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About the Author

Aravind Jukanti obtained both his bachelor's (1993–1997) and master's degrees (1997–1999) in agriculture from Acharya N.G. Ranga Agricultural University, Hyderabad, India. During his master's, his specialization was in "Genetics and Plant Breeding," working on protein and other quality aspects in baby corn maize. Later, he shifted to Montana State University (MSU), Bozeman, USA, on a full scholarship to pursue his PhD in wheat genetics. The title of his dissertation at MSU was "Molecular and Biochemical Characterization of Wheat (*Triticum aestivum* L.) Polyphenol Oxidases." Aravind Jukanti reported and characterized the wheat PPO multigene family. He was also successful in identifying the most important wheat kernel PPO probably involved in the undesirable darkening reaction. After graduating in 2005, he worked as a postdoctoral fellow with Dr. A.M. Fischer at MSU up to 2007, mostly working on grain protein content and nitrogen remobilization in barley. He moved to the lab of Dr. J. Jaworski during the winter of 2007 at Donald Danforth Plant Science Center (DDPSC), St. Louis, Missouri, USA. At DDPSC, he worked on proteomic aspects of triacylglycerol biosynthesis. He moved back to India in 2009; worked in the chickpea team as a breeder at the "International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India"; and later joined the "Indian Council of Agricultural Research (ICAR)" as a "Senior Scientist." He is presently working as a senior rice breeder at ICAR-IIRR, Hyderabad, India.

Introduction 1

Polyphenol oxidases (PPOs) are nuclear-encoded, copper-containing enzymes with diverse phylogenetic distribution among plants, animals, fungi, and bacteria (Yoruk and Marshall 2003; Mayer 2006). In addition to their ubiquitous nature, PPOs are reported in several plant tissues (Vamos-Vigyazo 1981; Sherman et al. 1991, 1995; Yoruk and Marshall 2003). Interestingly, multigene families have been reported for several plant PPOs, but not much is known about their specific biological functions (Anderson et al. 2006). PPOs are often referred to as tyrosinase, phenolase, catecholase, cresolase, polyphenolase, or catechol oxidase (Whitaker 1994, 1996; Yoruk and Marshall 2003). PPOs are also sometimes differentiated into monophenol monooxygenase (EC 1.14.18.1) and diphenol:oxygen oxidoreductase (EC 1.10.3.2) for enzyme nomenclature purposes (Mayer 2006), but in this book, the common term polyphenol oxidase (PPO) will be used. Plant PPOs are implicated in different biological roles, but they are best known for their involvement in the undesirable browning of plant products. The undesirable browning reaction has attracted attention of researchers especially plant breeders, plant physiologists, and food scientists. The oxidation of phenolic compounds by PPOs to o-quinones followed by their nonenzymatic polymerization or condensation with nucleophiles leads to undesirable discoloration of plant products (Mathew and Parpia 1971; Vamos-Vigyazo 1981; Whitaker 1995; Yoruk and Marshall 2003). Interestingly, browning reaction may be useful, viz., in fermentation process of tea (Subramanian et al. 1999) or protein preservation in forage crops (Lee et al. 2004; Sullivan and Hatfield 2006). But mostly the oxidative discoloration observed in several plant-based products is considered negative since they cause deterioration of quality by altering the product's organoleptic and nutritional properties (Vamos-Vigyazo 1981; Mathies and Whitaker 1984; Zawistowski et al. 1991; Martinez and Whitaker 1995). Further, the discoloration of food products negatively impacts the consumer acceptance, storage/shelf life, and their value.

The active site of the enzyme consists of two copper atoms each with three conserved histidine residues (Lerch 1983; Huber et al. 1985). Although a sequential mechanism of binding of substrates is indicated, the order of removal products is

