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ORIGINAL PAPER

The presentation of a new inhibitor to prevent enzymatic browning in mushroom, banana, and apple

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Abstract The aim of this study was to evaluate a computer-based method to find a new inhibitor for polyphenol oxidase (PPO) in banana, apple, and mushroom. First, the sequence of PPOs was separately obtained from Protein Data Bank, and their homology was investigated. Next, the same structure of their active site was found, and it was interacted with various phenolic and benzoic compounds by a molecular dynamic software. Moreover, the inhibition of enzymatic browning was also investigated at different laboratory conditions. This study showed that histidine–leucine–phenylalanine–histidine was in all types of PPOs. Also, molecular dynamic simulation showed that (3*S*)-2-(3,4-dihydroxyphenyl)-3,5,7-chromanetriol (DHPC) is the best compound to interact with PPOs. Based on experimental tests, DHPC had the highest efficacy at 4 °C. The decrease in inhibition of enzymatic browning was seen with the increase in temperature. Also, the decrease in pH led to increase in enzymatic browning. It could be concluded that DHPC is a good inhibitor for enzymatic browning. It seems that this compound can be used in different fruits and vegetables to inhibit enzymatic browning.

Keywords Browning · Enzyme · Inhibition · Polyphenol oxidase

Introduction

Peeling, cutting, and crushing lead to changes of physiological and biochemical properties of food products (Moelants et al. 2014; Niemira and Fan 2014). These changes are important causes of loss quality in fruits and vegetables, i.e., appearance, nutritional value, and marketability (Artes and Allende 2014; Rico et al. 2007; Zhang et al. 2015). Theoretically, the enzymatic browning in fruits and vegetables is due to activation of diphenol oxidase, polyphenol oxidase (PPO), catecholase, or tyrosinase (Mishra et al. 2013). The enzyme is found in many plant tissues, especially those that produce brown filaments (Giri 2014; Zhang et al. 2015). PPO catalyzes two basic reactions, including hydroxylation and oxidation. In hydroxylation, monophenols are converted to diphenol. And in oxidation, diphenols are converted to ortho-quinones. Ortho-quinones can be polymerized and create the high molecular weight compound, melanin (Bajwa et al. 2015; Corzo-Martınez et al. 2012). Melanin can also react with various amino acids and proteins and leads to brown color. Enzymatic browning causes the deterioration of fruits and vegetables, resulting in large economic losses (Dodd 2014). The browning of injured fruit tissues can cause

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Table 1 The full sequences of polyphenol oxidase used in this study

Sample	Sample sequence	Polyphenol oxidase protein sequences	GenBank
Apple (*Malus domestica*)	A1	MTSLSPPVVTTPTVPNPATKPLSPFSQNNSQVSLLTKPKRSFARKVSCKATNNDQ NDQAQSKLDRRNVLLGLGGLYGVAGMGTDPFAFAKPIAPPDVSKCGPADLPQ GAVPTNCCPPPSTKIIDFKLPAPAKLRIRPPAHAVDQAYRDKYYKAMELMKAL PDDDPRSFKQQAAVHCAYCDGAYDQVGFPELELQIHNSWLFFPFHRYYLYFFE KILGKLINDPTFALPFWNWDSPAGMPLPAIYADPKSPLYDKLRSANHQPPTLVD LDYNGTEDNVSKETTINANLKIMYRQMVSNSKNAKLFFGNPYRAGDEPDPGG GSIEGTPHAPVHLWTGDNTQPNFEDMGNFYSAGRDPIFFAHHSNVDRMWSIW KTLGGKRTDLTDSDWLDSGFLFYNENAELVRVKVRDCLETKNLGYVYQDVD IPWLSSKPTPRRAKVALSKVAKKLGVAHAAVASSSKVVAGTEFPISLGSKISTV VKRPKQKKRSKKAKEDEEEILVIEGIEFDRDVAVKFDVYVNDVDDLPSGPDKT EFAGSFVSVPHSHKHKKKMNTILRLGLTDLLEEIEAEDDDSVVVTLVPKFGAV KIGGIKIEFAS	AAA69902.1
	A2	MTSLSPPVVTTPTAPNPDTKPLSPFSQNNSQVSLLTKPKRSLGREVSCNATNND QFDQAQSKLDRRNVLLGLGGLYGVAGMVTDPRGFGKSIAPPDVSKCGPGD LPQGAVPTNCCPPPSTKIIDFKLPAPANLRIRPPAHAVDQAYRDKYYKAMEL MKALPDDDSRSFKQQGAVHCAYCDGAYDQVGFPELELQLHNSWLFFPFHR YYLYFCEKILGNLINDPTFALPFWNWDSPAGMPLPAIYADPKSPLYDKLRSA KHQPPTLVDLDYNGTEDNVSKETTINANLKIMYRQMVSNSKNAKLFFGNPY RAGDEPDPGGGSIEGTPHAPVHLWTGDNTQPNFEDMGNFYSAGRDPIFFAH HSNVDRMWSIWKTLGGKRADLTDSDWLDSGFLFYNENAELVRVKVRDCL ETKHLGYVYQDVDIPWLSSKPTPRRAEVALSPIAKKLGVAHPAVASSSKVV AGTEFPINLGSKISTVVKRPKQKKRSKKAKEDEEEILVIEGIEFDRDVAVKFD VYVNDVDDLPSGPDKTEFAGSFVSVPHSHKHKKKMNTTLRLGLTDLLEEIE AEDDDSVVVTLVPKFGAVKIGGIKIEFAS	BAA21676.1
	A3	MASMSAPLVTSATSIIPTTFLSPFSQKYHRISSFGNPRHSNLQAVSCKATNNSSD QNKNPSTSSNDHDHENPSPVNLDRRNVLIGLGSLYGGVAGLGSDPFAVAKP VSPPDLAKCGAADFPSGAVPTNCCPPTSQKIVDFKFPSPTKLRVRPAAHTVD KAYIEKYSKAIELMKALPDDDPRSFTQQADLHCAYCDGAYDQVGFPNLELQ IHQSWLFFPFHRYYLYFHERILAKLIDDPTFALPFWNWDAPAGMQLPALFAN PDSPLYDELRADSHQPPTLIDLDFNGTDETMSKDAQIDANLKIMYRQMVSNS KKPLLFFGSPYRAGTEPDPGAGSIETTPHGPVHTWTGDNTQPNFEDMGNFYS AARDPIFFSHHSNIDRMWNIWKSIGTKNKDINDTDWLDTGFLFYDKNAELVR VTVRDTLDNKKLGYTYEDVEIPWLKSRPTPRRTKLARKAKAAGVAKAAGV AKAAETTSSGKVVAGKDFPINLETKISTVVSRPKPKKRSKKEKEDEEEILVIQ GIELDKDVAVKFDVYVNDVDDEDAAPSGPDKSEFAGSFVSVPHKQKEKSK SCLRLGLTDLLEDLGAEDDESVVVTLVPRYGAQAVKIGSIKIEFLA	AGU01537.1

