amino acid

There are three main methods of soybean protein hydrolysis: Exogenous enzymatic hydrolysis, hot water treatment and germination treatment.

Wang et al. (2005b) studied the degree of protein hydrolysis in soybean milk by three exogenous enzymes, Neutrase, Alcalase and Protamex. The results showed that Alcalase was the best enzyme to hydrolyze protein, and the optimum conditions were 55 °C, 0.1 mg/mL (enzyme concentration in soybean milk), hydrolyzing for 4 hours. Song & Lu (2021) utilized non-starch polysaccharide enzymes and proteases for optimal combinatorial enzymatic treatment of soybean meal, in which macromolecular proteins were degraded into small molecular peptides to improve protein digestibility and utilization, at the same time eliminate anti-nutritional factors. The results showed that the content of peptides in soybean meal was 18.34%, and the removal rates of non-starch polysaccharide, soybean globulin and β-soybean globulin were 24.91%, 97.61% and 96.60%, respectively. Yin et al. (2021) explored the optimal technological conditions for preparing bioactive polypeptides from Papain hydrolyzed soybean clear liquid, and evaluated the antioxidant capacity of the bioactive polypeptides. The results showed that the optimal conditions for enzymatic hydrolysis of soybean clear solution were as follows: 2.0% papain, pH 5.0, temperature 53 °C and time 7 h, and the polypeptide yield was 115.50%. The concentration for 50% of maximal effect (EC50) of ABTS was 1.871 mg/mL and the EC50 for DPPH was 6.459 mg/mL, it indicated that the polypeptide sample had certain antioxidant activity. On the whole, however, there are still many problems to be solved in exogenous enzymatic hydrolysis of soybean protein. Firstly, the exogenous enzymatic hydrolysis process of soybean protein is complex, the speed of exogenous enzymatic hydrolysis is fast, and the amino acid composition and content of the products are changing constantly, so it is difficult to control the hydrolysis process. Second, the existing exogenous enzymatic hydrolysis effect is difficult to meet the needs of production and processing. Third, the selection of exogenous enzymes needs to ensure the high-quality functional characteristics of soybean protein substrates, and the cost is high.

Liu & Li (2013) studied the effect of soybean milk heat preservation on the quality of soybean yoghurt, and the results showed that the soybean milk heat preservation treatment could improve the quality of soybean yoghurt, but the effect was not very significant.

Sun (2013) studied the change of the nutrition and functional substance in the process of soybean germination with the varieties of Kennong 29, 30 and Heinong 53, and found that the protein content decreased by 12.80%, 14.47% and 11.14% respectively. Paucar-Menacho et al. (2010a) found that germination activates endogenous proteases, degrades macromolecular peptides and promotes the catabolism of other components, which leads to a significant increase in soybean peptides content after germination. Wilson et al. (1986) found that the peptide bonds of glycinin and B-globulin were broken by germination, and that protein degradation and non-protein nitrogen synthesis remained dynamic equilibrium during germination. Xu et al. (2006) found that soybean globulin degradation occurs in the first stage of seed germination. Godfrey & West (1983) showed that after germination, soybean protein degrades to produce antioxidant peptides that inhibit angiotensin-converting enzyme production and promote phagocytic lysis. In addition, Li & Wang (2009) found that the contents of soluble protein, ammonia nitrogen and peptide in 0 ~ 96 h after germination of soybean seeds were higher than those of non-germinated seeds, and the peak of soluble protein appeared at 9 ~ 12 h after seed germination, but the contents of amino acids and peptides increased during 96 h. At the same time, the activities of protease and peptidase increased significantly from 39 to 72 h after seed germination, and the changes of protease and peptidase activities were positively correlated with the contents of soluble protein, ammonia nitrogen and peptides.

From above studies, we could conclude that during germination, the endogenous hydrolase and protease are activated due to the enhanced respiration of soybean, and the macromolecular peptides and protein are decomposed into small peptides and free amino acids, plays a key role in the degradation of soy protein. Therefore, we can get more peptides and amino acids by thoroughly mastering the characteristics and mechanism of endogenous hydrolases and proteases, and controlling the degradation of soybean protein in combination with germination conditions. We can also study the genes of endogenous hydrolase and proteinase, and improve soybean varieties by modern biological technology, so as to obtain new soybean varieties suitable for using germination to obtain functional nutritional factors.

