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Metabolic Biosynthesis of Potato (Solanum tuberosum L.) Antioxidants and Implications for Human Health

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METABOLIC BIOSYNTHESIS OF POTATO (*Solanum tuberosum* L.) ANTIOXIDANTS AND IMPLICATIONS FOR HUMAN HEALTH

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Abstract

Potato (Solanum tuberosum L.) is common, affordable, readily stored, easily prepared for consumption, and nutritious. For these reasons, potato has become one of the top five crops consumed worldwide. Consequently, it is important to understand its contribution to both our daily and long-term health. Potato is one of the most important sources of antioxidants in the human diet. As such, it supports the antioxidant defense network in our bodies that reduces cellular and tissue toxicities that result from free radical-induced protein, lipid, carbohydrate, and DNA damage. In this way, potato antioxidants may reduce the risk for cancers, cardiovascular diseases, diabetes, and even radiation damage. A better understanding of these components of potato is needed by the food industry, health professionals, and consumers. This review provides referenced summaries of all of the antioxidant groups present in potato tubers and updated schematics including genetic regulation for the major antioxidant biosynthesis pathways. This review complements current knowledge on the role of potato in human health. We hope it will provide impetus towards breeding efforts to develop cultivars with increased antioxidant capacity as "functional foods" and encourage potato consumers and processors to work towards preservation of antioxidant capacity in cooked potato and potato products.

This review complements current knowledge on the role of potato in human health and provides impetus to the development of increased antioxidant capacity in cultivated potato and in diets containing potato or potato products (Camire et al., 2009). This review is not intended to replace the numerous reviews on antioxidants in foods but only to summarize known potato-based antioxidants.

POTATO AS A SOURCE OF ANTIOXIDANTS

Higher plants produce 5,000 to 25,000 primary and secondary metabolites (Trethewey, 2004). Secondary metabolites are typically biosynthesized in specific cells or tissues and then stored in other tissues, but some metabolites are ubiquitously produced then transferred to a final storage place via phloem or xylem (Wink, 2010). Hydrophilic substances are most often stored in vacuoles while the lipophilic metabolites are stored in plastids, latex, resin ducts, oil cells, or the cuticle (Wink, 2010). In general, as opposed to primary metabolites, that have essential metabolic functions in plants, secondary metabolites protect plants against abiotic (environmental extremes, heavy metals, etc.) and biotic (insect, fungi, microbial, etc.) stressors.

Dietary antioxidants are defined by the Food and Nutrition Board of the National Academy of Sciences (National Academy of Science, 1998) as a food substance that can significantly decrease the adverse effects of either reactive oxygen species (ROS) or reactive nitrogen species (RNS) on normal physiological function in humans. In recent decades, there has been an explosion of research indicating that the intake of plant secondary metabolites with

antioxidant capability is related to the human health benefits associated with fruit and vegetable intake. Consumption of antioxidant plant secondary metabolites has been highlighted as an important means of fighting the oxidative stress resulting from excessive formation of ROS and RNS. It is the accumulation of high levels of ROS and RNS that overwhelms the antioxidant defense network and ultimately leads to cellular and tissue toxicities resulting from free radical-induced protein, lipid, carbohydrate, and DNA damage. Such macromolecular damage has been related to adverse health outcomes and complications from a variety of environmental toxic exposures such as environmental pollutants like cigarette smoke and age-related human diseases including the metabolic syndrome, diabetes, various forms of dementia, arthritis, cancer, and cardiovascular disease (Ames and Gold, 1991; Gaziano et al., 1992; De Stefani et al., 1999; Ford et al., 2003; Hou, 2003; Devasagayam et al., 2004; Huber et al., 2006). Improved outcomes following radiation exposure could also be expected through antioxidant consumption (Okunieff et al., 2008).

It is important to note that plant secondary metabolites exert a variety of antioxidant functions due to their widely diverse structures. The health benefits of consuming plant antioxidants result from the synergistic and additive effects of multiple secondary metabolites providing complementary antioxidant activities, as opposed to the antioxidant effects of only a single bioactive component. Both water soluble (i.e., vitamin C) and lipid soluble micronutrients (i.e., vitamins A and E, and β-carotene) obtained from plants play a critical role in maintaining the antioxidant defense system by direct non-enzymatic scavenging of free radicals. A large number of plant secondary metabolites in the form of phenolics do not act directly as antioxidants in humans as they are too poorly bioavailable to exert direct free radical-scavenging

activities (Halliwell and Gutteridge, 2007). An important mechanism of antioxidant efficacy with phenolics is their biotransformation during intestinal digestion and metabolism, which generates metabolites and degradation products that regulate genes that contribute to the defense against oxidative stress. In that regard, the antioxidant response element (ARE) transduction pathway, which is controlled by redox-sensitive transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), has been indicated to up-regulate the synthesis of many important antioxidant detoxifying enzymes and endogenous antioxidants in response to intestinally-generated metabolites of phenolics (Mattson et al., 2007).

Unfortunately, many of the so-called fruit and vegetable "super antioxidant functional foods" identified as naturally high in antioxidant compounds, including blueberry and raspberry, are only produced seasonally and often by few specialized growers in limited amounts. Furthermore, they are highly perishable and as a consequence largely unavailable or costly, which leads to a relatively infrequent intake and ultimately limits their health benefits (Wu et al., 2004). Although potatoes (*Solanum tuberosum* L.) contain relatively lower levels of antioxidants compared with berries, potatoes have the distinction of being available globally. They are common and affordable in the developing world and can be stored for long periods of time (Wu et al., 2004; Andre et al., 2007; Love and Pavek, 2008). As well, potato is one of the top five crops consumed worldwide, and on a volume basis, it is estimated to be one of the most important sources of antioxidants in the human diet (Lachman and Hamouz, 2005). As such, many recent studies have determined the antioxidant levels of potato genotypes and the inheritance of some of these antioxidant-producing genes has been investigated in breeding studies (Brown et al., 2003; Brown, 2005; Lachman and Hamouz, 2005; Andre et al., 2007).

ANTIOXIDANT GROUPS AND RELATED PATHWAYS IN POTATO

There are three major groups of antioxidants present in potato, as in most plants. These classifications are based on metabolic relationships and structural composition, although all antioxidants are part of a larger complex and interconnected system of biochemical defence present in plants. The first group contains the aromatic antioxidants, with similar aromatic ring structure and closely related metabolic pathways. In the second group are the isoprenoid antioxidants, united by the plastid location of synthesis and lipid-solubility. In the third group, functionality is related to ascorbate and glutathione functions in a redox system of compound-recycling. These three groups are outlined in sequence and a general overview of the relationships between the antioxidant pathways is depicted in Figure 1.

Aromatic Antioxidants

Three separate pathways are believed to generate the majority of the aromatic antioxidants in higher plants (Hemingway and Laks, 1992). These consist of the shikimate pathway producing tyrosine, phenylalanine, and tryptophan (Figure 2) and kynurenine from tryptophan (Figure 3); the phenylpropanoid pathway (Figure 4) producing cinnamic acid and its derivatives; and the flavonoid pathway (Figure 5) producing an array of diverse flavonoid compounds and anthocyanins (Hemingway and Laks, 1992; Schijlen et al., 2004). All phenolics in plants are generated from the common intermediate, phenylalanine, or the close precursor shikimic acid.

Despite the common separation of these three pathways in the literature, it is important to point out that some researchers have conceptualized these three components as part of a larger

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metabolic unit (Hanson and Zucker, 1963; Hemingway and Laks, 1992). While these studies appear to have been largely overlooked, the rationale for this concept is strong. For example, these three pathways are integrated in producing precursors for one another and share similar responses to environmental stressors (Morris et al., 1989; Yao et al., 1995; Grace and Logan, 2000; Schijlen et al., 2004). Furthermore, when one pathway is suppressed it affects the expression of subsequent pathways. For example, in a study by Yao et al. (1995) transgenic potato plants with an extra shikimate pathway branch point acted as metabolic sinks for tryptophan, a shikimate end-product. This resulted in a marked decline in phenylalanine-derived phenolics, a direct product of the phenylpropanoid pathway. These three pathways are outlined below, starting with the shikimate pathway and their relationships noted.

The Shikimate Pathway

The shikimate pathway (Figure 2) is the initial pathway for aromatic antioxidant biosynthesis, as it does not receive precursor compounds from either the phenylpropanoid or flavonoid pathways (Bentley, 1990; Herrmann and Weaver, 1999). The shikimate pathway was once believed to be part of a dual pathway system located in both the cytoplasm and the plastids. Such dual pathways are common in plants, where an alternate occurs if one pathway is deactivated (Morris et al., 1989). More recently, the shikimate pathway was determined to be entirely plastidic in plant cells (Bentley, 1990; Herrmann, 1995; Schmid and Amrhein, 1995; Weaver and Herrmann, 1997).

Through a series of seven metabolic steps, the shikimate pathway consumes carbon metabolites, in the form of phosphoenolpyruvate and erythrose 4-phosphate, and produces chorismate, which is subsequently transformed into one of three aromatic amino acids

(phenylalanine, tryptophan, and tyrosine) as described in the sequence of Figure 2, modified

from Bentley (1990), Schmid and Amrhein (1995) and Herrmann and Weaver (1999). These

three aromatic amino acids are then used in other metabolic pathways in the plant to become

thousands of aromatic secondary metabolites, many of which demonstrate significant antioxidant

activity (Bentley, 1990; Herrmann, 1995; Schijlen et al., 2004). Some of the better known

antioxidant products include vitamin K, folate, and ubiquinone (Bentley, 1990). Apart from

products derived from chorismate, every intermediate in the shikimate pathway has the potential

to become a branch point for further metabolic pathways (Herrmann and Weaver, 1999). This

metabolic flexibility helps the shikimate pathway generate an astounding diversity of secondary

metabolites in response to numerous external and internal factors. In that regard, the antioxidant

kynurenic acid is a metabolite of tryptophan that is formed along the tryptophan–kynurenine

pathway in plants (Figure 3). N-formyl-kynurenine is formed from tryptophan via indole-2,3-

dioxygenase and tryptophan-2,3-dioxygenase and . Kynurenine formylase then converts N-

formyl kynurenine to L-kynurenine. Kynurenic acid is subsequently generated from kynurenine

by kynurenine aminotransferase (Nemeth et al., 2005; Turski et al., 2011). Recently potatoes

have been noted to contain relatively high concentrations of kynurenic acid among other tested

foods (Turski et al., 2012). In vitro studies have indicated that kynurenic acid is a powerful ROS

scavenging agent (Lugo-Huitrón et al., 2011). All three end products (kynurenic acid, 3-hydroxy-

L-kynurenine, and xanthurenic acid) are antioxidants and anti-inflammatory compounds (KEGG,

2013).

Environmental factors, particularly environmental stress, promote shikimate pathway

activity (Bentley, 1990; Herrmann, 1995). For example, excessive light, nitrogen or phosphorus

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starvation, amino acid limitations, disease, or insect damage, are all promoters of this pathway (Dyer et al., 1989; Dean et al., 1992; Weaver and Herrmann, 1997; Jones et al., 2002; Reyes and Cisneros-Zevallos, 2003). In another example, potato cultivars vulnerable to black spot disease (a non-pathogenic disease which is caused by mechanical stress; bruising) produced 55% more tyrosine than resistant potato cultivars (Dean et al., 1992). However, some plant pests have evolved methods to undermine shikimate pathway defences (Jones et al., 2002). For instance, the potato cyst nematode (*Globodera pallida*) produces the enzyme chorismate mutase, which disrupts defence chemicals synthesized by the shikimate pathway in its host, potato.

No universal suppressor or activator of the shikimate pathway has yet been found among the higher plants (Bentley, 1990; Herrmann, 1995). The end-products of the shikimate pathway function as suppressors in many other plant biosynthesis pathways, but have varied species-specific effects on shikimate pathway activity (Bentley, 1990; Yao et al., 1995). In potatoes, 3-deoxy-*D*-arabino-heptulosonate-7-phosphate synthase (DAHP synthase), the first enzyme in the shikimate pathway, is stimulated by tryptophan (a shikimate pathway end-product), Mn^{2+}, and sub-lethal doses of the broad-spectrum herbicide glyphosate, and strongly inhibited by Fe^{2+}, Zn^{2+}, and Cu^{2+} (Pinto et al., 1986; Pinto et al., 1988; Bentley, 1990; Schmid and Amrhein, 1994). Potato is uncommon among higher plants in having two isozymes of DAHP synthase; DAHP-Mn^{2+} synthase and DAHP-Co^{2+} synthase (Morris et al., 1989, Bentley, 1990; Schmid and Amrhein, 1994). While DAHP-Mn^{2+} synthase can only be activated by Mn^{2+}, DAHP-Co^{2+} synthase is promoted by Co^{2+}, Mg^{2+}, or Mn^{2+} (Schmid and Amrhein, 1994). As knowledge of the shikimate pathway expands, particularly in relation to its up-regulation in response to

environmental stress, it may be possible to manipulate the pathway to predictably increase

antioxidant levels in potato.

The Phenylpropanoid Pathway

The phenylpropanoid pathway involves a combination of three metabolic steps that

utilise an aromatic precursor from the shikimate pathway, phenylalanine, to produce a wide

range of phenolics with diverse structures (Castelluccio et al., 1995; Coquoz et al., 1998; Grace

and Logan, 2000) as described in Figure 4. Phenylalanine ammonia-lyase (PAL) is the rate-

limiting enzyme in the secondary metabolic pathway that leads to the production of phenolic

compounds. Many of these end-products and pathway-intermediates have documented

antioxidant activity, including chlorogenic acid, salicyclic acid, cinnamic acid, and ferulic acid;

while the phenylpropanoid pathway is also responsible for structural phenolics such as lignin

(Castelluccio et al., 1995; Coquoz et al., 1998; Grace and Logan, 2000). Many phenolics have

limited absorption as they are typically present as esters with glucose, hydroxyfatty acids, sterols

and alcohols. Due to the very low bioavailability and low human tissue concentrations it is

unlikely that phenolics exert significant direct antioxidant scavenging effects apart from within

the gut lumen where higher phenolic concentrations are found (Schaffer and Halliwell, 2012).