Table 1 continued

Sample	Sample sequence	Polyphenol oxidase protein sequences	GenBank
	A4	MTSLSPPVVTTPTVPNPDTKPLSPFSQNNSQVSLLTKPKRSFGRKVSCKDTNNDEID QAQSKLERRNVLLGLGGLYGVGGMDTDPRGWGKAIAPPDVSKCGPADLPQG GVPTICCPPRSTKIIDFKLPAPAKLRIRPPAHAGDQAYRDKHYKAMELMKALP DDDPRSFKQQGAVHCAYCDGAYDQVGFPELELQIHNSWLFFPLHRYYLYFFE KILGKLINDPTFAGPFWNWDSPAGMPLPAIYADPKSPLYDKLRSAQHQPPTLV DLDYNGTEDNVSKETTINANLKIMYRQMVSNSKNAKLFFGNPYRAGDEPDPG GGSIEGTPHAPVHLWTGDNTQPNFEDMGNFYSAGRDPIFFAHHSNVDRMWSI WKTLGGKRADLTDSDWLDSGFLFYNENAELVRVKVRDCLETKNLGYVYQD VDIPWLSSKPTPRRAKVALSKIAKKLGVAHAAVASSSKVVAGTEFPINLGSKIS TVVKRPKQKKRSKKAKEDEEEILVIEGIEFDRDVAVKFDVYVNDVDDLPSGP DKTEFAGSFVSVPHSHKHKKKMNTILRLGLTDLLEEIEAEDDDSVVVTLVPKF GAVKIGGIKIEFAS	BAA21677.1
Banana (*Musa acuminata*AAA Group)	B1	MVSLPKATLPLSSLSPPSNSNSNSNSFACAFHFSYPDRRRHAHPKISCKASDEHE MTANAKLDRRDVLVGLGGLCGAAAGLGIDSKALGNPIQAPDLTKCGPADLP TGATPTNCCPPYFPDKKIIDFKRPPNSSPLRVRPAAHLVDSDYLDKYKKAVEL MRALPADDPRNFMQQANVHCAYCDGAYDQIGFPNLELQVHNSWLFFPWHR FYLYFHERILGKLIGDDTFALPFWNWDAPGGMKLPSIYADPSSSLYDKFRDA KHQPPVLVDLDYNGTDPSFTDAEQIDQNLKIMYRQVISNGKTPLLFLGSAYR AGDNPNPGAGSLENIPHGPVHGWTGDRSQPNLEDMGNFYSAGRDPIFFAHH SNVDRMWYLWKKLGGKHQDFNDKDWLNTTFLFYDENADLVRVTLKDCL QPEWLRYDYQDVEIPWLKTRPTPKALKAQKTAAKTLKATAETPFPVTLQSA VSTTVRRPKVSRSGKEKEEEEEVLIVEGIEFDRDYFIKFDVFVNATEGEGITP GASEFAGSFVNVPHKHKHSKKEKKLMTRLCLGITDLLEDIGAEDDDSVLVT IVPKAGKGKVSVAGLRIDFPN	AHH92831.1
Banana Predicted: polyphenol oxidase I, chloroplastic-like (*Musa acuminata* subsp. Malaccensis)	B2	MEGKRWLSLLLLVLVLVGISMDLPREAPAASSNILKSSSARIPVNPQGGEQRDG SKSKGIPLKANLSVCHASFSDARPVYCCPAWKDADQTLLDFEFPDPSSPVRIR RPAHLVDEEFVAKYERAVAIMKQIPPDHPHNFWRQANMHCLYCTGAYDQM NSSALFKIHRSWLFFPWHRAFIYFHERILGKFMGDDTFALPYWSWDTPEGMW FPDIYRKGALNETERDAIHLREAAVDDFDYVDHDLASDVQIADNLAFMYHQ MISGAKKTELFMGCKLRSGVEGWCDGPGTIEAAPHNTLHSWVGNRYNPERE NMGAFYSAARDEVFFAHHSNIDRMWTVWKKLHGDKPEFVDQEWLESEFTF YDENVRLRRIKVRDVLNIDKLRYRYEDIDMPWLAARPKPSVHPKIARDILKK RNGEGVLRMPGETDRSQLSEYGSWTLDKTITVRVDRPRINRTGQEKEEEEEI LLVYGIDTKRSRFVKFDVFINVVDETVLSPKSREFAGTFVNLHHVSRTKSHD DGGMDSKMKSHLKLGISELLEDLEADEDDSIWVTLVPRGGTGVNTTVDGV RIDYMK	NCBI reference sequence: XP_009380367.1