Gaba synthesis controlled by glutamate decarboxylase

Gaba is a new soybean functional factor discovered by Nagatoishi & Aida (2007). Studies have shown that soybean Gaba can be increased by germination (Guo et al., 2011a). Li et al. (2009) found that after 48 hours of germination, the Gaba content in soybean was about 7 times higher than that of non-germinated soybean. Guo et al. (2011b) found that after germinating at 30 °C and pH 4.1 for 2 days, the Gaba content of soybean was 12.5 times higher than that of non-germinated soybean. Zhao (2012) found that the Gaba content in soybean seeds was lower at the initial germination stage, but increased gradually after the sprout length was 3 cm. Wang et al. (2015a) compared the Gaba content in germination and non-germination of three domestic soybean varieties (ZH13, ZH30, ZH42), and found that the Gaba content in germination of ZH13 increased by 36.7 times, the most significant change. Cao et al. (2018) studied the relationship between Gaba and glutamic acid in germinated brown rice, demonstrating that Gaba formation is regulated by Glutamate decarboxylase activity and has a significant positive correlation with glutamic acid content. Zhang & Xu (2008) found that the Gaba content of soybean was the highest ($0.601\ mg^*g^{-1}$) after 7 days of Germination, however, Gaba content decreased with the prolonging of germination time, which was due to the slow down of glutamic acid synthesis in substrate and the increase of the possibility of forming succinic semi-aldehydes under the action of Gaba transaminase after Gaba enrichment.

The effect of Glutamate decarboxylase on GABA in the above report is related to the germination of brown rice, but no relevant research on soybean germination has been reported. Therefore, the mechanism of Glutamate decarboxylase during soybean germination needs to be further studied.

Soybean agglutinin controlled by transcription factors

Agglutinin is a powerful binding protein first discovered by Stillmark in 1889 that inhibits protein digestion and utilization, and in 1954 Boyd et al renamed it agglutinin (Chen et al., 1977). Agglutinins interfere with the secretion of enzymes by binding to glycoproteins, glycolipids, or polysaccharides, and agglutinin is lectin, thus could be alergen to some people. Plant Hemagglutinin (PHA) is one of the major anti-nutritional factors in legumes. Many studies show that germination treatment can effectively reduce the content of soybean agglutinin. Pan (2004) and Chen et al. (1977) found that the content of agglutinin was only 3.7% of that in non-germinating soybean after 4 days of germination. Guo et al. (2008) found that the content of agglutinin in soybean decreased by 16.5% after 4 days of germination. Paucar-Menacho et al. (2010b) found that the agglutinins in soybean decreased by 9.9% after germination at 30 °C for 21 h, but by 72.6% after germination at 25 °C for 72 h. Paucar-Menacho et al. (2010a) found that soybean agglutinins decreased by 58.7% after germinating at 25 °C for 42 h.

Lu (2021) studied the phenotype, transcriptome sequencing, transcription factors screening and QPCR validation of PHA content traits in bean pod. Six transcription factors, ERF1, WRKY22, WRKY33, SKP1, CBF3D and MYC2, were found to be involved in PHA synthesis. However, it has not been reported whether these transcription factors change during soybean germination and whether the change has an effect on the content of Soybean agglutinins.

Trypsin inhibitor

Soybean Trypsin inhibitor is an anti-nutritional factor, mainly Kunitz and Bowman-Brik inhibitors, which are polypeptides containing 181 and 71 amino acid residues respectively. Rooke et al. (1998) found that the Kunitz type inhibitor content in soybean did not change obviously at the beginning of germination, and began to decrease at the 8th day of germination, and reached the lowest level at the 14th day of germination. Savelkoul et al. (1992) found that the Trypsin inhibitor content of Dutch soybean decreased by 25.5% after 7 days of germination. Dikshit & Ghadle (2003) found that the Trypsin inhibitor content of soybean variety macc-13 decreased by 51.68% after 72 hours of germination. Kumar et al. (2006) found that the trypsin inhibitor was more sensitive to heat treatment, and after 6 days of germination, the trypsin inhibitor in soybean decreased by 32%. Paucar-Menacho et al. (2010b) found that the content of Bowman-Brik inhibitor decreased by 12.4% at 30 °C for 63 h and 27.0% at 25 °C for 72 h, and deduced that the change of Bowman-Brik inhibitor content was due to the effect of germination temperature and time on the activity of soybean endogenous protease. Jiang et al. (2013) found that endogenous protease during soybean germination significantly reduced the content of Trypsin inhibitor in soybean milk, and compared with non-germinated materials, the Trypsin inhibitor activity (Tia) decreased significantly to less than 10% at 28 hours of germination, and its maximum Trypsin inhibitor decreased to 91.7%. Table 2 shows that the Tia in raw and cooked soymilk is affected by different germination time.