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not fully understood (Wilcox et al. 1985; Janovitz-Klapp et al. 1990; Whitaker 1994). PPOs can catalyze two different reactions: (i) *o*-hydroxylation of monophenols (monophenolase activity) and (ii) oxidation of diphenols to *o*-quinones (diphenolase activity; Mason 1957; Mayer and Harel 1979; Janovitz-Klapp et al. 1990). Some plant PPOs exhibit both types of activities, while others have either of the activities (Yoruk and Marshall 2003). But usually the diphenolase activity is the predominant form in higher plants. Some discrepancies in reaction properties of PPOs have been reported but were later shown to be due to differences in extraction/purification procedures or assay methods utilized (Sanchez-Ferrer et al. 1989b; Wesche-Ebeling and Montgomery 1990; Espin et al. 1997). Accurate determination of PPO activity is very crucial to study its properties and function, since peroxidase (EC 1.11.1.7) also can catalyze the oxidation of *o*-diphenols to *o*-quinones in presence of hydrogen peroxidase (Vamos-Vigyazo 1981; Miller et al. 1990; Nicolas et al. 1994; Richard-Forget and Guillard 1997). Some methods are suggested to detect/reduce the peroxidase interference (Mayer and Harel 1979; Nicolas et al. 1994; Richard-Forget and Guillard 1997). Different methods that quantify oxygen consumption, color formation, and product formation are available to determine the PPO activity (Yoruk and Marshall 2003).

The properties of PPOs have been extensively studied in several plant species, and they vary accordingly. Though the plant PPOs are nuclear encoded, they are plastidial enzymes. The synthesis and transport of plant PPOs to chloroplasts, their actual site, are complex processes, but it has the features of import of nuclear-encoded proteins to their respective organelles (Mayer 2006). Interestingly, the location of fungal PPOs is not yet clear, though it appears to be cytoplasmic. Rast et al. (2003) reported that at least some fungal PPOs are cell wall bound, i.e., in the extracellular matrix. Multiple forms of PPOs have been found in several plant species. These multiple forms exhibit distinct differences in their physicochemical and enzymatic properties. Despite the available evidence, there are some conflicting reports regarding the number of molecular forms of PPOs in some species/tissue. Production of artifacts, interconversion among the PPO forms, hormonal induction, and attachment of phenolic products or carbohydrates could result in multiple isoforms of PPOs (Yoruk and Marshall 2003).

The primary substrates of PPOs are different phenolic compounds; the concentration and types of phenols (mono-/di-/triphenols) vary significantly in plants. Substrate specificity of PPOs also varied with species and variety. Some phenolic substrates like catechol are major substrates in different crops like field bean, apple, and peach (Paul and Gowda 2000; Zhou et al. 1993; Flurkey and Jen 1980). Another important physiochemical property that varies significantly with different factors including plant source is the pH optimum (4.0–8.0). It is interesting to note that some PPOs (field bean, 4.0) show narrow pH optimum, while others (lettuce, 5.0–8.0) have a wider pH range (Yoruk and Marshall 2003). Multiple pH optima have also been observed in some plant species. Temperature is another key factor that considerably affects the PPO activity. The optimum temperature varies widely but is mostly in the range of 20–45 °C with a few exceptions like strawberry and

cucumber (50 °C; Serradell et al. 2000; Miller et al. 1990). Thermal stability of PPOs depends upon several factors including temperature and exposure time.

Several of the plant and fungal PPOs characterized were observed to be latent (van Gelder et al. 1997; Mayer 2006), and these are not involved in catalyzing phenols. The extent of latency of plant PPOs varies widely with species and tissues. The latent PPOs can be activated by different methods, like acid and base treatment (Kenten 1957), frost and aging (Lieberei and Biehl 1978, Meyer and Biehl 1980), alcohols (Guillard and Richard-Forget 1997; Espin and Wichers 1999; Onsa et al. 2000), mild heat treatment (Sheptovitsky and Brudvig 1996), and exposure to strong detergents (van Gelder et al. 1997; Chazarra et al. 2001; Okot-Kotber et al. 2002; Jukanti et al. 2003). Sodium dodecyl sulfate (SDS) has been extensively used for activation of latent PPOs in several plant species (Sanchez-Ferrer et al. 1989a, b, 1990; Moore and Flurkey 1990; Jimenez and Garcia-Carmona 1996; Laveda et al. 2000; Jukanti et al. 2003). Additionally, PPOs (both plant and fungal) have also been activated by partial proteolytic degradation (Gandía-Herrero et al. 2005; Jukanti et al. 2006; Mayer 2006). Several studies were conducted to understand the mechanism of proteolytic activation (Robinson and Dry 1992; Rathjen and Robinson 1992; Dry and Robinson 1994). The molecular weight of different forms of plant PPOs is in the range of 32–200 kDa but majority of them in the range of 35–70 kDa (Flurkey 1986; Sherman et al. 1991; Van Gelder et al. 1997; Yoruk and Marshall 2003).