Table 1 continued

Sample	Sample sequence	Polyphenol oxidase protein sequences	GenBank
	B3	MSLLLNSSLTGASSACLLRREKCRRRGRGHVHGVTCHQGGNDDRREAARQQR SRLLLDRRDMLLGGLGGLYGVTAGPKVLAEPIMPPDLSKCHDANAPALDN HCCPPYSGSETILEYDFPATPLRVRRPAHLVKDDQEYMDKYKEAVRRMKNL PAEHPWNYYQQANIHCQYCNDAYYQQNTDDVPVQVHFSWIFLPWHRYYL HFYERILGKLIDDDTFTIPFWNWDTKDGMTFPAIFQDAASPLYDPKRDQRH VKDGAILDLKYAYTENTASDSEIIRENLCFIQKTFKHSLSLAELFMGDPVRA GEKEIQEANGQLEVIHNAAHMWVGEPDGYKENMGDFSTAARDSVFFCHH SNVDRMWDIYRNLRGNSVEFNDKDWLHSTFLFHDENEQLVKVKIQDCLNP TKLRYTFEQVPLPWLGNINCQKTAETKSKSTAELSLKRVGEFGTTPKALDA SNPLRVIVARPKKNRKKKEKQEKVEVLQIKDIKVTTNETARFDVYVAVPYG DLAGPDYGEFVGSFVRLAHRKKGSDGTEEQGPKKKGKLKLGITALLEDIDA EDADKLVVTLVLRTGSVTVGGVSIKLLQTDTPAVI	NCBI reference sequence: XP_009388329.1
	B4	MALQLNSSFTGASSACLLHRERSRRLNVPVVTCRQGNNDDRSDAARQQKSPS LLDRRDMLLGLGGLYGLTAGPKVLAKPIMPPDLSKCHDAKAPALDNHCCP PYNPSETISEYGFPATPLRVRRPAHLVKDDQEYLDKYKEAVRRMKNLPADH PWNYYQQANVHCQYCNYAYYQQNTDDVPVQVHFSWIFLPWHRYYLHFY ERILGKLIDDDTFTIPFWNWDTKDGMTFPAIFHEESSPLSDTKRDQRHVKDG KIVDLKYAYTENPASNSEIIRENLCFIQKTFKHSLSLAELFMGDPVRAGEKEI QEANGQLEVIHNAVHMWVGEPCGYKENMGDFSTAARDSVFFSHHSNVDRL WEIYRNLRGNRIEFEDNDWLDSTFLFYDENEKLVKVKMGDCLNPTKLRY TFEQVPLPWLGKINCQKTTETKSKSTTEMSLTRVGEFGTTPKALDASNPLR VIVARPKKNRKKKEKQEKVEVLQINDIKVTTNETARFDVYVTVPYGDL	NCBI reference sequence: XP_009388330.1
Mushroom tyrosinase (*Agaricus bisporus*)	M1	MSLIATVGPTGGVKNRLNIVDFVKNEKFFTLYVRSLELLQAKEQHDYSSFFQL AGIHGLPFTEWAKERPSMNLYKAGYCTHGQVLFPTWHRTYLSVLEQILQG AAIEVAKKFTSNQTDWVQAAQDLRQPYWDWGFELMPPDEVIKNEEVNIT NYDGKKISVKNPILRYHFHPIDPSFKPYGDFATWRTTVRNPDRNRREDIPG LIKKMRLEEGQIREKTYNMLKFNDAWERFSNHGISDDQHANSLESVHDD IHVMVGYGKIEGHMDHPFFAAFDPIFWLHHTNVDRLLSLWKAINPDVWV TSGRNRDGTMGIAPNAQINSETPLEPFYQSGDKVWTSASLADTARLGYSY PDFDKLVGGTKELIRDAIDDLIDERYGSKPSSGARNTAFDLLADFKGITKE HKEDLKMYDWTIHVAFKKFELKESFSLLFYFASDGGDYDQENCFVGSINA FRGTAPETCANCQDNENLIQEGFIHLNHYLARDLESFEPQDVHKFLKEKGL SYKLYSRGDKPLTSLSVKIEGRPLHLPPGEHRPKYDHTQARVVFDDVAVH VIN	CAA11562.1

Table 1 continued

Sample	Sample sequence	Polyphenol oxidase protein sequences	GenBank
Polyphenol oxidase (*Agaricus bisporus* var. bisporus H97)	M2	MSHLLVSPLGGGVQPRLEINNFVKNDRQFSLYVQALDRMYATPQNET ASYFQVAGVHGYPLIPFNDAVGPTEFSPFDQWTGYCTHGSTLFPTW HRPYVLILEQILSGHAQQIADTYTVNKSEWKKAATEFRHPYWDWA SNSVPPPEVISLPKVTITTPNGQKTSVANPLMRYTFNPVNDGGFYGP YNQWDTTLRQPDSTGVNAKDNVNRLTSVLKNAQASLTRATYDM FNRVTTWPHFSSHTPASGGSTSNSIEAIHDNIHVLVGGNGHMSDPS VAAFDPIFFLHHANVDRLIALWSAIRYDVWTSPGDAQFGTYTLRY KQSVDESTDLAPWWKTQNEYWKSNELRSTESLGYTYPEFVGLDM YNKDAVNKTISRKVAQLYGPQRGGQRSLVEDLSNSHARRSQRLAK RSRLGQLLKGLFSDWSAQIKFNRHEVGQSFSVCLFLGNVPEDPREWL VSPNLVGARHAFVRSVKTDHVAEEIGFIPINQWIAEHTGLPSFAVDLV KPLLAQGLQWRVLLADGTPAELDSLEVTILEVPSELTDDEPNPRSRPP RYHKDITHGKRGGCREA	NCBI reference sequence: XP_006463026.1
Tyrosinase (*Agaricus bisporus* var. bisporus H97)	M3	MSLIATVGPTGGVKNRLNIVDFVKNEKFFTLYVRSLELLQAKEQHDYS SFFQLAGIHGLPFTEWAKERPSMNLYKAGYCTHGQVLFPTWHRTYL SVFEQILQGAAIEVANKFTSNQTDWIQAAQDLRQPYWDWGFELMPP DEVIKNEEVNITNYDGKKISVKNPILRYHFHPIDPSFKPYGDFATWRT TVRNPDRNRREDIPGLIKKMRLEEGQIREKTYNMLKFNDAWERFSN HGISDDQHANSLESVHDDIHVMVGYGKIEGHMDHPFFAAFDPIFWL HHTNVDRLLSLWKAINPDVWVTSGRNRDGTMGIAPNAQINDETPLE PFYQSEDKVWTSASLADTARLGYSYPDFDKLVGGTKELIRDAIDDL IDERYGSKPSSGARNTAFDLLADFKGITKEHKEDLKMYDWTIHVAF KKFELKESFSLLFYFASDGGDYDQENCFVGSINAFRGTTPETCANCQ DNENLIQEGFIHLNHYLARDLESFEPQDVHKFLKEKGLSYKLYSRED KSLTSLSVKIEGRPLHLPPGEHRPKYDHTQDRVVFDDVAVHVIN	NCBI reference sequence: XP_006459626.1
Tyrosinase (*Agaricus bisporus*)	M4	MSDKKSLMPLVGIPGEIKNRLNILDFVKNDKFFTLYVRALQVLQAR DQSDYSSFFQLGGIHGLPYTEWAKAQPQLHLYKANYCTHGTVLF PTWHRAYESTWEQTLCEAAGTVAQRFTTSDQAEWIQAAKDLR QPFWDWGYWPNDPDFIGLPDQVIRDKQVEITDYNGTKIEVENPIL HYKFHPIEPTFEGDFAQWQTTMRYPDVQKQENIEGMIAGIKAAAP GFREWTFNMLTKNYTWELFSNHGAVVGAHANSLEMVHNTVHFLI GRDPTLDPLVPGHMGSVPHAAFDPIFWMHHCNVDRLLALWQTMN YDVYVSEGMNREATMGLIPGQVLTEDSPLEPFYTKNQDPWQSDD LEDWETLGFSYPDFDPVKGKSKEEKSVYINDWVHKHYGFVTTQT ENPALRLLSSFQRAKSDHETQYALYDWVIHATFRYYELNNSFSIIF YFDEGEGCTLESIIGTVDAFRGTTSENCANCARSQDLIAEGFVHLN YYIGCDIGQHADHEDDAVPLYEPTRVKEYLKKRKIGCKVVSAEGE LTSLVVEIKGAPYYLPVGEARPKLDHEKPIVILDDIIHRVN	ADE67053.1

