

Soybean secondary metabolites and flavors: The art of compromise among climate, natural enemies, and human culture

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Abstract

Soybean is a food crop in high demand in Northeast Asia. Besides protein and oil, soybean is also a rich source of health-beneficial secondary metabolites such as flavonoids, terpenes, and alkaloids. The long history of soybean domestication resulted in a rich collection of soybean germplasms, which could be generally categorized as wild, land-race, and cultivated soybeans. Previous research has shown that soybean seeds from diverse genetic backgrounds exhibited different metabolite profiles. Germplasms originating from different geographical regions, i.e., at different latitudes and longitudes, probably experienced different selective pressures and evolved different secondary metabolite profiles. Domestication has generally led to a reduction in secondary metabolite contents in seeds since many of these compounds are related to the bitter taste or other agronomic traits that may hinder the ease of farming and harvest. These selection forces have possibly rendered the different flavors of soybean germplasms. Due to the popularity of soy food products, the post-domestication selection of soybean based on flavor is a common phenomenon. In Northeast Asian countries, soy foods such as soy milk, tofu, and fermented soy products are popular. Based on the consumer preference for the flavors of these products, soybean germplasms with different metabolite profiles are selected for different commercial uses. However, the breeding of soybeans for maximizing health benefits and for the preferred flavors of food products may create contradictions. Industrial methods to remove undesirable flavors and molecular breeding to produce cultivars with desired metabolite profiles may be the solution.



1. Introduction

Soybean (*Glycine max* [L.] Merr.) is an important legume crop, the seeds of which serve as human food, animal feed and a source of edible oil (Hartman, West, & Herman, 2011). Soybean is also important in the fossil fuel-free oil production for the biofuel industry (Koc, Abdullah, & Fereidouni, 2011), the production of industrial biomaterials and for the pharmaceutical industry (Chung & Singh, 2008). It is essential in sustainable agriculture because of its ability to enrich soil with nitrogen through biological nitrogen fixation, thus reducing the reliance on chemical fertilizers and their impact on soil quality and greenhouse gas emissions from their production and application (Stagnari, Maggio, Galieni, & Pisante, 2017).

Demands for soybeans are high in Northeast Asian countries including China, Japan, the Republic of Korea, and Russia, where in 2019, the quantity of soybean imports ranked in the top five among major crops (Fig. 1) (FAO, 2021). Besides proteins and oils, soybean is a reservoir of a rich variety of secondary metabolites, some of which are of high nutraceutical/pharmaceutical value to humans. Examples include isoflavones that act as phytoestrogen and are abundantly found in soybean (Messina, 1999; Messina, Persky, Setchell, & Barnes, 1994). Unlike primary metabolites, secondary metabolites are not critical for essential life processes like growth and development. However, these metabolites offer plants adaptive traits and enhance their ecological fitness (Ku, Contador, et al., 2020). The molecular structures and cellular profiles of secondary metabolites in plants are often specific to taxonomically related groups, except for some key enzymes that may have a wider distribution in the plant kingdom through horizontal gene transfer mediated by endophytic bacteria or fungi (Wink, Botschen, Waterman, Schäfer, & Waterman, 2010). By acting both as defence chemicals against natural enemies and signaling molecules mediating communications between plants and symbiotic microorganisms, secondary metabolites promote the physiological fitness of plants within their local communities (Wink et al., 2010). To combat against natural enemies such as herbivores, many of the secondary metabolites have unpleasant flavors.

Being a popular part of the human diet, soybean has undergone domestication with the conscious selections for desirable traits. For legumes in general, since many of the secondary metabolites are related to the bitter taste of seeds or other agronomic traits that may hinder the ease of farming and harvest, domestication has generally led to a reduction in secondary metabolites

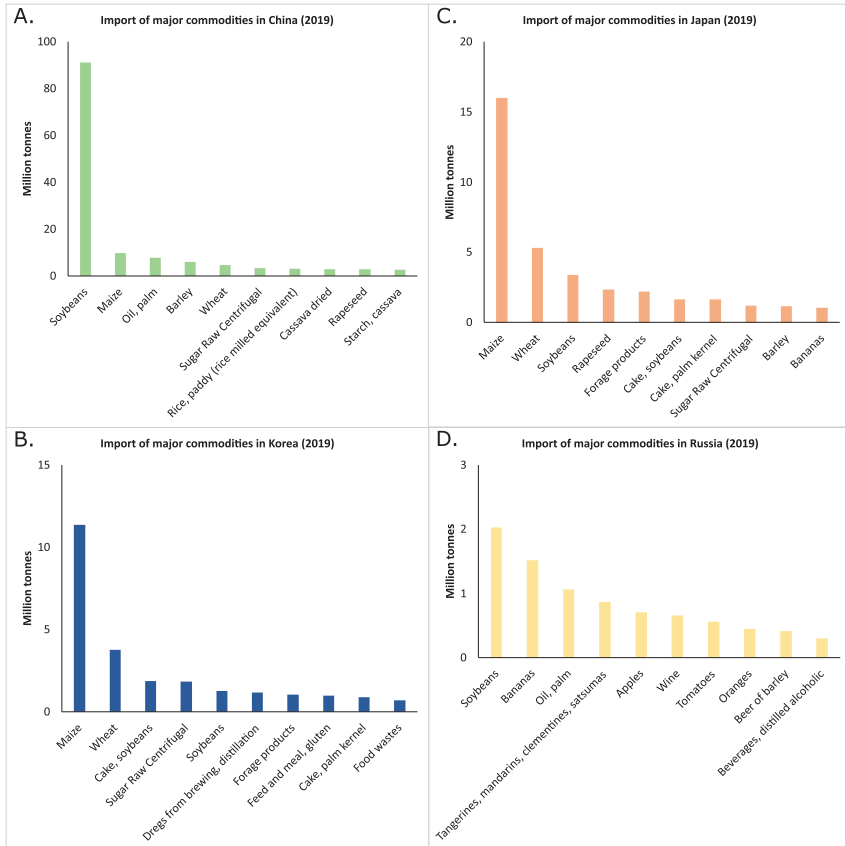


Fig. 1 Import of major commodities in (A) China, (B) Korea, (C) Japan, and (D) Russia in 2019 (FAO, 2021).

in cultivated germplasms and has created a bottleneck in genetic diversity when compared to their wild progenitors (Ku, Contador, et al., 2020). This may have caused the loss of potentially beneficial secondary metabolites among the cultivar populations. On the other hand, the elimination of secondary metabolites that give undesirable tastes from soybean cultivars would be preferable for increasing the acceptance of soy products in the consumer market. Understanding the shifts in secondary metabolite compositions in soybean as influenced by human selection and environmental adaptations, and their underlying genetic basis, might help to build (or re-build) favorable soybean secondary metabolite profiles that fit the needs of environmental adaptations, nutritional value, and flavor. This could be accomplished by exploiting the genetic richness of the wild populations.



2. The diversity of soybean germplasms

Metabolite profiling and genetic analyses of soybean have shown the genetic diversity among germplasms collected from different parts of the world, including China, South Korea, North Korea, Japan, United States, Brazil, Pakistan, Russia, Ghana, South Africa, Zambia, Ethiopia, Cameroon and Nigeria (Chander et al., 2021; Karikari, Bhat, Denwar, & Zhao, 2020; Malik et al., 2017; Valliyodan et al., 2021). The diverse genetic backgrounds of soybean germplasms imply correspondingly diverse metabolite profiles. This speculation is supported by previous findings showing that soybean germplasms with a long history of breeding selection for specific traits have diverse metabolite profiles (Kusano et al., 2015). In another study, it was also demonstrated that the metabolite profiles of soybean seeds from diverse genetic backgrounds showed accession-dependent genetic diversity (Lin et al., 2014). For example, a major difference in seed metabolite profiles was observed between black and yellow soybean seeds (Jo et al., 2021). This seed color-associated difference in metabolite profiles is thought to be a consequence of the differential accumulation of flavonoids and anthocyanins in the epidermal layer of the seed coat. Interestingly, soybean seeds with black seed coats and green cotyledons tend to have higher anthocyanin and phenolics with health benefits than the more commonly cultivated soybean with yellow seed coats and yellow cotyledons (Jo et al., 2021). This creates a niche for black soybean in pharmaceutical applications. The diverse secondary metabolite compositions in cultivated and wild soybean germplasms have been previously reviewed (Ku, Contador, et al., 2020).



3. Major biochemical pathways for the synthesis of secondary metabolites in soybean, and their molecular structures and nutraceutical/ pharmaceutical values

Enzymes involved in the biosynthetic pathways of secondary metabolites are possible targets for genetic engineering. The biosynthetic pathways also hint at the nutraceutical values of the secondary metabolites, which are usually related to the molecular structure of these metabolites.

3.1 Flavonoids

Flavonoids are a group of naturally occurring polyphenols that are widely distributed in the plant kingdom. Based on the variations in their molecular

structure, flavonoids are classified into several sub-groups including flavones, flavonols, flavanones, flavanonols, anthocyanidins, flavanols, and isoflavones (Ku, Ng, et al., 2020). Different modifications within each sub-group further increase the functional diversity of flavonoids. Among the various sub-groups, isoflavones are most abundant in soybean and render it the main food source of this widely adopted health supplement (Křížová, Dadáková, Kašparovská, & Kašparovský, 2019). There are three major isoflavones in soybean, namely genistein, daidzein and glycitein. The aglycone form, which is also known as the free form without any conjugate, is biologically active while isoflavone glycosides and their glycoconjugates confer stability and solubility for storage in seeds (Ku, Ng, et al., 2020). The amino acids, phenylalanine and tyrosine, are the precursors of secondary metabolite biosynthesis in the phenylpropanoid pathway (Dong & Lin, 2020). Their conversion into various secondary metabolites in this pathway is illustrated in Fig. 2. Phenylalanine is first converted into S-(cinnamoyl)-CoA by phenylalanine ammonia lyase (PAL) together with 4-coumarate-CoA ligase. Naringenin chalcone and isoliquiritigenin are generated by chalcone synthase (CHS) or chalcone reductase (CHR). Chalcone isomerase (CHI) then converts them into naringenin and liquiritigenin, which are then converted into the isoflavonoids, genistein, daidzein and glycitein, which are particularly enriched in soybean seeds (Fig. 2).

Due to the structural similarity between flavonoids (particularly genistein and daidzein) and mammalian estradiol, they are widely recognized as phytoestrogens that display therapeutic and chemopreventive effects on hormone-related cancers, such as ovarian and breast cancers, with epidemiological evidence (Lee, Kim, & Song, 2012; Nagata et al., 2014). Genistein, among the soy isoflavones, has been demonstrated to be the most significant contributor to the anticancer property of soybean (Ahmad et al., 2014). It is found to exert pleiotropic effects in modulating genes in multiple signaling pathways that control cell cycle and apoptosis (Banerjee, Li, Wang, & Sarkar, 2008). Apart from the anti-carcinogenic effects, flavonoids are reported to be antioxidants with free radical-scavenging abilities, exhibiting protective effects against oxidative stress caused by the accumulation of reactive oxygen species (ROS) (Mira et al., 2002). Other beneficial physiological effects of soy isoflavones include anti-inflammatory properties, and preventive effects on many degenerative and chronic diseases such as cardiovascular diseases, obesity, diabetes, and osteoporosis (Křížová et al., 2019). Owing to the high nutraceutical values brought forth by flavonoids, soybean has been regarded as a functional food that can promote human health through dietary consumption.