Enzymatic browning caused by PPOs has significant economic impact on plant products (cereal, fruit, and vegetables) and seafood like shrimp/lobsters, thereby necessitating its control. Over the years, several compounds and approaches have been identified that control or prevent the enzymatic browning. Though several inhibitors or approaches are available, their effectiveness varies; therefore, specific control is required for a particular/individual PPO system (Ferrar and Walker 1996). Physical treatments like heating, freezing, and refrigeration could be used as alternative methods to control adverse browning (Ashie et al. 1996; Kim et al. 2000). Food safety is a major concern while using inhibitors to control browning reaction as some of them could be harmful. Therefore, identification of natural inhibitors specifically in the case of fruits and vegetables is very important. Some natural inhibitors like honey (Chen et al. 2000) and few others have been shown to be promising, but they are yet to be used on a commercial scale. But controlled level of PPO expression through DNA-/RNA-based strategies is the safest and most promising approach to reduce/control the browning reaction without any adverse health concerns. Advanced molecular tools have been utilized in manipulating the expression levels of PPOs; these studies will be discussed in detail in other chapters.

A role in plant defense is often been suggested for plant PPOs due to their induction upon wounding, pathogen attack, or insect infestation in addition to various abiotic/biotic stresses or various signaling compounds (Constabel et al. 1995; Thipyapong and Steffens 1997; Maki and Morohashi 2006). Despite PPOs' implication in plant defense mechanisms, most part of the early research focused on correlative studies. But recent progress has enabled in deciphering and understanding at least some molecular mechanisms of PPO action in important functions including plant defense (e.g., Thipyapong et al. 2004a). With recent advances in molecular

techniques, it is now possible to study/examine the role of specific PPO genes in response to injury, pest/pathogen attack, or abiotic stresses. Further, in some recent studies, the PPO expression levels were manipulated to study the PPO action or mechanisms to pathogen attack, water stress, and salinity (Thipyapong et al. 2004a [plant defense], b [water stress]; Liang et al. 2006). Induction of PPO has also been reported in fungi upon bacterial infection (Soler-Rivas et al. 2000). There is no doubt that these studies have given some insights of PPO action, but there is still no direct explanation for the underlying mechanism(s).

In plants, PPOs are mostly known for their role in unacceptable browning of products and to some extent for involvement in plant defense. However, precisely what roles do these enzymes play in plant metabolism is still very unclear. Majority of the characterized plant PPOs have diphenolase/catechol oxidase activity, but some have monophenolase/tyrosinase activity (Sullivan 2015). Though it was suggested that PPOs could be involved in production of caffeic acid from *p*-coumaric acid due to the tyrosinase activity (Vaughan and Butt 1969), it was later proved to be otherwise (Schoch et al. 2001; Franke et al. 2002). However, due to the occurrence of multiple forms of PPOs in several species, it is possible that PPOs are capable of performing important roles in plant metabolism. Some recent studies have demonstrated the possible role of PPOs in specific cases including (i) betalain biosynthesis (Gandia-Herrero and Garcia-Carmona 2013), (ii) tyrosine metabolism (Araji et al. 2014), (iii) lignin biosynthesis (Cho et al. 2003), and (iv) aurone biosynthesis (Sato et al. 2001). Additionally, the availability of different advanced analyses (genomic, transcriptomic, metabolomic, and proteomic) can help in studying and understanding the role(s) and function of PPOs in plants and other organisms.

In addition to plants, PPOs are also reported in seafood products like shrimp (Simpson et al. 1988; Rolle et al. 1991; Chen et al. 1997) and lobster (Chen et al. 1991a; Ali et al. 1994). Enzymatic browning also called as melanosis is a major concern in these economically important products as they are highly vulnerable to browning reaction. PPO inhibition studies have been a major area of research in the seafood products (Chen et al. 1991b, 1993; Kim et al. 2000). The seafood like shrimp is treated with chemical preservatives/melanosis inhibitors (e.g., sulfites) in the processing facilities to control the undesirable enzymatic reaction and extend their shelf life (Montero et al. 2001). Kim et al. (2000) described PPOs in seafood and their impact on quality. Animal PPOs are involved in the biosynthesis of melanin, the pigment of hair and skin (Hill 1992). Additionally, they are responsible for cuticular hardening, wound healing, and defense reactions in crustaceans and insects (Ferrer et al. 1989; Gillespie et al. 1997; Sugumaran 1998).

