Open Access Contents
Int. J. Drug Dev. & Res.
| October - December 2013 | Vol. 5 | Issue 4 | ISSN 0975-9344 |
www.ijddr.in

Computational approach for enzymes present in *Capsicum annuum*: A review

Shivendu Ranjan

Nandita Dasgupta

Arkadyuti Roy Chakraborty

Melvin Samuel S

Pradeep Singh Jadon

Ramalinagam Chidambaram*
School of Bio Sciences and Technology, VIT University, Tamilnadu, India

Corresponding Authors:
Ramalingam Chidambaram
Professor, School of Biosciences & Technology
VIT University, Vellore-14
Tamilnadu, India
email: shivenduranjan2013@vit.ac.in,

Abstract:
Capasicum annuum or sweet bell pepper is one of the more economical and agriculturally viable vegetable grown all over the world owing to its antioxidant and other medicinal properties. This review highlights the essential enzymes present and its mode of action using bioinformatics online tools *viz.* uniprot, swissprot and Brenda enzyme db and ExPAsy protein databases. The enzymes *viz.* peroxidase, polyphenol oxidase, tyrosinase, catecholase, Pectin esterase, Catalase, 9-lipoxygenase, L-asparaginase, Polygalactouronase, Capsanthin and Ribulose-Phosphate 3-Epimerase contribute to its properties by various molecular mechanisms. Understanding of these mechanisms will be helpful for application of these enzymes in food processing and in the production of food ingredients. The increasing sophistication of processing industries creates a demand for a broad variety of enzymes with characteristics compatible with food processing conditions for e.g. shelf life of fruits and vegetables can be increased by decreasing the levels of peroxidase and polyphenoloxidase.

Keywords: BRENDA Enzyme DB, *Capasicum annuum*, Enzymes, ExPAsy protein DB.

Review Paper

Introduction

Capsicum annuum (Sweet bell pepper) is a common ingredient of staple food. In Brazil and in some other countries, the sweet bell pepper is considered one of the more important commodities in terms of production volume and commercially (De et al., 2009). There are various species from *Capsicum* genera and all are widely cultivated throughout world. Most enzymes studied have higher specific activity and protein content in green than in the red pepper (Mateos et al., 2003). It's medicinal and antioxidant properties are the main attributes to be focused on research purposes. Further, sweet bell pepper is also a good source of bioactive compounds, such as the carotenoids (Kim et al., 2004). These substances can substantially reduce the risk of developing degenerative diseases such as macular degeneration in women (Moller et al., 2006). It also inhibit carcinogenic activity (Nishino et al., 2002) and reduce (50% reduced rate) posterior subcapsular cataracts in subjects with higher plasma lutein concentration (Gale et al., 2003). An inclusion complex of red bell pepper pigments with β-cyclodextrin is used a as natural colorant in yogurt (Lidiane et al., 2013). Recent trends of research is continuously shifting towards nanotechnology (Kingsley *et al.,* 2013), computational approach. Computational approach should be applied to understand biological properties and its mechanism *e.g.* antimicrobial effect of garlic and cinnamon (Shivendu *et al.,* 2012a), antibacterial effect of marigold leaves (Nandita *et al.,* 2012), blood coagulation property of marigold leaves

(Shivendu *et al.,* 2012b). This review provides some essential information about the enzymes present in *capsicum annuum* retrieved from online database *viz.* swissprot, uniprot, ExPAsy, and BRENDA ENZYME DB.

UniProt (launched on 15 December 2003) comprises of three components: Firstly, the UniProt Knowledgebase which will continue the work of Swiss-Prot, TrEMBL and PIR by providing an expertly curated database; Secondly, the UniProt Archive (UniParc) which is updated with novel sequences daily; Thirdly, the UniProt non-redundant reference databases (UniProt NREF), which provide concise views on top of the UniProt Knowledgebase and UniParc (Rolf et al., 2004). Swiss-Prot is the leading universal curated protein sequence database, which contained as of November 2003 (release 42.6) contains 140,000 curated sequence entries from over 8300 different species. Being non-redundant, means that all reports for a given protein are merged into a single entry, and is highly integrated with other databases (Gasteiger et al., 2001). ExPASy is the SIB Bioinformatics Resource Portal which provides access to scientific databases and software tools in different areas of life sciences including proteomics, genomics, phylogeny, systems biology, population genetics, transcriptomics etc (Panu et al., 2012). BRENDA (BRaunschweig ENzyme DAtabase) was created in 1987 at the German National Research Center for Biotechnology at Braunschweig and is currently being maintained by the University of Cologne (Priti et al., 2003).