Phenylpropanoid pathway

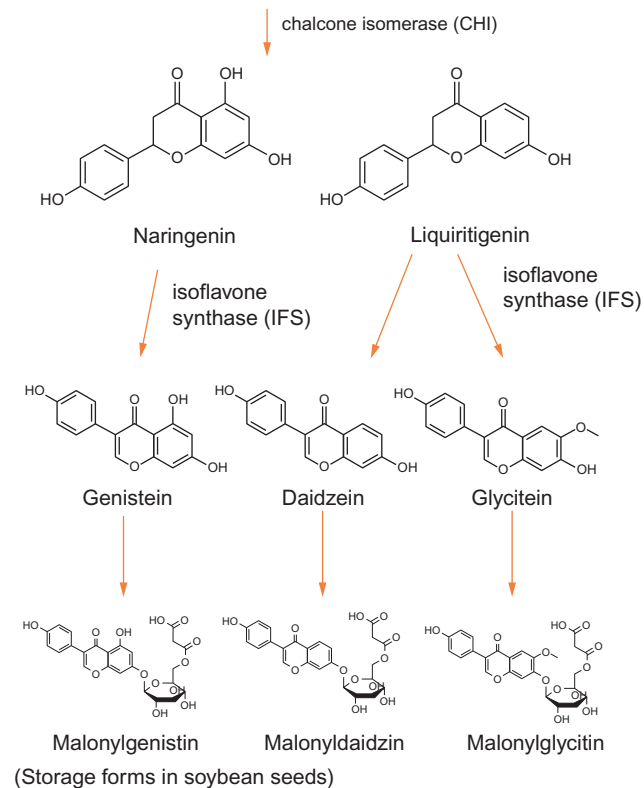
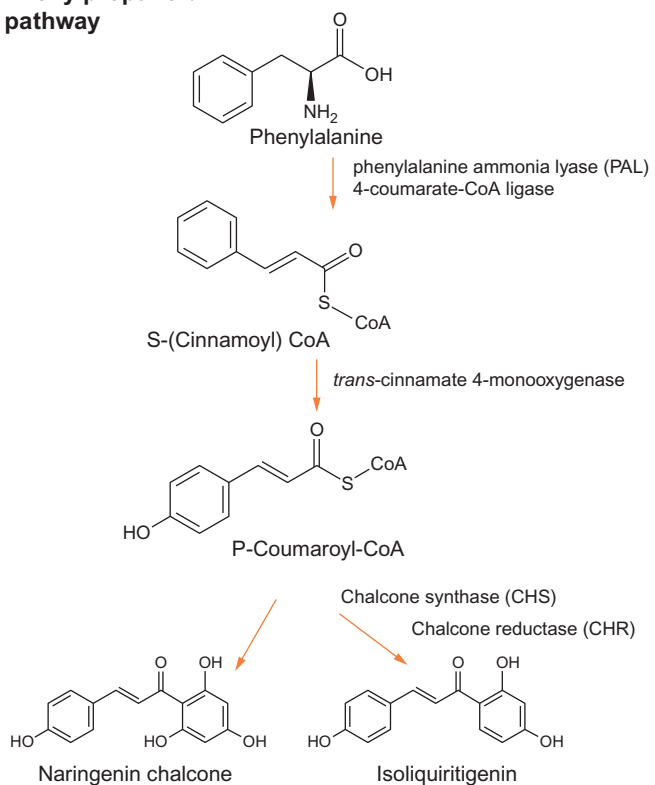


Fig. 2 The phenylpropanoid pathway for flavonoid biosynthesis.

In the plant, flavonoids help mediate interactions with its abiotic and biotic environments. For symbiotic interactions, soy isoflavones act as signaling molecules to initiate legume-rhizobium communications for the formation of nitrogen-fixing nodules (Kosslak, Bookland, Barkei, Paarent, & Appelbaum, 1987). Microbial infections or pathogen attacks also stimulate the biosynthesis and accumulation of soybean isoflavones such as glyceollin, an isoflavonoid phytoalexin, to develop resistance against biotic threats (Araújo et al., 2015; Sepiol, Yu, & Dhaubhadel, 2017). In the face of abiotic stresses, flavonoids protect plants from UV damage by absorbing UV radiation and quenching the resulting reactive oxygen species (ROS) (Kumar & Pandey, 2013). Their antioxidant properties also allow soybean to combat oxidative stresses caused by high salinity (Wu et al., 2008).

In tracking the effect of domestication on flavonoid contents in soybean, the average total seed isoflavone level in wild soybean (*Glycine soja*) was found to be higher than that in the cultivated accessions (Wang, Zhao, & Gai, 2010). In general, wild soybeans possess higher antioxidant content than cultivated ones, and such differences highly correlate to the seed total flavonoid levels (Li et al., 2016). Interestingly, a negative correlation was found between the total isoflavone levels in cultivated soybean and the longitudes and latitudes across different geographic regions of China in which these cultivars were grown, while no such correlation was found in wild accessions, indicating that climate, and possibly human preference, could have contributed to the selection pressure on soybean during the domestication process (Wang et al., 2010).

3.2 Terpenes

3.2.1 Saponins

Saponins are triterpenes or sterol glycosides present in a number of food plants such as legumes, ginseng, garlic, and tea (Oakenfull, 1981), and soybean is one of their major dietary source for both human food and animal feed. Five bioactive saponins have been isolated from soybean, complete with elucidated structures. Soyasaponin I, II and III are group B saponins while soyasaponin A₁ and A₂ are group A saponins (Kitagawa, Saito, Taniyama, & Yoshikawa, 1985; Kitagawa, Yoshikawa, & Yosioka, 1976). Group B saponins are the primary saponins in intact soybeans and generally exist in the 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP)-conjugated form (Kudou et al., 1993). Saponins are derived from the terpenoid (isoprenoid) pathway. Two pathways, the mevalonate pathway and the non-mevalonate pathway (Fig. 4), are involved in isoprenoid biosynthesis.

In the mevalonate pathway, acetyl-CoA is converted into isopentenyl pyrophosphate (IPP), which could be converted to dimethylallyl pyrophosphate (DMAPP) through a reversible reaction. DMAPP is then converted to isoprene (Fig. 3). The non-mevalonate pathway is an alternative IPP and DMAPP biosynthetic pathway (Fig. 4). The mevalonate and non-mevalonate pathways both produce the same products but through different enzymatic reactions. While the former pathway takes place in the cytoplasm of plant cells, the latter pathway occurs in bacteria, eukaryote parasites, and the plastids of plant cells (Banerjee & Sharkey, 2014).

The amphiphilic nature of saponin molecules, which is contributed by the hydrophobic triterpene and the hydrophilic sugar, allows them to act as a natural surfactant to enhance the water and nutrient uptake by plants (Fournier, Erdman, & Gordon, 1998). Moreover, in response to abiotic stresses, the accumulation of saponin would be induced to protect plants from oxidative stress (Wu et al., 2008). For example, the group B saponins were found to be one of the key secondary metabolites that conferred salt tolerance in soybean, making saponins important secondary metabolites in soybean (Wu et al., 2008). The anti-herbivory properties of saponins make them natural insect repellents that protect the plant from biotic threats (De Geyter, Lambert, Geelen, & Smagghe, 2007).

When comparing between wild and cultivated soybean germplasms, clear variety-specific differences could be observed in their saponin profiles. In a study using 800 *Glycine max* and 329 *Glycine soja* germplasms, saponin type AaBc was found predominantly in wild soybeans but it only accounted for 0.3% of total saponin in the cultivated accessions, in which saponin type Aa was predominant (Tsukamoto, Kikuchi, Harada, Kitamura, & Okubo, 1993). In another study on 3025 wild soybean accessions (*Glycine soja*), a large variation in saponin contents was observed (Krishnamurthy, Chung, & Singh, 2013). However, it appeared that group B saponins, especially saponin Bc and Bg, were lost during soybean domestication (Tsukamoto et al., 1993). Therefore, the diverse genetic backgrounds governing saponin compositions in the wild soybean germplasms (*Glycine soja*) could be employed in engineering the cultivated accessions to produce the beneficial group B saponins.

In terms of nutraceutical/therapeutic value, saponins are highly beneficial to humans. Like flavonoids, saponins (particularly those in the DDMP-conjugated form) can act as antioxidants through their high ability to scavenge free radicals (Yoshiki, Kahara, Okubo, Sakabe, & Yamasaki, 2001). Other benefits including anti-inflammatory, anticarcinogenic, hypocholesterolemic,

**Terpenoid backbone biosynthesis
mevalonate pathway**

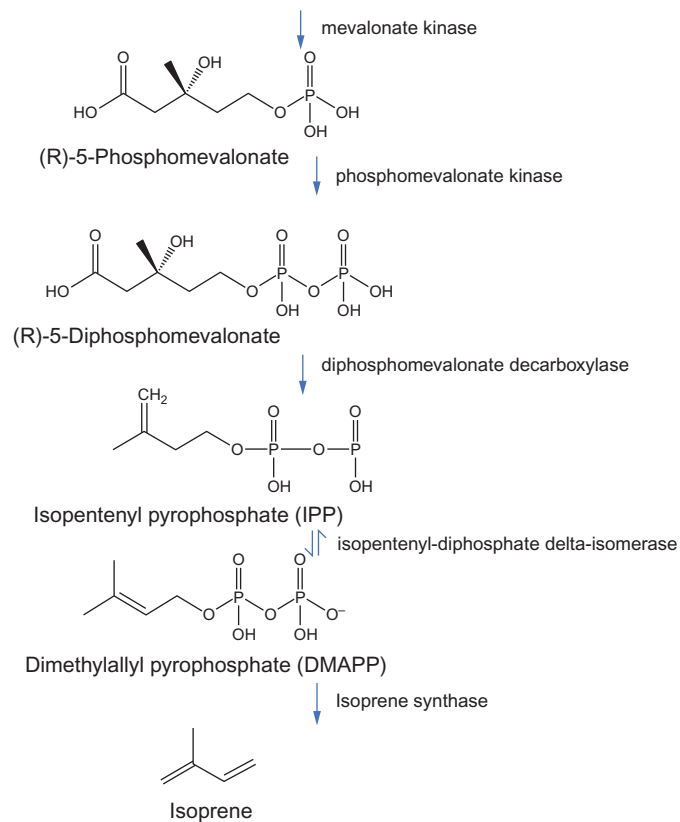
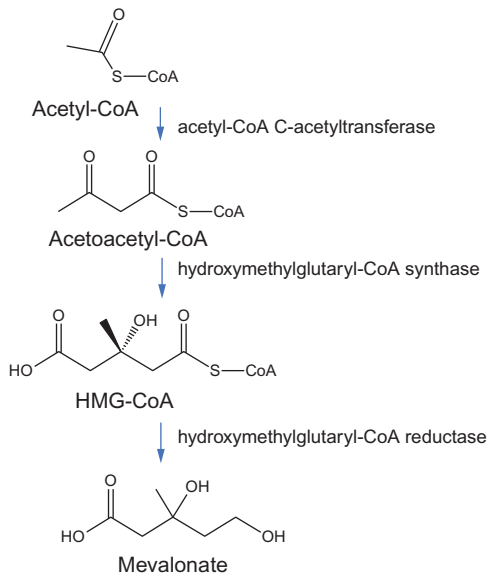


Fig. 3 The mevalonate pathway for isoprene biosynthesis.