The first report of PPOs was by Bertrand (1896), and since then there have been several hundred papers addressing different aspects of PPOs. Detailed work on PPOs was made possible due to the pioneering work of Keilin and Mann (1938) and Kubowitz (1938) with regard to enzyme isolation. Later it was Mason (1956, 1966) who described the structure, possible functions, and importance of number of copper atoms at the active site. He also stated the necessity to understand the nature of PPO isozymes and monophenolase/diphenolase activity. Now it is an established fact that both monophenolase and diphenolase activities are the features of PPOs.

Several comprehensive reviews on plant PPOs including Mayer and Harel (1979), Steffens et al. (1994), Yoruk and Marshall (2003), and Mayer (2006) have been published. The authors have reviewed and discussed the various attributes including functions, structure, multiplicity, induction, and molecular properties of PPO. The latest review by Sullivan (2015) discussed the specific role of PPOs in biosynthesis of metabolites through both monophenolase and diphenolase activities. Although significant new observations regarding the different aspects of PPOs have been elucidated, certain problems like the function (including the mechanism), location, expression, in vivo activation, and molecular control have not yet been unambiguously resolved. Hence, the major focus of future work should be to understand the mechanisms/function and comparatively less to studies on presence of PPOs in a species. The use of advanced biological tools coupled with newer approaches (transcriptomic, proteomic, and metabolomic) will definitely aid in effectively addressing the pertinent problems of PPOs in the near future. In this book, I have made a sincere effort in presenting the different attributes of PPOs in a most comprehensive way by including the latest findings about plant PPOs in particular.

References

Ali MT, Marshall MR, Wei CI, Gleeson RA (1994) Monophenol oxidase activity from the cuticle of Florida Spiny lobster (*Panulirus argus*). J Agric Food Chem 42:53–58

Anderson JV, Fuerst EP, Hurkman WJ, Vensel WH, Morris CF (2006) Biochemical and genetic characterization of wheat (*Triticum* spp.) kernel polyphenol oxidases. J Cereal Sci 44:353–367

Araji S, Grammer TA, Gertzen R, Anderson SD, Mikulic-Petkovsek M, Veberic R, Phu ML, Solar A, Leslie CA, Dandekar AM, Escobar MA (2014) Novel roles for the polyphenoloxidase enzyme in secondary metabolism and the regulation of cell death in walnut. Plant Physiol 164:1191–1203

Ashie INA, Simpson BK, Smith JP (1996) Mechanisms for controlling enzymatic reactions in foods. Crit Rev Food Sci Nutr 36:1–30

Bertrand G (1896) Sur une nouvelle oxydase, ou ferment soluble oxidant, d'origine végétale. C R Acad Sci Paris 122:1215–1217

Chazarra S, García-Carmona F, Cabanes J (2001) Hysteresis and positive cooperativity of iceberg lettuce polyphenol oxidase. Biochem Biophys Res Commun 289:769–775

Chen JS, Rolle RS, Marshall MR, Wei CI (1991a) Comparison of phenol oxidase activity from Florida Spiny lobster and Western Australian lobster. J Food Sci 56:154–157

Chen JSJS, Wei C, Rolle RS, Otwell WS, Balaban MO, Marshall MR (1991b) Inhibitory effect of kojic acid on some plant and crustacean polyphenol oxidases. J Agric Food Chem 39:1396–1401

Chen JS, Balaban MO, Wei C, Gleeson RA, Marshall MR (1993) Effect of carbon dioxide on the inactivation of Florida Spiny lobster polyphenol oxidase. J Sci Food Agric 61:253–259

Chen JS, Charest DJ, Marshall MR, Wei CI (1997) Comparison of two treatment methods on the purification of shrimp polyphenol oxidase. J Sci Food Agric 75:12–18

Chen L, Mehta A, Berenbaum M, Arthur RZ, Engeseth NJ (2000) Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. J Agric Food Chem 48:4997–5000

Cho MH, Moinuddin SGA, Helms GL, Hishiyama S, Eichinger D, Davin LB, Lewis NG (2003) (+)-Larreatricin hydroxylase, an enantio-specific polyphenol oxidase from the creosote bush (Larrea tridentata). Proc Natl Acad Sci USA 100:10641–10646