Peroxidase (EC number: 1.11.1)

It belongs to a large Family of Class III Plant Peroxidases i.e. Oxidoreductases. It acts on peroxide as an acceptor. Further peroxidases (POD) are haem-containing enzymes that use hydrogen peroxide as the electron acceptor to catalyse a number of oxidative reactions. Most haem peroxidases follow the given reaction scheme:

$Fe^{3+} + H_2O_2 \rightarrow [Fe^{4+}=O]R'$ (Compound I) $+ H_2O$

$[Fe^{4+}=O]R' +$ substrate $\rightarrow [Fe^{4+}=O]R$ (Compound II) + oxidised substrate

$[Fe^{4+}=O]R +$ substrate $\rightarrow Fe^{3+} + H_2O +$ oxidised substrate

The enzyme reacts with one equivalent of H_2O_2 to give $[Fe^{4+}=O]R'$ (compound I). This is a oxidation/reduction reaction involves transfer of two electrons where H_2O_2 is reduced to water and the enzyme is oxidised. One oxidising equivalent resides on iron, giving the oxyferryl intermediate (Nelson et al., 1994), while in many peroxidases the porphyrin (R) is oxidised to the porphyrin pi-cation radical (R'). Compound I then oxidises an organic substrate to give a substrate radical (Li and Poulos, 1994).

POD mainly oxidises phenols to phenoxy radicals, which participate in reactions where polymers and oligomers are produced which are further responsible for enzymatic browning and off-flavor development (Morimoto et al., 2000). It is involved in photorespiration and assimilation of symbiotically induced nitrogen in plant-specific functions. Recent postgenetic approaches in transcriptome and proteome showed that plant peroxisomes have more important roles in various metabolic processes including biosynthesis of plant hormones (Mano and Nishimura, 2005).

POD can be found in plants, animals and microbes. It is one of the most thermostable enzymes performing single electron oxidation on a wide variety of compounds in the presence of hydrogen peroxide. POD reduces H_2O_2 to water while oxidizing a variety of other substrates. The catalytic process of POD occurs in a multi step

reaction. This is shown in the following scheme, where S stands for substrate and S• represents the corresponding radical. AH_2 and AH• represent a reducing substrate and its radical product, respectively (Lengauer and Rarey, 1996).

$$POD + H_2O_2 \longrightarrow \text{Compound I} + H_2O$$
$$\text{Compound I} + S \longrightarrow \text{Compound II} + S^{\bullet}$$
$$\text{Compound II} + S^{\bullet} \longrightarrow POD + S^{\bullet}$$

A simplified equation for peroxidase activity may be given as:

$$H_2O_2 + 2AH_2 \longrightarrow 2H_2O + AH^{\bullet}$$

Furthermore, the related activity of PPO and POD is due to the generation of hydrogen peroxide during the oxidation of phenolic compounds in PPO-catalyzed reactions (Francisco and Juan, 2001; Khan and Robinson, 1994; Sugai and Tadini, 2006). Peroxidise is the enzyme responsible for off-flavour development, so its inactivation will inhibit any such off-flavour development (Yemenicioglu et al., 1999).

Polyphenol Oxidase (EC number: 1.14.18.1)

It also belongs to a large family of Class III Plant Peroxidases i.e. Oxidoreductases enzyme family so its mode of action is similar to that of POD i.e. acts on paired donors with O_2 as the oxidant and incorporation or reduction of oxygen. The incorporated oxygen need not be derived from O_2; or with another compound as a single donor, and incorporation of one oxygen atom into the other donor (Francisco and Juan, 2001; Khan and Robinson, 1994). Polyphenol oxidase (PPO) is a dicopper-containing enzyme which is involved in the oxidation of the polyphenols from plants. PPO shows a higher activity with diphenols (Onsa et al., 2000). Two kinds of reactions catalyzed by PPO are the hydroxylation of monophenols to o-diphenol and the oxidation of o-diphenol to o-quinone (Francisco and Juan, 2001) schematic reaction of which is shown in Figure 1 (Prontipa et al., 2010).