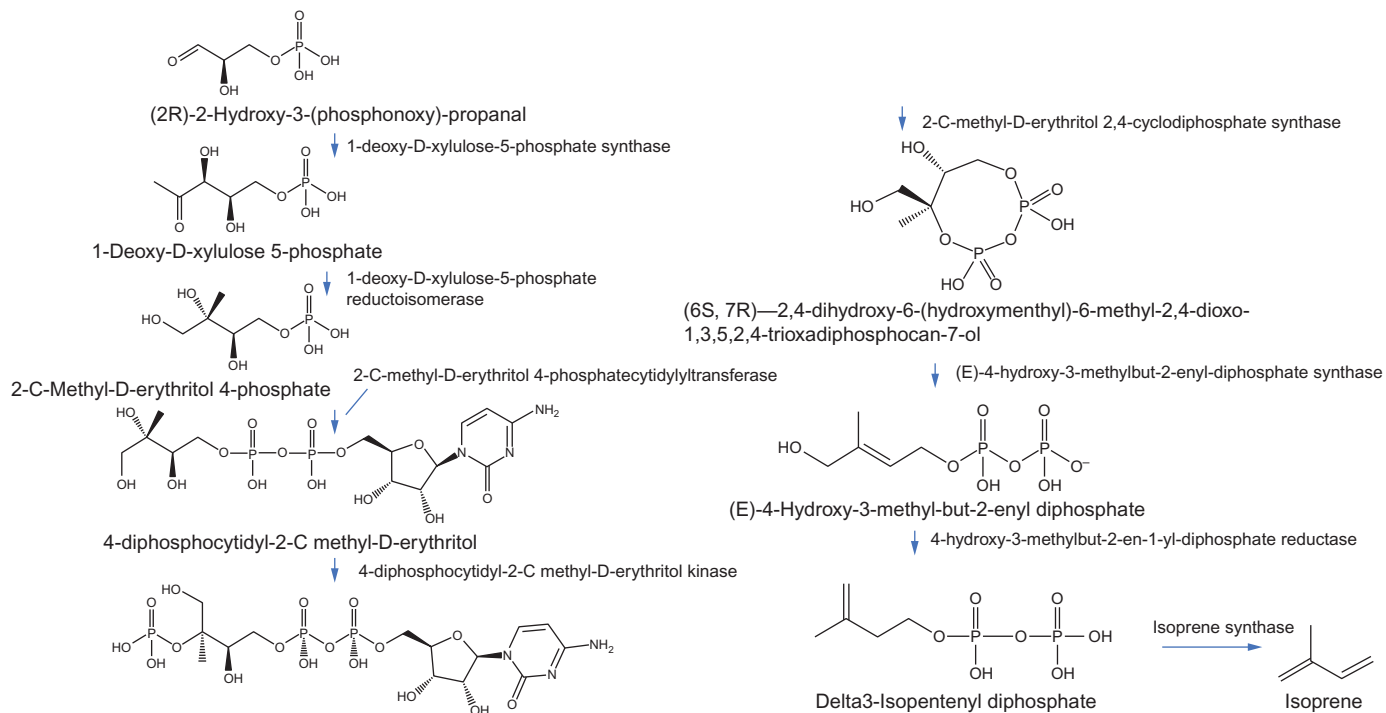


Fig. 4 The non-mevalonate pathway for isoprene biosynthesis.

immunostimulatory, hepatoprotective, and antidiabetic abilities have also been reported with soyasaponins (Fournier et al., 1998; Surh et al., 2001). Although saponins from other plants, such as alfalfa, were found to have strong hemolytic activities and were relatively toxic to some poikilothermic animals such as fish and frog, saponins derived from soybeans were found to be non-toxic and innocuous to mice, chicks, and rats, even when these animals were fed soyasaponins at levels three times higher than the natural level in soybean (Ishaaya, Birk, Bond, & Tencer, 1969). Thus, the dietary intake of soyasaponins should pose no threat to human health (Oakenfull & Sidhu, 1990).

3.2.2 Phytosterols

Phytosterols are a group of steroidal triterpenes naturally occurring in plants (Wang, Yao, & Wei, 2011). The four major types of soybean phytosterols include β -sitosterol, campesterol, stigmasterol, and brassicasterol (Wang et al., 2011). Differences among these phytosterols are mainly due to the presence/absence of a double bond at C22 and the types of substituents at C24 of the molecule (Wang et al., 2011).

Soybean oil deodoriser distillate (SODD) is the main source for commercial phytosterol extraction (Benites, Concha, Reis, & De Oliveira, 2009). The conversion of soybean phytosterols to therapeutic steroidal hormones has long been a practice of the pharmaceutical industry (Wang et al., 2011). The typical A-ring structure with a 5,6-double bond and a 3 β -hydroxyl group makes soybean phytosterols an ideal raw material for producing steroidal hormones. Almost all kinds of steroidal hormones, including adrenocortical hormones and sex hormones, could be generated from the intermediates derived from soybean phytosterols (Wang et al., 2011).

Because of the structural similarity between phytosterols and cholesterol, the direct intake of soybean sterols through diet can help reduce the absorption of cholesterol in the intestines, thereby lowering the level of low-density lipoproteins in the serum, and eventually preventing atherosclerosis or other cardiovascular diseases in human (Ostlund, Racette, & Stenson, 2003). Other beneficial properties of phytosterols include anti-inflammatory, antipyretic, antineoplastic, and immunomodulating activities (Shi, Nam, & Ma, 2010).

Like cholesterol in animals, phytosterols are responsible for regulating the fluidity and maintaining the normal functions of the plant cell membrane. This includes cellular proliferation and differentiation (Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000), which explains the increase in phytosterol content in soybean seeds during germination (Shi et al., 2010).

3.3 Alkaloids

Alkaloids are a group of nitrogen-containing heterocyclic secondary metabolites with great structural diversities. Being in the legume family that possesses nitrogen-fixing ability, soybean can therefore produce more alkaloids than food crops belonging to other families (Wink, 2013).

Alkaloids are mainly derived from lysine, nicotinate, and ornithine, which are all derivatives of L-aspartate. For lysine-derived alkaloids, L-aspartate is first converted to L-lysine, which is then converted to cadaverine and indolizidine by the activities of lysine decarboxylase and lysine 6-dehydrogenase, respectively. Cadaverine is converted to quinolizidine alkaloids or piperidine alkaloids by a series of enzymatic reactions. The quinolizidine ring is then formed from the cyclization of cadaverine (Suzuki, Koike, Murakoshi, & Saito, 1996). Once the quinolizidine skeleton is formed, various alkaloid products can be generated by enzymes such as hydroxylases and dehydrogenases. For nicotinate-derived alkaloids, quinolinate is converted from L-aspartate by quinolinate synthase. Using quinolinate as the precursor, nicotinate is synthesized by nicotinate-nucleotide pyrophosphorylase, and then eventually converted to pyridine alkaloids through a series of enzymatic reactions. In the ornithine pathway, L-glutamate is first converted into L-ornithine, and then through decarboxylation into putrescine. Next, putrescine is converted to pyrrolizidine alkaloids, pyrrolidine alkaloids or tropane alkaloids (Lichman, 2021). The biosynthesis of alkaloids is illustrated in Fig. 5.

Quinolizidine alkaloids (QAs) are mainly found in *Lupin* spp. and have been employed in pharmaceutical uses due to their antibacterial, antiviral, antipyretic, and cytotoxic activities (Saito & Murakoshi, 1995). In bitter *Lupinus angustifolius* accessions, the activity of lysine decarboxylase is high in leaves, stems, pedicels, and pods while the level of QAs is high in the phloem (Otterbach, Yang, Kato, Janfelt, & Geu-Flores, 2019). It was therefore suggested that QAs are synthesized in other tissues and then transported to seeds (Otterbach et al., 2019).

QA biosynthesis in soybean has not been fully characterized. However, both known and novel alkaloids have been isolated from soybean greens (Wang et al., 2016). The known alkaloids include gensenine, (1S,3S)-1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid, (1R,3S)-1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid, and indole-3-carboxylic acid. The newer alkaloids include soyalkaloid A and isoginsene (Wang et al., 2016). Likewise, lysine decarboxylase has been characterized and isolated from soybean (Kim, Kim, & Cho, 1998). In another study, lysine decarboxylase activities were detected in the extracts of soybean root and hypocotyl (Matsuzaki & Soybean, 2009).

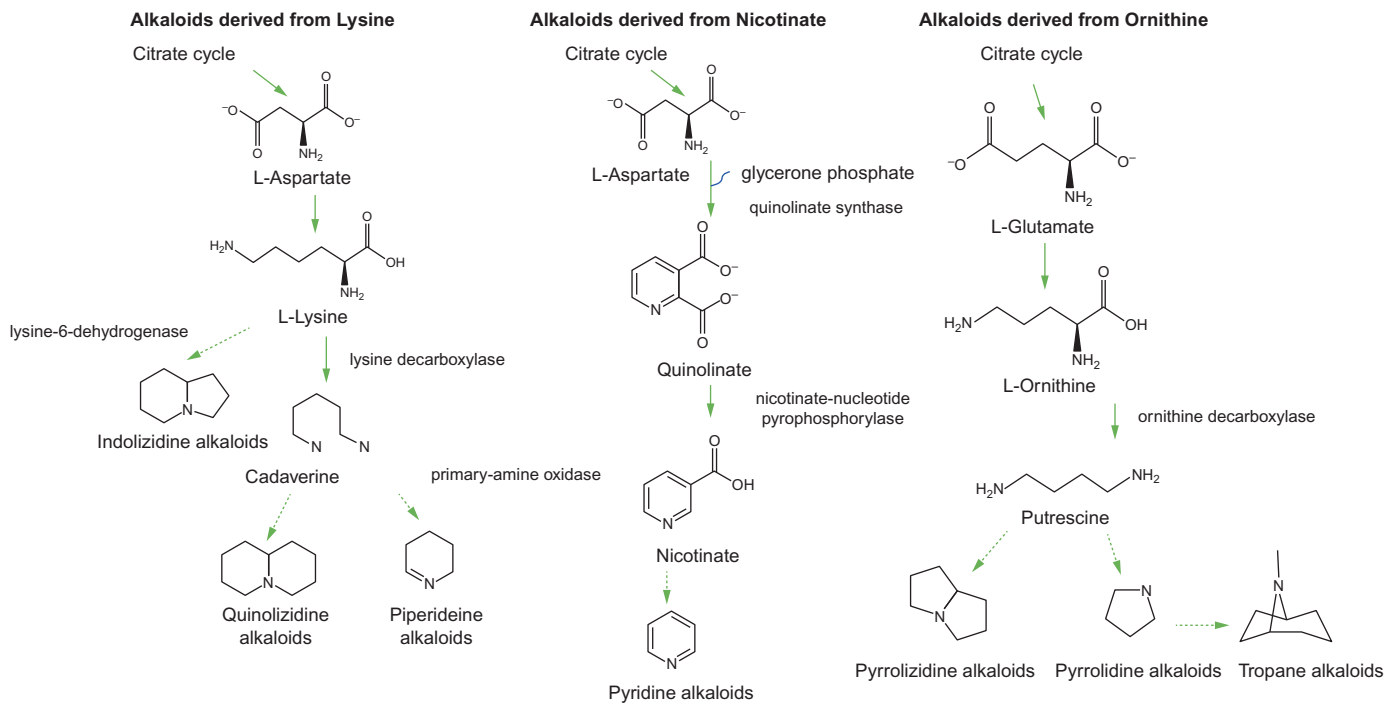


Fig. 5 The pathway for alkaloid biosynthesis.

Many alkaloids are reported to have pronounced pharmaceutical values. They possess anticancer activities (Gupta et al., 2015), anti-malarial activities (Onguéné et al., 2013), neuroprotective potential of preventing stroke by facilitating blood circulation in the brain (Kumar & Khanum, 2012), and potential as therapeutic drugs for targeting neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease (Amirkia & Heinrich, 2014). In addition, the newly identified soyalkaloid A was found to be able to ameliorate concanavalin A-induced hepatic injury (Wang et al., 2016).

Many alkaloids are neuromodulators or neurotoxins and are thought to have evolved in plants for defence against pathogens and herbivores (Wink, 2013). In one study on the causal constituents of insect resistance in soybean cultivars, quinic acid, which is derived from quinine, is identified to be one of the chemicals with dramatically different levels between the insect-resistant and insect-susceptible lines (Tester, 1977).



4. The influence of climate on soybean secondary metabolite production

4.1 Impacts of latitude on secondary metabolites in soybean: Photoperiod and temperature

Geographical regions at different latitudes have different daylengths and temperatures. Daylength, a measurement of the photoperiod during which plants receive light radiation from the sun, has been known to affect the vegetative period and the time to flowering. Average daily temperature also affects the timing for planting and the rate of growth within a growing season. Evidence showed that planting soybean too early in the season when daylength was short led to premature flowering and reduced yield (Board & Hall, 1984; Islam, Fujita, & Zheng, 2019), while planting too late in the season with shortening daylength was also enough to cause premature flowering and reduced yield (Board & Hall, 1984; Islam et al., 2019). Planting soybean at warmer temperatures was found to reduce the time to first flowering compared to planting at cooler temperatures, and the temperature effects were more prominent under short daylength than under long daylength (Board & Hall, 1984).