The in vivo induction of antioxidant capacity in humans following intake of phenolics has

recently been related to their activation of adaptive cellular stress responses including the ARE

pathway (Mattson et al., 2007).

The evolutionary basis of the phenylpropanoid pathway in plants is still obscure. The

structural function of the lignins produced in the phenylpropanoid pathway has led to speculation

that the evolution of this pathway allowed for the structural features that first promoted land-colonization by early plants (Douglas, 1996).

As noted with the shikimate pathway, environmental stress strongly affects phenylpropanoid pathway activity (Grace and Logan, 2000; Solecka and Kacperska, 2003). In particular, the phenylpropanoid pathway may mediate the immune response of plants to fungal infection (Hahlbrock and Scheel, 1989). In response to stress, the primary enzyme involved in the phenylpropanoid pathway, PAL, is increased, which subsequently causes the phenylpropanoid pathway products to build-up in stressed tissues (Solecka and Kacperska, 2003). This allows free radical-scavenging phenolics and structural phenolics to flood stressed tissues, providing both structural support and relief from free radical oxygen species in those areas (Koes et al., 1994). Interestingly, phenolics also accumulate in plant tissues exposed to excess carbohydrates, suggesting that the phenylpropanoid pathway could also function to divert excess photosynthate into stable product pools (Grace and Logan, 2000). However, as with the shikimate pathway, not all stressors affect all plant species in the same way. For instance, Reyes and Cisneros-Zevallos (2003) found that although wounding caused an expected build-up of phenolic compounds in potato tubers, other known stressors such as methyl jasmonate, ethylene, temperature, and light, did not. This is despite the fact that these stress factors have proven to cause a build-up of phenolics in studies done with other plant species (Hahlbrock and Scheel, 1989; Reyes and Cisneros-Zevallos, 2003). As well, certain elicitors, such as β-1,3-oligosaccharide, changed the proportion of particular end products in potato, while not affecting the overall level of phenolics produced (Matsuda et al., 2005).

Although few studies have focused on increasing the antioxidant activity of potatoes by regulating the shikimate pathway, this has been accomplished very successfully in potato with the phenylpropanoid pathway. Lukaszewicz et al. (2002) were able to up-regulate the production of phenolics (particularly chlorogenic acid) to produce 45% more antioxidant activity in transgenic potato plants by increasing the production of a certain protein labelled 14-3-3. Similarly, they found that repressing the expression of this protein also depressed antioxidant activity and phenolic levels in transgenic plants (Lukaszewicz et al., 2002). Also with potatoes, simple illumination was linked to transient increase in phenolics (particularly chlorogenic acid) through direct stimulation of PAL (Lamb and Rubery, 1976; Hahlbrock and Scheel, 1989; Lewis et al., 1998). The up-regulation of these compounds has also been linked to increased resistance to the aggressive fungal disease of potato, Late Blight (*Phytophthora infestans*) (Yao et al., 1995; Wang et al., 2008). A similar study noted that potatoes treated with a PAL-inhibiting compound, L-2-aminooxy-3-phenylpropionic acid, and then exposed to an avirulent race of *P. infestans* showed a complete lack of resistance response to the fungus (Hahlbrock and Scheel, 1989).

Phenolic acids have also been associated with cold tolerance and temperature-related accumulation (Reyes et al., 2004). When potatoes were grown under field conditions, colder temperatures with longer days increased phenolic acid content by 1.4 times in tubers, while a second study noted that inhibiting the phenylpropanoid pathway also reduced the ability of potato plants to put on fresh leaf weight or accumulate dry matter under cold-stressed conditions (Solecka and Kacperska, 2003; Reyes et al., 2004). As with the shikimate pathway, clues to up-regulate the phenylpropanoid pathway have not yet been thoroughly explored towards commercial application.

The Flavonoid Pathway

The phenylpropanoid pathway is involved in the synthesis of flavonoids. With the precursors malonyl-CoA and *p*-coumaroyl-CoA (derived from the carbohydrate and phenylpropanoid pathways, respectively) and an initiating enzyme of chalcone synthase (CHS), the flavonoid pathway (Figure 5) synthesizes a variety of secondary metabolites with documented antioxidant activity due to their polyphenolic structure. Most flavonoid compounds have greater antioxidant capacity on a molar basis than the better-known antioxidant compounds such as vitamins E and C (Rice-Evans et al., 1995; Rice-Evans et al., 1997; Schijlen et al., 2004). However, the micromolar tissue concentrations of flavonoids in human tissues are considered too low to directly affect in vivo antioxidant capacity (Schaffer and Halliwell, 2012). On the other hand, there is increasing evidence that flavonoids can enhance the expression of endogenous antioxidant systems such as catalase and superoxide dismutase and enzymes involved with glutathione metabolism that is likely modulated via ARE signaling transduction pathways (Schaffer and Halliwell, 2012). Flavonoids are among the most numerous of the secondary metabolites, with over 6,000 described so far, and more being identified in every new study (Schijlen et al., 2004). Unlike the structurally-diverse end-products of the shikimate and phenylpropanoid pathways, all flavonoids are structurally similar; with a pair of six-carbon aromatic rings connected by a hetero cycle with three carbon atoms (Schijlen et al., 2004). Modifications of the three-carbon hetero cycle lead to the different classes of flavonoids, such as flavones, flavanones, isoflavones, flavonols, flavan-3-ols and anthocyanidins (Schijlen et al., 2004) (Figure 5). Other flavonoid groups include condensed tannins, xanthones and aurones.

Products of the flavonoid pathway are critical to a plethora of essential plant activities (Koes et al., 1994). For example, while chloroplasts are dependent on energy-providing light for photosynthesis, anthocyanins help shield DNA and photosynthetic pigments from UV damage. Anthocyanins also provide the colour displays which attract pollinators, and disseminators to indehiscent fruit, while flavonoids provide necessary compounds for pollen and pollen tube development in some plants (Coe et al., 1981; Mo et al., 1992; van der Meer et al., 1992; Koes et al., 1994). Flavonoids include key compounds released by leguminous roots to regulate the *nod* genes used by nitrogen-fixing symbiotic bacteria, enabling the symbiotic association between legumes and bacteria (Peters et al., 1986; Zaat et al., 1987; Koes et al., 1994). The vast array of functions provided by compounds produced from the flavonoid pathway, as well as the distribution of those functions and their respective biosynthetic pathways in the plant kingdom, suggests a step-wise sequential evolution for the flavonoid pathway among plants (Koes et al., 1994).

The flavonoid pathway is very well understood (particularly in comparison to research concerning the shikimate and phenylpropanoid pathways), and this has allowed for the identification of the DNA-binding proteins responsible for pathway regulation, the location of the genes responsible for these proteins, and has even enabled the synthesis of these proteins in vitro (Holton and Cornisch, 1995; Ranish and Hahn, 1996; Schijlen et al., 2004). Molecular tools developed from these studies have now enabled genetic modification of the flavonoid pathway in numerous crop plants, including potato (Sévenier et al., 2002; Schijlen et al., 2004). As a result, the almost limitless modification of the flavonoid pathway in plants is now possible, including the up-regulation or down- regulation of potato genes (Rommens et al., 2008; Jung et al., 2009),

or the introduction of novel genes to synthesize novel compounds never before found in particular plant species (Schijlen et al., 2004). Anthocyanin content in potatoes can be greatly increased through transgenic manipulations (Lorenc-Kukuła et al., 2005; Payyavula et al., 2012; Wei et al., 2012). In a study involving up-regulation of genes in potato, the increased expression of a leaf colour gene (from the MYC type R gene family) and a colourless gene (from the MYB type CI gene) resulted in a large accumulation of both kaempferol in the tubers and anthocyanin in the peel (De Vos et al., 2000; Schijlen et al., 2004). However, mixed results occurred when novel genes, from various origins, were introduced into potatoes in an attempt to up-regulate existing genes (Schijlen et al., 2004). While *Petunia* cDNA increased accumulation of petunidin and pelargonidin-type anthocyanins, the cDNA from barley was non-functional in potato (Stobiecki et al., 2002; Lukaszewicz et al., 2004; Schijlen et al., 2004). Similarly, anthocyanins were depressed when transgenic plants were engineered with an anti-sense construct of chalcone synthase (CHS) *Petunia* cDNA (Stobiecki et al., 2002; Schijlen et al., 2004). As well, potatoes engineered to express the *Petunia* cDNA encoding the dihydroflavonol-4-reductase (DFR) enzyme showed a 3-fold increase in petunidin derivatives and a 4-fold increase in pelargonidin derivatives, while transgenic plants engineered with a corresponding anti-sense DFR construct demonstrated a significant decrease in anthocyanin levels (Lukaszewicz et al., 2004). A similar study noted a modest increase in anthocyanins in transgenic potatoes expressing chalcone isomerase (CHI) enzyme *Petunia* cDNA genes (Stobiecki et al., 2002). Interestingly, transgenic plants with anti-sense constructs of the CHI *Petunia* cDNA did not show any evidence of CHI suppression suggesting that the gene was not homologous enough to participate in suppression or, as previously documented, that chalcones were spontaneously converting to naringenin in the

absence of CHI (Stobiecki et al., 2002; Schijlen et al., 2004). The homologous structure of the flavonoid pathway in all plants enables the insertion of foreign genes that can create entire branches of the flavonoid pathway not normally seen in the recipient plant (Schijlen et al., 2004). For example, the insertion of two genes from the iris and pansy genome into the rose genome produced the first blue rose, once the unobtainable "holy grail" of rose breeding, as the blue anthocyanin delphinidin is not naturally genetically present in roses (Tanaka, 2006). This amazing success with foreign gene insertion into roses suggests the possibilities of other complex gene insertions into the flavonoid pathway. In the future, flavonoid pathway manipulations may lead to completely novel compounds in potato plants.

As with the shikimate and phenylpropanoid pathways, environmental factors regulate flavonoid pathway activity (Schijlen et al., 2004). In particular, microbial elicitors and UV radiation strongly up-regulated the pathway (Koes et al., 1994; Lewis et al., 1998; Schijlen et al., 2004). Most plants show little or no anthocyanin biosynthesis in the absence of UV radiation (Lewis et al., 1998). However, potatoes are a marked exception to this rule; potato tubers can accumulate high levels of anthocyanins despite forming in the dark underground (Lewis et al., 1998). Deeply coloured potatoes start anthocyanin synthesis at tuber initiation and a near-constant concentration of anthocyanins is synthesized as the tubers enlarge (Lewis et al., 1999). Cultivars with less color showed a lower initial level of anthocyanin biosynthesis, which builds up over time until a maximum concentration (related to tuber weight) is reached (Lewis et al., 1999). Flavonoid synthesis follows a similar pattern in the deeply coloured and paler cultivars but with overall lower concentrations compared with anthocyanins (Lewis et al., 1999). In all cases, anthocyanin accumulation begins at the stolon end of the tuber and gradually moves

across the tuber to the rose end (Lewis et al., 1999). At tuber maturity, the concentration is relatively similar at both ends (Lewis et al., 1999). Cold temperatures tend to increase anthocyanin concentrations. For example, cold storage (at 4°C) for 6 months increased anthocyanin concentrations, which were greater at the rose end compared with the stolon end (Lewis et al., 1999). Similarly, the relatively colder and longer days of Colorado-grown compared with Texas-grown tubers resulted in 2.5-fold greater anthocyanin concentrations (Reyes et al., 2004). Interestingly, cold temperatures were also linked to increased activity in the phenylpropanoid pathway in potato tubers as mentioned above (Solecka and Kacperska, 2003; Reyes et al., 2004).

The deep purple-fleshed potato cultivars are rich in anthocyanins (Hamouz et al., 2011; Burgos et al., 2013), for example, these reached 418 mg/100 g fresh weight (FW) in the cultivar Guincho, with high antioxidant activity (Burgos et al., 2013). Anthocyanin contents vary significantly among blue fleshed cultivars (Hamouz et al., 2011). High positive correlations between antioxidant capacity and total anthocyanins/phenolics suggest that anthocyanins and phenolic compounds are mainly responsible for the antioxidant capacity in colored potatoes (Reyes et al., 2005). Therefore, visual selection of purple/blue fleshed potatoes or genetically regulated anthocyanin pathways (Lukaszewicz et al., 2004; Lorenc-Kukuła et al., 2005; Rommens et al., 2008; Zhang et al., 2009; Wei et al., 2012) can effectively improve the antioxidant capacity. Field- and storage-based studies such as these provide clues to complex environmental regulation of antioxidant levels in potato. A better understanding of these mechanisms may enable environmental manipulation to boost antioxidant levels during field culture or improve post-harvest handling of potato tubers destined for human consumption.