The browning process can be caused by both enzymatic and non-enzymatic biochemical reactions. Polyphenol oxidase (PPO) and peroxidase (POD) are two well known enzymes involved in the browning process (Sapers, 1993; Chen et al., 2000; Deepa et al., 2007). PPO catalyzes the conversion of phenolic compounds to quinones and assists their products' polymerization in the presence of oxygen leading to the formation of undesirable brown pigments and off-flavored products. The browning of injured, peeled or diseased fruit tissues can causes undesirable changes in quality during handling, processing or storage (Yemenicioglu et al., 1999). Different forms of PPO are found in *Capsicum annuum*, such as tyrosinase (Nisit et al., 2007), cathecolase (Deepa et al., 2007).

Tyrosinase/Cresolase/Phenolase (EC 1.14.18.1)

Generally known as Tyrosinase but can also be named as monophenol monooxygenase, phenolase, monophenol oxidase, cresolase, monophenolase, tyrosine-dopa oxidase, monophenol monooxidase, monophenoldihydroxyphenylalanine oxygen oxidoreductase, *N*-acetyl-6-hydroxytryptophan oxidase, monophenol, dihydroxy-L-phenylalanine oxygen oxidoreductase, o-diphenol oxygen oxidoreductase, phenol oxidase. Its synthetic name is L-tyrosine,L-dopa:oxygen oxidoreductase (BRENDA, 2013; ExPAsy, 2013).

It is type III copper protein found in a variety of bacteria, fungi, plants, insects, crustaceans, and mammals, which is involved in the synthesis of betalains and melanin. The enzyme, which is activated upon binding molecular oxygen, can catalyse both a monophenolase reaction cycle (reaction 1) or a diphenolase reaction cycle

(reaction 2). During the monophenolase cycle, one of the bound oxygen atoms is transferred to a monophenol (such as L-tyrosine), generating an *o*-diphenol intermediate, which is subsequently oxidized to an *o*-quinone and released along with a water molecule. The enzyme remains inactive in its deoxy state and is further restored to the active oxy state by the binding of a new oxygen molecule. During the diphenolase cycle the enzyme binds to an external diphenol molecule (such as L-dopa) and oxidizes it to an *o*-quinone that is released along with a water molecule, leaving the enzyme in the intermediate met state. The enzyme then binds to a second diphenol molecule and repeats the process, thereby ending in a deoxy state (Rolff et al., 2011; Steiner et al., 1996).

Reaction (1)

L-tyrosine + O_2 = dopaquinone + H_2O (overall reaction)

(1a) L-tyrosine + ½ O_2 = L-dopa

(1b) L-dopa + ½ O_2 = dopaquinone + H_2O

Reaction (2)

2 L-dopa + O_2 = 2 dopaquinone + 2 H_2O

Catechol oxidase/ Catecholase (EC 1.10.3.1**)**

Generally known as Catechol oxidase but can additionally called as diphenol oxidase, *o*-diphenolase, polyphenol oxidase, pyrocatechol oxidase, dopa oxidase, catecholase, *o*-diphenol oxygen oxidoreductase and *o*-diphenol oxidoreductase, but the synthetic name is 1,2-benzenediol:oxygen oxidoreductas (BRENDA, 2013; ExPAsy, 2013).

It is also a type 3 copper protein that catalyses the oxidation of catechol (i.e., *o*-diphenol) to the corresponding *o*-quinone exclusively. The enzyme also acts on a variety of substituted catechols. Unlike tyrosinase which can catalyse both the monooxygenation of monophenols and the oxidation of catechols (Mayer and Harel, 1979; Gerdemann et al., 2002).

2 catechol + O_2 = **2** 1,2-benzoquinone + **2** H_2O

Pectinesterase (EC 3.1.1.11)

Pectinesterase which can also be called as pectin demethoxylase, pectin methoxylase, pectin methylesterase, pectase, pectin methyl esterase and pectinoesterase. The Systematic name is pectin pectylhydrolase (BRENDA, 2013; ExPAsy, 2013). Pectin in presence of water is converted into *n* methanol and pectate with the help of enzyme pectin esterase (Figure 2).

pectin + *n* H_2O = *n* methanol + pectate

It is involved in the regulation of the cell wall rigidity and also plays a key role in process of fruit ripening (Castro et al., 2004). Temperature stability is indicated by presence of 75% enzyme activity at 55°C, 10% activity activity activity at 75°C and 5% at 85°C, maximum stability of it is found upto a maximum temperature of 95°C (Mejia-Cordova et al., 2005).

7. Catalase (Ec 1.11.1.6)

The enzyme Catalase is also referred as equilase, caperase, optidase, catalase-peroxidase, CAT and its s**ystematic name is** hydrogen-peroxide:hydrogen-peroxide – oxidoreductase (BRENDA, 2013; ExPAsy, 2013).