Besides influencing the time to flowering and yield, latitude and longitude, which are associated with daylength and temperature, also have a significant effect on secondary metabolite profiles. The diverse seed isoflavone profiles among wild soybean accessions originating in different geographical areas in Korea have been documented (Tsukamoto et al., 2018).

Using geographical data and orthogonal partial least-squares discriminant analyses on soybeans grown at different longitudes and latitudes in China, a study demonstrated that growing soybean at high longitudes yields seeds with higher isoflavone contents. It also showed that soybean grown at higher latitudes had higher accumulation of anthocyanins (Liu et al., 2017). The same study illustrated that enhanced biosynthesis of isoflavones occurred in high-longitude environments whereas a low-longitude environment promoted anthocyanin biosynthesis (Liu et al., 2017). Existing evidence suggests that total phenolic and isoflavone contents in soybean seeds from cooler regions are higher than those from warmer regions (Król-Grzymała & Amarowicz, 2020). However, there is a trade-off between desired metabolite profiles and yield because higher average daytime temperatures within physiological limits are beneficial to soybean yield (Zheng, Chen, & Han, 2009). Higher mean daily maximum temperature during pod filling leads to grain yield increase by a range of 6–10% for each 1 °C rise. This can lead to more than 22% of total yield increase per growing season at the optimal growth temperature during the pod filling stage (Zheng et al., 2009).

Taking daylength and temperature into consideration, it is thus important to identify soybean accessions suitable for cultivation in specific geographical regions. Soybean cultivars of different maturity groups have different photoperiod responses for the adaptation to different daylength conditions (Yang et al., 2019). It is possible to breed early-maturing soybean accessions to be grown under short daylength without compromising the yield. Similarly, it will be of interest to exploit the influences of temperature and latitude as a means to tailor soybean breeding to enhance the production of health-beneficial secondary metabolites.

4.2 The role of secondary metabolites in facilitating adaptations to climate change

Climate change is characterized by the increased frequencies and intensities of climate-related disasters including floods, heat waves and drought (Pareek, Dhankher, & Foyer, 2020). These stresses have a detrimental effect on agricultural production and food security, not only because they lead to low crop yields for human food and animal feed, but also because such low yields drive up agricultural commodity prices, which in turn exacerbates the impoverished state of the livelihoods of poor communities. It is thus important to exploit the vast genetic differences between cultivated crops and their wild relatives as a means to re-introduce genes into cultivated crops to enhance their adaptation to the stresses associated with climate change.

Primary and secondary metabolites are differentially accumulated in soybean leaves in response to drought and heat stress (Das, Rushton, & Rohila, 2017). Metabolites associated with carbohydrate, amino acid and peptide metabolism, such as purine and pyrimidine biosynthesis, were impacted by drought and heat stress (Das et al., 2017). Sugar and nitrogen metabolism were also shown to play important roles in regulating the responses to drought and heat stress in soybean (Das et al., 2017).

The consequence of flooding is hypoxia in crops since the water overload limits gas exchange between the atmosphere, the soil and the plant. By contrasting the metabolite profiles of two soybean germplasms with different responses to flooding stress, it was shown that flooding highly affected primary and secondary metabolites in soybean plants. The most affected primary and secondary metabolites were those involved in carbon and nitrogen metabolism; and those involved in the phenylpropanoid pathway, respectively (Coutinho et al., 2018). Integrating the knowledge from metabolomics and proteomics can allow us to accurately pinpoint the key regulators of flooding responses by crop plants, including soybean. Specifically, proteomic analyses have produced evidence strongly suggesting that the most affected proteins due to flooding are related to development, protein synthesis or degradation, and secondary metabolism (Komatsu, Makino, & Yasue, 2013). These proteins were found to be involved in pathways consistent with those uncovered by metabolic profiling of flooding response (Coutinho et al., 2018). Given that genome sequencing of cultivated soybean accessions (*Glycine max*) and their wild relatives (*Glycine soja*) has been proposed as a tool for revealing the mechanisms underlying processes involved in regulating potential pathways for adaptation to climate change (Li et al., 2017), it should be possible to translate this information to comparative proteomic and metabolite profiling to shed light on the genes, proteins and metabolites that are important for the adaptation to climate change. This would require screening contrasting (sensitive vs tolerant) lines and analyzing their genomic variations, as well as the transcriptomic, proteomic and metabolomic responses to drought, heat and flooding stresses.

Hence, such analyses emphasize the relevance of omics approaches to the development of new soybean varieties that can adapt to climate change. Furthermore, it is evident that secondary metabolites in the phenylpropanoid and anthocyanin biosynthesis pathways play a critical role in conferring tolerance to the three abiotic stresses characteristic of climate change, namely drought, heat and flooding.



5. The influence on soybean secondary metabolite production by natural enemies

5.1 Overview of the perception of biotic stress by plants

Plants are sessile and therefore are unable to physically evade microbial infections and herbivore attacks. Instead, plants have evolved a range of defence mechanisms in response to these biotic stimuli, such as the production of antimicrobial or toxic compounds, which are either constitutively produced or synthesized when challenged by biotic stresses. Phytohormones and secondary metabolites such as phenolics and flavonoids play an important role in plant defence responses.

Defence against pathogens starts with the recognition of non-self by plants. Molecules involved in non self-recognition have been extensively reviewed. In summary, fragments of flagellin, DNA, lipoproteins, and lipopolysaccharides from microbes serve as pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs). Recognition of PAMPs or MAMPs by the plant pattern recognition receptors (PRRs) would initiate the downstream defence responses (Ku, Cheng, et al., 2020; Ku, Contador, et al., 2020; Ku, Ng, et al., 2020; Medzhitov & Janeway, 1997). Herbivores produce a suite of herbivore-associated molecular patterns (HAMPs) similar to PAMPs and MAMPs, which induce a set of anti-herbivory defence responses (Felton & Tumlinson, 2008; Mithöfer & Boland, 2008). In plants, the perception of invasions, whether microbial or herbivore-related, rapidly activates ion flow mechanisms, modulates the production of reactive oxygen species (ROS), modifies primary metabolism and the biosynthesis of secondary metabolites, and induces the expressions of defence genes (Romero-puertas, Terrón-camero, Peláez-vico, Molina-moya, & Sandalio, 2021).

5.2 Soybean plants can distinguish between mechanical damage and herbivore attack

Plants have evolved sophisticated signaling networks to perceive and cope with herbivory. The proteomic analyses of the salivary secretion of green stink bug, *Nezara viridula*, identified a significant portion of the active enzymes to be associated with digestion, detoxification, and antioxidation (Giacometti et al., 2020; Lomate & Bonning, 2016). Soybean plants can distinguish between mechanical damage and herbivore attack. When developing soybean seeds are damaged, lignin accumulates in the lacerated area

and leads to seed cell wall thickening, but this only occurred in the presence of insect saliva, and not after mechanical damage (Giacometti et al., 2020).

In soybean, it has been shown that the damage by stink bugs is perceived by mitogen-activated protein kinases (MPKs), which triggered jasmonic acid (JA)/ethylene (ET)- and salicylic acid (SA)-regulated defences downstream through the MPK cascades (Giacometti et al., 2020). A similar pathway of defence response activation was also reported in *Nicotiana attenuate* upon herbivory attack (Wu, Hettenhausen, Meldau, & Baldwin, 2007). In soybean, upon stink bug feeding on the pod, the expressions of *MPK3*, *MPK4*, and *MPK6* in the seed were induced (Giacometti et al., 2016). In addition, the *MPK3*, *MPK4*, and *MPK6* proteins were activated by phosphorylation (Giacometti et al., 2016). On the other hand, mechanical damage alone did not elicit the same effects on the MPK genes and proteins as the damage due to stink bug feeding. Although mechanical damage also induced the expression of *MPK6* and phosphorylation of *MPK6*, it repressed the expression of *MPK3* and *MPK4* or induce their phosphorylation (Giacometti et al., 2016). In the same study, the genes involved in flavonoid synthesis in developing seeds, *phenylalanine ammonia-lyase 2* (*PAL2*), *chalcone synthase 7* (*CHS7*), and *isoflavone synthase 2* (*IFS2*), had increased transcript levels 72 hours after both stink bug feeding and mechanical damage (Giacometti et al., 2016). At the same time, the transcript levels of *PAL2* and *CHS7* were also inducible by methyl-jasmonate or SA treatment (Giacometti et al., 2016). Such phenomena hinted at the involvement of flavonoids in defence responses. In another study comparing the transcript profiles between aphid infestation-susceptible and aphid infestation-resistant soybean plants after aphid attack, it was found that aphids could suppress phytohormone signaling in the susceptible plants (Studham & MacIntosh, 2013). In the aphid-susceptible soybean plants, a significant induction of abscisic acid (ABA)-related transcripts was observed 7 days after aphid infestation (Studham & MacIntosh, 2013). It was hypothesized that aphids could induce ABA production which suppresses SA- and JA-mediated defence responses (Studham & MacIntosh, 2013).

5.3 Flavonoids play a role in the defence against herbivores

The correlation between flavonoid levels and herbivory deterrence has been investigated (Burden & Norris, 1992; Chiang, Norris, Ciepiela, Shapiro, & Oosterwyk, 1987; Hart, Kogan, & Paxton, 1983; Peruca et al., 2018; Piubelli, Hoffmann-Campo, De Arruda, Franchini, & Lara, 2003;

Zhou et al., 2011). Multiple isoflavones, for example, daidzin, 4',7-dihydroflavone, daidzein, and formononetin, were induced and accumulated in leaves following the *Spodoptera litura* larvae attack (Murakami et al., 2014; Nakata et al., 2016). In addition, malonyldaidzin, ononin, and malonylononin were accumulated in soybean leaves treated with *S. litura* gut content extracts. By applying ^{13}C -labeled phenylalanine together with gut content extracts to mechanically damaged leaves, ^{13}C was incorporated into the 4',7-dihydroxyflavone, daidzein, and formononetin found in the leaf extract, supporting the hypothesis that active flavonoid biosynthesis occurs at the site of elicitation (Murakami et al., 2014). The increase in selective *de-novo* biosynthesis of soybean isoflavones upon the gut content elicitation was previously reported (Nakata et al., 2016), suggesting a possible legume-specific defence response against *S. litura*. As the *S. litura* gut content does not have direct β -glucosidase activity, the accumulation of isoflavone aglycones might be a result of the enhanced hydrolysis of isoflavone glycosides. It has been demonstrated that *S. litura* could not metabolize daidzein, which could inhibit the growth of *S. litura* larvae (Zhou et al., 2011). Also, the herbivory-deterrent effect of daidzein was observed in experiments conducted with other Spodoptera members (Peruca et al., 2018). The accumulation of daidzein, without being converted to glyceollin, might serve as an adaptive anti-herbivory defensive strategy of soybean against chewing insects.

The presence of phytoalexins and phenolic compounds in the soybean plant might alter the host selection of Mexican bean beetle, *Epilachna varivestis* Mulsan (Burden & Norris, 1992; Chiang et al., 1987; Hart et al., 1983), and this antiherbivory effect was found to be dose-dependent (Burden & Norris, 1992). Soybean glyceollin reduced the efficiency of food utilization but did not affect the growth, development, and survival of Mexican bean beetle larvae (Hart et al., 1983). The elevated level of total phenolics correlated with the quantifiable and temporal patterns of active PAL and L-tyrosine ammonia-lyase (TAL) enzymes in affected tissues (Chiang et al., 1987). Besides the Mexican bean beetle, the application of glyceollin also showed a dose-dependent antiherbivory effect on the chrysomelids bean leaf beetle, *Cerotoma trifurcata* (Forster), and southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber as well (Fischer, Kogan, & Paxton, 1990). Different species of chewing insects elicit similar defence responses in the soybean plant, where feeding by one species of chewing insect could initiate a broad resistance response by the plant against other chewing insects as well, known as cross-resistance (Srinivas, Danielson, Michael Smith, & Foster, 2001).