Plastidic Isoprenoid Antioxidants

Numerous isoprenoid secondary metabolites with antioxidant activity are synthesized in plant plastids including the important lipid-soluble compounds; the tocopherols and carotenoids (Smirnoff, 2005; DellaPenna and Pogson, 2006). The pathways that biosynthesize tocopherol and carotenoids are interrelated, leading some researchers to suggest grouping these two pathways together under an umbrella term of plastidic isoprenoid synthesis (Smirnoff, 2005; DellaPenna and Pogson, 2006). For instance, the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway (the non-mevalonate pathway) provides the necessary precursor isopentylpyrophosphate for conversion to geranylgeranyl disphosphate (GGDP), a precursor to both the carotenoid pathway (DellaPenna and Pogson, 2006) (Figure 6) and the tocochromanol pathway that produces tocopherol (Figure 7). There is also evidence that changes to one of the isoprenoid biosynthetic pathways affects the other (DellaPenna and Pogson, 2006). For example, Römer et al. (2002) found that increasing zeaxanthin content in potato tubers inadvertently caused a 2- to 3-fold increase in α-tocopherol (DellaPenna and Pogson, 2006). Co-regulation of the carotenoid and tocochromanol pathways is hypothetical at this point as their relationship is not well described in the literature. As such, the carotenoid and tocochromanol pathways are considered separately here.

The Carotenoid Pathway

The carotenoids are lipophilic pigments that include over 700 known compounds. In the human diet, α-carotene, lutein, zeaxanthin, lycopene, β-cryptoxanthin, fucoxanthin, astaxanthin are the most important among which are β-carotene (precursor to vitamin A) and lycopene (DellaPenna and Pogson, 2006; Lu and Li, 2008) have received the most attention that is related

in part to their high antioxidant potential. Carotenoids have a potent antioxidant capability to directly quench ROS such as singlet oxygen, superoxide anion and lipid peroxyl radicals via several mechanisms. In plants, carotenoids serve a variety of functions. They act as accessory pigments in photosynthesis providing red and yellow colouration to tissues and serve as chloroplast antioxidants (Smirnoff, 2005; DellaPenna and Pogson, 2006; Lu and Li, 2008). The metabolic steps and associated genes of the carotenoid pathway (Figure 7) are well understood and have been successfully manipulated in vitro (Ducreux et al., 2005; Lu and Li, 2008). Unfortunately, the factors involved in pathway regulation in the potato are less well understood (Ducreux et al., 2005; Lu and Li, 2008). Two studies examined the expression of the carotenoid pathway during tuber development and observed the involvement of zeaxanthin epoxidase in the suppression and regulation of carotenoid synthesis based on gene activity, which led them to hypothesize that this enzyme could be directly related to the regulation of the carotenoid pathway in potato (Morris et al., 2004; DellaPenna and Pogson, 2006). As well, a recent review on potato cultivars and carotenoid accumulation also identified a correlation between the transcript levels of the phytoene synthase (PSY) enzyme and the carotenoid content across 20 different potato cultivars (DellaPenna and Pogson, 2006). Interestingly, this transcript activity did not seem to act on the carotenoid pathway directly but instead caused the increased transcription of a carotenoid storage protein, fibrillin, suggesting that increased carotenoid levels were observed because of an increased capacity of the tuber to store the synthesized compounds (Morris et al., 2004; DellaPenna and Pogson, 2006).

Despite potential limitations posed by the lack of thorough knowledge of regulators involved in the carotenoid pathway, numerous studies have proven successful in up-regulating

existing enzymes to promote greater carotenoid levels (Lu and Li, 2008). For example, transgenic potato tubers enhanced with an *Erwinia uredovora* crtB gene (responsible for encoding PSY) boosted carotenoid levels 6.5-fold compared with controls; with β-carotene and lutein reaching levels 11- and 19-fold greater, respectively, compared with controls (Ducreux et al., 2005). Diretto et al. (2006, 2007) demonstrated in a series of studies that silencing non-heme β-carotene hydroxylases CHY1 and CHY2, as well as silencing the first step in the lutein biosynthesis (LCY-e) branch of the carotenoid pathway, produced relatively high levels of β-carotene, compared with the usual very low concentrations in potato tubers. By silencing CHY1 and CHY2, an increase in β-carotene values of 38-fold and total carotenoids of 4.5-fold was observed, while silencing LCY-e resulted in a 14-fold increase in β-carotene and a 2.5-fold increase in total carotenoids compared with controls (Diretto et al., 2006 and 2007).

Numerous studies have also succeeded in up-regulating specific carotenoid compounds or introducing entirely novel ones into a carotenoid profile (DellaPenna and Pogson, 2006). Potato tubers typically accumulate the carotenoids violaxanthin and lutein (Römer et al., 2002; DellaPenna and Pogson, 2006). However, Van Eck et al. (2007) noted that halting the conversion of β-carotene to zeaxanthin in potato tubers by silencing the β-carotene hydroxylase gene (bch) resulted in an increase of β-carotene from trace amounts up to 331 µg/g fresh mass (Van Eck et al., 2007). Römer et al. (2002) demonstrated that zeaxanthin production could also be up-regulated. They created transgenic potatoes with sense and antisense factors of zeaxanthin epoxidase, inhibiting the transformation of zeaxanthin to violaxanthin. From this, they noted an accumulation of zeaxanthin of 4- to 130-fold greater than controls depending on the transgenic line and the duration of tuber development (Römer et al., 2002). Bub et al. (2008) went a step

further and proved that potato tubers could be genetically engineered to produce large amounts of zeaxanthin that was bioavailable in the human body.

Studies looking at the up-regulation of carotenoid compounds are particularly important in potato as potatoes traditionally have lesser concentrations of carotenoids relative to other crop plants (Morris et al., 2004). In fact, the greatest levels of carotenoids found in potato tubers were present during early tuber development with relative concentration of accumulated carotenoids decreasing as the tubers accumulate dry mass (Morris et al., 2004).

The evolution of the carotenoid pathway is a complex one. Similar to the shikimate pathway, several key enzymes involved in the carotenoid pathway are also found in cyanobacteria, bacteria, and fungi (Sandmann, 2002). However, unlike the conserved nature of the shikimate pathway in both plants and cyanobacteria, numerous evolutionary steps have resulted in a range of genetic diversity between the carotenoid pathways in microorganisms and plants (Sandmann, 2002). For instance, the plant-specific enzyme capsanthin capsorubin synthase, involved in converting violaxanthin to capsorubin among other things, is believed to have formed from a gene duplication of a lycopene cyclise gene, an enzyme with a very similar genetic code in both cyanobacteria and plants (Sandmann, 2002). For other enzymes, such as PSY, a clear line of evolution can be traced from archea and specific groups of bacteria to cyanobacteria and green algae, and finally to higher plants (Sandmann, 2002). However, despite the apparent diversification of the carotenoid pathway in plants, it is now believed that the precursors of the carotenoid pathway were acquired in a similar way to the shikimate pathway, through opportunistic plants absorbing endosymbiotic cyanobacteria into their own cells (Sandmann, 2002).

The existence of the carotenoid pathways across a wide range of species including microorganism and plants underline the importance of carotenoids for these organisms. A carotenoid-deficient mutant of the extremely radioresistant bacterium *Deinococcus radiodurans* became approximately 100-fold more sensitive to radiation than the wild type (Carbonneau et al., 1989). Similar results were obtained from carotenoid-less transgenic *D. radiodurans* in which the *crt*B gene encoding phytoene synthase was knocked out (Xu et al., 2007). The phytoene synthase is the enzyme controlling the entry step of all carotenoid pathways (Carbonneau et al., 1989) (Figure 7: enzyme a). This is strong evidence that carotenoids, and likely also other antioxidants, play an important role in protecting cells from radiation and other stress damage by scavenging stress- induced oxidative free radicals. Although radiation caused DNA damage can be inheritable and increase cancer risk, protein oxidation is found to be likely the leading cause of irradiation-induced cell death (Daly et al., 2007; Krisko and Radman, 2010). Many antioxidant nutrients and phytochemicals have antimutagenic properties (Weiss and Landauer, 2003). Rats fed with β-carotene (50 mg/kg diet) for 1 week were found to be more tolerant to radiation (Ben-Amotz et al., 1996).

The Tocochromanol Pathway

The tocochromanols are divided into two groups, one with 4 tocopherols and the other with 4 tocotrienols, synthesized exclusively by photosynthetic organisms, all of which are responsible for varying levels of vitamin E activity (Sattler et al., 2003; DellaPenna and Pogson, 2006). Vitamin E plays an important antioxidant role in humans as potent lipid-soluble free radical-scavenging and chain-breaking antioxidant. Tocopherols and tocotrienols share a

common structure consisting of a hydrophobic isoprenoid tail and a chromanol head, but differ in the degree of saturation and the source of their tails (Smirnoff, 2005). For instance, the tails of tocopherols are derived from phytyl diphosphate and are saturated, while the tails of tocotrienols are derived from geranylgeranyl diphosphate and are unsaturated (Smirnoff, 2005). The shikimate pathway provides ρ-hydroxyphenylpyruvate (derived from tyrosine) to produce the hydrophilic chromanol head, while the MEP provides the isoprenoid tail (Grusak and DellaPenna, 1999; Ajjawi and Shintani, 2004; Smirnoff, 2005; DellaPenna and Pogson, 2006). In plants, the tocochromanols protect against oxidative stress in the lipids of photosynthetic membranes and plant seeds, are involved in primary carbohydrate metabolism, and stabilize membrane structure through polyunsaturated fatty acyl chains (Sattler et al., 2003; Smirnoff, 2005; Dφrmann, 2007). It has also been noted that environmental stressors such as high light and drought cause a build-up of tocopherol compounds, suggesting that tocopherol may also be involved in plant defence (Munne-Bosch et al., 1999; Collakova and DellaPenna, 2003; Smirnoff, 2005). However, it has also been noted in *Arabidopsis* that tocopherol-deficient mutants demonstrated the same photosynthetic levels and plant viability as control plants with normal tocopherol expression, suggesting that in its absence other compounds may contribute antioxidant properties (Porfirova et al., 2002).

As with the carotenoid pathway, all core pathway enzymes involved in tocochromanol biosynthesis (Figure 8) have been identified and isolated in the lab (Chen et al., 2006; DellaPenna and Pogson, 2006). As well, numerous regulatory enzymes have been identified in the tocochromanol pathway including the ρ-hydroxyphenolpyruvate dioxygenase (HPPD) enzyme (responsible for catalyzing the conversion of the shikimate-derived ρ-

hydroxyphenylpyruvate to homogentisic acid) and homogentisate phytlytransferase (HPT) (responsible for the conversation of homogentisic acid to phytyl-diphosphate) (Chen et al., 2006). This has allowed for genetic manipulation of the pathway for increased production of tocochromanols as well as manipulation of the ratio of tocopherol forms (particularly to α-tocopherol, which presents the greatest antioxidant activity) and modifying the tocopherol:tocotrienol ratio (Smirnoff, 2005; Chen et al., 2006; DellaPenna and Pogson, 2006). For instance, Römer et al. (2002) were able to produce potato tubers with 2- to 3-fold more α-tocopherol than control tubers by suppressing the conversion of zeaxanthin to violaxanthin with both antisense and co-suppression constructs of zeaxanthin epoxidase. However, not all transgenic studies with potatoes have proven completely effective and there may in fact be an upper limit to lipid-soluble antioxidants in potato tubers. Crowell et al. (2008) observed in their study that an attempt to increase levels of tocopherol in potato tubers showed an increase in α-tocopherol in the leaves and stem parts of the plant by 266% and 106% by inserting an *Arabidopsis thaliana* HPPD and HPT gene, respectively. However, the tubers showed between 10- to 100-fold less α-tocopherol than the aerial parts of the plant, suggesting a biological limitation on the accumulation of α-tocopherol in the potato tuber despite up-regulation of the biosynthetic pathway in the plant (Crowell et al., 2008). Interestingly, a relationship between aromatic acid antioxidant pathways and the tocochromanol pathway was demonstrated using genetically engineered tobacco plants (Rippert et al., 2004; Smirnoff, 2005). When a yeast prephenate dehydrogenase gene was inserted, it caused up-regulation of the precursor to the tocochromanol pathway, ρ-hydroxyphenylpyruvate. This caused the shikimate pathway to divert

away from the phenylpropanoid pathway by converting prephenate directly to p-hydroxyphenylpyruvate.

As with many other antioxidant pathways, the distribution of the tocochromanol pathway in both plant and animal groups, as well as its location in plant cells, has been used as an indication of the pathway's possible evolution (Sattler et al., 2003). Currently, tocopherol synthesis has only been observed in plants, algae, and some cyanobacteria (Sattler et al., 2003). It was noted that not only are tocopherol biosynthetic enzymes in plants nuclear-encoded while the majority of the tocopherols and biosynthetic enzymes are located in plastid membranes, but Sattler et al. (2003) noted that tocopherol cyclase (responsible for catalyzing the formation of tocopherols) appeared to be evolutionarily conserved between cyanobacteria and plants (Goksoyr, 1967). This led researchers to theorize that, as with many antioxidant pathways, the pathway responsible for tocopherol synthesis was acquired by plants through endosymbiotic cyanobacteria (Goksoyr, 1967; Sattler et al., 2003). However this is a poorly covered area in the literature and more studies comparing the genetics of the cyanobacteria and plant-derived tocochromanol enzymes will hopefully become available with the expansion of plant genetic engineering.