It is an oxidoreductase by classification and its mode of action is the same as others mentioned previously i.e. it reduces H_2O_2 to water (**2** H_2O_2 = O_2 + **2** H_2O) (Dounce, 1983). This enzyme can also act as a peroxidase for which several organic substances like ethanol can act as a hydrogen donor. A manganese protein containing Mn^{III} in the resting state, which also belongs here, is often called pseudocatalase (Kono and Fridovich, 1983). It is found in stems, leaves, roots and developing fruits of capsicum annuum and serves to protect cells from the toxic effects of hydrogen

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., October -December 2013, 5 (4): 88-97

peroxide by reducing it to water. There are 492 amino acids present in it (Kwon and An, 2001).

Linoleate 9S-lipoxygenase /9-lipoxygenase (EC 1.13.11.58)

The above enzyme can also be termed as 9-lipoxygenase, 9*S*-lipoxygenase, linoleate 9-lipoxygenase, LOX1 (gene name), 9S-LOX, and the synthetic names is linoleate:oxygen 9*S*-oxidoreductase (BRENDA, 2013; ExPAsy, 2013). It converts linolate to 9-hydroperoxy-10,12-octadecadinoate in presence of oxygen. (Gigot et al., 2010)

Overall Reaction:

Linoleate + O_2 = (9*S*,10*E*,12Z)-9-hydroperoxy-10,12-octadecadienoate

It contains a nonheme iron and a common plant lipoxygenase that oxidizes linoleate and alpha-linolenate, the two most common polyunsaturated fatty acids in plants by inserting molecular oxygen at the C9 position with (S)-configuration. The enzyme plays a physiological role during the early stages of seedling growth (Bannenberg et al., 2009).

In capsicum annuum it regulates defense and cell death response to microbial pathogens. The optimum temperature for its activity is 25°C with an optimum pH of 6 ranging upto a mximum of pH 6.5 (Hwang and Hwang, 2010).

L-Asparaginase/ L-asparaginase (EC 3.5.1.1)

Its accepted name is asparaginase and can be also called as asparaginase II, L-asparaginase, colaspase, elspar, leunase, crasnitin and a-asparaginase. The synthetic name is L-asparagine amidohydrolase (BRENDA, 2013; ExPAsy, 2013). It catalyzes the reaction in which asparaginase is involved in cells (Figure 3) overall reaction is as; L-asparagine + H_2O = L-aspartate + NH_3 (Narta et al., 2007). It is responsible for asparagine degradation (Yao et al., 2005) and is involved in the interconversion of aspartate and asparagines (Falzone et al., 1988).

It is found in Capsicum annuum, Datura inoxia, Pisum sativum, Solanum lycopersicum, Tamarindus indica, Vigna unguiculata, Withania somnifera etc with the function of asparatate degradation and is also involved in the interconversion of aspartate and asparagines (Oza et al., 2009).

Polygalactouronase/PG (EC 3.2.1.15)

It can also be called as pectin depolymerise, pectinase, endopolygalacturonase, pectolase, pectin hydrolase, pectin polygalacturonase, endo-polygalacturonase, poly-a-1,4-galacturonide glycanohydrolase, endogalacturonase and endo-D-galacturonase. Its synthetic name is poly (1, 4-a-D-galacturonide) glycanohydrolase (BRENDA, 2013; ExPAsy, 2013). It is involved in the random hydrolysis of (1→4)-a-D-galactosiduronic linkages in pectate and other galacturonans. It splits pectin chain by adding a molecule of water and breaks the linkage between two galacturonan units. These enzymes catalyze reactions that break a- 1,4-glycosidic bonds (Huang et al., 1990).

PG activity increases during ripening. Ripening specific pectin methylesterase, active in vivo, appears to enhance PG-mediated pectin ultra-degradation, resulting in cell wall dissolution and emergence of deciduous fruit traits. Some pectin methylesterase isozymes are apparently inactive in vivo, particularly in green fruit and throughout ripening in the 'Hard Pick' line, thereby limiting PG-mediated pectin depolymerization which results in moderately difficult fruit separation from the calyx (Rancibia and Motsenbocker, 2006). It is also involved in homogalacturonan degradation (Willats et al., 2001). Exo-poly-a-D-

galacturonosidase (EC 3.2.1.82) (exoPG) hydrolyzes peptic acid from the non-reducing end, further releasing digalacturonate (He and Collmer, 1990). It is found in Capsicum frutescens and capsicum annuum with the similar functions of ripening (Gong et al., 2008).