A1–A4 = apple, B1–B4 = banana, M1–M4 = mushroom

Table 2 The homology between different polyphenol oxidases used in this study

Enzyme sequence	Identity %
A1–A2	95.3 % identity in 593 residues overlap; score: 3008.0; gap frequency: 0.0 %
A1–A3	69.7 % identity in 617 residues overlap; score: 2144.0; gap frequency: 4.7 %
A1–A4	95.8 % identity in 593 residues overlap; score: 3016.0; gap frequency: 0.0 %
A2–A3	67.1 % identity in 617 residues overlap; score: 2064.0; gap frequency: 4.7 %
A2–A4	94.8 % identity in 593 residues overlap; score: 2994.0; gap frequency: 0.0 %
A3–A4	66.9 % identity in 617 residues overlap; score: 2049.0; gap frequency: 4.7 %
B1–B2	44.3 % identity in 524 residues overlap; score: 1067.0; gap frequency: 5.3 %
B1–B3	43.8 % identity in 536 residues overlap; score: 1028.0; gap frequency: 4.1 %
B1–B4	42.4 % identity (67.8 % similar) in 509 aa overlap (19-512:6-503)
B2–B3	39.6 % identity in 502 residues overlap; score: 844.0; gap frequency: 4.6 %
B2–B4	36.9 % identity (66.7 % similar) in 496 aa overlap (21-488:26-499)
B3–B4	87.6 % identity (94.7 % similar) in 508 aa overlap (1-508:1-504)
M1–M2	40.2 % identity in 376 residues overlap; score: 719.0; gap frequency: 3.2 %
M1–M3	98.4 % identity in 556 residues overlap; score: 2957.0; gap frequency: 0.0 %
M1–M4	48.8 % identity in 578 residues overlap; score: 1342.0; gap frequency: 5.2 %
M2–M3	39.9 % identity in 376 residues overlap; score: 722.0; gap frequency: 3.2 %
M2–M4	37.0 % identity in 387 residues overlap; score: 563.0; gap frequency: 6.2 %
M3–M4	49.3 % identity in 578 residues overlap; score: 1357.0; gap frequency: 5.2 %
A1–B1	62.2 % identity in 556 residues overlap; score: 1747.0; gap frequency: 4.1 %
A1–B2	42.8 % identity in 484 residues overlap; score: 947.0; gap frequency: 3.7 %
A1–B3	41.5 % identity in 540 residues overlap; score: 945.0; gap frequency: 4.8 %
A1–B4	42.4 % identity in 467 residues overlap; score: 859.0; gap frequency: 4.3 %
A2–B2	41.7 % identity in 484 residues overlap; score: 921.0; gap frequency: 3.7 %
A2–B3	40.3 % identity in 539 residues overlap; score: 910.0; gap frequency: 4.5 %
A2–B4	40.9 % identity in 464 residues overlap; score: 816.0; gap frequency: 4.3 %
A3–B1	59.5 % identity in 555 residues overlap; score: 1697.0; gap frequency: 5.2 %
A3–B2	42.8 % identity in 500 residues overlap; score: 930.0; gap frequency: 5.6 %
A3–B3	41.4 % identity in 551 residues overlap; score: 875.0; gap frequency: 7.1 %
A3–B4	40.7 % identity in 477 residues overlap; score: 807.0; gap frequency: 5.5 %
A4–B1	60.3 % identity in 556 residues overlap; score: 1680.0; gap frequency: 4.1 %
A4–B2	41.9 % identity in 484 residues overlap; score: 921.0; gap frequency: 3.7 %
A4–B3	40.1 % identity in 539 residues overlap; score: 900.0; gap frequency: 4.5 %
A4–B4	40.6 % identity in 463 residues overlap; score: 804.0; gap frequency: 3.9 %
A1–M1	50.0 % identity in 30 residues overlap; score: 88.0; gap frequency: 0.0 %
A1–M2	54.2 % identity in 24 residues overlap; score: 82.0; gap frequency: 0.0 %
A1–M3	50.0 % identity in 30 residues overlap; score: 88.0; gap frequency: 0.0 %
A1–M4	50.0 % identity in 30 residues overlap; score: 89.0; gap frequency: 0.0 %
A2–M1	50.0 % identity in 30 residues overlap; score: 88.0; gap frequency: 0.0 %
A2–M2	54.2 % identity in 24 residues overlap; score: 82.0; gap frequency: 0.0 %
A2–M3	50.0 % identity in 30 residues overlap; score: 88.0; gap frequency: 0.0 %
A2–M4	50.0 % identity in 30 residues overlap; score: 89.0; gap frequency: 0.0 %
A3–M1	38.1 % identity in 63 residues overlap; score: 91.0; gap frequency: 4.8 %
A3–M2	58.3 % identity in 24 residues overlap; score: 84.0; gap frequency: 0.0 %
A3–M3	38.1 % identity in 63 residues overlap; score: 91.0; gap frequency: 4.8 %
A3–M4	46.7 % identity in 30 residues overlap; score: 84.0; gap frequency: 0.0 %
A4–M1	50.0 % identity in 30 residues overlap; score: 88.0; gap frequency: 0.0 %
A4–M2	54.2 % identity in 24 residues overlap; score: 82.0; gap frequency: 0.0 %
A4–M3	50.0 % identity in 30 residues overlap; score: 88.0; gap frequency: 0.0 %
A4–M4	50.0 % identity in 30 residues overlap; score: 89.0; gap frequency: 0.0 %
B1–M1	50.0 % identity in 30 residues overlap; score: 83.0; gap frequency: 0.0 %