The Ascorbic Acid and Glutathione Redox System

Ascorbic acid (AA), glutathione (GSH), and vitamin E are reported to be the most important antioxidant compounds in mammalian cells (Winkler et al., 1994). They are linked by a series of coupled oxidation-reduction reactions (Figure 9), which allows the reduced forms of these antioxidants to be continually reformed from their oxidized forms (Winkler et al., 1994). GSH is oxidized to glutathione disulfide (GSSG) in the process of its antioxidant function and

this reaction also acts directly to reduce the oxidized form of AA, dehydro-L-ascorbic acid (DHA), back to its reductant form (Winkler et al., 1994; Potters et al., 2002). As AA then becomes oxidized in its function as an antioxidant, it reduces the oxidized tocopheryl radical to the reductant form vitamin E (Winkler et al., 1994; Smirnoff, 2005). These reductant forms then exist as pools in plant tissues to be used whenever needed (Potters et al., 2002). This redox reaction (Figure 9) allows these three reductants to function much more efficiently as antioxidant compounds than compounds not involved in redox recycling, as their redox regeneration allows them to be used almost continually, while the antioxidant capacity of alternate compounds is limited to existing intercellular concentrations (Winkler at al., 1994). As well, this cyclic redox reaction means that any methods implemented to increase either AA or GSH antioxidant activity in plant tissues can work either by directly increasing synthesis activity in the pathway or increasing the recycling of the oxidized version of the compound to the antioxidant-ready reduced form (Love and Pavek, 2008). Despite this close association, there has been no concentrated effort to consider both pathways as one metabolic unit as with the aromatic acid antioxidant pathways (Winkler et al., 1994). This is most likely a result of evidence that alternate enzymes can reduce either DHA or GSSG when necessary, which puts into question the depth of the relationship between AA and GSH (Winkler et al., 1994). As with previously mentioned pathway groups, AA and GSH are considered separately here to coincide with the literature.

Ascorbic acid (Vitamin C) Synthesis

Ascorbic acid (AA), vitamin C, is an important antioxidant compound, efficiently scavenging toxic ROS and RNS in both plants and animals, and preventing scurvy disease in humans (Arrigoni and De Tulllio, 2002). The antioxidant function of ascorbate is related to its

inhibition of the oxidation of other compounds by sacrificially acting as a reducing agent, which results in the formation of the poorly reactive ascorbyl radical (semidehydroascorbate) followed by further oxidation to generate dehydroascorbic acid (Smirnoff, 2011). It is one of the most universally distributed antioxidant compounds, being found in all chlorophyll-containing eukaryotes (Horemans et al., 2000; Pallanca and Smirnoff, 2000; Smirnoff et al., 2001; Love and Pavek, 2008). More importantly, humans are unable to synthesize AA because they lack L-gulono-lactone oxidase (GulL-ox) (Arrigoni and De Tullio, 2000). The complete function of AA in plants is still not fully determined, although to date it has been linked with general antioxidant activity, redox control and pH-mediated modulation of PSII activity, the signal transduction network linking flowering time, developmental senescence, programmed cell death and pathogen response, assistance in the synthesis of ethylene, gibberellins, anthocyanins, and hydroxyproline (Arrigoni and De Tulllio, 2002; Hancock et al., 2003; Barth et al., 2006). It also acts as a precursor for specific processes leading to the biosynthesis of organic acids (Debolt et al., 2007).

The complete biosynthetic pathway of AA has only recently been elucidated. However, there still exists minor debate as to certain key enzymes involved and potential alternate pathways (Smirnoff et al., 2001; Valpuesta and Botella, 2004). The most recently determined pathway (Smirnoff et al., 2001) can be seen in Figure 10. Given the novel status of the complete AA pathway, it should come as no surprise that very little is known concerning natural or artificial expression regulation in AA biosynthesis (Smirnoff et al., 2001; Ishikawa et al., 2006). A feedback inhibition form of regulation for the AA pathway has been recently hypothesized based on AA inhibition of the initial enzyme in the ascorbate-dedicated reactions, guanosine diphosphate-mannose-3',5'-epimerase (Wolucka and Van Montagu, 2003; Smirnoff, 2005).

However, antisense constructs of alternate enzymes involved in the pathway, including L-galactose dehydrogenase and L-galactonolactone dehydrogenase, produced plants with lower AA levels, suggesting additional methods of pathway regulation (Tabata et al., 2001; Gatzek et al., 2002; Smirnoff, 2005). Despite this limited knowledge, recent studies have been making inroads into the possible regulation of this pathway.

In potatoes, AA is actively synthesized in cut tubers in response to wounding (Johnson and Schaal, 1957; Ôba et al., 1994). However, a recent study by Tedone et al. (2004) has demonstrated that AA synthesized in foliar tissue is transported to developing tubers, suggesting that either AA is both transported and synthesised directly in tuber tissues, or perhaps that AA can only be synthesized in tubers when the stored tuber is no longer connected to foliar tissues. This study also suggests that this relationship may allow for the increase in tuber AA by increasing foliar AA. However no studies to date seem to have carried this hypothesis further (Tedone et al., 2004). As well, there has been moderate success with the limited number of genetic studies which have been conducted, suggesting there may be potential for genetic regulation (Ishikawa et al., 2006). One of the few studies that has been done with potato noted an antisense suppression of *vtc1* and GDP-manpyrophosphorylase by 40% and 50% respectively in potato that resulted in a reduction of 30% and 44% respectively in wild-type AA content (Conklin et al., 1999; Keller et al., 1999; Smirnoff et al., 2001). The location of AA biosynthesis in plants is also still under debate. However, recent research presented the possibility that ascorbic acid is synthesized directly in the phloem (Hancock et al., 2003; Tedone et al., 2004). Results such as these indicate that there may be many successful attempts at increasing cellular AA in the future.

As mentioned previously, AA exists in a reduced state in the plant that is part of a redox reaction that contains DHA and the intermediate monodehydro-L-ascorbate (MDHA) (Figure 11) (Potters et al., 2002). As a part of the redox reaction, MDHA can be reduced by both the enzyme MDHA reductase with electrons taken from NADPH or by ferredoxin alone (Miyake and Asade, 1995; Asada, 1999; Potters et al., 2002). DHA is thought to be reduced primarily by DHA reductase and electrons donated from GSH (Potters et al., 2002). However, several studies have reported that alternate proteins (including one isolated from potato tubers) have demonstrated DHA reductase activity and other studies even argue for a non-enzymatic direct reaction between DHA and GSH, suggesting that numerous methods may exist in plants for returning DHA to a reduced state (Dipierro and Borracino, 1991; Winkler et al., 1994; Potters et al., 2002).

AA and its oxidation products are present in potato tubers and the tuber concentrations of AA during development have been documented (Love and Pavek, 2008). Levels are low in newly formed tubers and increase throughout the growing period of the plant, reaching their greatest level during the last months of growth (Mondy and Munshi, 1993b; Love and Pavek, 2008). Unfortunately from that point onward, AA levels will decrease continuously especially within the first 4-10 weeks (Shaker et al., 1978; Perkins et al., 1990; Mondy and Munshi, 1993b; Dipierro and De Leonardis, 1997; Love and Pavek, 2008).

As with many antioxidants, environmental conditions are linked to regulating AA synthesis in plants (Love and Pavek, 2008). Light in particular promotes the synthesis of AA in leaves, which is not surprising given the known role of AA in photoprotection (Smirnoff et al., 2001; Love and Pavek, 2008). As well, DHA recycling is promoted through the up-regulation of dehydoascorbate reductase by atmospheric ozone, sulphur dioxide, and UV-B radiation (Conklin

et al., 1996; Chen et al., 2003; Yoshida et al., 2006; Love and Pavek, 2008). Increasing nitrogen fertilizer rates have been shown to both increase and decrease AA in tubers (Augustin, 1975; Mondy et al., 1979; Love and Pavek, 2008). Augustin (1975) showed that increasing nitrogen fertilizer rates caused a 15-20% decrease in tuber AA, while Mondy et al. (1979) showed opposite results - a 28% increase in tuber AA. One of the only studies looking at irrigation and AA in potato tubers noted a 15-20% decrease in AA content as irrigation increased (Zhang et al., 1997). As well, boron foliar sprays have been linked in potato to significant increases in tuber AA (Mondy and Munshi, 1993a). In squash (*Cucurbita pepo*), boron deficiency was linked to AA deficiency (Lukaszewski and Blevins, 1996; Smirnoff et al., 2001). Excessive potassium fertilizer has also been shown to significantly increase AA levels in tubers (Mondy and Munshi, 1993c). As well, gamma irradiation decreased AA in tubers (Cotter and Sawyer, 1960).

Unlike for many antioxidant pathways, there has been a documented effort to translate AA research into usable methods for increasing vitamin C in potato tubers for the industry (Love and Pavek, 2008). There exists a long history of studies examining vitamin C content in potato tubers which have shown to vary across cultivars (Love et al., 2004; Love and Pavek, 2008). Love and Pavek (2008) suggested that commonly available potato germplasm can be used to create breeding programs to rapidly increase vitamin C content in potatoes (Love et al., 2004; Love and Pavek, 2008). However, as also pointed out by Love and Pavek (2008), vitamin C has yet to be viewed as an important trait for potato breeders, making its increase by current breeding programs not very likely.

Glutathione

GSH is a reduced tripeptide non-protein thiol present in all plants (Xiang and Oliver, 1998). It is formed in a two-step reaction utilizing ATP and the compounds glutamate, cysteine, and glycine (Figure 12) (Meister and Anderson, 1983; Xiang and Oliver, 1998). GSH is then oxidized to form GSSG, which utilizes an NADPH-derived source of electrons and the enzyme GSSG reductase to reduce back to GSH (May et al., 1998; Potters et al., 2002). In plants, GSH and GSSG are responsible for numerous processes, including but not limited to antioxidant defence, sulphur uptake by roots, the detoxification of xenobiotics and air pollutants (such as ozone), redox buffering, pathogen defence controlling cellular heavy metal concentrations by providing the necessary precursor for phytochelatins biosynthesis, and aiding the adaptation of plants to environmental stressors (Alscher, 1989; Noctor and Foyer, 1998; Xiang and Oliver, 1998).

The complete pathway for GSH biosynthesis has been elucidated and appears to be common to all organisms which produce GSH (Noctor and Foyer, 1998). In plants, GSH synthesis seems to be controlled by a feedback inhibition of γ-glutamylcysteine synthetase (γ-ECS) by GSH (Schneider and Bergmann, 1995; Noctor and Foyer, 1998). As well, regulation of the GSH pathway has been achieved both through environmental manipulation and through genetic regulation (Alscher, 1989; Xiang and Oliver, 1998). GSH synthesis is controlled environmentally by activation or suppression of the enzyme responsible for catalyzing GSH synthesis from γ-glutamylcysteine (Figure 12 enzyme a) and glycine; GSH synthetase (Alscher, 1989). Studies have shown GSH synthetase to be light and pH sensitive (needing light to function and a pH of between 8.5 and 9), as well as requiring between 10-30 mM of Mg^{2+} (Lee and Halliwell, 1986; Alscher, 1989). In fact, several studies have noted that in the absence of light the GSH precursor

γ-glutamylcysteine (γ-EC) accumulated to very high levels in foliar tissues (Noctor et al., 1997a; Noctor and Foyer, 1998). This accumulation was halted by the addition of photorespiration products (such as glycine) (Noctor et al., 1997b; Noctor and Foyer, 1998). In potatoes, environmental treatments have also been shown to increase intercellular GSH deposition and expression, including sulfate, H_2S, ethylene chlorohydrin, gamma irradiation, and the herbicide 3-aminotriazole (Guthrie, 1932; Cotter and Sawyer, 1960; de Kok and Kuiper, 1986; Buwalda et al., 1993; Stroiński and Zielezińska, 1997; Noctor and Foyer, 1998). Cadmium chloride has also been shown to affect the activity of GSH reductase, from increasing its activity to inhibiting its activity completely, depending on the concentration of the cadmium ions, the potato cultivar, and the incubation period of the treatment (Stroiński et al., 1999). At the same time, compounds shown to increase activity in other antioxidant pathways, such as H_2O_2, have been shown to have no effect on GSH expression or cause cellular decreases (Lappartient and Touraine, 1997; Noctor and Foyer, 1998; Xiang and Oliver, 1998). Treatments involving the addition of compounds involved in the GSH synthesis pathway have been shown to affect cellular GSH levels as well (Noctor and Foyer, 1998). Supplying cysteine to the system increases GSH concentrations, while supplying glutamine has no visible effect on GSH levels (Noctor and Foyer, 1998). As well, Xiang and Oliver (1998) showed that both GSH and GSSG exposure have no effect on the cellular levels of GSH. In the lab, GSH expression and cellular deposition have increased by manipulating the genes responsible for the synthesis of GSH reductase, glutathione synthase, and γ-glutamylcysteine synthetase (Xiang and Oliver, 1998). As well, insertion of genes from bacterial species showed significant success in increasing GSH levels in potatoes (Harms et al., 2000; Stiller et al., 2007). Stiller et al. (2007) created chimeric 'White Lady' potato tubers with a

SAT-coding *cysE* gene from *Escherichia coli* that demonstrated 1.5-fold higher glutathione levels than the non-transformed control plants. A second study by Harms et al. (2000) involving the same gene and gene source inserted into potato showed a 2-fold increase in GSH levels compared to controls.