From the above reports, we can see that the Trypsin inhibitor content of soybean is significantly affected by germination. The main reason may be that the endogenous protease related to soybean Trypsin inhibitor is activated, this protease activity is sensitive to environmental temperature, water content and other conditions. Therefore, further research can be conducted on the mechanism of endogenous proteases related to soybean Trypsin inhibitor content.

3.2 Soybean lipid metabolism

Soybean lipid

In recent years, soybean lipid has been widely used in various fields, especially in the petroleum industry. Soybean lipid, which accounts for 18-25%, provides sufficient vegetable oil and is the main source of daily edible oil. Soybean fatty acids mainly include oleic acid (OA), linoleic acid (LA), linolenic acid (Lla), palmitic acid (PA) and stearic acid (SA), of which linoleic acid is the main component (Choi et al., 2021) . The genetic mechanism of soybean fatty acid content is affected by temperature, and linolenic acid and oleic acid content may change at different temperatures (Zhou, 2013). Regular consumption of unsaturated lipid in soy can lower cholesterol, prevent and reduce coronary heart disease, hypertension, diabetes complications and blood clots (Yang et al., 2020).

Sun (2013) studied the 120-hour germination process of soybean varieties Heinong 53, Kennong 30 and Kennong 29. The results showed that the total lipid content decreased by 6.83%, 8.35% and 8.83%, respectively, and the saturated fatty acid content increased from 14.66% to 15.93%, the contents of palmitic acid and linoleic acid in unsaturated fatty acids decreased by 0.67% and 2.98%, respectively, and this change may be due to the hydrolysis of the unsaturated fat in the lipid by the lipase. Wang & Ju (2018) studied the change of matter in the process of soybean germination with the varieties of Tiefeng 31, Zhongli sprouted bean and Dongnong 6903, and found that with the extension of germination time, the lipid content decreased by 10.35%, 9.88% and 10.14%, respectively, and deduced that during the germination of soybean seeds, the lipid is hydrolyzed by lipase and oxidized by Lipoxygenase to small molecules.

Lipoxygenase

Lipoxygenase (LOX) is a single polypeptide-chain protein that is ubiquitous in plants and animals and is most active in soybeans (Huang et al., 2007). LOX accounts for 1 to 2 percent of the total protein in soybeans (Li, 2015). Volatiles produced by LOX oxidation of soybeans, such as 2-pentyl Furans, are associated with undesirable soybean odor (Kaczmarska et al., 2021). LOX can oxidize the unsaturated fat in soybean milk to form the bad flavor (Zeng et al., 2019). Xing et al. (2020) studied the soybean which germinated for 20h and found that germination could decrease LOX activity significantly, and the similar results were also reported in Jiang et al. (2006), Suberbie et al. (1981), Fu et al. (2006), Paucar-Menacho et al. (2010b), and Bordingnon et al. (1995). Hildebrand & Hymowitz (1983) found that the LOX isozymes L-2 and L-3 activity decreased significantly in soybean after germination for 24 hours. Kumar et al. (2006) studied the effect of germination temperature on the activity of lipoxygenase isozyme, two Indian soybean genotypes at different temperatures (25 °C and 35 °C) were cultured in seed germination apparatus for 144 h, and the activity of lipoxygenase isozymes in germinating seedlings was detected every 24 h. The results showed that lipoxygenase-I and lipoxygenase-II + III degraded continuously in 144 h, and the degradation rate of both lipoxygenase-I and lipoxygenase-II + III was faster at higher germination temperature (35 °C) .