Constabel CP, Bergey DR, Ryan CA (1995) Systemin activates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadecanoid defense signaling pathway. Proc Natl Acad Sci U S A 92:407–411

Dry IB, Robinson SP (1994) Molecular cloning and characterization of grape berry polyphenol oxidase. Plant Mol Biol 26:495–502
Espin JC, Ochoa M, Tudela J, Garcia-Canovas F (1997) Monophenolase activity of strawberry polyphenol oxidase. Phytochemistry 45:667–670
Espin JC, Wichers HJ (1999) Kinetics of activation of latent mushroom (*Agaricus bisporus*) tyrosinase by benzyl alcohol. J Agric Food Chem 47:3503–3508
Ferrar PH, Walker JRL (1996) Inhibition of diphenol oxidases: a comparative study. J Food Biochem 20:15–30
Ferrer OJ, Koburger JA, Simpson BK, Gleeson RA, Marshall MR (1989) Phenol oxidase levels in Florida Spiny lobster *(Panulirus argus*): relationship to season and molting stage. Comp Biochem Physiol 93B:595–599
Flurkey WH (1986) Polyphenoloxidase in higher plants. Plant Physiol 81:614–618
Flurkey WH, Jen JJ (1980) Purification of peach polyphenol oxidase in the presence of added protease inhibitors. J Food Biochem 4:29–41
Franke R, Humphreys JM, Hemm MR, Denault JW, Ruegger MO, Cusumano JC, Chapple C (2002) The *Arabidopsis* REF8 gene encodes the 3-hydroxylase of phenylpropanoid metabolism. Plant J 30:33–45
Gandia-Herrero F, Garcia-Carmona F (2013) Biosynthesis of betalains: yellow and violet plant pigments. Trends Plant Sci 18:334–343
Gandía-Herrero F, Jiménez-Atiénzar M, Cabanes J, García-Carmona F, Escribano J (2005) Evidence for a common regulation in the activation of a polyphenol oxidase by trypsin and sodium dodecyl sulfate. Biol Chem 386:601–607
Gillespie JP, Kanost MR, Trenczek T (1997) Biological mediators of insect immunity. Annu Rev Entomol 42:611–643
Guillard FA, Richard-Forget F (1997) Polyphenoloxidases from William pear (*Pyrus communis* cv. Williams): activation, purification and some properties. J Sci Food Agric 74:49–56
Hill HZ (1992) The function of melanin or six blind people examine an elephant. BioEssays 14:49–56
Huber M, Hintermann G, Lerch K (1985) Primary structure of tyrosinase from *Streptomyces glaucescens*. Biochemist 24:6038–6044
Janovitz-Klapp AH, Richard FC, Goupy PM, Nicolas JJ (1990) Kinetic studies on apple polyphenol oxidase. J Agric Food Chem 38:1437–1441
Jimenez M, Garcia-Carmona F (1996) The effect of sodium dodecyl sulphate on polyphenol oxidase. Phytochemistry 42:1503–1509
Jukanti AK, Bruckner PL, Habernicht DK, Foster CR, Martin JM, Fischer AM (2003) Extraction and activation of wheat polyphenol oxidase by detergents: biochemistry and applications. Cereal Chem 80:712–716
Jukanti AK, Bruckner PL, Fischer AM (2006) Molecular and biochemical characterisation of polyphenol oxidases in developing kernels and senescing leaves of wheat (*Triticum aestivum*). Funct Plant Biol 33:685–696
Keilin D, Mann T (1938) Polyphenol oxidase: purification, nature and properties. Proc R Soc Lond Ser B 125:187–205
Kenten RH (1957) Latent phenolase in extracts of broad-bean *(Vicia faba* L.) leaves. 1. Activation by acid and alkali. Biochem J 67:300–307
Kim J, Marshall MR, Wei C (2000) Polyphenol oxidase. In: Haard NF, Simpson BK (eds) Seafood enzymes utilization and influence on postharvest seafood quality. Marcel Dekker, New York, pp 271–315
Kubowitz F (1938) Spaltung und resynthese der polyphenoloxydase und des haemocyanin. Biochem Z 299:32–57
Laveda F, Nunez-Delicado E, Garcia-Carmona F, Sanchez-Ferrer A (2000) Reversible sodium dodecyl sulfate activation of latent peach polyphenol oxidase by cyclodextrins. Arch Biochem Biophys 379:1–6