Given the broad distribution of GSH synthesis across kingdoms, the evolutionary origins of GSH have been often examined. As a result, the evolution of GSH is one of the most well understood antioxidant pathways both from a phylogeny and genetic perspective. One of the earliest theories involving GSH synthesis focused on its capacity to detoxify thiol-reactive by-products of oxygen and suggested that GSH may have evolved as a consequence of increasing atmospheric oxygen levels in the very earliest stages of evolution (Fahey et al., 1987; Frova, 2003). One of the first tests of this theory was the Fahey et al. (1987) study, which assumed that GSH synthesis most likely evolved first in phototrophic eubacteria in conjunction with increasing oxygen levels on the planet. As one of the primary functions of GSH is its antioxidant activity to thiol-reactive by-products of oxygen, Fahey et al (1987) found that all eukaryotic phototrophs in their study (including green and purple bacteria, cyanobacteria, and eukaryotic algae) contained GSH, supporting their hypothesis that GSH synthesis evolved in conjunction with the oxi-detoxification of the earth (Fahey et al., 1987). More recently, studies have examined the amino acid sequence, genomes, and crystalline structure of GSH S-transferase (GST), which is responsible for catalyzing the reaction between GSH and many toxic products of xenobiotic or biotic origins in numerous eukaryotes and prokaryotes across numerous kingdoms to create a most parsimonious tree indicating the likely evolutionary steps of GSH synthesis (Buetler, 1992; Snyder and Maddison, 1997; Sheehan et al., 2001; Frova, 2003). In fact, GST

relationships have been so thoroughly studied across all aerobic organisms that over 100 different sequences of GST have been identified and the protein superfamily has been given its own nomenclature (Snyder and Maddison, 1997; Sheehan et al., 2001; Frova, 2003). This research has helped determine that GSH synthesis most likely evolved in microorganisms as a direct response to increasing atmospheric oxygen and the organism of origin has since been traced to being likely before the prokaryotic and eukaryotic split (Frova, 2003). Gene studies have also identified that GST has most likely been evolving through a process of gene amplification followed by diversification, which has allowed for the incredible diversity of genetics and functions seen in GSTs today (Armstrong, 1998; Sheehan et al., 2001). Research in this area is very deep and beyond the focus of this review to discuss further. For excellent review articles of this topic, the reader is encouraged to see Sheehan et al. (2001) and Frova (2003).

Known Antioxidant Compounds in Potato

The biosynthesis pathways responsible for synthesis of antioxidant compounds may be more understood in potato. However, the complete identity of all compounds that can be generated by these pathways is much less understood. To facilitate future studies of antioxidant compounds in potato, and other plants, an annotated list was compiled by the authors from literature citations of all identified antioxidant compounds in potato (Table 1 and 2). Despite all efforts, this list is likely incomplete, with some antioxidant groups in particular (such as the anthocyanins) expected to have many more compounds which have yet to be discovered.

MAJOR FACTORS AFFECTING ANTIOXIDANT CAPACITY OF POTATO

The Effect of Cooking Methods on Antioxidant Capacity in Potato

Knowledge of the possible pathways and compounds responsible for antioxidant activity in potato is certainly important to understanding the contribution of potato to human nutrition. However, it is likely that the total antioxidant capacities and profiles of potatoes as reported in the vast majority of studies is not truly representative of the antioxidant content of the potatoes ultimately being consumed as part of the human diet. This is because, while it is standard in the field of nutrition to utilize raw plant material in nutrient analyses, potatoes are not commonly consumed raw.

A variety of cooking methods are used for potato tubers and each has the potential to modify the antioxidant profile of the potato product consumed (Brown, 2005). As such, it is also imperative when discussing antioxidant capacity in potato to examine the existing knowledge of how cooking methods may affect the antioxidant concentrations in a cooked product. This is a brand new area of research, so few concrete conclusions have been reached (Brown, 2005). However, a complex relationship of interactions between the numerous pathways responsible for antioxidant capacity and the cooking methods utilized (boiling, baking frying, microwaving being the most common) is believed likely.

Antioxidant capacity of potatoes can decrease or increase after cooking depending on the cooking procedures. Sevcik et al. (2009) did not detect significant changes of antioxidant capacity between boiled and fresh potato samples (with the peel) when these were boiled in water for 12 min, but detected 11% decrease after cooking by microwave and 29% decrease after frying in vegetative oil. Different antioxidants are affected differently during cooking. Although

caffeic acid was drastically decreased in boiled tubers, the concentrations of total phenolics and antioxidant activity in boiled tubers were either higher or not significantly changed compared with raw tubers in four native Andean accessions (Burgos et al., 2013). The antioxidant capacity in the anthocyanins purified from purple potatoes was increased after heating at high temperatures (100-150 °C), indicating antioxidant activities of degradation products of these anthocyanins (Nayak et al., 2011). The carotenoids lutein and zeaxanthin concentrations in boiled tubers were not affected or were higher than the concentrations in raw tubers (Burgos et al., 2012).

The goal of examining the effects of cooking methods on antioxidant levels in potato tubers would ultimately be to suggest methods which preserve the greatest potential antioxidant capacity in consumed potato foods. While different cooking methods have very different effects on antioxidants within potato cultivars, the ultimate effect of each cooking method depends on the type of antioxidants being assessed (Burgos et al., 2009; Xu et al., 2009; Blessington et al., 2010; Natella et al., 2010). It would appear that each major pathway is affected by different cooking methods in different ways. For example, the best cooking method for preserving ascorbic acid levels seems to be boiling potatoes, while several other studies have noted that boiling significantly decreases total antioxidant capacity and total phenolics relative to other cooking methods (Burgos et al., 2009; Blessington et al., 2010; Natella et al., 2010). In addition, different cultivars seem to respond to the same cooking methods in different ways. Almost all studies examined observed genotype-level differences in the response of antioxidant levels to cooking methods (Han et al., 2004; Brown et al., 2008; Burgos et al., 2009; Blessington et al., 2010; Navarre et al., 2010). Interestingly, some cooking methods have been noted to increase

antioxidant activities in certain situations. For example, Blessington et al. (2010) noticed that baking, frying, and microwaving potatoes caused significant increases in tuber levels of chlorogenic acid, caffeic acid, (-) epicatechin, ρ-coumaric acid, and vanillic acid. Natella et al. (2010) found a more complex relationship between antioxidants and cooking methods, noting that while microwaving increased tuber total phenols, it also decreased total antioxidant capacity. Still, others who were investigating the same cooking methods and response variables observed universal decreases, albeit some cooking methods causing greater decreases than others (Tudela et al., 2002; Xu et al., 2009). The developmental age of the tuber seems to have an effect with cooking actually increasing the recoverable amounts of chlorogenic acid and rutin in new potatoes from two different cultivars (Navarre et al., 2010).

Independent of the method of cooking used or pathway involved, the effect of each cooking method on antioxidant levels in potatoes can be affected by several factors including growing location. This underlines the role of environmental conditions in overall antioxidant capacity (Brown et al., 2008). Far more research needs to be done in this area before any concrete conclusions can be made especially considering the complex picture painted by the limited work that has been done so far.

SUMMARY

Potato stem tubers have become one of the world's most popular food items and are now grown in most countries globally. Where potato is a staple, it has an important role to play in both phytonutrient delivery and as a dietary source of diverse antioxidant compounds. These antioxidant substances serve to protect the body through multiple antioxidant activities. In so doing, they may prevent damage related to radiation exposure or pollutants and offer protection

from a long list of age-related and inflammatory diseases, particularly cancers, cardiovascular diseases, and, diabetes.

We could all benefit from identification of relatively more nutritious potato cultivars that are grown and handled to optimize their phytonutrient status and cooked in a manner that will enhance their dietary contribution. However, there is little information available to researchers or consumers on potato cultivars with the greatest range and concentration of antioxidant compounds. The marketplace is responding to consumer demand for cultivars with highly pigmented skin and flesh colours. Still, this remains a relatively tiny portion of the fresh potato market. Although research into the field of potato-based antioxidants has increased significantly in recent decades, there remain many knowledge gaps. An understanding of complex and inter-related biosynthetic pathways, environmental triggers, and regulators are important to breeding potato for specific traits such as increased overall antioxidant capacity or greater content of one or more specific antioxidant(s). To enable a better understanding of these pathways and processes, the major antioxidant biosynthesis pathways were summarized with particular emphasis on pathway constituents known to be present in potato tubers. A greater understanding is required of the effects of practical growing, handling, and storage methods to maximize phytonutrients and particularly antioxidant capacity in commercial cultivars. Also, food handling and cooking procedures that preserve antioxidant content or reduce the loss of these important compounds should be further explored and their effects on the biochemical pathways of interest described. Research in this area is still in its infancy. Both cooking methods and genotype were reviewed for their effects on antioxidant pathways and found to impact differently on the various pathways. We underline the importance of breeding efforts to develop cultivars with increased

antioxidant capacity and the need for information for those growing, preparing and handling potato towards preservation of antioxidant capacity in cooked potato and potato products.

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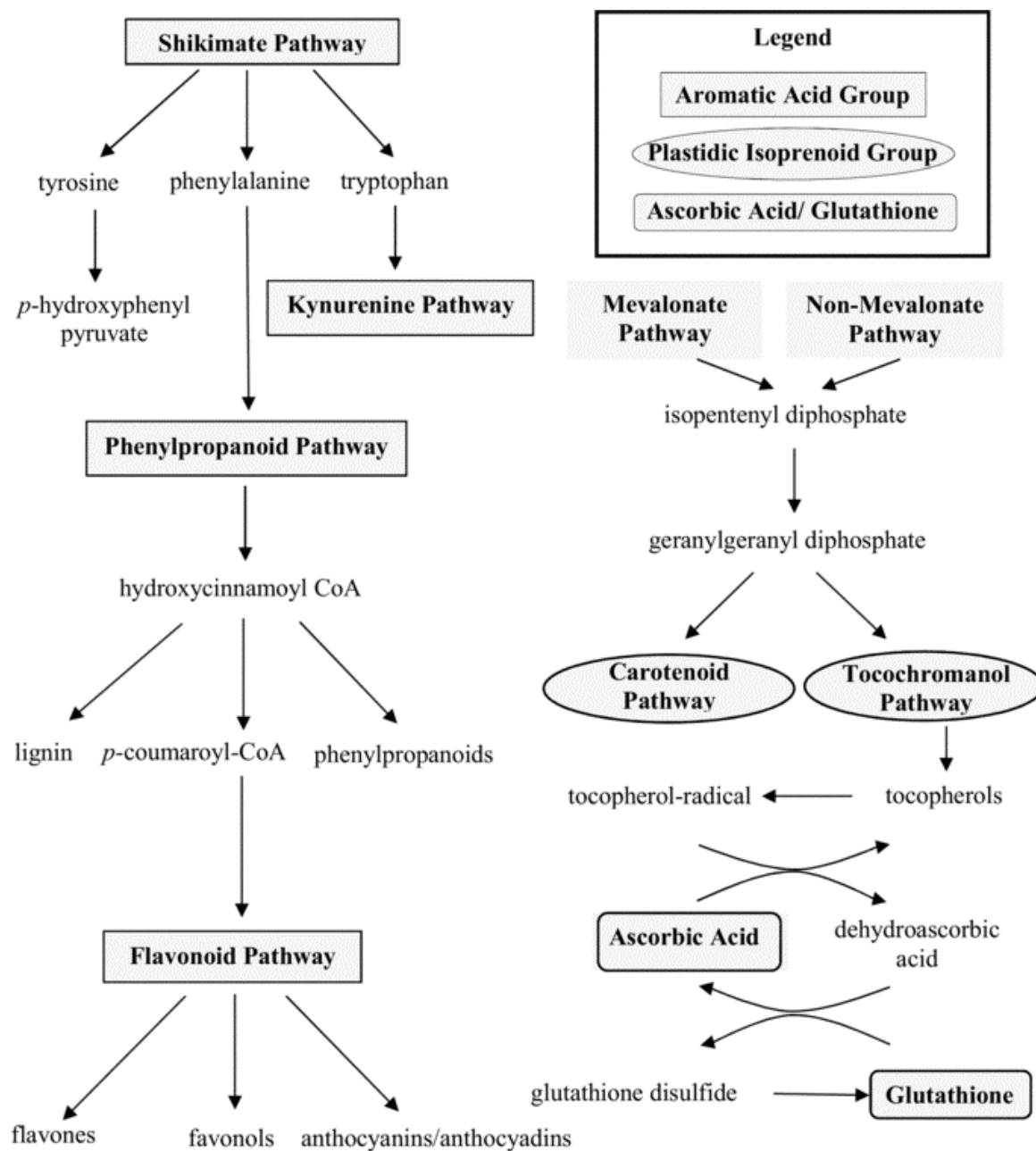
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Figure 1. General overview of the complex relationships between the antioxidant pathways. Pathway titles are shaded and different pathways are made in different shapes as illustrated in the legend.