Table 2. Tia in raw and cooked soybean milk was affected by different germination time[a] (Jiang et al., 2013).

Germination time (h)	TIU/g		mgTI/g		Residue%	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
0	56,245 ± 2741	8280 ± 485	29.60 ± 1.44 A	4.36 ± 0.26 a	100 ± 4.9	14.7 ± 0.9
28	40,552 ± 325	4685 ± 885	21.34 ± 0.17 B	2.47 ± 0.45 b	72.1 ± 0.6	8.3 ± 1.5
50	43,001 ± 2121	5397 ± 497	22.63 ± 1.12 B	2.84 ± 0.26 b	76.5 ± 3.8	9.6 ± 0.9
72	45,243 ± 1244	5894 ± 18	23.81 ± 0.65 B	3.10 ± 0.01 b	80.4 ± 2.2	10.5 ± 0.0

[a]Results are the means of two replicates with standard deviation.Values marked with the same letter in the same column are not significantly different in mgTI/g ($p<0.05$).TIU, trypsin inhibitor units.

Xing et al. (2020) further qualitatively and Quantitative analysis the two main bean odors of the passivated germinated soybean by headspace solid-phase microextraction-gas chromatography-mass spectrometry, the results showed that there was a significant positive correlation between the activity of Lipoxygenase and the content of bean odor. So we can infer that the LOX content and activity of soybean and its products could be decreased by germination to improve the edible value and sensory quality.

Isoflavone metabolism and multi-enzyme system

Isoflavone is a kind of flavonoid, which is a kind of secondary metabolite formed in the growth of soybean, and mainly in the form of antioxidants (Luo & Zhang, 2019). There are 12 known Isoflavones, divided into 4 groups: Aglycone (Genistein, Daidzein, Glycitin), Malonyl-Glucoside (Daidzin, Genistin, Glycitein), Glycoside (Daidzin, Glycitein, Genistin) and Acetyl-Glucoside (Daidzin, Genistin, Glycitein) (Wang et al., 2021). The content of Isoflavone in dry soybean seeds is only 1~5 mg×g^{-1}, which is not enough to meet the daily needs of human and animal. Six Isoflavones monomers can be detected in dormant or germinated soybean, and their forms or mutual transformation are shown in Figure 2 (Teng, 2019).

Zeng et al. (2014) found that the total Isoflavones content of soybean reached the highest when the sprout length was 3 cm after germination. Zhao (2012) found that the isoflavone content reached the highest value (13 μg*g^{-1}) when the length of soybean sprouts was 5 cm. Wang et al. (2015b) germinated the high quality domestic soybean variety Zhonghuang 13 at 30 °C, 95% relative humidity and in the dark, and found that the Isoflavone content was the highest (3.796 mg*g^{-1}) on the third day of germination, but with the extension of germination time, the Isoflavone content showed a decreasing trend. Sun (2013) also found that the content of Isoflavone increased at first and then decreased with the prolonging of germination time, and considered that the endogenous glycosidase was activated by germination and converted glycosides and aglycones into each other. Wang et al. (2022) found that soybean germination could increase the content of Isoflavone component Genistein and soybean Gansui. Huang et al. (2017) found that the Isoflavone aglycones were more than 700% in black soybean after 3 days of germination, and considered that it may be due to the hydrolysis of soybean glucoside resulting from germination, thus increasing the Isoflavone aglycone content.

Previous studies have shown that L-Phenylalanine metabolism is the most important pathway for Isoflavones biosynthesis. This metabolic process can produce important secondary metabolites such as Flavone and Lignin (Yu et al., 2000; Li et al., 2007). Daidzein, daidzein and genistein, the three glycogen Isoflavones in soy, are synthesized directly from plant L-Phenylalanine, other types of Isoflavones are synthesized by glycosylation or malonyl glycosylation of the metabolite after transport to Golgi apparatus (Graham, 1991).