Soybean aphid is one of the common herbivores that can colonize soybean for a long term. Strategies must be adopted by the aphid to suppress the soybean defence responses (Kanobe, McCarville, O'Neal, Tylka, & MacIntosh, 2015; Liehti & Farmer, 2002). Metabolomic and transcriptomic analyses on aphid-infested soybean revealed the upregulation of the phenylpropanoid pathway and the accumulation of isoflavonoids, such as daidzein, formononetin, and genistein (Hohenstein et al., 2019). Mass spectrometry imaging showed that isoflavone accumulation due to aphid infestation was localized in mesophyll cells and the epidermis (Hohenstein et al., 2019; Klein et al., 2015). This suggests that the accumulation of isoflavonoids is a part of the non-phloem defence against phloem-sucking aphid colonization. Polyunsaturated fatty acids are the precursors of jasmonic acid (JA) which is involved in plant response to herbivory (Liehti & Farmer, 2002). Herbivore attack might have resulted in the reduced levels of polyunsaturated fatty acids, particularly linoleic and linolenic acids in soybean, with a concomitant increase in palmitic acid in leaves, and stearic and oleic acid in seeds upon aphid colonization (Kanobe et al., 2015). As the remodeling of fatty acid composition was not observed in root colonization by soybean cyst nematode (SCN) and brown stem rot fungus, this observation appeared to be specific to aphid infestation in soybean. Alterations in fatty acid composition might be one of the counter-strategies for aphids to suppress the effectiveness of JA-mediated defence responses by modulating fatty acid desaturase (FAD) 2 and FAD6 activities in soybean (Kanobe et al., 2015). Moreover, SA was also accumulated in the form of pipercolic acid, suggesting the active regulation by aphids of soybean defence via the SA pathway (Klein et al., 2015).

Upon damage to developing seeds due to feeding by the southern green stink bug, elevated levels of daidzein, which is the immediate precursor of glyceollin, and genistein were observed (Piubelli et al., 2003). After the sting bug injury, an increase in the isoflavone concentration was observed. However, glyceollin was not detectable. It was hypothesized that the midgut bacterial community of stink bugs could play a role in deactivating the chemical defences of soybean developing seeds as well. Some of the bacterial community isolates showed *in-vitro* β -glucosidase and raffinase activities, which enable the degradation of galactosyl derivatives and increase the digestibility of soybean plants. Enzymes in midgut isolates might also degrade isoflavonoids and deactivate soybean protease inhibitors, helping aphids tolerate soybean defences and feed on the plant (Medina et al., 2018).

5.4 Phytohormones mediate the accumulation of secondary metabolites during defence responses

The signaling cascade for non-self-recognition can be mediated by various phytohormones, including SA, JA, and ET (Bari & Jones, 2009). These hormones can then trigger biochemical and molecular strategies to deter pathogen colonization and insect herbivory (Bari & Jones, 2009; Ku, Sintaha, Cheung, & Lam, 2018), which often involve the accumulation of secondary metabolites.

The induction of JA upon necrotrophic pathogen infection, insect attack and wounding has been recently reviewed (Ku et al., 2018). In soybean, it was reported that methyl jasmonate treatment on the cotyledon enhanced the light-induced accumulation of malonyl-genistin (Graham & Graham, 1996). When methyl jasmonate was applied together with *Phytophthora sojae* cell-wall glucan elicitor in the light, the accumulation of malonyl-daidzin was increased (Graham & Graham, 1996). However, the induced accumulation of malonyl-genistin and malonyl-daidzin were both inhibited by the application of abscisic acid (ABA) (Graham & Graham, 1996).

Attack by *Aphis glycines* has been shown to increase the accumulation of daidzein and genistein in soybean leaves, and this effect could be further enhanced by the application of beta-aminobutyric acid (BABA), which provided additional upregulation on isoflavone and callose biosynthesis genes, leading to greater accumulation of genistein and callose, and the subsequent increased resistance to *A. glycines* (Yao, Zhong, Wang, Yan, & Wu, 2020).

5.5 The accumulation of secondary metabolites and their role in the defence against pathogenic microbes

In response to the fungus, *Phakopsora pachyrhizi*, that causes Asian soybean rust, soybean produces a vast number of secondary metabolites, such as terpenes and glyceollin. (Parmezan et al., 2020; Silva et al., 2020, 2021; Tremblay, Hosseini, Alkharouf, Li, & Matthews, 2010; van de Mortel et al., 2007). For example, *P. pachyrhizi* inoculation was shown to induce daidzein and genistein accumulation in soybean plants, and the application of quercetin or kaempferol could inhibit *P. pachyrhizi* spore germination (Lygin et al., 2009). It is hypothesized that terpene production might also contribute to the resistance against *P. pachyrhizi* in soybean, and its accumulation is temporally regulated by the *Rpp* genetic background (Parmezan et al., 2020). Temporal variations in the expressions of soybean genes in the flavonoid biosynthetic pathway and of the WRKY family of

transcription factors upon *P. pachyrhizi* infection were observed (van de Mortel et al., 2007). In a global gene expression analysis, in the resistant soybean plants, 12 h after *P. pachyrhizi* infection when fungal germination and penetration were initiated, a number of leaf genes peaked in expression, then dropped back down to levels similar to those of the mock-inoculated control (van de Mortel et al., 2007). However, in the susceptible plants, the expression levels of this set of genes remained unchanged until 96 hours after *P. pachyrhizi* infection. Some of the genes displaying such differential expression pattern between the resistant plants and the susceptible plants upon *P. pachyrhizi* infection are involved in the flavonoid biosynthetic pathway (van de Mortel et al., 2007).

The necrotrophic plant pathogen, *Sclerotinia sclerotiorum*, induces oxidative stress in the host, resulting in pathogenesis (Chen, Ullah, Reichelt, Gershenson, & Hammerbacher, 2019). For example, *S. sclerotiorum* was found to secrete quercetin dioxygenase to degrade flavonols in Arabidopsis (Chen et al., 2019), and in soybean, wounding and *S. sclerotiorum* infection in stems induced the accumulation of isoflavones, especially daidzein and genistein, in leaves (Wegulo, Yang, Martinson, & Murphy, 2005). By integrating the results from transcriptomic and metabolomic analyses, it was found that the antifungal activities in soybean upon *S. sclerotiorum* infection were achieved by reprogramming the phenylpropanoid pathway to modulate the redox capacity (Ranjan et al., 2019). In particular, fluctuations in the cellular levels of dehydroascorbic acid (DHA) and trans-4-hydroxy-L-proline suggested the involvement of antioxidants in the resistance response to *S. sclerotiorum* (Ranjan et al., 2019). Moreover, during *S. sclerotiorum* infection, the accumulation of jasmonoyl-L-isoleucine (JA-Ile), which is the bioactive form of jasmonate, was observed earlier in the resistant soybean lines than in the sensitive line. At 6 and 12 h after inoculation, the level of JA-Ile was higher in the resistant line than in the sensitive line (Ranjan et al., 2019). However, at 72 h after inoculation, the level of JA-Ile was higher in the sensitive line than in the resistant line (Ranjan et al., 2019). Such a phenomenon indicates the importance of JA signaling to the resistance against *S. sclerotiorum* (Ranjan et al., 2019).

5.6 Secondary metabolites play a role in beneficial plant-microbe interactions

Flavonoids and isoflavonoids play a critical role in the interactions between plants and microbes, including both pathogens and beneficial symbionts (Phillips & Kapulnik, 1995; Stafford, 1997). In contrast to the inhibitory

effect on the pathogenic fungi *P. pachyrhizi*, flavonoids stimulated the germination of arbuscular mycorrhizal fungus spores and enhanced hyphal growth to facilitate nutrient transports between the rhizosphere and soybean roots (Abdel-Lateif, Bogusz, & Hocher, 2012). Soybean plants use flavonoids and isoflavonoids as chemoattractants and inducers to attract rhizobia toward their roots and to activate the nod genes cassette for nodulation (Spaank, 1995). Plant growth-promoting rhizobacteria (PGPR) could trigger soybean secondary metabolism through microbe-associated molecular patterns (MAMP) systemic induction. PGPR was shown to have successfully primed the plant against *Xanthomonas axonopodis* pv. *glycines*. Although not all PGPR strains could trigger a significant induction of isoflavones, MAMPs from PGPR had shown a cross-resistance defensive metabolism in soybean, suggesting the involvement of other non-isoflavone elements in regulating the defence against this pathogen (Algar et al., 2014). PGPR could also enhance the plant's resistance against soybean cyst nematode (SCN) through reprogramming the biosynthesis of secondary metabolites, such as phenylpropanoids, alkaloids, and fatty acids (Kang, Zhu, Wang, Chen, & Duan, 2018).

5.7 The negative roles of secondary metabolites in plant defence

The susceptibility of soybean plants is associated with the types and amounts of secondary metabolites induced in the plant according to the particular type of stress and to specific herbivores. Compared to the *Euschistus heros*-resistant soybean cultivar (Dowling), the susceptible cultivar (Williams 82) had a higher level of sugars and isoflavones, and was preferred by *Euschistus heros* for oviposition (Silva, O-Panizzi, Blassioli-Moraes, & Panizzi, 2013). In a feeding choice test, the same amount of the phytoalexin, glyceollin, had a greater deterring effect on Mexican bean beetle larvae and adults than on soybean looper (Hart et al., 1983). Transcriptomic and biochemical differences were observed and reported between the resistant and susceptible plants, suggesting the contribution of genetic variations to the resistance against herbivores and pathogens (Chiang et al., 1987; Chiozza, O'Neal, & MacIntosh, 2010; Parmezan et al., 2020; Silva et al., 2013; Studham & MacIntosh, 2013; van de Mortel et al., 2007).

Several genetic variations between the resistant and susceptible soybean plants were comprehensively discussed in insect feeding studies. Aphids feed on the soybean phloem that provides carbohydrates and nitrogenous compounds. A positive correlation between available phloem nitrogen in

soybean and aphid abundance was demonstrated (Chiozza et al., 2010; Walter & DiFonzo, 2007). In particular, susceptible soybean plants had a higher level of α -aminobutyric acid, asparagine, glutamine, glutamic acid, histidine, proline, and serine (Chiozza et al., 2010). Such nutritional quality could have influenced the determination of host suitability by the insect (Chiozza et al., 2010). In another study, transcriptome analyses revealed that many defence-related genes were constitutively expressed in the *Rag1*-carrying resistant soybean line, but were activated only during aphid infestation in the susceptible soybean line (Studham & MacIntosh, 2013).



6. Secondary metabolites in soy food products: The balance between nutrients and flavor

6.1 Secondary metabolites as anti-nutrients

Despite being the “super food” that provides valuable nutrients, soybean also contains several anti-nutritional factors that may counteract its nutritional quality.

6.1.1 Trypsin inhibitor

Trypsin inhibitors (TIs) are considered a key anti-nutritional factor in soybean. There are two major protease inhibitors in soybean, the Bowman-Birk trypsin inhibitor (BBI) and the Kunitz trypsin inhibitor (KTI) (Liener, 1995). Consumption of these TIs in relatively high concentrations from raw soy flour was found to cause pancreatic hyperplasia and hypertrophy in rats, weakening their growth (Liener, Nitsan, Srisangnam, Rackis, & Gumbmann, 1985). In rats, prolonged feeding with soy flour containing high TIs eventually led to the development of neoplastic and hyperplastic nodules, including carcinomas, in pancreas (McGuinness et al., 1980).

Fortunately, both TIs could be inactivated by heat or other pre-processing methods such as fermentation and germination (Dixit, Kumar, Rani, Manjaya, & Bhatnagar, 2011). However, valued soy proteins and essential amino acids could become insolubilized by the heat treatment and therefore lost in the final soya products (Dixit et al., 2011). New treatments, for example, gamma irradiation, could be considered for better conservation of nutritional attributes (Dixit et al., 2011). Although it has been recognized as safe to treat food with gamma irradiation (Ravindran & Jaiswal, 2019), such treatment may bring forth other concerns. It has been reported that gamma irradiation leads to the reduction of phenolic compounds and tannin besides the trypsin inhibiting activity in raw and cooked

soybean (de Toledo, Canniatti-Brazaca, Arthur, & Piedade, 2007). In general, gamma irradiation is also known to reduce the vitamin contents in food products, such as vitamins A, B₁, C, D, E, and K (Kilcast, 1994; Lima, Vieira, Santos, & Mendes de Souza, 2018; Ravindran & Jaiswal, 2019). Using irradiation rather than heat treatment needs careful consideration as radiation does destroy nutrients like vitamins.