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Figure 2. Shikimate pathway (modified from Bentley, 1990; Schmid and Amrhein, 1995; Weaver and Hermann, 1997; KEGG, 2013). Enzymes involved in the pathway are: a. 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthase, b. 3-dehydroquinate synthase, c. 3-dehydro quinate dehydratase, d. shikimate NADP oxidoreductase, e. shikimate kinase, f. 5-*enol*pyruvul shikimate-3-phosphate synthase, g. chorismate synthase, h. chorismate mutase, i. prephenate aminotransferase, j. arogenate dehydratase, k. arogenate dehydrogenase, l. anthranilate synthase, m. anthranilate phosphosribosyl transferase, n. anthranilate phosphosribosyl isomerase, o. indole-3-glycerol phosphate synthase, and p. tryptophan synthase.
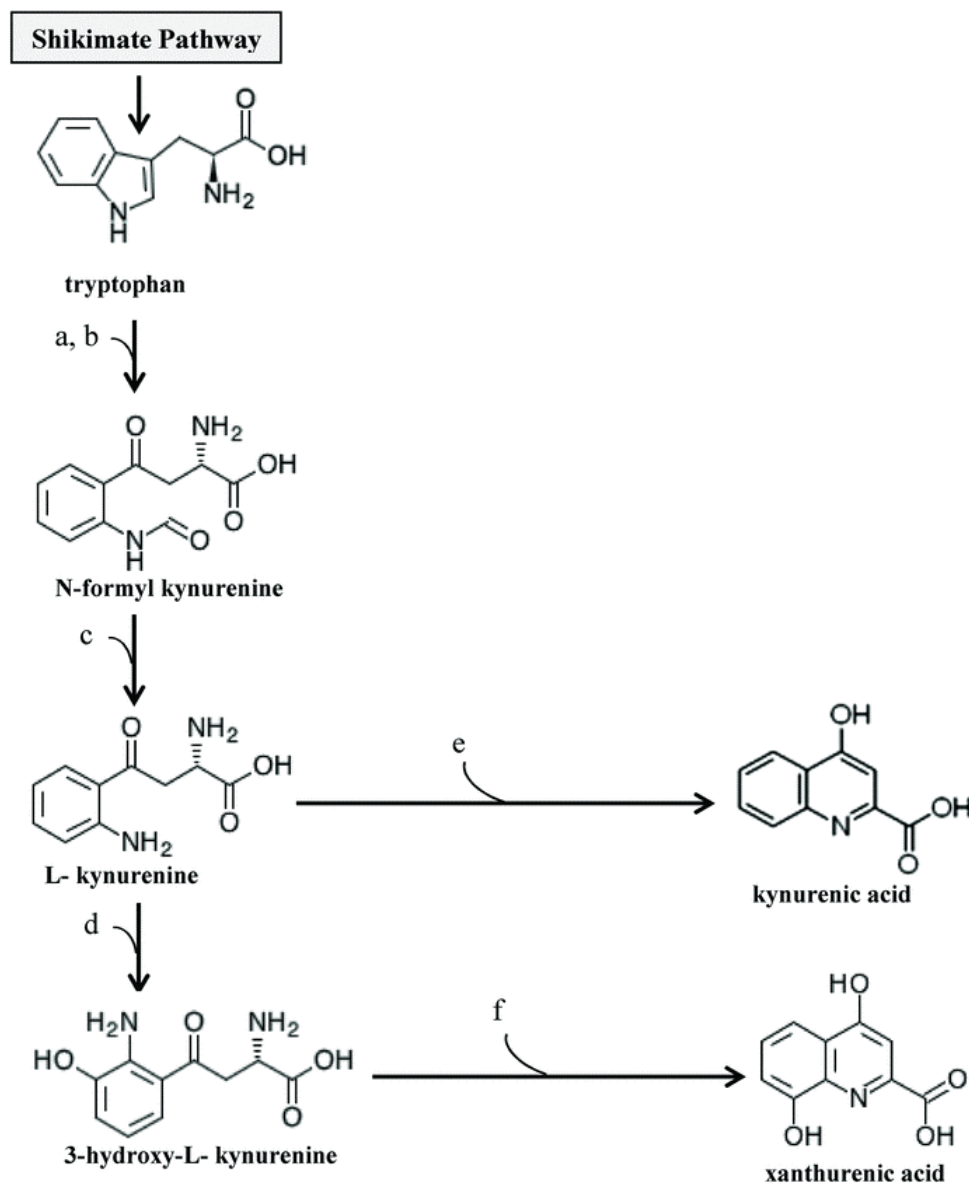
Figure 3. Kynurenine pathway compiled from tryptophan catabolism pathway (KEGG, 2013).

Enzymes involved in the pathway are: a. indoleamine 2, 3-dioxygenase, b. tryptophan 2, 3-

dioxygenase, c. kynurenine formylase, d. kynurenine 3-hydroxylase, e. kynurenine

aminotransferase, and f. kynurenine oxoglutarate transaminase

Figure 4. The most prominent branches of the phenylpropanoid pathway, as currently understood (Lee et al. 1995; Dixon et al., 2002; Costa et al., 2003; KEGG, 2013). Enzymes involved in the pathway are represented as follows: a. phenylalanine ammonia-lyase, b. cinnamate 4-hydroxylase, c. tyrosine ammonia-lyase, d. 4-coumarate:CoA ligase, e. cinnamoyl-CoA reductase, f. cinnamoyl alcohol dehydrogenase, g. *p*-coumarate 3-hydroxylase, h. caffeoyl-CoA *O*-methyltransferase, i. *p*-coumaroyl-CoA 3-hydroxylase and associated reductase, j. ferulate 5-hydroxylase and associated reductase, k. caffeate *O*-methyltransferase, l. benzoic acid 2-hydroxylase, m. peroxidase, and n. catalase-peroxidase.
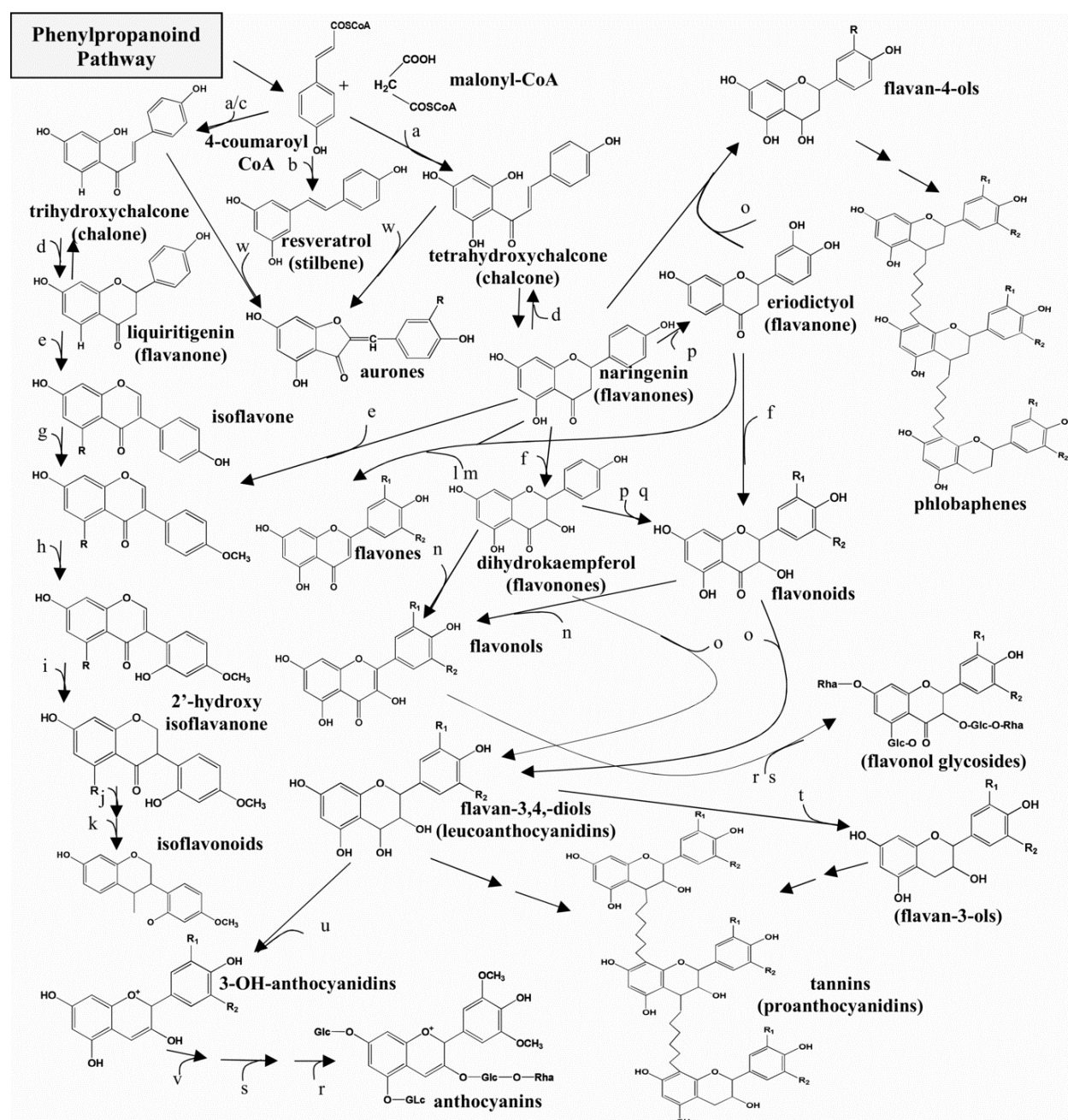
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Figure 5. Overview of the main branches of the flavonoid pathway (Dixon et al., 2002; Schijlen et al., 2004). Enzymes involved in the pathway are as follows: a. chalcone synthase, b. stilbene synthase, c. chalcone reductase, d. chalcone isomerase, e. isoflavone synthase, f. flavanone 3-hydroxylase, g. isoflavone *O*-methyltransferase, h. isoflavone 2'-hydroxylase, i. isoflavone reductase, j. vestitone reductase, k. 7,2' –dihydroxy-4'-methoxyisoflavanol dehydratase, l and m. flavone synthases, n. flavonol synthase, o. dihydroflavonol 4-reductase, p. flavonoid 3'-hydroxylase, q. flavonoid 3',5'-hydroxylase, r. rhamnosyl transferase, s. uridine diphosphoglucose -flavonoid glucosyltransferase, t. leucoanthocyanidin reductase, u. leucoanthocyanidin dioxygenase, v. *O*-methyltransferase, and w. aureusidin synthase.
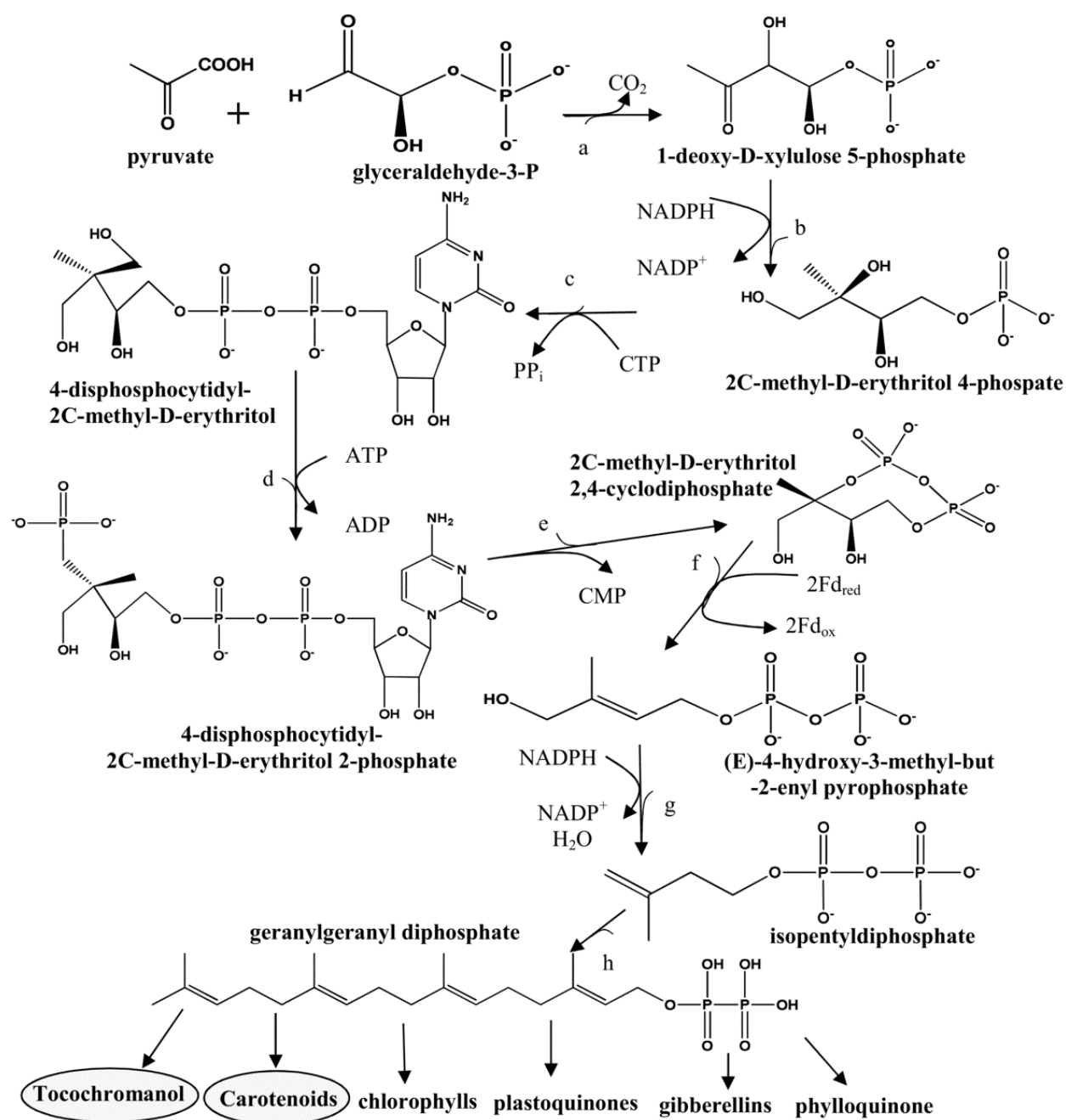
Figure 6. The non-mevalonate pathway produces geranylgeranyl diphosphate, the common precursor for both carotenoids and tocopherols (DellaPenna and Pogson, 2006; KEGG, 2013). Some studies include this pathway as part of the carotenoid pathway in plants. Enzymes indicated in this pathway are as follows: a. l -deoxy-D-xylulose 5-phosphate synthase, b. l -deoxy-D-xylulose 5-phosphate reductoisomerase, c. 4-diphosphocytidyl -2C -methyl-D-erythritol synthase, d. 4-diphosphocytidyl-2C-methyl-D-erythritol kinase, e. 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, f. (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate synthase, g. (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate reductase, and h. isopentyl diphosphate isomerase.
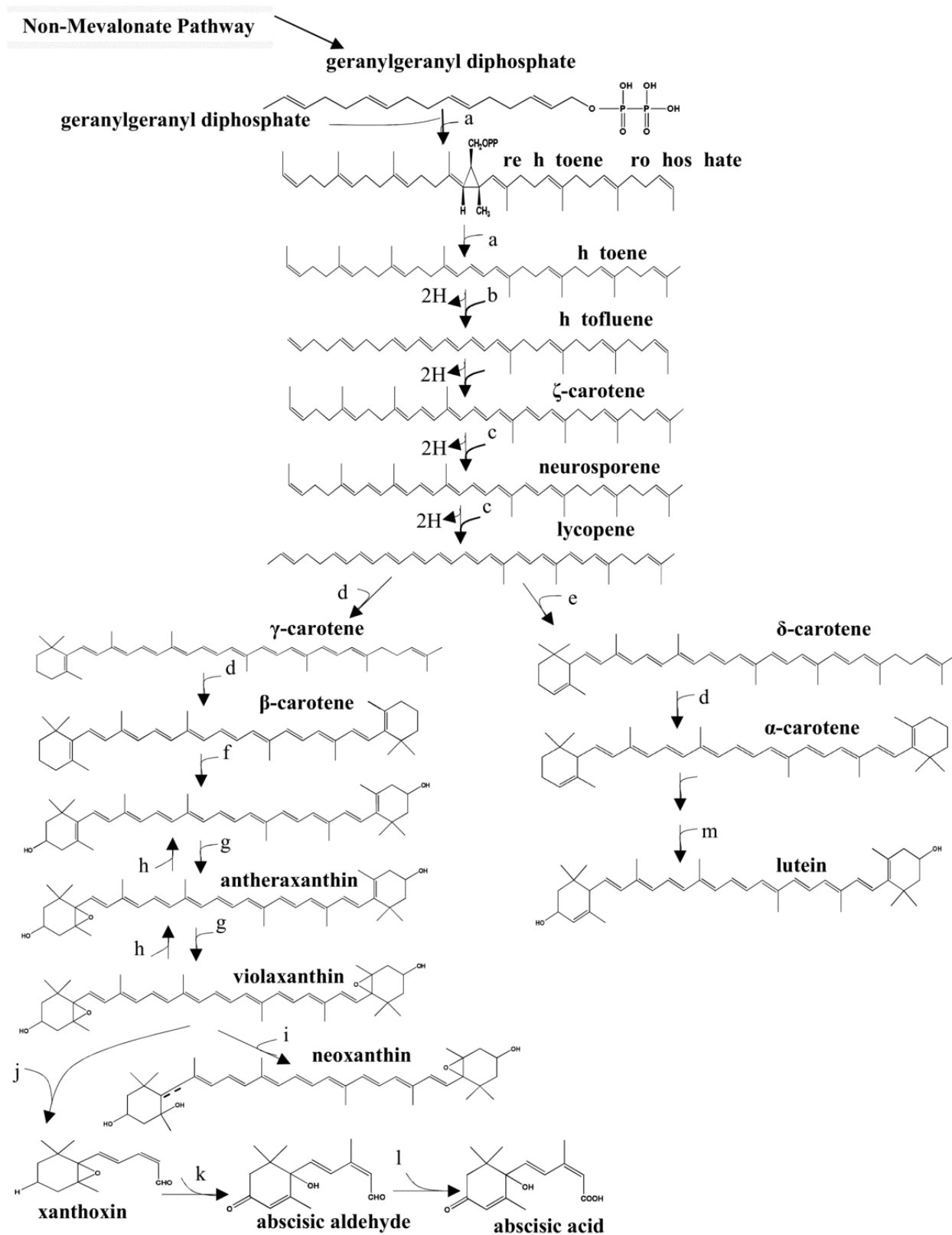
Figure 7. Major branches of the carotenoid pathway in plants (Fraser and Bramley, 2004).