Table 2 continued

Enzyme sequence	Identity %
B1–M2	58.3 % identity in 24 residues overlap; score: 80.0; gap frequency: 0.0 %
B1–M3	50.0 % identity in 30 residues overlap; score: 83.0; gap frequency: 0.0 %
B1–M4	50.0 % identity in 30 residues overlap; score: 82.0; gap frequency: 0.0 %
B2–M1	35.3 % identity in 51 residues overlap; score: 86.0; gap frequency: 3.9 %
B2–M2	44.4 % identity in 27 residues overlap; score: 76.0; gap frequency: 0.0 %
B2–M3	35.3 % identity in 51 residues overlap; score: 86.0; gap frequency: 3.9 %
B2–M4	38.2 % identity in 34 residues overlap; score: 80.0; gap frequency: 0.0 %
B3–M1	35.0 % identity in 60 residues overlap; score: 71.0; gap frequency: 3.3 %
B3–M2	38.2 % identity in 55 residues overlap; score: 82.0; gap frequency: 7.3 %
B3–M3	35.0 % identity in 60 residues overlap; score: 71.0; gap frequency: 3.3 %
B3–M4	32.7 % identity in 55 residues overlap; score: 75.0; gap frequency: 7.3 %
B4–M1	36.7 % identity in 60 residues overlap; score: 75.0; gap frequency: 3.3 %
B4–M2	40.0 % identity in 55 residues overlap; score: 88.0; gap frequency: 7.3 %
B4–M3	36.7 % identity in 60 residues overlap; score: 75.0; gap frequency: 3.3 %
B4–M4	36.4 % identity in 55 residues overlap; score: 80.0; gap frequency: 7.3 %

undesirable quality changes during handling, processing, and storage (Ali et al. 2015; Quevedo et al. 2014a, b). To prevent PPO activity in fruits and vegetables, many efforts have been previously done. One of them is the use of reducers which revive o-quinones precursors and convert them into non-colored compounds (Wu 2014; Zhou et al. 2015). The inhibitors of PPO are classified into two categories, including competitive and non-competitive. Importantly, competitive inhibitors interact with the copper site, and non-competitive inhibitors interact with the phenolic site (Ackaah-Gyasi et al. 2015; Boeckx et al. 2015). For example, bisulfite is a competitive inhibitor, and L-cysteine is a non-competitive inhibitor (Ali et al. 2014; Saeidian 2014).

To find an inhibitor for a specific enzyme, a computer-based method (CBM) has been introduced (Jiménez-Atiénzar et al. 2004; Ma et al. 2014). Although researchers have been studied on different inhibitors (Altunkaya and Gökmen 2008; Kuijpers and Vincken 2013), most of them are toxic and can change texture and taste of fruits. Here, a CBM was used to find a new inhibitor for PPO in two types of fruits (banana and apple) and one type of mushroom. Moreover, the efficacy of inhibitor was checked by some experiments. This article carried out in the Pajoohesh Medical Lab, Yazd, in 2015.

Materials and methods

Materials

(3*S*)-2-(3,4-dihydroxyphenyl)-3,5,7-chromanetriol (DHPC) was purchased from Merck, Germany. Apple, banana, and mushroom were provided from different shops of Yazd, Iran.

Table 3 The average homology between different polyphenol oxidases

Sample	Mean of identity (%)
Apple	81.6
Banana	49.1
mushroom	52.6
Apple–Banana	65.35
Apple–mushroom	73.47
Banana–mushroom	50.85

Simulation study

PPO sequences of apple, banana, and mushroom were separately obtained from Protein Data Bank (PDB), http://www.rcsb.org/pdb (Table 1). In order to check homology of these sequences, an online software was used, http://www.isb-sib.ch. Then, the place of all histidines (H) and all phenyl alanines (F) was highlighted. Then, the quantity of HH, FF, HF, FH, H1–20H, F1–20F, H1–20F, and F1–20H was quantified. In the next level, the best sequence was selected, based on its frequency. Then, this sequence was interacted with various phenolic and benzoic compounds by a molecular dynamic (MD) software, Ascalaph Designer. Finally, the average of intermolecular energy was calculated for each compound.