Lee MRF, Winters AL, Scollan ND, Dewhurst RJ, Theodorou MK, Minchin FR (2004) Plant-mediated lipolysis and proteolysis in red clover with different polyphenol oxidase activities. J Sci Food Agric 84:1639–1645

Lerch K (1983) *Neurospora* tyrosinase: structural, spectroscopic and catalytic properties. Mol Cell Biochem 52:125–138

Liang M, Haroldsen V, Cai X, Wu Y (2006) Expression of a putative laccase gene, ZmLac1, in maize primary roots under stress. Plant Cell Environ 29:746–753

Lieberei R, Biehl B (1978) Activation of latent phenolase from spinach chloroplasts by ageing and by frost. Phytochemistry 17:1427–1429

Maki H, Morohashi Y (2006) Development of polyphenol oxidase activity in the micropylar endosperm of tomato seeds. J Plant Physiol 163:1–10

Martinez MV, Whitaker JR (1995) The biochemistry and control of enzymatic browning. Trends Food Sci Technol 6:195–200

Mason HS (1956) Structures and functions of the phenolase complex. Nature 177:79–81

Mason HS (1957) Mechanisms of oxygen metabolism. Adv Enzymol 19:79–231

Mason HS (1966) Preliminary remarks on polyphenoloxidase. In: Peisach J, Aisen P, Blumberg WE (eds) The biochemistry of copper. Academic, New York, pp 339–341

Matheis G, Whitaker JR (1984) Modification of proteins by polyphenol oxidase and peroxidase and their products. J Food Biochem 8:137–162

Mathew AG, Parpia HAB (1971) Food browning as a polyphenol reaction. Adv Food Res 29:75–145

Mayer AM (2006) Polyphenol oxidases in plants and fungi: going places? A review. Phytochemistry 67:2318–2331

Mayer AM, Harel E (1979) Polyphenol oxidases in plants. Phytochemistry 18:193–215

Meyer H, Biehl B (1980) Activities and multiplicity of phenolase from spinach chloroplasts during leaf ageing. Phytochemistry 19:2267–2272

Miller AR, Kelley TJ, Mujer CV (1990) Anodic peroxidase isoenzymes and polyphenol oxidase activity from cucumber fruit: tissue and substrate specificity. Phytochemistry 29:705–709

Montero P, Lopez-Caballero ME, Perez-Mateos M (2001) The effect of inhibitors and high pressure treatment to prevent melanosis and microbial growth on chilled prawns (*Penaeus japonicus*). J Food Sci 66:1201–1206

Moore BM, Flurkey WH (1990) Sodium dodecyl sulfate activation of a plant polyphenoloxidase. Effect of sodium dodecyl sulfate on enzymatic and physical characteristics of purified broad bean polyphenoloxidase. J Biol Chem 265:4982–4988

Nicolas JJ, Richard-Forget FC, Goupy PM, Amiot M, Aubert SY (1994) Enzymatic browning reactions in apple and apple products. Crit Rev Food Sci Nutr 34:109–157

Okot-Kotber M, Liavoga A, Yong K-J, Bagorogoza K (2002) Activation of polyphenol oxidase in extracts of bran from several wheat (*Triticum aestivum*) cultivars using organic solvents, detergents, and chaotropes. J Agric Food Chem 50:2410–2417

Onsa GH, Saari N, Selamat J, Bakar J (2000) Latent polyphenol oxidases from sago log *(Metroxylon sugu):* partial purification, activation, and some properties. J Agric Food Chem 48:5041–5045

Paul B, Gowda LR (2000) Purification and characterization of a polyphenol oxidase from the seeds of field bean *(Dolichos lablab)*. J Agric Food Chem 48:3839–3846

Rast DM, Baumgarten D, Mayer C, Hollenstein GO (2003) Cell wall-associated enzymes in fungi. Phytochemistry 64:339–366

Rathjen AH, Robinson SP (1992) Aberrant processing of polyphenol oxidase in a variegated grapevine mutant. Plant Physiol 99:1619–1625

Richard-Forget FC, Guillard FA (1997) Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* Cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. J Agric Food Chem 45:2472–2476

Robinson SP, Dry IB (1992) Broad bean leaf polyphenol oxidase is a 60 Kilodalton protein susceptible to proteolytic cleavage. Plant Physiol 99:317–323