Enzymes are as indicated: a. phytoene synthase, b. phytoene desaturase, c. ζ-carotene desaturase, d. lycopene β-cyclase, e. lycopene ε-cyclase, f. β-ring hydroxylase, g. violaxanthin deepoxidase, h. zeaxanthin epoxidase, i. neoxanthin synthase, j. 9-cis-epoxycarotenoid dioxygenase, k. aldehyde oxidase, l. NAD^+ oxidoreductase, and m: ε-ring hydroxylase.

Figure 8. Tocochromanol pathway (Hunter and Cahoon, 2007). Enzymes involved in the pathway are as follows: a. geranylgeranyl diphosphate reductase, b. tocopherol cyclase, c. p-hydroxyphenyl-pyruvate dioxygenase, d. homogentisate phytyltransferase, e. methyl phytyl benzoquinone methyl transferase, f. γ-tocopherol methyl transferase, g. 2-methyl-6-solanyl-1,4-benzoquinone methyl transferase, h. homogentisate geranylgeranyl transferase, i. 2-methyl-6-geranylgeranyl benzoquinol methyltransferase, j. tocotrienol cyclase, k. γ-tocotrienol methyl transferase, and l. homogentisate solanyl transferase.
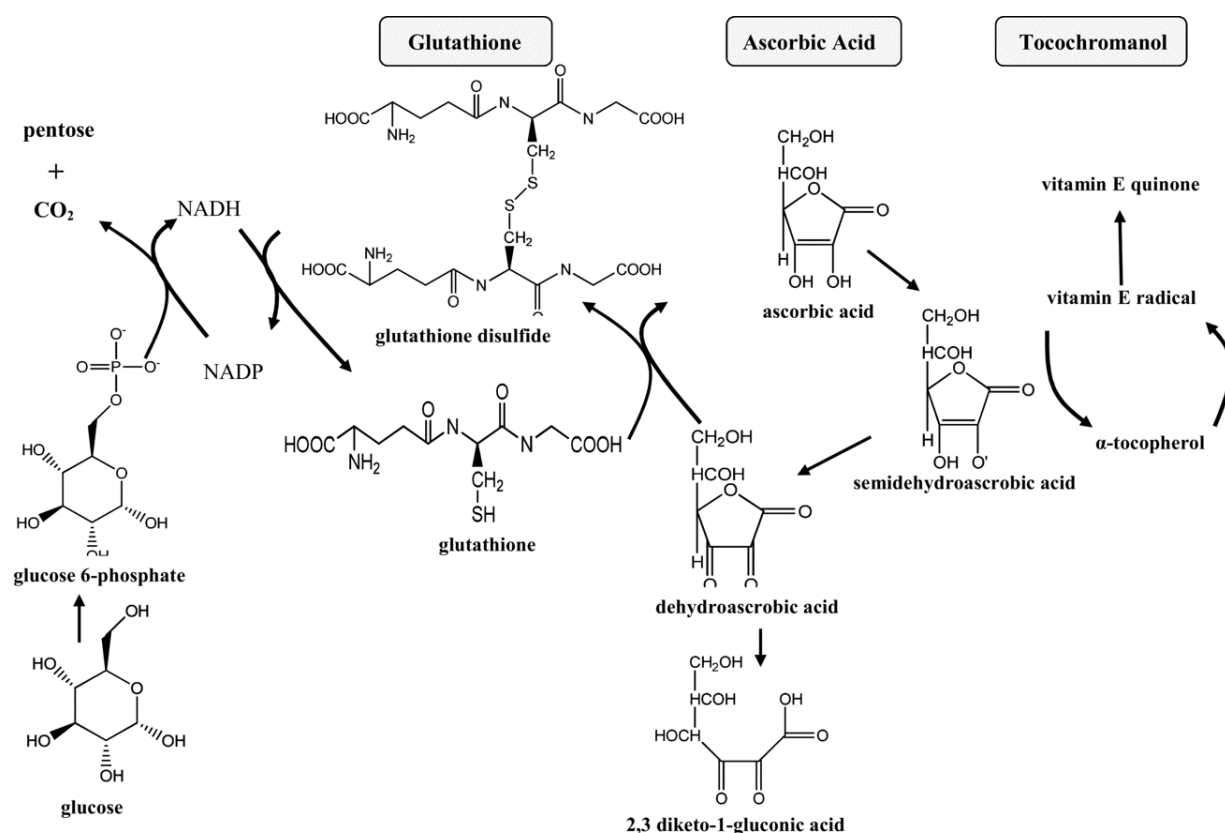
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Figure 9. The cyclic redox relationship between the glutathione, ascorbic acid, and tocochromanol pathways.
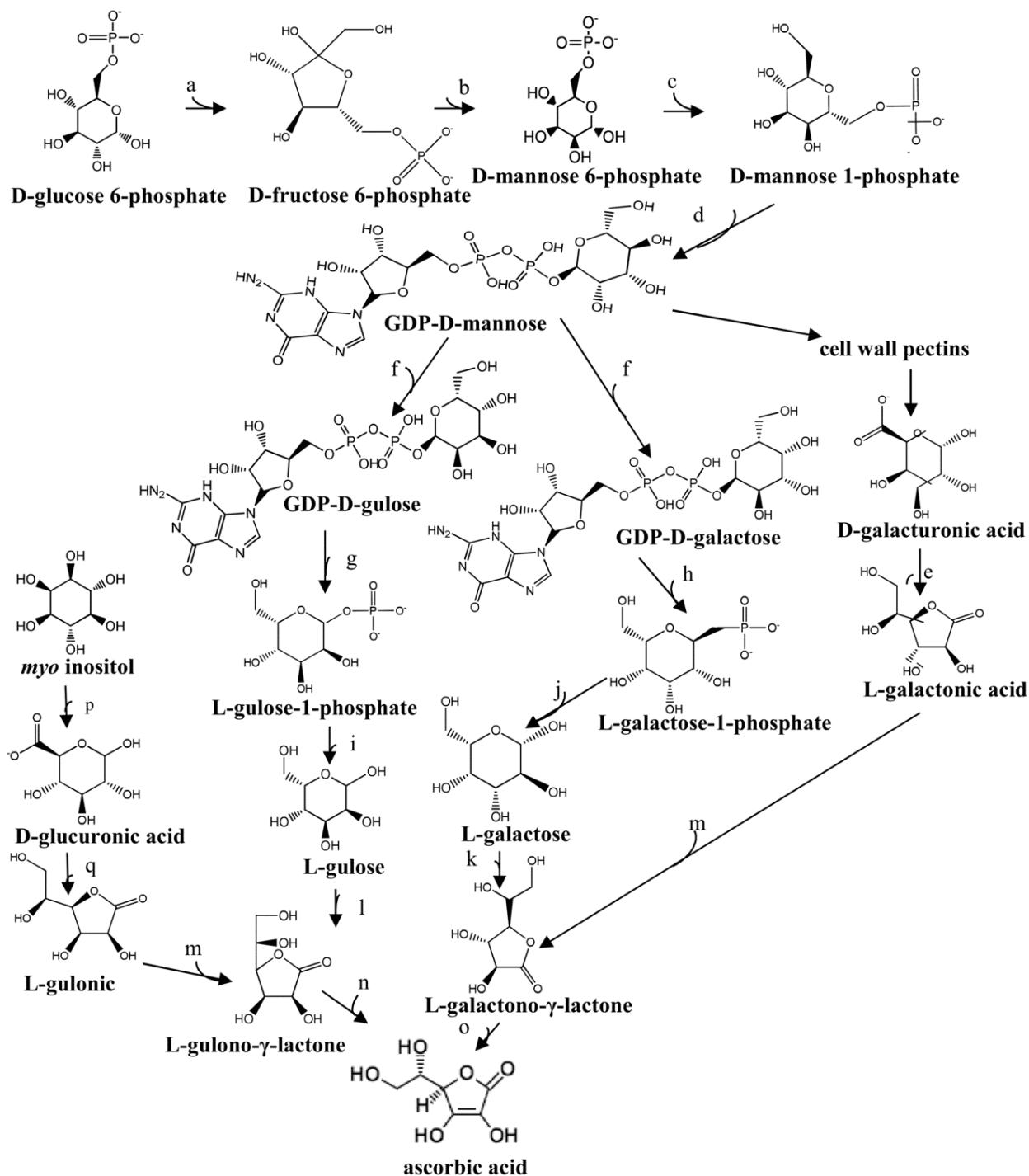
Figure 10. Composite overview of all known pathways responsible for synthesizing ascorbic acid in plants (Noctor and Foyer, 1998; KEGG, 2013). Enzymes are represented as follows: a. phosphogluco isomerase, b. phosphomannoisomerase, c. phosphomannomutase, d. GDP-D-mannose pyrophosphorylase, e. D-galacturonic acid reductase, f. GDP-mannose 3',5'-epimerase, g. GDP-L -gulose pyrophosphatase, h. GDP-L -galactose pyrophosphatase, i. L-gulose-l-phosphate phosphatase, j. L-galactose-l-phosphate phosphatase, k. L-galactose dehydrogenase, l. L-gulose dehydrogenase, m. aldonolactonase, n. L-gulono-γ-lactone oxidase, o. L-galactono-γ-lactone dehydrogenase, p. *myo*-inositol oxygenase, and q. D-glucuronic acid reductase.
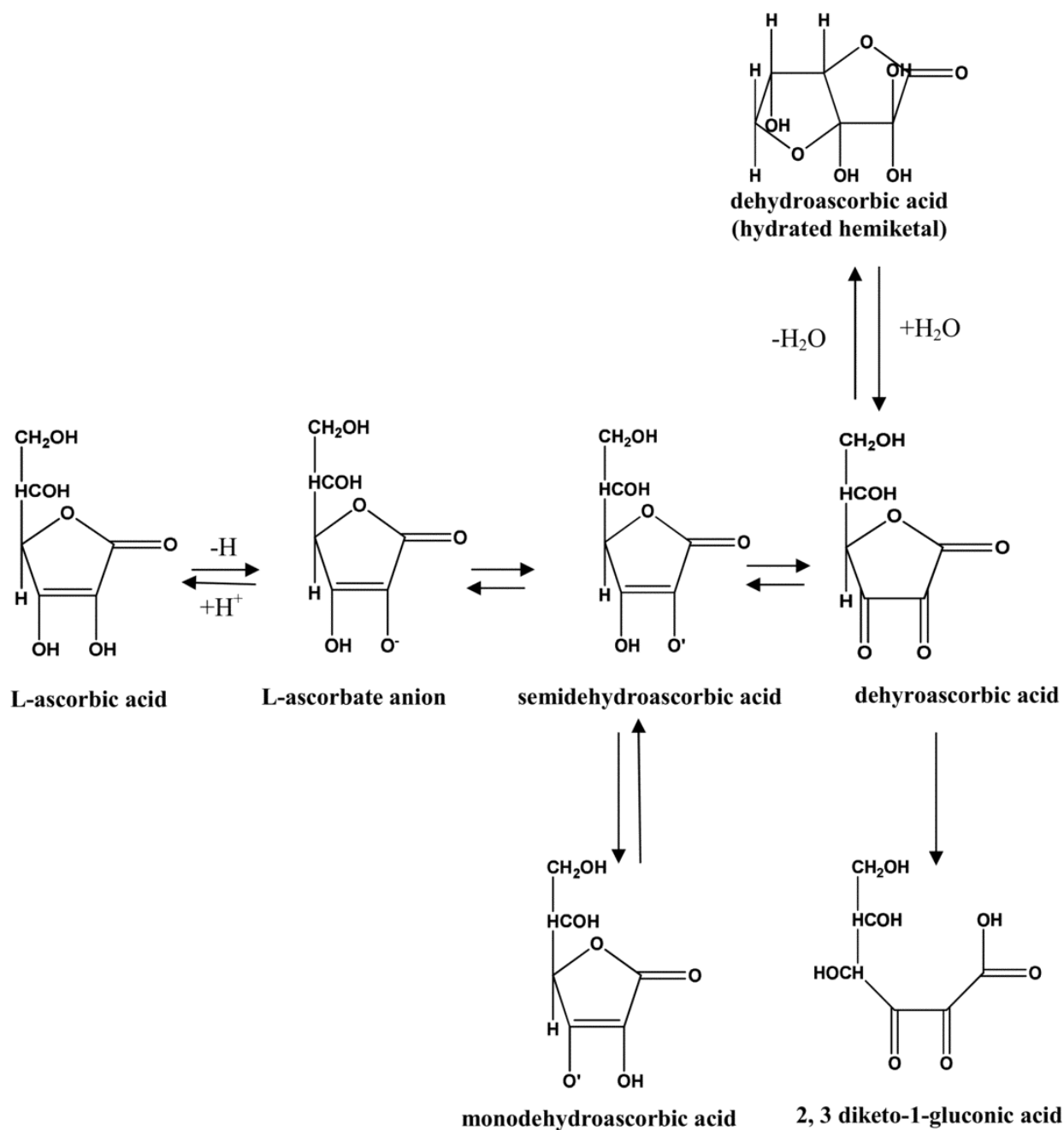
Figure 11. The redox reaction of ascorbic acid in plants (Potters et al., 2002).
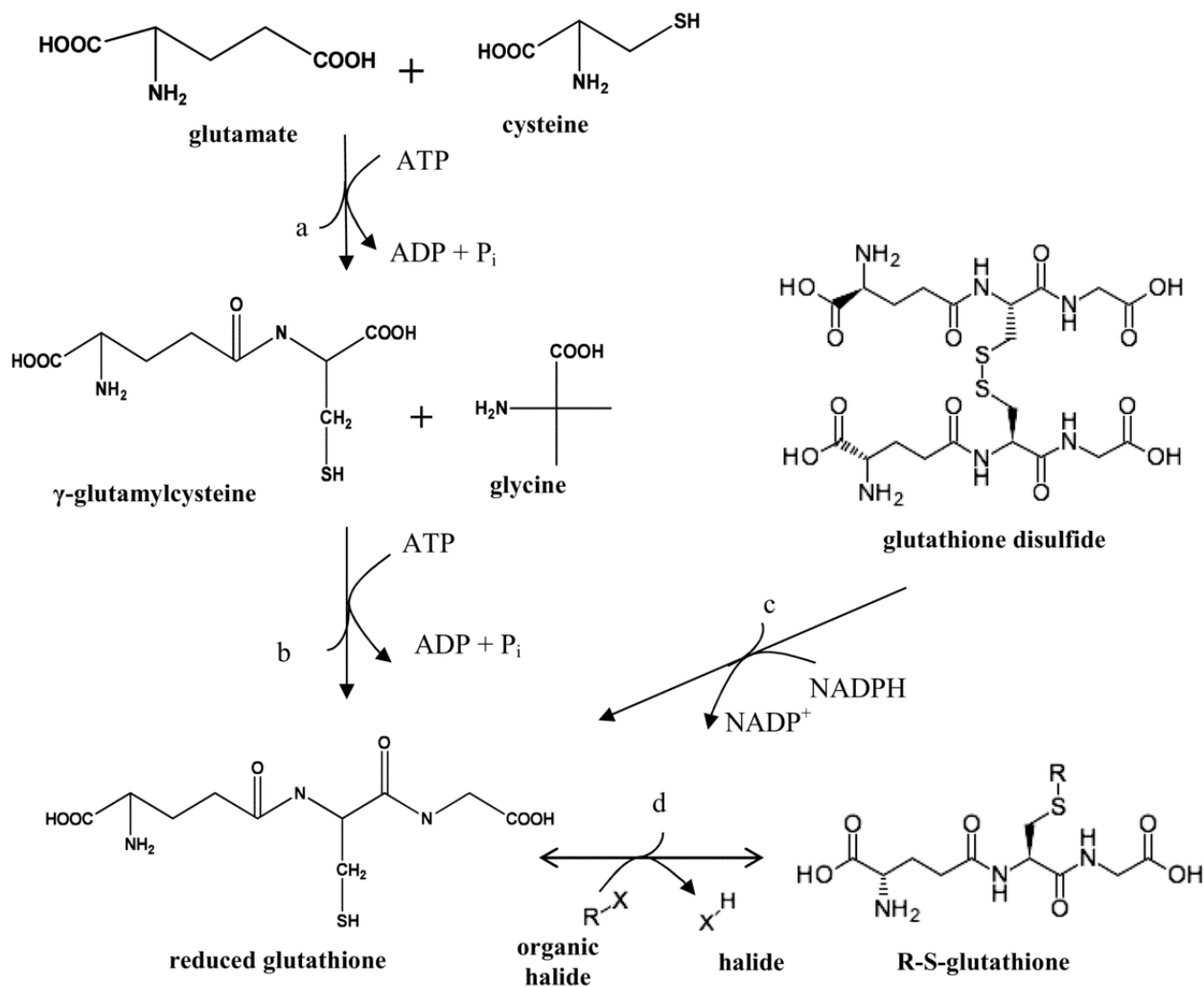
Figure 12. The glutathione biosynthesis pathway in plants (Noctor et al., 1998; KEGG, 2013).