In contrast to KTI, BBI survives in gastric juice (Messina & Barnes, 1991). Despite being an anti-nutritional factor, BBI was found to exhibit anticarcinogenic activities through its ability to inhibit chymotrypsin, in addition to the original substrate trypsin (Fournier et al., 1998). The lowest effective concentration for its chemoprotective activity can be easily achieved through dietary intake of soybean, based on the level used in animal studies (0.1% of total diet) (Messina & Barnes, 1991). Therefore, it is a fine balance between destroying the anti-nutritional properties and retaining the dosage required for beneficial effects when treating soy products to remove TIs.

TIs protect plants against insect feeding and soil pathogens (Marchetti et al., 2000). To understand the effect of domestication on TI contents in soybean, genomic and proteomic profiles of wild, landrace and cultivated soybean accessions were studied (Natarajan, Xu, Bae, & Bailey, 2007). The levels and variations of the protein profiles of KTIs were found to be higher in wild soybean germplasms than cultivated ones (Natarajan et al., 2007). During soybean domestication, the levels and the complexity of KTIs in seeds may have been reduced or lost.

6.1.2 Phytic acid

Phosphorus (P) in soybean seed is mostly stored in the form of phytic acid, an organic phosphate with the structural formula, myo-inositol 1,2,3,4,5,6-hexaphosphate (Raboy, 1997). Due to the strong ability of phytic acid to chelate metal ions, it is primarily deposited as a mixture of phytic salts of various mineral cations, particularly potassium and magnesium (Raboy, 1997). In developing seeds, the concentrations of inorganic P and minerals are important for regulating enzymatic activities involved in critical metabolic processes, such as protein and starch metabolism, for early seed growth and germination. Since the reserve levels of P and metal ions in the seed are often severalfolds greater than the optimal levels, the deposition and remobilization of phytic acid represent important mechanisms for the storage and retrieval of P and mineral ions (Raboy, 1997). It was found that metal cations were deposited to phytic acid at the aleurone layer after they had completed their physiological roles in seed development (Ogawa, Tanaka, & Kasai, 1979).

Phytic acid concentration is high in oilseeds, averaging 1.5% dry weight (Erdman, 1979). Unfortunately, phytic acid P is nutritionally unavailable to nonruminants (Erdman, 1981). It has therefore been regarded as an anti-nutritional factor in the diet, for its excretion as the salts of essential mineral ions might lead to both mineral and P deficiencies in human populations (Erdman, 1981). Conflicting results were often produced regarding the anti-nutritional property of phytic acid. In a study that fed rats with soy-derived meals, a reduction in zinc and iron availability was detected (Liener, 1995), while an experiment with human subjects conducted by USDA did not find such any significant reduction in these minerals (Miles et al., 1987). Despite being an anti-nutrient, the dietary intake of phytic acid was found to suppress iron-catalyzed oxidative damage in the colon, hence there is growing interest in phytic acid being an agent against colon cancer (Graf & Eaton, 1993). Considering the potential benefits and harm that dietary phytic acid might bring to human, its quantitative variations among cultivated soybean lines deserve much attention.

In a study on 38 cultivated soybean germplasms, the proportion of seed total P represented by phytic acid varied from 67% to 77%, with no significant difference among the germplasms (Raboy, Dickinson, & Below, 1984). As for seeds of wild soybean germplasms, the concentrations of phytic acid were generally 30% greater than those in cultivated soybeans (Raboy et al., 1984). Since phytic acid accounted for a relatively constant portion of the total P among all the germplasms, and the total P in seeds was found to be influenced by both heritable multi-genic controls and the environmental availability of P to the developing plants, these factors also accounted for the variations in phytic acid P among the germplasms (Raboy, 1997). Phytic acid accounts for more than 70% of total P in the seed of maize (Raboy et al., 2000). In a study that searched for non-lethal homozygous maize mutants with low phytic acid contents, two mutants named *lpa-1* and *lpa-2* were identified (Raboy et al., 2000). Both of them displayed a wild-type kernel phenotype under a reduction in phytic acid P and an increase in inorganic P (Raboy, Young, & Gerbasi, 1994). This indicates that the sequestration of P as phytic acid is not essential for P homeostasis, and the highly correlated relationship between seed total P and phytic acid P is not an absolute requirement for the normal development and function of the seed (Raboy, 1997). This study might provide insights for engineering low-phytic acid mutants of soybean with wild-type viability, or provide a genetic marker for screening desirable phytic acid P contents in soybean.

6.2 Secondary metabolites and soybean flavor

6.2.1 Lipoxygenases as off-flavor generators

Since lipoxygenases initiate the production of off-flavor compounds, they are regarded as anti-nutritional factors. Lipoxygenases (LOXs) belong to a group of non-heme iron-containing dioxygenases widely distributed in animals, plants, and fungi (Lenis, Gillman, Lee, Shannon, & Bilyeu, 2010). In soybean, up to 2% of seed proteins are represented by lipoxygenases (Lenis et al., 2010). Lox-1, Lox-2, and Lox-3 are the three major isozymes present as storage proteins in the mature soybean seed, and each one is encoded by a unique gene (Wang, Croft, Järlfors, & Hildebrand, 1999). Lox-1 is more active on linoleic acid (LA) and is categorized as Type I LOX with an optimal pH around 9; Lox-2 and Lox-3 are more active on methyl linoleate and are categorized as Type II LOX with an optimal pH between 6.5 and 7 (Dixit et al., 2011). The pH-dependent activities of LOXs give hints on the design of soy food processing methods. Lox-2, although classified in a different category from Type I LOX, can also oxygenate esterified polyunsaturated fatty acid (PUFA) units in biomembranes (Robinson, Wu, Domoney, & Casey, 1995). All soybean lipoxygenase isozymes are monomeric and contain one iron atom per molecule of protein for their enzymatic function (Donnelly & Robinson, 1995).

Lipoxygenases catalyze the oxidation of PUFAs that contain a *cis*, *cis*-1, 4-pentadiene moiety to produce an initial free radical, which mediates subsequent oxygenation and conjugates to form hydroperoxyl unsaturated acids (Donnelly & Robinson, 1995). These initial products would then be degraded by hydroperoxide lyases and isomerases to generate a variety of aldehydes, ketones and alcohols, also known as the green leaf volatiles (GLVs). Many of them are of low flavor thresholds and give an objectionable rancid flavor and aroma (Robinson et al., 1995).

The physiological role of LOX in soybean seed development remains unclear as no adverse effects were found on the crop performance of the LOX1/2/3 triple null mutant (Wang et al., 1999). The three isoforms disappear during the first day of seed germination, and the absence of substantial oxygenation events on PUFAs suggests that they are not involved in lipid mobilization during germination (Wang et al., 1999). Three new LOX isozymes, LOX 4-6, appear in the cotyledon of germinating soybean (Wang et al., 1999). These LOX isozymes are believed to be involved in plant growth, wounding, and defence against pathogens and herbivores (Wang et al., 1999).

The ability of lipoxygenases in catalyzing free radical formation also makes them an undesirable component in soybean products

(Donnelly & Robinson, 1995). The initial free radical might also trigger chain reactions such as the subsequent oxidation of non-PUFA constituents such as vitamin A and β -carotene, resulting in altered sensory quality (flavor and aroma), loss of essential nutrients and overall reduction in the quality of food (Donnelly & Robinson, 1995). Since most lipoxygenases are able to withstand thermal processing, they remain sufficiently active to affect the quality of soy products after long-term storage (Donnelly & Robinson, 1995). In intact cells, LA and LOX are separated in different subcellular compartments. However, the processing of soy products requires crushing and homogenization of soybean seeds and subsequently results in the mixing of the enzyme with its substrate (Mellor, Bligh, Chandler, & Hodgman, 2010). This allows the endogenous LOX to initiate a series of lipid oxidation reactions and eventually produce a range of volatile aldehydes (hexanal and nonenal) and ketones, the off-flavor compounds that give soybean product an unpleasant grassy-beany flavor and aroma (Robinson et al., 1995).

The presence of these objectionable flavors and aroma greatly reduces product desirability, particularly in beverages made from crushed soybeans (Mellor et al., 2010). N-hexanal, among the off-flavor compounds, is the one of particular interest to food manufacturers in their attempt to improve soy product flavor (Mellor et al., 2010). Some processing methods have been developed to reduce the off-flavor of soy milk, but the trade-off is the introduction of an undesirable chalky texture to soybean products (Iassonova, Johnson, Hammond, & Beattie, 2009). Soybean cultivars with low LOX content are therefore in demand in the food market. However, removing LOX will not totally resolve the off-flavor issue because soybean unsaturated fatty acids have self-oxidative mechanisms that do not require participation of LOX but need oxygen presence (Vercellotti, St. Angelo, & Spanier, 1992). Therefore, removing oxygen in the vessel by deaeration (Chew & Nyam, 2020) during processing will greatly improve the off-flavor issue.

6.2.2 The bitter tastes of flavonoids, saponins, and alkaloids

Despite being beneficial phytonutrients that promote health in human populations, plant-based flavonoids, saponins and alkaloids are mostly bitter. They trigger the bitter rejection response in humans and are routinely removed in debittering processes by the food industry (Drewnowski & Gomez-Carneros, 2000).

In a study on the non-volatile minor constituents responsible for the undesirable bitter taste in soy flour, three isoflavones, glycitein 7- β -O glucoside, daidzein and genistein, were identified to additively contribute to the

objectionable flavor of soybean products (Huang, Hsieh, & Chang, 1982). Glycitein 7- β -O glucoside, in particular, possesses herb-like bitterness and astringency (Huang et al., 1982).

In another similar study on ground soybean, both group A and group B saponins, together with isoflavones, contributed significantly to the undesirable bitter and astringent taste (Okubo et al., 1992). Among these molecules, group A soybean saponins had the most intense undesirable bitter taste when conjugated to glycosides (Okubo et al., 1992). While the degradation of soybean saponins to the aglycone form reduced such an undesirable taste, the reverse trend was observed in isoflavone glycosides (Okubo et al., 1992).

Being toxic secondary metabolites that confer herbivory resistance on plants, alkaloids also contribute to the bitter taste of plants (Drewnowski & Gomez-Carneros, 2000). The instinctive rejection by humans due to the bitter taste of alkaloid-containing crops could help to avoid suffering from acute toxicity from consuming an excessive amount of the alkaloids (Wink, 2013).

The physiological roles, nutritional values, and flavors of secondary metabolites in soybean are summarized in Table 1.



7. Natural and human selections of soybean

Considering their physiological roles in soybean plants and nutritional values and effects on flavors in soy foods, secondary metabolites have experienced heavy natural and human selective pressures. While natural selection has worked on improving the adaptations and ecological fitness of the soybean plant to its environment, human selection has focused on enhancing the ease of cultivation, harvest, improving yield, catering to consumer preferences and, ultimately, profitability (Ku, Contador, et al., 2020). Since secondary metabolites are linked to many domestication-related traits, human selection on the crop performance of soybean could indirectly mean the selection of secondary metabolite profiles (Ku, Contador, et al., 2020).

7.1 Natural selection: Adaptations to the environment

In terms of environmental adaptations, both natural and artificial selections favor a more effective response to climate change or abiotic stresses and more favorable interactions with other organisms in the local community, including symbiosis with beneficial rhizobia and fungi, and defence responses against pathogens and herbivores. However, the growing of soybean in fields with favorable cultivation conditions and protection from farmers against crop diseases and herbivory have largely diminished the pressure of natural

Table 1 Summary of the physiological roles, nutritional values, and flavors of secondary metabolites in soybean.