Table 4 The quantity of different sequences, containing histidines (H) and phenyl alanines (F)

Sequences	Quantity	Sequences	Quantity	Sequences	Quantity	Sequences	Quantity
FF	12	FH	10	HF	7	HH	12
F1F	11	F1H	12	H1F	5	H1H	9
F2F	8	F2H	12	H2F	6	H2H	3
F3F	12	F3H	12	H3F	2	H3H	12
F4F	5	F4H	9	H4F	12	H4H	3
F5F	9	F5H	4	H5F	12	H5H	4
F6F	8	F6H	11	H6F	4	H6H	6
F7F	8	F7H	7	H7F	2	H7H	4
F8F	10	F8H	4	H8F	4	H8H	12
F9F	10	F9H	3	H9F	1	H9H	6
F10F	4	F10H	0	H10F	4	H10H	5
F11F	4	F11H	3	H11F	1	H11H	4
F12F	8	F12H	1	H12F	5	H12H	2
F13F	8	F13H	1	H13F	1	H13H	2
F14F	4	F14H	2	H14F	3	H14H	4
F15F	4	F15H	3	H15F	2	H15H	2
F16F	3	F16H	2	H16F	3	H16H	3
F17F	1	F17H	1	H17F	1	H17H	0
F18F	7	F18H	0	H18F	1	H18H	0
F19F	6	F19H	2	H19F	1	H19H	1
F20F	3	F20H	0	H20F	0	H20H	5

Table 5 The selected sequences, containing histidines (H) and phenyl alanines (F)

	H3H	H8H	H4F	H5F	F1H	F2H	F3H	F3F
A1	HAPVH	HNSWLFFPFH	HNSWLF	HRYYLYF	FAH	FPFH	FFPFH	FALPF
	HSHKH					FFAH	FFAHH	FAGSF
						FAHH		
A2	HAPVH	HNSWLFFPFH	HNSWLF	HRYYLYF	FAH	FPFH	FFPFH	FALPF
	HSHKH					FFAH	FFAHH	FAGSF
						FAHH		
A3	HGPVH	HQSWLFFPFH	HRISSF	HRYYLYF	FSH	FSHH	FFPFH	FLSPF
			HQSWLF			FPFH	FFSHH	FALPF
						FFSH		FAGSF
A4	HSHKH	HNSWLFFPLH	HNSWLF	HRYYLYF	FAH	FAHH	FFAHH	FAGP
	HAPVH					FPLH	FFPLH	FAGSF
						FFAH		
B1	HGPVH	HFSYPDRRRH	HNSWLF	HNSWLFF	FAH	FAHH	FFPWH	FACAF
	HKHKH	HNSWLFFPWH				FPWH	FFAHH	FAGSF
						FFAH		FYLYF
								FALPF
B2	HNTLH	HRSWLFFPWH	HRSWLF	HRSWLFF	FAH	FKIH	FFPWH	FAGTF
		HNFWRQANMH		HGDKPEF		FPWH	FIYFH	
						FMYH	FFAHH	
						FAHH	FVNLH	
						FFAH		

Table 5 continued

	H3H	H8H	H4F	H5F	F1H	F2H	F3H	F3F
B3	HNAAH	HFSWIFLPWH	HFSWIF	HRYYLHF	FKH	FCHH	FLPWH	FTIPF
			HSTFLF		FCH	FLFH	FFCHH	FPAIF
						FFCH		FSWIF
								FVGSF
B4	HNAVH	HFSWIFLPWH	HFSWIF	HRYYLHF	FKH	FSHH	FLPWH	FTIPF
					FSH	FFSH	FFSHH	FPAIF
								FSWIF
M1	HDDIH	HGQVLFPTWH	HGQVLF	HPIDPSF	FIH	FWLH	FPTWH	FSLLF
			HDYSSF	HDYSSFF		FSNH	FWLHH	FFAAF
								FDPIF
M2	HFSSH	HGSTLFPTWH	HGSTLF	HEVGQSF	FRH	FSSH	FPTWH	FDPIF
	HDNIH			HTGLPSF	FLH	FLHH	FFLHH	
						FFLH		
M3	HDDIH	HGQVLFPTWH	HGQVLF	HDYSSFF	FIH	FWLH	FPTWH	FFAAF
			HDYSSF	HVAFKKF		FSNH	FWLHH	FDPIF
				HPIDPSF				FSLLF
M4	HYKFH	HLYKANYCTH	HGTVLF	HPIEPTF	FVH	FSNH	FWMHH	FSIIF
	HNTVH	HGTVLFPTWH	HNTVHF			FWMH	FPTWH	FEGDF
			HKHYGF					FDPIF

Table 6 The average of intermolecular energy and number of hydrogen bonding between HLFH and some phenolic and benzoic compounds

	Intermolecular energy	No. of hydrogen bonding
(+)-Epicatechin	−0.05	3
(3*S*)-2-(3,4-dihydroxyphenyl)-3,5,7-chromanetriol	−7.37	3
[3,4-Dihydroxy(2H3)phenyl](2H2)acetic acid	−0.05	2
[3,4-Dihydroxy(5-2H)phenyl]acetic acid	−0.03	3
{4-[(*Z*)-hydroxy(oxonio)methyl]phenyl}oxonium	−0.00	3
2,3-Dihydroxybenzoate	−0.04	3
2,3-Dihydroxybenzoic acid	−1.80	1
2-Hydroxy(1–14C)benzoic acid	−1.76	2
2-Hydroxy(2H4)benzoic acid	−0.40	3
2-Hydroxy(carboxy-11C)benzoic acid	−0.45	2
3,4,5-Trihydroxy(1,3,5-13C3)benzoic acid	−0.11	3
3,4,5-Trihydroxy(2H2)benzoic acid	−0.77	2
3,4,5-Trihydroxybenzoic acid	−0.01	3
3,4-Dihydroxy(2–2H)benzoic acid	−0.09	2
3,4-Dihydroxy(5–2H)benzoic acid	−0.32	2
3,4-Dihydroxy(6–2H)benzoic acid	−0.08	3
3,4-Dihydroxybenzeneacetate	−2.48	3
3,4-Dihydroxybenzoate	−0.97	2
3,4-Dihydroxybenzoic acid	−0.75	2
3,4-Dihydroxyphenylacetic acid	−0.02	1
3-Hydroxybenzoate	−0.94	3
3-Hydroxybenzoic acid	−0.02	2
3-Hydroxybenzoic acid	−1.57	2
4-(2H3)methyl(2H3)benzene-1.2-(2H2)diol	−0.05	2