The primary substrate of the L-Phenylalanine pathway, Phenylalanine (Phe), is L-Phenylalanine to Cinnamate by the action of L-Phenylalanine lyase (PAL), the synthesis of coumaryl CoA was catalyzed by Cinnamic acid 4-hydrocylase (C4H) and 4-Coumarate: CoA ligase (4CL) . At this point, part of coumaroyl CoA was catalyzed by Chalcone synthase (CHS) and Chalcone reductase (CHR) to produce Isoliquiritigenin. The isomerization

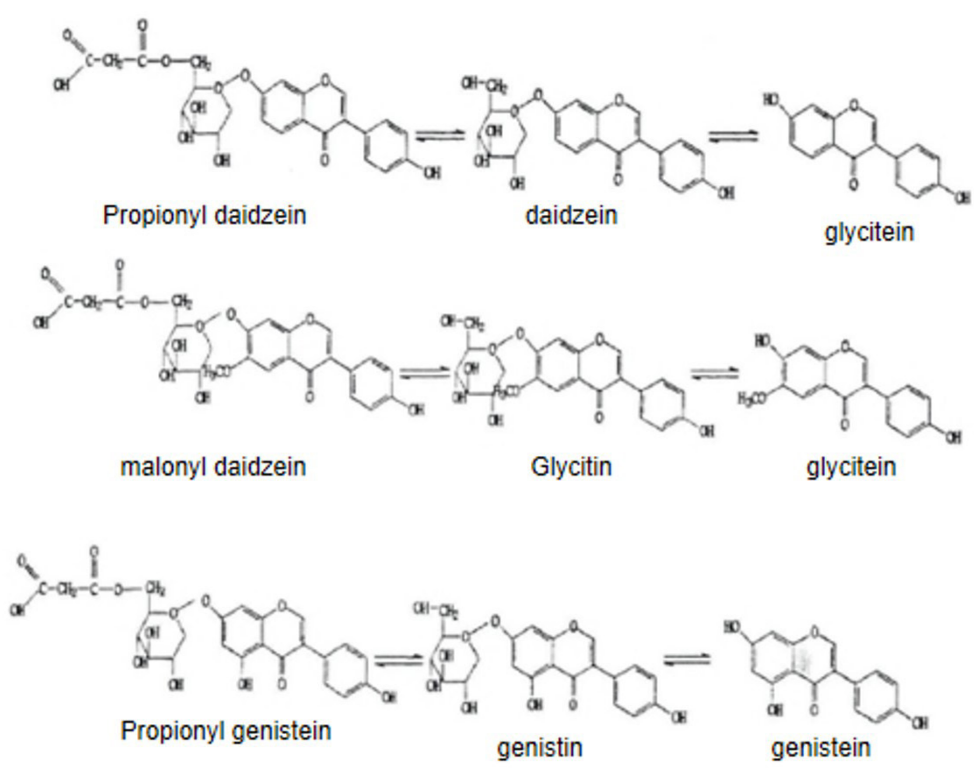

Figure 2. Schematic diagram of mutual transformation of three series of isoflavones (Teng, 2019).

of iso-glycyrrhizin with Chalcone isomerase (ChI) catalyzes the formation of Liquiritigenin, which is then converted into daidzein or daidzein. Another part of coumaroyl CoA was catalyzed by chalcone synthase to produce Naringenin, and then to produce genistein by Isoflavone synthase (IFS) (Yu et al., 2000; Latunde-Dada et al., 2001; Akashi et al., 1999; Jung et al., 2000).

To sum up, the synthesis of Isoflavones consists of two stages, the L-Phenylalanine pathway and the flavonoid synthesis pathway, which are influenced by several enzymes. In the L-Phenylalanine pathway, PAL is the rate-limiting enzyme, C4H and 4CL are the key enzymes affecting the synthesis of isoflavone precursors, while in the flavonoid pathway, CHS, CHR and CHI enzymes are closely related to the synthesis of Isoflavones, while IFS enzymes are directly involved in the synthesis of isoflavones. The synthesis of Isoflavones is regulated by multiple genes. *IFS* is the most important gene affecting the synthesis of isoflavones, and *CHS*, *CHR* and *ChI* are the most important genes affecting the Isoflavones synthesis of isoflavones. Genes such as *PAL*, *C4H* and *4CL* affect Isoflavones production by regulating L-Phenylalanine metabolism in plants. Other genes, such as *F3H (Flavanone 3-hydroxylase)*, indirectly affect isoflavone production by inhibiting other branches of the L-Phenylalanine pathway (Yu et al., 2003).