Capsanthin (EC 5.3.99.8)

Its accepted name is capsanthin/capsorubin synthase and some other names are as CCS, ketoxanthophyll synthase, capsanthin-capsorubin synthase and Systematic name is violaxanthin-capsorubin isomerise (BRENDA, 2013; ExPAsy, 2013). Capsanthin catalyses two reactions from substrate antheraxanthin and violaxanthin which produce capsanthin and capsorbin respectively (Bouvier et al., 1994).

This multifunctional enzyme is induced during the chromoplast differentiation in plants; the gene is specifically expressed during chromoplast development in fruits thereby accumulating ketocarotenoids but not in mutants impaired in this biosynthetic step (Bouvier et al., 1994). In capsicum annuum it is involved in apsanthin and capsorubin biosynthesis (Al-Babili et al., 2000). It has relation with molecular genetics as y locus in pepper is responsible for capsanthin-capsorubin synthase which ultimately relates to the color of capsicum annuum (Popovsky and Paran, 2000). The capsanthin-capsorubin synthase gene is activated specifically during the final stages of Capsicum fruit ripening (Lefebvre et al., 1998).

Ribulose-Phosphate 3-Epimerase (EC 5.1.3.1)

Its accepted name is ribulose-phosphate 3-epimerase but follows some other names such as phosphoribulose epimerase, erythrose-4-phosphate isomerise, phosphoketopentose 3-epimerase, xylulose phosphate 3-epimerase, phosphoketopentose epimerase, ribulose 5-phosphate 3-epimerase, D-ribulose phosphate-3-epimerase, D-ribulose 5-phosphate epimerase, D-ribulose-5-P 3-epimerase, D-xylulose-5-phosphate 3-epimerase, pentose-5-phosphate 3-epimerase and Systematic name is D-ribulose-5-phosphate 3-epimerase (BRENDA, 2013; ExPAsy, 2013). Enzyme ribulose-phosphate 3-epimerase is involved in the conversion of D-ribulose 5-phosphate into D-xylulose 5-phosphate as shown in Figure 4 (Thom et al., 1998)

In capsicum it is mainly involved in the pentose-phosphate pathway, although it has many functions like the Bifidobacterium shunt, Calvin-Benson-Bassham cycle, D-arabinose degradation II, formaldehyde assimilation II (RuMP Cycle), formaldehyde assimilation III (dihydroxyacetone cycle), heterolactic fermentation, pentose phosphate pathway (non-oxidative branch) and the ribitol degradation (Thom et al., 1998).

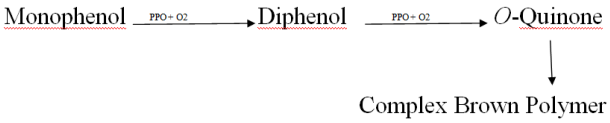


Figure 1: Hydroxylation of monophenols to diphenols and to o-quinonne

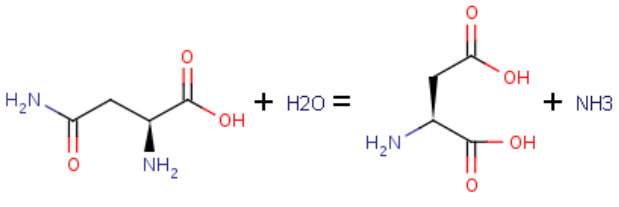
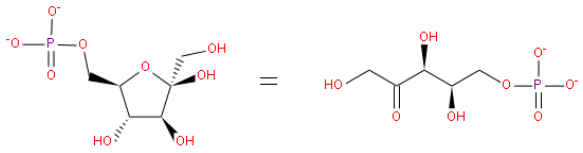