Table 6 continued

	Intermolecular energy	No. of hydrogen bonding
4-(2H3)methyl-1.2-benzenediol	−0.01	3
4-Hydroxy(2H4)benzoic acid	−1.71	3
4-Hydroxy(carboxy-11C)benzoic acid	−0.10	2
4-Hydroxy(carboxy-13C)benzoic acid	−0.53	3
4-Hydroxy(carboxy-13C)benzoic acid1	−0.02	3
4-Hydroxy(carboxy-14C)benzoic acid	−0.27	2
4-Hydroxybenzoate	−1.29	2
4-Hydroxybenzoic acid	−0.48	2
4-Methyl-1,2-(2H3)benzenediol	−0.00	2
4-Methyl-1,2-(3H3)benzenediol	−0.26	2
4-Methylcatechol	−0.01	2
D-(+)-Catechin	−0.07	2
DL-CATECHIN	−0.07	3
Gallate	−0.22	1
Gallic acid	−0.28	3
Guaiacol	−2.54	3
o-Hydroxybenzoic acid	−0.00	3
p-Hydroxybenzoic acid	−0.03	2
Protocatechuic acid	−0.14	2
Pyrogallol	−0.01	2
Salicylate	−1.53	2
Salicylic acid	−0.73	2

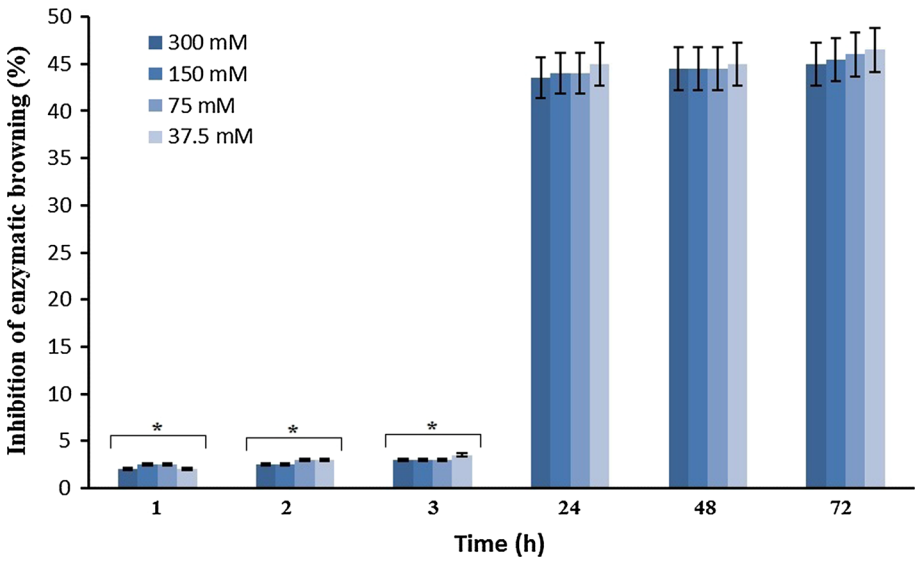

Fig. 1 The inhibition of enzymatic browning at 4 °C. *$P < 0.05$ compared with 24, 48, and 72 h. $n = 3$

Experimental study

First, serial concentrations (37.5, 75, 150, and 300 mM) of DHPC were prepared in distilled water. In the next step, apple, banana, and mushroom were cut and separately held in petri dish. Then, 100 µL of DHPC was added to all slices and incubated for 1, 2, 3, 24, 48, and 72 h. Temperature and pH were separately adjusted as follows:

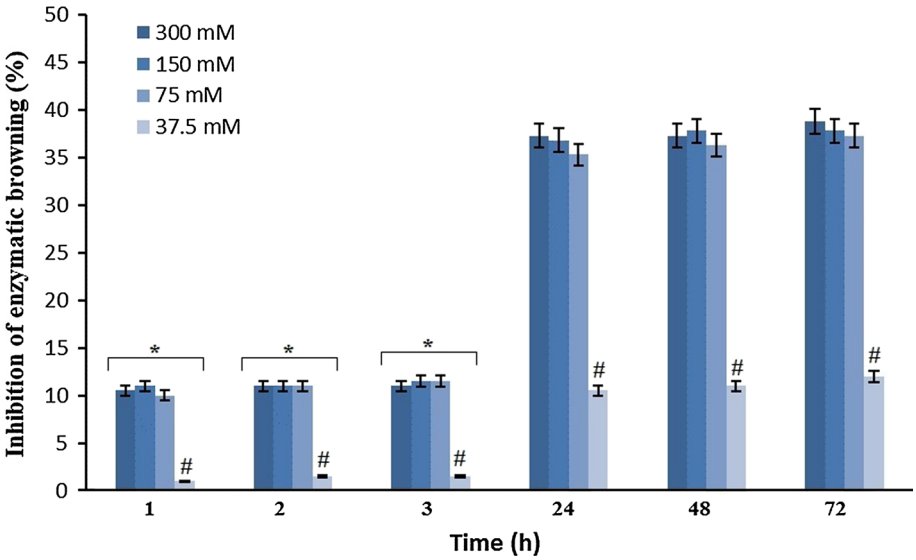

Fig. 2 The inhibition of enzymatic browning at 25 °C. $^{*}P < 0.05$ compared with 24, 48, and 72 h. $^{\#}P < 0.05$ compared with 300, 150, 75 mM, $n = 3$