3.3 Glycometabolism

Metabolisms of oligosaccharides and dietary fibre

Soybean oligosaccharides and dietary fiber are both beneficial carbohydrates for human body (Gu, 2009).

Soy oligosaccharides are carbohydrates made from stachyose, sucrose and raffinose, which have a slightly sweet flavor that reduces fat and prevents arteriosclerosis. Yuan & Zhao (2002) found that the germination process can lead to a gradual decrease in soybean oligosaccharide content. The main regulatory enzymes in soybean oligosaccharide synthesis include inositol -1-phosphate synthase, raffinose synthase, inositol galactoside synthase and Stachyose synthase. Among them, stachyose synthase (STS) gene and INOSITOL-1-PHOSPHATE synthase (MIPS1) gene control sucrose and stachyose content, galactoside synthetase and Raffinose synthetase are key enzymes in the biosynthesis pathway of sucrose to raffinose galactoside series oligosaccharides (Qiu et al., 2014). The expression of oligosaccharide-regulated enzymes during soybean germination has not been reported.

Dietary fiber is a complex mixture consisting mainly of hemicellulose, gum, cellulose, pectin and β-glucan with mixed bonds (Wu et al., 2012). It alters peripheral tissue sensitivity to insulin, raises serum insulin levels and regulates blood glucose levels (Sun & Yu, 2021). Mai et al. (2011) found that dietary fiber can inhibit the absorption of fatty substances such as cholesterol and triglyceride, lower blood lipids, reduce hunger, reduce weight loss, and promote gastrointestinal peristalsis, improve defecation function. Ma et al. (2020) found that when the dietary fiber content was 1.4%, the prepared high-fiber soybean had good texture, elasticity and tenderness, it is concluded that dietary fiber has a good promoting effect on the quality of soybean products. Wang et al. (2014) in the experiment found that the soybean cellulose showed the trend of rising first and then decreasing with the germination time, and reached the maximum value at 60 hours. At present, researches on dietary fiber of beans mainly focus on the extraction and modification of soybean and soybean dregs dietary fiber (Li & Kang, 2015). There is no report on the mechanism of soybean germination affecting the structure and content of dietary fiber.

Soybean saponin synthesis controlled by β-aromatinol Synthetase

Compared with the United States and Canada genotypes, the soybean from China has the highest saponin concentration, but only 0.32% (Wang, 1996).The molecular structure of soybean saponin is composed of oligosaccharides and oleanocarbene (Yuan & Zhao, 2002). It consists of six sapogenins and six monosaccharides, including β-d-galactose, β-d-xylose, α-l-rhamnose, α-l-arabinose, β-d-glucose, β-d-Glucuronic acid, and acetyl soyasaponin (Zhu et al., 2005). The types of saponins are classified into steroidal saponins and triterpenoid saponins (Wu et al., 2010)· Soybean saponins are amphiphilic compounds that possess three natural saponins, namely Saponins A, saponins B, and Saponins E, and consist of nonpolar triterpene saponins and oligosaccharides (Wang et al., 2005a; Zhang et al., 2011). It has anti-thrombotic, antioxidant, lipid and alcohol lowering, and Lipid peroxidation properties (Shi, 2007).

The cyclization of 2,3-oxide squalene is a key step in soybean saponin biosynthesis (Haralampidis et al., 2002). Squalene oxide cyclase in soybean is β-amyrin synthase (βAS) (Kushiro et al., 1998). βAS is a rate-limiting enzyme in soybean saponin synthesis. Takagi et al. (2011) cloned two βAS genes named GMBAS1 and GMBAS2, respectively. In order to reduce the bitterness and astringency of soybean processed food, the content of soybean saponins was decreased by silencing GMBAS1 and GMBAS2 genes by RNA interference, but the transgenic lines can still grow normally. This indicated that the soybean Saponin in soybean seed did not affect the normal growth and development of soybean.