Figure 2: Overall reaction in which enzyme asparaginase is involved



D-ribulose 5-phosphate = D-xylulose

Figure 3: Overall reaction in which ribulose-phosphate 3-epimerase is involved

References

1) Al-Babili S, Hugueney P, Schledz M, Welsch R, Frohnmeyer H, Laule O, Beyer P (2000) Identification of a novel gene coding for neoxanthin synthase from Solanum tuberosum. FEBS Lett 485(2-3):168-72.
2) Bannenberg G, Martinez M, Hamberg M, Castresana C (2009) Diversity of the enzymatic activity in the lipoxygenase gene family of *Arabidopsis thaliana*. Lipids 44:85–95.
3) Bouvier F, Hugueney P, d'Harlingue A, Kuntz M, Camara B (1994) Xanthophyll biosynthesis in chromoplasts: isolation and molecular cloning of an enzyme catalyzing the conversion of 5,6-epoxycarotenoid into ketocarotenoid. Plant J 6: 45–54.
4) Gale CR, Hall NF, Phillips DIW, Martyn CN (2003) Lutein and zeaxanthin status and risk of age-related macular degeneration. Investigative Ophthalmology Visual Science 44: 2461–2465.
5) Castro SM. Van Loey A, Saraiva JA, Smout C, Hendrickx M (2004) Activity and process stability of purified green pepper (*Capsicum annuum*) pectin methylesterase. J Agric Food Chem: 52: 5724-5729.
6) Cédric Gigot, Marc Ongena, Marie-Laure Fauconnier, Jean-PaulWathelet, Patrick Du Jardin, Philippe Thonart (2010) The lipoxygenase metabolic pathway in plants: potential for industrial production of natural green leaf volatiles. Biotechnol Agron Soc Environ 14(3): 451-460.
7) Chen L, Mehta A, Berenbaum M, Zangerl AR, Engeseth NJ (2000) Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. J Agric Food Chem 48(10): 4997-5000.
8) De Azevedo-Meleiro CH, Rodriguez-Amaya DB (2009) Qualitative and quantitative differences in the carotenoid composition of yellow and red peppers determined by HPLC-DAD-MS. J Sep Sci 32(21): 3652-3658.
9) Dounce AL (1983) A proposed mechanism for the catalatic action of catalase. J Theor Biol 105(4): 553-67.
10) Falzone CJ, Karsten WE, Conley JD, Viola RE (1988) L-aspartase from *Escherichia coli*: substrate specificity and role of divalent metal ions. Biochemistry 27(26): 9089-93.
11) Francisco ATB, Juan Carlos E (2001) Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J Sci Food Agric 81: 853–876.
12) Gasteiger E, Jung E, Bairoch A (2001) SWISS-PROT: connecting biomolecular knowledge via a protein database. Curr Issues Mol Biol 3 (3); 47-55.
13) Gerdemann C, Eicken C, Krebs B (2002) The crystal structure of catechol oxidase: new insight into the function of type-3 copper proteins. Acc Chem Res 35: 183–191.
14) Gong Z, Yang R, Li D (2008) Specific expression gene from *Capsicum annuum* after inoculated with Phytophthora capsici.; Submitted (DEC-2008) to the EMBL/GenBank/DDBJ databases.
15) He SY, Collmer A (1990) Molecular cloning, nucleotide sequence, and marker exchange mutagenesis of the exo-poly-alpha-D-galacturonosidase-encoding pehX gene of *Erwinia chrysanthemi* EC16. J Bacteriol 172: 4988-4995.
16) Huang JH, Schell MA (1990) DNA sequence analysis of pglA and mechanism of export of its polygalacturonase product from *Pseudomonas solanacearum*. J Bacteriol 172: 3879-3887.
17) Hwang IS, Hwang BK (2010) The pepper 9-lipoxygenase gene CaLOX1 functions in defense and cell death responses to microbial pathogens. Plant Physiol 152: 948-967.
18) Kengo Morimoto, Kenji Tatsumi, Ken-Ichi Kuroda (2000) Peroxidase catalyzed co-polymerization of pentachlorophenol and a potential humic precursor. Soil Biology & Biochemistry 32: 1071-1077.
19) Khan AA, Robinson DS (1994) Hydrogen donor specificity of mango isoperoxidases. Food Chem. 49: 407–410.
20) Kono Y, Fridovich I (1983) Isolation and characterization of the pseudocatalase of *Lactobacillus plantarum*. J Biol Chem 258: 6015–6019.
21) Kwon SI, An CS (2001) Molecular cloning, characterization and expression analysis of a

catalase cDNA from hot pepper (*Capsicum annuum L.*). Plant Sci 160(5): 961-969.

22) Lefebvre V, Kuntz M, Camara B, Palloix A (1998) The capsanthin-capsorubin synthase gene: a candidate gene for the y-locus controlling the red fruit colour in pepper. Plant Mol Biol 36: 785-789.

23) Lengauer T, Rarey M (1996) Review Computational methods for biomolecular docking. Curr Opin Struct Biol 6(3): 402-406.

24) Li H, Poulos TL (1994) Structural variation in heme enzymes: a comparative analysis of peroxidase and P450 crystal structures. Structure 2(6): 461-464.