Secondary metabolites	Physiological role in plant	(Anti-)Nutritional value for humans	Flavor	References
Flavonoids	<ul style="list-style-type: none"> – Initiate symbiotic interactions with rhizobia – Abiotic and biotic stress resistance 	<ul style="list-style-type: none"> – Phytoestrogen with anticarcinogenic effects – Antioxidant – Anti-inflammatory effect – Prevention of degenerative and chronic diseases 	Bitter and astringent	Ahmad et al. (2014), Banerjee et al. (2008), Carter et al. (2018), Huang et al. (1982), Kosslak et al. (1987), Křížová et al. (2019), Kumar and Pandey (2013), Lee, Kim, and Song (2012), Mira et al. (2002), Nagata et al. (2014), Sepiol et al. (2017), Wu et al. (2008)
Saponins	<ul style="list-style-type: none"> – Natural surfactant – Abiotic and biotic stress resistance 	<ul style="list-style-type: none"> – Antioxidant – Anti-inflammatory, anticarcinogenic, hypocholesterolemic, immunostimulatory, hepatoprotective, and antidiabetic effects 	Bitter and astringent	Fournier et al. (1998), De Geyter et al. (2007), Okubo et al. (1992), Surh et al. (2001) Wu et al. (2008), Yoshiki et al. (2001)
Alkaloids	<ul style="list-style-type: none"> – Defence against pathogens and herbivores 	<ul style="list-style-type: none"> – Anticancer activities – Anti-malarial activities – Preventing stroke by facilitating blood circulation in brain – Therapeutic effects in neurodegenerative diseases 	Bitter	Gupta et al. (2015), Huang et al. (1982), Kumar and Khanum (2012), Onguéné et al. (2013), Wink (2013)

Continued

Table 1 Summary of the physiological roles, nutritional values, and flavors of secondary metabolites in soybean.—cont'd

Secondary metabolites	Physiological role in plant	(Anti-)Nutritional value for humans	Flavor	References
Phytosterols	<ul style="list-style-type: none"> – Stabilizing plant cell membrane – Controlling cellular proliferation and differentiation 	<ul style="list-style-type: none"> – Precursors of steroidal hormones – Reducing serum cholesterol levels – Anti-inflammatory, antipyretic, antineoplastic, and immunomodulating activities 	Not determined	Ostlund et al. (2003) ; Piironen et al. (2000) , Shi et al. (2010) , Wang et al. (2011)
Trypsin inhibitor	<ul style="list-style-type: none"> – Defence against insects and pathogens 	<ul style="list-style-type: none"> – BBI exhibits anticarcinogenic activities – As anti-nutrients: Prolonged consumption of high concentration of raw soybean may lead to development of neoplastic and hyperplastic nodules, including carcinomas, in pancreas 	Not determined	Fournier et al. (1998) , Marchetti et al. (2000) , McGuinness et al. (1980)
Phytic acid	<ul style="list-style-type: none"> – Phosphorus storage in developing seed 	<ul style="list-style-type: none"> – Anti-colonic cancer – As anti-nutrients: May lead to mineral and P deficiencies 	Not determined	Erdman (1981) , Graf and Eaton (1993) , Raboy (1997)
Lipoxygenase	<ul style="list-style-type: none"> – Storage protein 	<ul style="list-style-type: none"> – As anti-nutrients: Leading to loss of essential nutrients 	Generating off-flavor compounds such as aldehydes	Donnelly and Robinson (1995) , Wang et al. (1999)

selection from abiotic and biotic stresses (Ku, Contador, et al., 2020). Since the secondary metabolites that facilitate ecological adaptations were no longer needed to enhance crop yield or performance in agricultural lands, they were no longer prioritized in the human selection process and might instead be lost due to the undesirable properties they confer on the soybean plant or soy products. For example, the bitter-tasting genistin and group B saponins are found to be key secondary metabolites that were associated with increased salt tolerance and a distinguishing feature between wild and cultivated soybeans (Wu et al., 2008). In cases where it is necessary to cultivate soybean in areas with adverse environmental conditions, such as high latitudes or high salinity, or where the threat from diseases is serious, the wild progenitors can provide the genetic resources for reintroducing the ability to produce these favorable secondary metabolites into cultivars.

7.2 Human selection: Seed traits, nutritional values, and flavors

In some instances, human selection might introduce additional pressure or have a completely opposite direction of selection to that of natural selection during soybean domestication. Domestication-related traits mainly concern the yield and harvestability of crops. Yield-related parameters include leaf length, leaf width, pod length, number of seeds per pod, 100-seed weight, days to flowering, and floral color, and those related to harvestability include pod shattering and germination rate (Lo et al., 2018). Other agronomic traits selected for due to human preferences include seed size, appearance, taste, and digestibility (Ku, Contador, et al., 2020). Many of these agronomic traits are regulated by secondary metabolites. For example, as discussed in Section 6.2, the flavor of soybean seeds is affected by the off-flavor generating lipoxygenases and the bitter-tasting flavonoids, saponins and alkaloids. Other examples of agronomic traits controlled by flavonoids include tissue color and seed coat color, which are related to the ease of sowing and religious concerns (Abbas et al., 2017; Heiser, 1988). Floral pigmentation, a trait related to both insect pollination and human esthetic preferences, is also determined by flavonoids (Tanaka, Sasaki, & Ohmiya, 2008). The digestibility of seeds is influenced by all flavonoids, saponins, and alkaloids (Gupta, 1987).

7.2.1 Simultaneous selection for large seeds with high oil but low protein contents

The focus of crop domestication has been on the improvement of yield and harvestability (Dehaan et al., 2016). Larger seeds are directly related to

higher yield and easier harvest. Domestication-related loci associated with seed size were identified in soybean (Zhou, Jiang, et al., 2015; Zhou, Wang, et al., 2015), and It has been suggested that soybean domestication is directly related to the increase in seed size (Wang et al., 2020).

Soybean has been subjected to the simultaneous selection for larger seed size, higher oil content, and lower protein content, and that this was achieved by the selection for the gene, *GmSWEET10a*, which encodes a sugar transporter (Wang et al., 2020). An increased expression of *GmSWEET10a* leads to an enhanced flux of sugars from the maternal tissue to the embryo, thus facilitating cell division and expansion in the embryo and consequently an increased seed size. The increase in sugar supply to the embryo also enhances lipid synthesis. However, the nitrogen supply may become a limiting factor for these processes. The result is a reduction in the protein content (Wang et al., 2020). The selection for *GmSWEET39* for higher oil but lower protein seed contents was suggested to have followed a similar path (Zhang et al., 2020). These findings are consistent with a previous report highlighting the negative correlation between oil and protein contents in soybean seed (Patil et al., 2018).

The selection for desirable levels of oil content in soybean seeds during domestication has been discussed (Zhou, Jiang, et al., 2015; Zhou, Wang, et al., 2015; Zong et al., 2017). The human selection for soybeans with high oil contents was suggested to begin as early as 7500 years B.P. (C-14 ages where the year 1950 is used as “present”), with the high-oil content soybean cultivars established by 4000 years B.P. (Zong et al., 2017). Later, it was found that the increase in soybean seed oil content as a result of domestication had resulted from the artificial selection for the gene *GmOLEO1*, which encodes the oleosin protein for oil body formation (Zhang et al., 2019). It was also found that the quantitative trait loci (QTLs) related to protein, oil, and sucrose contents were found to overlap with those QTLs governing domestication-related traits such as plant maturity and seed color (Patil et al., 2018).

While soybean cultivars have been reported to have a wide range of seed sugar levels (Hou et al., 2009), the process of selecting for seed sugar contents during domestication is less clear than that for seed oil content. In a study attempting to identify the molecular footprints of soybean domestication, a gene related to carbohydrate metabolism was found to be related to domestication (Li et al., 2013). However, genes related to carbohydrate metabolism were found to exhibit significant variations in their DNA methylation levels during soybean domestication (Shen et al., 2018). Although sugar transporters, such as *GmSWEET10a* (Wang et al., 2020)

and *GmSWEET39* (Zhang et al., 2020), have been reported to be selected for during domestication, it has been suggested that such selections were indirect results of the selection for lipid synthesis and therefore the seed lipid content (Wang et al., 2020).

7.2.2 Selection for seeds with reduced flavonoids in the seed coat

Seed color, including seed coat color and hilum color, is a domestication related trait (Zhou, Jiang, et al., 2015; Zhou, Wang, et al., 2015). Wild soybean varieties tend to have black seed coats while cultivated varieties tend to have yellow seed coats (Wang et al., 2018). It has been suggested that seeds with light colors were selected during domestication for easier sowing and religious reasons (Heiser, 1988). The loss of seed coat color is a result of the selection for the dominant allele, *I*, which silences *Chalcone Synthase* (*CHS*) alleles and thus inhibits the flavonoid pathway in the seed coat (Paauw, Koes, & Quattrocchio, 2019; Tuteja, Zabala, Varala, Hudson, & Vodkin, 2009). In a study comparing among the metabolic profiles of a cultivated, a landrace and a wild accession, epicatechin was only found in the landrace and the wild accessions but not the cultivated one (Yun, Kang, Kim, Kim, & Kim, 2020). Since epicatechin is related to the hardness of the seed coat, it was suggested that the existence of epicatechin in the landrace and wild accessions renders the hardness and the decreased germination rates of the seeds (Yun et al., 2020).

Besides “domestication,” the concept of “post-domestication selection” has also been introduced (Abbo et al., 2014). The appearance and taste of crops could be regarded as being subjected to post-domestication improvement (Abbo et al., 2014).

7.3 Controversy on the selection for off-flavor-producing metabolites in soybean

Glycosides, including those of saponins and isoflavones, have been identified to contribute to the bitterness and astringency of soybean seeds, with group A saponins being the major contributors (Okubo et al., 1992). The *Sg-1* locus was found to regulate the structural diversity of saponins in soybean (Sayama et al., 2012), and is situated in a genomic region under selection during soybean domestication (Zhou, Jiang, et al., 2015; Zhou, Wang, et al., 2015). Nevertheless wild soybeans do not necessarily have higher saponin contents than cultivated ones (Ku, Contador, et al., 2020). During domestication, it is not clear whether soybeans with low levels of saponins were actively selected for. From the perspective of the

food industry, it is reasonable to select soybean varieties with low seed saponin levels to rid soybean seeds of bitterness and astringency. However, from the perspective of nutraceutical benefits, there may be reasons to select soybean varieties with high seed saponin levels.

In short, domestication has selected for a more pleasant taste, a better yield, and a more esthetically favorable appearance in the soybean seed. However, such alterations in the characters of the plant often contradict with natural selection for better defence against natural enemies, and higher rates of dispersal and survival of seeds. This genomic evolution due to artificial selection results in a shift in secondary metabolite profiles and generally reduces the genetic diversity and the availability of secondary metabolites in soybean cultivars. However, sometimes due to the close proximity of certain QTLs or genes for secondary metabolite biosynthesis to those responsible for other domestication-related traits, concomitant selections for secondary metabolites may also have happened (Heiser, 1988).



8. Possible post-domestication selection of soybean seeds based on their uses in food products

As discussed above, commercial considerations possibly play a role in human selection on soybeans. Isoflavones in soybeans are related to the bitter taste of soy food products. Specifically, it has been suggested that daidzein and genistein are responsible for the objectionable flavor of soy milk (Matsuura, Obata, & Fukushima, 1989). Genistein was found to stimulate the human bitter receptors hTAS2R14 and hTAS2R39 (Roland et al., 2011). Besides isoflavones, other contents, such as protein, lipid, and sugar, also influence the taste of the soy food products.