The germination treatment had a great effect on the content of soybean saponin. Peng et al. (2011) found that with the extension of germination time, the content of soybean saponin increased and reached the highest level in 72 hours. Huang et al. (2017) found that the content of total saponins in soybean after 3 days Germination treatment was 3 times higher than that in soybean without germination, but the content of total saponins in black soybean decreased slightly. Paucar-Menacho et al. (2010b)found that the content of soyasaponins in Brazilian brs258 increased from 7.4 $mg \cdot g^{-1}$ to 23.5 $mg \cdot g^{-1}$ at 30 °C for 63 h. Shimoyamada & Okubo (1991) found that the content of soybean saponin after germination 8 days was 8 times higher than that before germination. Jyothi et al. (2007) also found that soybean saponin content increased from 2.8% to 8.9% after 4 days of germination. Chen & Chang (2015) found that the soybean saponin content increased by 11% after 72 h germination treatment. Saponin A has obvious bitter and astringent taste, and the ratio of saponin B to saponin A can be used as one of the evaluation indexes of soybean product quality, which will directly affect the taste and flavor of soybean. Li (2018) measured the contents of saponin A and saponin B, and found that the ratio of saponin B to saponin A increased gradually during germination.

In conclusion, the next step is to increase the ratio of saponin B to saponin A during soybean germination by utilizing the different functions of βas genes.

3.4 Metabolism of vitamins and minerals

Vitamin C and ROS-related enzymes

Vitamin C (VC), also known as L-ascorbic acid (AA), is a water-soluble vitamin, found in daily fruits and vegetables, in redox metabolic reaction plays a regulatory role, lack of it can cause scurvy. Some studies showed that germination significantly increased the content of VC in soybean. Kaushik et al. (2010) found that the VC content in dry soybean was very low and increased obviously after germination. Fordham et al. (1975) found that the VC content of soybean after germination for 5 days was 0. 20 mg · g^{-1} FW, the content of cotyledon was the highest, the next was bud. Peng et al. (1958) found that the length and temperature of the sprouts significantly affected the content of VC in Mung Bean sprouts. Song et al. (2013) found that with the increase of soybean sprout length, the content of VC increased, and when the sprout length was 30 mm, the content of VC reached the maximum (0.1169 mg · g^{-1}). Pan (2004) found that germinated for 3 days, the VC content of soybean decreased to 0. 25 mg · g^{-1}, and germinated for 5 days, the content of VC decreased to 0.21 mg*g^{-1}. Du et al. (2015) also found that with the increase of germination length, the content of VC in soybean increased, and when the sprout length was 5 cm, the content of VC in soybean sprout reached 120. 09 mg per 100 g. Huang et al. (2014) monitored the change of VC content in soybean at different germination time, and found that the VC content in soybean reached the maximum value of 5.27 mg*g^{-1} on the second day of germination, and decreased by 37% on the 5th day compared with the 2nd day. Xu (2003) considered that due to the activation of galacturonolactone dehydrogenase after germination treatment, the synthesis of VC was promoted.

Reactive oxygen species (ROS) are produced by plant cells that are metabolically active. The concentration of reactive oxygen species may determine their role, for example, they may be involved in signal transduction or cause oxidative damage to various cellular components. To ensure intracellular homeostasis and minimize the negative effects of excessive ROS, plant cells have evolved a complex antioxidant system that includes ascorbic acid (Bilska et al., 2019). Ascorbic acid is a versatile non-enzymatic antioxidant with not only the potential to scavenge ROS, but also to modulate some essential functions of plants under both stress and non-stress conditions (Akram et al., 2017). Both ascorbic acid (AA) and Dehydroascorbic Acid (DhAA) inhibit ROS in plants and animals (Dewhirst & Fry, 2018). All seeds, not just soybeans are low or do not have free AA, it is mainly in dehydro-ascorbic acid form DhAA. Thus when water enters during imbibition, DhAA easily and promptly hydrolyses to AA, which is an important factor in maintaining of oxidative balance during germination, since it is process highly relaying on oxidative processes and ROS formation. Therefore, ROS-related enzymes such as Superoxide dismutase, ascorbic Peroxidase, Glutathione reductase, mitochondrial, Dehydroascorbic Acid reductase, and Dehydroascorbic Acid reductase are worthy of further study after germination.