25) Lidiane Martins MG, Nicolly P, Valéria GC, Deborah QF, Kátia G, de Lima A (2013) Inclusion complexes of red bell pepper pigments with β-cyclodextrin: Preparation, characterisation and application as natural colorant in yogurt. Food Chemistry. doi: 10.1016/j.foodchem.2012.09.065.

26) Mano S, Nishimura M (2005) Plant peroxisomes. Vitam Horm 72: 111-54.

27) Mateos RM, León AM, Sandalio LM, Gómez M, del Río LA, Palma JM (2003) Peroxisomes from pepper fruits (*Capsicum annuum L.*): purification, characterisation and antioxidant activity. J Plant Physiol 160(12): 1507-16.

28) Mayer AM, Harel E (1979) Polyphenol oxidases in plants. Phytochemistry 18(2): 193–215.

29) Mejia-Cordova SM, Montanez JC, Aguilar CN, de la Luz Reyes-Vega M, de la Garza H, Hours RA, Contreras-Esquivel (2005) Extraction of pectinesterase from Jalapeno chili pepper (*Capsicum annuum*) and its thermal stability. Food Sci. Biotechnol. 14: 185-189.

30) Moeller SM, Parekh N, Tinker L, Ritenbaugh C, Blodi B, Wallace RB, Mares JA, CAREDS Research Study Group (2006) Associations between intermediate age-related macular degeneration and lutein and zeaxanthin in the Carotenoids in Age-related Eye Disease Study (CAREDS): ancillary study of the Women's Health Initiative. Arch Ophthalmol. 124(8): 1151-1162.

31) Narta UK, Kanwar SS, Azmi W (2007) Pharmacological and clinical evaluation of lasparaginase in the treatment of leukemia. Crit Rev Oncol Hematol 61 (3): 208-221.

32) N Deepa, Charanjit Kaur, Binoy George, Balraj Singh, Kapoor HC (2007) Antioxidant constituents in some sweet pepper (*Capsicum annuum L.*) genotypes during maturity. LWT - Food Science and Technology 40 (1): 121–129.

33) Kingsley JD, Shivendu R, Nandita D, Proud S (2013) Nanotechnology for tissue engineering: Need, techniques and applications. Journal of pharmacy research 7: 200-204.

34) Nandita D, Shivendu R, Proud S, Rahul J, Swati M, Arabi MSMA (2012) Antibacterial activity of leaf extract of Mexican marigold (Tagetes erecta) against different gram positive and gram negative bacterial strains. Journal of Pharmacy Research 5(8): 4201-4203.

35) Nelson RE, Fessler LI, Takagi Y, Blumberg B, Keene DR, Olson PF, Parker CG, Fessler JH (1994) Peroxidasin: a novel enzyme-matrix protein of Drosophila development. EMBO J 13(15): 3438-3447.

36) Nishino H, Murakoshi M, Ii T, Takemura M, Kuchide M, Kanazawa M, Mou XY, Wad S, Masuda M, Ohsaka Y, yogosawa S, Satomi Y, Jinno K (2002) Carotenoids in cancer chemoprevention. Cancer Metastasis Rev 21: 257-264.

37) Nisit Kittipongpatana, Piyamart Maneerat, Patchanee Pattanakitkosol, Ornanong S Kittipongpatana (2007) Effect of Some Factors on the Growth of *Capsicum annuum L.* Cell Suspension Culture and Biotransformationof Hydroquinone to Arbutin. CMU J Nat Sci 6(2): 207-218.

38) Onsa GH, bin Saari N, Selamat J, Bakar J (2000) Latent polyphenol oxidases from sago log (Metroxylon sagu): partial purification, activation, and some properties. J Agric Food Chem 48(10): 5041-5045.

39) Oza VP, Trivedi SD, Parmar PP, Subramanian RB (2009) *Withania somnifera* (Ashwagandha): a novel source of L-asparaginase. J Integr Plant Biol 51: 201-206.

40) Panu Artimo, Manohar Jonnalagedda, Konstantin Arnold, Delphine Baratin, Gabor Csardi, Edouard de Castro, Séverine Duvaud, Volker Flegel, Arnaud Fortier, Elisabeth Gasteiger, Aurélien Grosdidier, Céline Hernandez, Vassilios Ioannidis, Dmitry Kuznetsov, Robin Liechti, Sébastien Moretti, Khaled

Mostaguir, Nicole Redaschi, Grégoire Rossier, Ioannis Xenarios, Heinz Stockinger (2012) ExPASy: SIB bioinformatics resource portal. Nucleic Acids Res doi: 10.1093/nar/gks400.