Generally, high protein and high sucrose are desirable characteristics for food-grade soybeans (Jegadeesan & Yu, 2020), although most soybeans are used for animal feed (Voorra, Larrea, & Bermudez, 2020). Soybean provides the second most important source of vegetable cooking oil (Voorra et al., 2020) as well as being consumed in various cuisines. When soybean seeds are made into different food products, there are different considerations for the seed nutrient content. For example, in European countries, the most commonly consumed soybean-based foods are in the form of soy milk and bean sprouts (Keinan-Boker et al., 2002). Soy milk has been used as a major alternative to cow's milk, due to the prevalence of allergies toward cow's milk protein (casein), as well as lactose intolerance (Vanga & Raghavan, 2018). Lactose intolerance has been recognized as a common phenomenon

among Asians since 1960s, and therefore there have been concerns about the consumption of cow's milk and other milk products (Davis & Bolin, 1967). Lactose intolerance refers to lactose malabsorption combined with symptoms (Jellema, Schellevis, van der Windt, Kneepkens, & van der Horst, 2010). In a global analysis, the world lactose malabsorption prevalence in children and adults aged 10 years or older was predicted to be 68% (Storhaug, Fosse, & Fadnes, 2017). Besides lactose intolerance, cow's milk allergy (CMA) also raises health concerns. Generally, about 2–3% infants and 0.5–3% adults are allergic to cow's milk, although the percentages vary among different populations (Jooste, Anelich, & Motarjemi, 2014). In the early 1900s, soy milk, which is technically an aqueous extract of soybean, was introduced as an “artificial milk” (Berk, 1992). In the 1930s, Dr. Harry W. Miller, an American physician-missionary, promoted the consumption of soy milk in China in the attempt to improve the nutritional status of the population, especially infants and small children, during food shortages. However, soy milk was not popularized in Hong Kong until after World War II. A beverage company, Hong Kong Soya Bean Products Co. Ltd, promoted soy milk as a soft beverage instead of as an “artificial milk” (Berk, 1992). Despite its popularity in Hong Kong, soy milk was not widely accepted by customers in the West (Berk, 1992). The “beany taste” of soy milk has been considered to be a major hurdle of soy milk promotion (Berk, 1992). Soy protein allergy is another hurdle despite of high quality protein with nine essential amino acids (Cordle, 2004; Singer, Zhang, Mian, & Huang, 2019). Besides the preference of soy milk to cow's milk in Asian countries, soybean is in high demand in Asian countries as a common ingredient in their cuisines. Examples of soybean-based foodstuffs in Asian countries include tofu, soy sauce, *jang*, and *natto*.

In the following sections, common soybean-based food products will be used as examples to illustrate the artificial selection for specific seed content compositions.

8.1 Soy milk

Ketones and aldehydes, which are produced by the oxidation of soybean oil, have been suggested to be the source of the “beany taste” in soy milk (Berk, 1992). Specifically, these compounds include hexanal, hexanol, *trans*-2-nonenal, 1-octen-3-ol, *trans,trans*-2,4-decadienal, benzaldehyde, 2-pentyl furan, 1-octen-3-one, *trans,trans*-2,4-nonadienal, *trans*-2-hexenal,

trans-2-nonenal, and dimethyl trisulfide (Li et al., 2019; Yu, Liu, Hu, & Xu, 2018; Yuan & Chang, 2007), and their levels in soy milk are influenced by processing methods and soybean varieties.

Different soybean cultivars give different levels of off-flavor compounds in soy milk (Yu et al., 2018). Among 26 soybean cultivars from China processed using the same technology, the total off-flavor compounds in the soy milk, including hexanal, hexanol, benzaldehyde, 1-octen-3-ol, 1-octen-3-one, 2-pentylfuran, *trans*-2-nonenal, *trans,trans*-2-4-nonadienal, and *trans,trans*-2-4-decadienal, were found to range from 8.37 to 39.25 mg/L (Yu et al., 2018). The cultivar having the lowest level of total off-flavor compounds lacks lipoxygenases 1 and 2 (Yu et al., 2018). Lipoxygenase activity, protein content, and linoleic acid are positively correlated with the “beany taste” of soy milk (Yuan & Chang, 2007). It was demonstrated that soy milk produced from soybeans lacking lipoxygenases had less undesirable aroma and bitterness than that made from soybeans having functional lipoxygenases (Yang, Smyth, Chaliha, & James, 2016). A positive correlation between soybean protein content and hexanal in soy milk was shown. This is consistent with a previous study demonstrating that the protein level in soybean was positively correlated with the level of lipids, which was in turn positively correlated with that of volatile compounds formed from the lipids in soy milk (Min, Yu, Yoo, & St. Martin, S., 2005). Another report found that the concentrations of “beany taste”-causing compounds, including hexanal, *trans*-2-hexanal, and 3-methylbutyraldehyde, are significantly correlated with the contents of fatty acids specifically and not with the contents of total protein or total fat (Shi et al., 2015). The rationale behind such correlations remained unclear, but overall, for use in the food industry to produce soy milk with low levels of off-flavor compounds, soybean varieties with low levels of lipoxygenases, proteins and lipids tend to be selected.

8.2 Tofu

Tofu is a popular soy food and is usually consumed as a meat alternative (Sadler, 2004). Similar to soy milk, tofu produced from soybeans lacking lipoxygenases had reduced undesirable aromas and bitterness than that produced from soybeans having lipoxygenases (Yang et al., 2016). In addition to the taste, the firmness of tofu is also an important property (Lim, DeMan, Deman, & Buzzell, 1990). Coagulation is thus a critical procedure for tofu production, and tofu processing leads to 44% reduction of isoflavones although the distribution of different forms of isoflavones was not affected (Wang & Murphy, 1996). Some protein-associated isoflavones may have

lost in the whey when Ca^{2+} in the coagulant reacts with the proteins during coagulation (Wang & Murphy, 1996). The lower levels of isoflavones, including free, glycosylated and malonylated isoflavones, in tofu compared to soybean flour was also demonstrated in another report (Liu, Chang, & Wiesenborn, 2005). The lower level of soysaponin I in tofu compared to soybean flour was also reported (Hu, Lee, Hendrich, & Murphy, 2002). Tofu may thus be a desirable soy food product if the flavors of isoflavones and saponins are of concern.

8.3 Fermented soybean products

Fermentation has been used as a means of food preservation (Ross, Morgan, & Hill, 2002), as well as for providing other benefits such as the generation of unique flavor and aroma (Ray & Joshi, 2014) and the production of antibiotics (Ross et al., 2002). Fermented soy products also bring forth health benefits such as alleviating the effects of diabetes mellitus, hypertension, cardiac disorders, and cancer-related issues (Jayachandran & Xu, 2019). Fermented soybean products have been widely adopted in Asian countries. Although these products have been regarded as ethnic foods in Asian countries, they are becoming more widely available in western countries.

8.3.1 Soy sauce

Soy sauce is produced by the fermentation of a mixture of defatted soybean meal and roasted wheat grits, using microorganisms such as *Aspergillus*, *Pediococcus*, *Saccharomyces*, and *Candida* spp. (Sugiyama, 1984; Wilson, 1995). Soy sauce is also called *shoyu*, *jiang-you*, and *kanjang* in Japan, China and Korea, respectively (Wilson, 1995). Soybeans with high protein and unsaturated fatty acid contents are preferred for soy sauce production (Wan, Wu, Wang, Wang, & Hou, 2013). During fermentation, the enzymatic activities on starch and protein result in the production of flavor compounds including alcohols, acids, esters, aldehydes, furans, and pyrazines (Zhao et al., 2018). The degradation of starch and protein in the mixture by microorganisms under high salt concentrations results in the unique aroma of soy sauce (Sugiyama, 1984; Wilson, 1995).

8.3.2 Jang

In Korea, the fermentation of soybean has been recorded since the Three Kingdom period (57 B.C.–A.D. 668) (Cwiertka & Moriya, 2008). A few centuries later, *kanjang* (soy sauce) and *doenjang* (fermented soybean paste)

were recorded (Cwiertka & Moriya, 2008). After the introduction of red peppers to Korea at the end of the sixteenth century, gochujang (fermented red pepper paste) was recorded (Cwiertka & Moriya, 2008). The aroma of kanjang is mainly contributed by glutamic acid and other free amino acids from the breakdown of soybean proteins by *Bacillus* spp. (Shin & Jeong, 2015). Doenjang is famous for its health benefits such as liver detoxification and tumor control (Shin & Jeong, 2015). These health beneficial effects are due to secondary metabolites including trypsin inhibitors, isoflavones, vitamin E, and linoleic acid (Shin & Jeong, 2015).

The long history of using soybean to produce these food products with very distinctive tastes and aromas may have contributed to the selection for soybean varieties having particular seed content compositions. For the production of *jang*, besides the yield (Ha et al., 2013; Kim, Baek, Han, et al., 2010; Kim, Baek, Ko, et al., 2010), other qualities including the levels of protein, sugar, total nitrogen content, total free amino acid level and glutamic acid content in the seed are also important considerations (Choi et al., 1999).

8.3.3 Natto

The unique tastes and aromas of fermented soybean products are the results of different combinations of metabolites. For natto production, soybean seeds having a pale yellow seed coat, a low ammonia-like flavor, as well as a high carbohydrate content for promoting microbial activities and sweetness are desired (Escamilla, Rosso, Holshouser, Chen, & Zhang, 2019). The awareness of the health benefits due to the isoflavone contents in natto also drives the selection for soybean varieties with high seed isoflavone contents (Ikeda et al., 2006).



9. Industrial methods and the selection of soybean varieties for soy food production

Industrial methods have been developed to minimize the unpleasant flavors of soy food products. Efforts have been paid to minimize the adverse effects from off-flavor components such as flavonoids and lipoxygenase. It was found that soaking, commonly the first step of soy milk production, induced an increase in daidzein and genistein in the soaked soybeans (Matsuura et al., 1989). The level of increase was dependent on the soaking temperature and pH, with 50 °C and pH6 being the most favorable conditions for the daidzein and genistein production (Matsuura et al., 1989).

Such a phenomenon is due to the activity of β -glucosidases which hydrolyze the isoflavones from the glucoside form to the aglycone form (Matsuura et al., 1989). To minimize the production of daidzein and genistein during soybean soaking, glucono- δ -lactone was proposed as an additive in the soaking water to inhibit the activities of β -glucosidases (Matsuura et al., 1989). Similarly, in another study comparing four different processing techniques, namely soaked soybean-boiling before filtration, soaked soybean-boiling after filtration, dry soybean-boiling (no soaking before grinding soybeans with water for boiling) before filtration, dry soybean-boiling (no soaking before grinding soybeans with water for boiling) after filtration, it was found that DS-BBF produced the least beany flavor in soy milk (Yu et al., 2018). Also, the levels of these compounds in soy milk were positively correlated with the soaking temperature (Li et al., 2019). Strategies have been designed to inactivate lipoxygenase during soy food processing. One example is employing thermal inactivation by superheated steam (Chong, Mah, Easa, & Tan, 2019). When applying these strategies to minimize the adverse effects on the flavors of soy products by the off-flavor components, attention must be paid to make sure other nutritional components, such as crude protein, shall not be negatively affected (Chong et al., 2019). With the advancement of soy food processing technologies enhances, there is more flexibility in choosing soybean germplasms for industrial uses. However, the processing technologies have to be tailor-made for each end-product in order to produce a favorable secondary metabolite profile as well as retaining the desirable nutritional components.



10. Conclusion

The composition of secondary metabolites in soybean is a result of both natural and human selections. While natural selection is mostly influenced by environmental factors such as adaptations to local climate and the prevalence of natural enemies, human selection has been focused on the appearance, yield, and harvestability of the seeds. Secondary metabolites, including flavonoids, terpenes, and alkaloids, play important roles in all these aspects. Meanwhile, these metabolites contribute to the flavors of soybean. As soybean is widely employed to produce food products such as soy milk, tofu and fermented soy products, the need for the post-domestication selection of secondary metabolites associated with the flavors of soybean cannot be ignored. Industrial methods have been developed to minimize the effects of off-flavor metabolites on soy food products.

However, the soybean processing conditions have to be tailored to each specific food product. Wild soybean accessions have diverse secondary metabolite compositions in the seed due to the diverse genetic backgrounds. Thus, wild soybean germplasms could serve as a huge gene pool for molecular breeding to develop soybean cultivars with desirable secondary metabolite profiles that fit different uses.

Acknowledgments

J.Y. Chu copy-edited this manuscript. Any opinions, findings, conclusions or recommendations expressed in this publication do not reflect the views of the Government of the Hong Kong Special Administrative Region or the Innovation and Technology Commission.

Funding

This research was funded by the Hong Kong Research Grants Council General Research Fund (14143916) to H.-M.L.

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