Phytic acid and mineral elements related to phytase and phosphatase

Phytic acid is a strong chelating agent, can chelate iron, calcium, magnesium and other metal ions, reduce the bioavailability of metallic elements. The content of phytic acid in soybean is as high as 2%, which leads to the decrease of absorption and digestibility of mineral elements and protein in human and animal (Persson et al., 1987). Miao (2003) found that germination reduced phytic acid in soybean by 62.80%. Wang et al. (2015c) found that the phytic acid content of soybean germinated for 6 days was 70% lower than that of non-germinated soybean, while the iron and zinc bioavailability were significantly increased. Beleia et al. (2010) considered that the decrease in phytic acid content was mainly due to the activation of endogenous phytase and phosphatase by germination. Under the action of Phytase and phosphatase, phytic acid and phytate were hydrolyzed in large quantities, releasing elements such as phosphorus, thus weakening the binding of mineral elements by phytic acid. Bove et al. (2001) pointed out that after more than 60% of the combined seed hydration in seed germination, the water uptake of the seeds entered a sluggish stage, and the metabolic activities within the seeds began to be active, and the ions in the osmotic active substances accumulated in large quantities, cell wall acidification promotes cell wall polymer loosening. At the same time, with the increase of H^+-ATPase activity, it further promotes the seed water absorption and weakens the restriction of the surrounding tissues (such as endosperm). Therefore, from the above reports, we can see that the mechanism of phytase and its interaction with phosphatase can lead to the degradation of phytic acid, releasing a large amount of Pi for ATP synthesis. This is the main reason for the rapid decrease of phytic acid after imbibition start.

4 Outlook

Soybean is an indispensable food and dish in our daily life, which contains rich nutrients and functional active substances. Soybean germination can improve the nutritional factors of soybean and reduce the anti-nutritional factors, which is very beneficial to improve the nutritional value and safety of soybean products. However, there are still some problems to be discussed in the practical research on improving the quality of soybean products by germination technology:

- (1) Since there is lack in literature describing technological aspects of seed germination, there are many valuable sources on germination physiology. Some of the pathways are same for all seeds, but some are species and genotype specific;
- (2) It is very important to study the effect of germination on the expression of key enzyme genes of soybean functional nutrient factors. Soy functional factors have a wide range of health care effects, including anticancer and antihypertensive effects, improvement in cardiovascular disease, type 2 diabetes and obesity (Pedrosa et al., 2021). Studies have shown that Isoflavones can reduce atrazine induced oxidative stress and inflammation, as shown by the accumulation of Malondialdehyde and Glutathione depletion, and increase the release of TNF A and IL-6 (Jang et al.,

2021), respectively, in the substantia Nigra, among them, Genistein and curcumin have inhibitory effects on prostate cancer cells (Aditya et al., 2013), and dietary isoflavones can also reduce osteoporosis-induced bone loss (Zheng et al., 2016), and also have anti-aging effects, it can be used in some skin care products with cosmetic effect. With the rapid development of modern biological technology, such as over-expression, gene knockout, proteomic transcriptome sequencing and other technologies have been widely used in plants, animals and microorganisms. How to use modern biological technology to study the key control enzyme gene expression mechanism of soybean functional nutrient factors during germination, so as to achieve"Directional" control of the content of functional nutrient factors. The study provided a possibility to improve the functional and nutritional quality of soybean;

- (3) In addition, given that sprouting increases the mineral content of calcium, iron, zinc and various vitamins in soybean products, certain mineral and vitamin deficiencies in the human body can be increased by dietary therapy with the consumption of sprouting soybean products, as a supplement to drug therapy, it has obvious practical value. In a sense, germination technology improves the nutritional value of soybean products, which is in line with People's idea of pursuing a healthy life and has a broad research prospect.

Ethical approval

Not Applicable.

Conflict of interest

All authors declare that they have no conflict of interest.

Availability of data and material

Not Applicable.

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Author contributions

Conceptualization, methodology, Jinting Lu, Ying Zhang; investigation, Jianghua Cheng, Yayuan Xu, Yujie Chen; writing-original draft preparation, writing review and editing, Ying Zhang, Jinting Lu; supervision, Ying Zhang, Kun Qian. All authors have read and agreed to the published version of the manuscript.

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