Enzymes involved in the reactions are as follows: a. γ-glutamylcysteine synthetase, b.

glutathione synthetase, c. glutathione disulfide reductase, and d. glutathione S-transferase.

Table 1. List of the flavonoid pathway secondary metabolites that were detected in potato

(*Solanum tuberosum* L.) cultivars and reported to have antioxidant activity.

Group	Antioxidant	Ref.
Anthocyanins	petunidin-3-*O*-[6-(4-ferulyl-*O*-α-rhamnopyranosyl)-β-glucopyranoside]-5-*O*-β-glucopyranosides	1
	malvidin-3-*O*-[6-(4-ferulyl-*O*-α-rhamnopyranosyl)-β-glucopyranoside]-5-*O*-β-glucopyranosides	1
	malvidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside (also called negretein)	2, 3, 4
	petunidin-3-rutinoside-5-glucoside	3, 4, 5
	petunidin-3-coumaroyl-rutinoside-5-glucoside	3
	pelargonidin-3-rutinoside-5-glucoside	3
	pelargonidin-3-coumaroyl-rutinoside-5-glucoside	3
	peonidin-3-rutinoside-5-glucoside	3
	peonidin-3-coumaroyl-rutinoside-5-glucoside	3, 5
	malvidin-3-rutinoside-5-glucoside	3, 4
	malvidin-3-coumaroyl-rutinoside-5-glucoside	3
	petunidin-3-*O*-[6-*O*-(4-*O*-E-caffeoyl-*O*-α-rhamnopyranosyl)-β-glucopyranoside]-5-*O*-b-glucopyranoside	6
	peonidin-3-*O*-[6-*O*-(4-*O*-E-caffeoy1-*O*-α-rhamnopyranosyl)-β-glucopyranoside]-5-*O*-b-glucopyranoside	6
	peonidin-3-*O*-[6-*O*-(4-*O*-E-p-coumaroyl-*O*-α-rhamnopyranosyl)-β-glucopyranoside]-5-O-β-glucopyranoside (also called peonanin)	6
	petunidin-3-*O*-[6-*O*-(4-*O*-E-*p*-coumaroyl-*O*-α-rhamnopyranosyl)-β-glucopyranoside]-5-*O*-β-g1ucopyranoside (also called petanin)	3, 5, 6
	pelargonidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside	3, 4
	pelargonidin-3-rutinoside	3
	peonidin-glycoside	3
	peonidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside	3, 4
	petunidin-3-(*p*-coumaroyl-rutinoside)-5-glucoside	3, 4
	pelargonidin *p*-coumaroyl 5-glucoside 3-rhamnosylglucoside	7
	cyanidin *p*-coumaroyl 5-glucoside 3-rhamnosylglucoside	7
	peonidin *p*-coumaroyl 5-glucoside 3-rhamnosylglucoside	7
	delphinidin *p*-coumaroyl 5-glucoside 3-rhamnosylglucoside	7
	petunidin *p*-coumaroyl 5-glucoside 3-rhamnosylglucoside	7
	malvidin *p*-coumaroyl 5-glucoside 3-rhamnosylglucoside	7
	pelargonidin 3-rhamnosylglucoside	7
	cyanidin 3-rhamnosylglucoside	7

	Ref.
delphinidin 3-rhamnosylglucoside	7
petunidin 3-rhamnosylglucoside	7
petunidin-3-caffeoyl-rutinoside-5-glucoside	4, 5
delphinidin-3-*p*-coumaroyl-rutinoside-5-glucoside	4, 5
cyanidin-3-*p*-coumaroyl-rutinoside-5-glucoside	5
delphinidin-3-rutinoside	5
petunidin-3-feruloyl-rutinoside-5-glucoside	4, 5
malvidin-3-rutinoside	4
peonidin-3-feruloyl-rutinoside-5-glucoside	4
malvidin-3-feruloyl-rutinoside-5-glucoside	4
petunidin-3-*p*-coumaroyl-rutinoside	4

Table 1. Continued.

Group	Antioxidant	Ref.
Flavanone	naringenin	3
Flavan-3-ol	eriodictyol	3
	catechin	3
Flavonol	epicatechin	3, 8
	kaempferol	3, 7
	myricetin	3, 7
	quercetin	3, 7
	quercetin-3-rutinoside (also called rutin)	5
	kaempferol-3-rutinoside	3, 5

1: Fossen and Andersen, 2000; 2: Chmielewska, 1936; 3: Lewis et al., 1998; 4: Hillebrand et al.,

2009; 5: Andre et al., 2007; 6: Fossen et al., 2003; 7: Harborne, 1960; 8: Crowell et al., 2008.

Table 2. List of secondary metabolites from different biosynthetic pathways that were detected in potato (*Solanum tuberosum* L.) cultivars and reported to have antioxidant activity.

Pathway	Antioxidants	Ref.
Carotenoids	β-cryptoxanthin	1
	lutein	1, 2, 3, 4, 5, 6, 7, 8, 9
	violaxanthin	1, 2, 3, 4, 5, 6, 7, 8
	zeaxanthin	1, 2, 3, 4, 5, 6, 7, 8
	canthaxanthin	9
	lutein-5,6-epoxide	8, 10
	β-carotene	1, 8, 11
	neoxanthin	8, 11, 23
	antheraxanthin	1, 21
Phenylpropanoid	5-*O*-caffeoylquinic acid (also called chlorogenic acid)	1, 12, 13, 14, 15
	3-*O*-caffeoylquinic acid (also called *neo*-chlorogenic acid)	1, 12
	4-*O*-caffeoylquinic acid (also called *crypto*-chlorogenic acid)	1, 12
	genic acid	14
	gallic acid	14
	t-cinnamic acid	16
	salicyclic acid	13, 16
	syringic acid	16
	gentisic acid	16
	sinapic acid	17
	protocatechuic acid	1, 14
	caffeic acid	1, 14, 15
	ferulic acid	1, 13, 15
	p-coumaric acid	13, 15
	p-hydroxybenzoic acid	13
	vanillic acid	13
Ascorbic Acid	ascorbic acid	18
	dehydroascorbic acid	18
Shikimate	folic acid	19
	tyrosine	1
	tryptophan	1
Tocochromanol	α-tocopherol	20, 21
Glutathione	glutathione	22

1: Andre et al., 2007; 2: Iwanzik et al., 1983; 3: Mazza and Miniati, 1993; 4: Rodriguez-Saona et al., 1998; 5: Fossen and Andersen, 2000; 6: Brown et al., 2003; 7: Fossen et al., 2003; 8: Brown, 2005; 9: Reddivai et al., 2007; 10: Kasim, 1967; 11: Tevin and Schonecker, 1986; 12: Ramamurthy et al., 1992; 13: Cvikrova et al., 1994; 14: Sotillo et al., 1994; 15: Leo et al., 2008; 16: Onyeneho et al., 1993; 17: Friedman, 1997; 18: Camire et al., 2009; 19: Kolasa et al., 1993; 20: Piironen et al., 1986; 21: Crowell et al., 2008; 22: Al-Saikhan et al., 2006; 23: Chmielewska, 1936.