41) Priti Pharkya, Evgeni V Nikolaev, Costas D Maranas (2003) Review of the BRENDA Database. Metabolic Engineering 5(2): 71–73.

42) Prontipa Nokthai, Vannajan Sanghiran Lee, Lalida Shank (2010) Molecular Modeling of Peroxidase and Polyphenol Oxidase: Substrate Specificity and Active Site Comparison. Int J Mol Sci 11 (9): 3266–3276.

43) Popovsky S, Paran I (2000) Molecular genetics of the y locus in pepper: its relation to capsanthin-capsorubin synthase and to fruit color. Theor Appl Genet 101: 86-89.

44) Rancibia RA, Motsenbocker CE (2006) Pectin methylesterase activity in vivo differs from activity in vitro and enhances polygalacturonase-mediated pectin degradation in tabasco pepper. J Plant Physiol 163: 488-496.

45) Rolf Apweiler, Amos Bairoch, Cathy H Wu, Winona C Barker, Brigitte Boeckmann, Serenella Ferro, Elisabeth Gasteiger, Hongzhan Huang, Rodrigo Lopez, Michele Magrane, Maria J Martin, Darren A Natale, Claire O'Donovan, Nicole Redaschi, Lai-Su L Yeh (2004) UniProt: the Universal Protein knowledgebase. Nucleic Acids Res doi: 10.1093/nar/gkh131.

46) Rolff M, Schottenheim J, Decker H, Tuczek F (2011) Copper-O_2 reactivity of tyrosinase models towards external monophenolic substrates: molecular mechanism and comparison with the enzyme. Chem Soc Rev 40: 4077–4098.

47) Shivendu R, Nandita D, Proud S, Madhumita R, Ramalingam C (2012a) Comparative study of antibacterial activity of garlic and cinnamon at different temperature and its application on preservation of fish. Advances in Applied Science Research 3(1): 495-501.

48) Shivendu R, Nandita D, Proud S (2012b) Time dependent and spectrophotometric analysis of blood clotting property of marigold leaf extract and comparative study of its antibacterial activity. J Biotechnol Biomater 2(6): 276

49) S Kim, J Park, IK Hwang (2004) Composition of main carotenoids in Korean red pepper (*Capsicum annuum, L.*) and changes of pigment stability during the drying and storage process. Journal of Food Science 69: 39 - 44.

50) Sapers GM (1993) Browning of foods: Control by sulfites, antioxidants, and other means. Food Technol 68:5–84.

51) Steiner U, Schliemann W, Strack D (1996) Assay for tyrosine hydroxylation activity of tyrosinase from betalain-forming plants and cell cultures. Anal Biochem 238: 72–75.

52) Sugai AY, Tadini CC (2006) Thermal inactivation of mango (*Mangifera indica* variety Palmer) puree peroxidase. Proceedings of 2006 CIGR Section VI International Symposium on Future of Food Engineering. Warsaw, Poland. 2006

53) The BRENDA enzyme database, Available online at http://www.brenda-enzymes.org/ (Retrieved date: 16 Feb 2013).

54) The ExPASy Enzyme database, Available online at http://www.enzyme.expasy.org/ (Retrieved date: 25 Feb 2013)

55) Thom E, Möhlmann T, Quick WP, Camara B, Neuhaus HE (1998) Sweet pepper plastids: enzymic equipment, characterisation of the plastidic oxidative pentose-phosphate pathway, and transport of phosphorylated intermediates across the envelope membrane. Planta 204: 226-233.

56) Willats WG, McCartney L, Mackie W, Knox JP (2001) Pectin: cell biology and prospects for functional analysis. Plant Mol Biol 47(1-2): 9-27.

57) Yao M, Yasutake Y, Morita H, Tanaka I (2005) Structure of the type I L-asparaginase from the hyperthermophilic archaeon *Pyrococcus horikoshii* at 2.16 angstroms resolution. Acta Crystallogr D Biol Crystallogr 61(3): 294-301.

58) Yemenicioglu A, Özkan M, Cemeroglu B (1999) Some characteristics of polyphenol oxidase and peroxidase from taro (*Colocasia antiquorum*). Tr J Agric Forestry 23: 425–430.

Article History:----------

Date of Submission: 22-04-2013

Date of Acceptance: 29-04-2013

Conflict of Interest: NIL

Source of Support: NONE