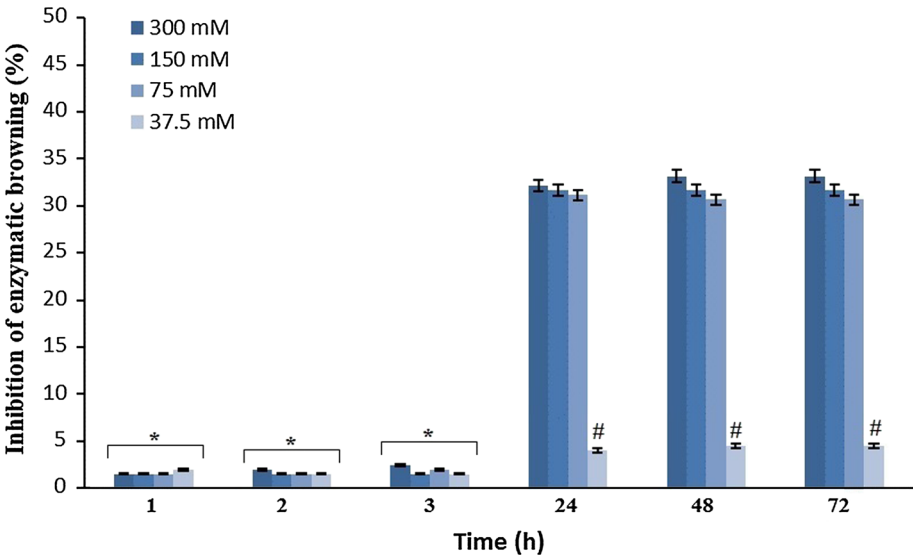

Fig. 3 The inhibition of enzymatic browning at 37 °C. $^{*}P < 0.05$ compared with 24, 48, and 72 h. $^{\#}P < 0.05$ compared with 300, 150, 75 mM, $n = 3$

At 4 °C with pH 7
At 25 °C with pH 7
At 37 °C with pH 7
At 37 °C with pH 5
At 37 °C with pH 9

After incubation, a picture was taken from each petri dish by a digital camera with resolution of 13 mega pixel. Then, all pictures were inserted in Photoshop CS6 software, and the color of slices was recorded. The total color was measured by Formula 1. Finally, the inhibition of enzymatic browning was calculated, according to Formula 2 (Holderbaum et al. 2010).

$$\sqrt{R^2 + G^2 + B^2} = \text{The total color} \quad (1)$$

where R, G, and B are red, green, and blue color, respectively.

$$I = \text{TC}_\text{c} - \text{TC}_\text{t}/\text{TC}_\text{c} \quad (2)$$

where (I) is the inhibition of enzymatic browning, TC_c is the total color in control, and TC_t is the total color in test.

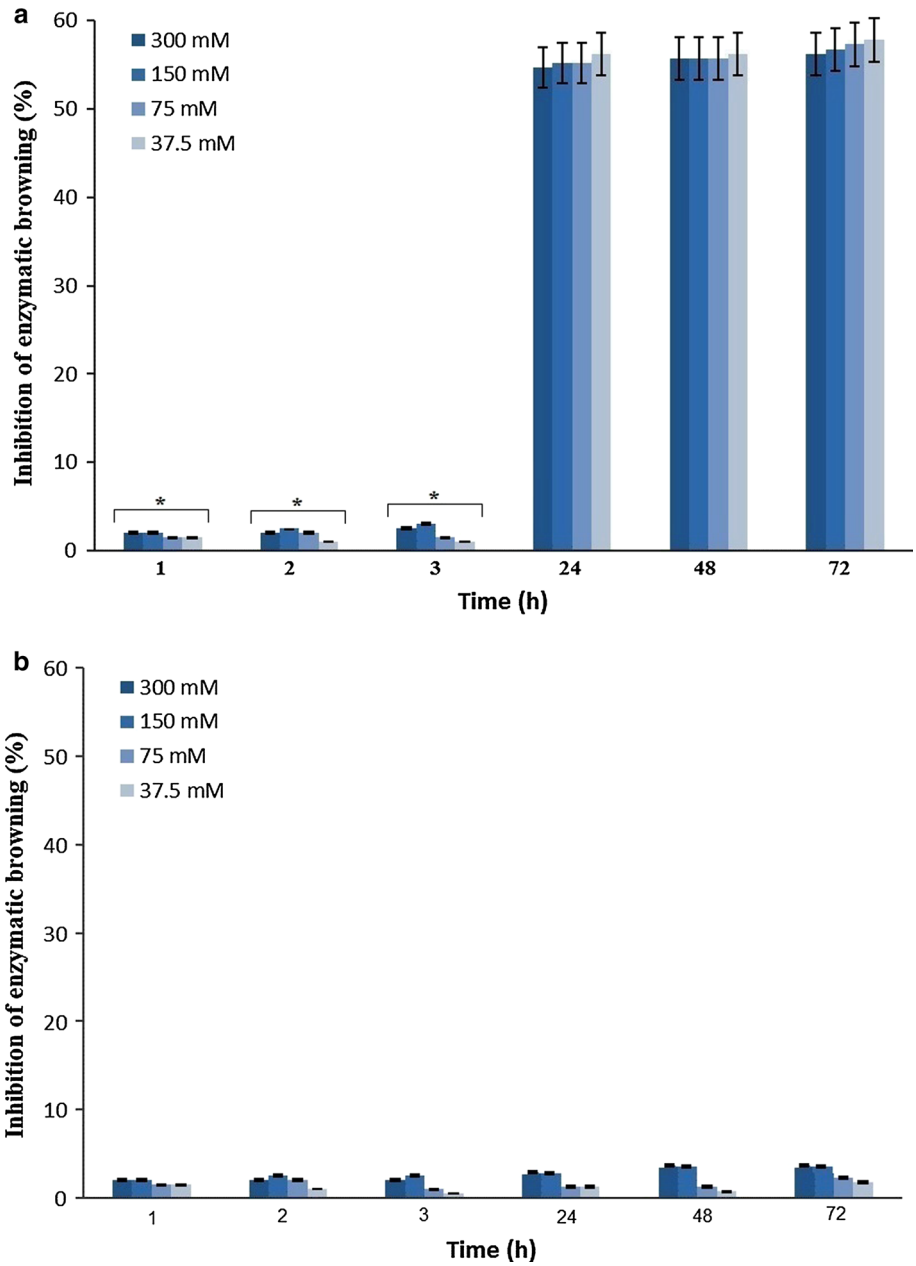

Fig. 4 The inhibition of enzymatic browning at pH 5 (**a**) and 9 (**b**). *$P < 0.05$ compared with 24, 48, and 72 h, $n = 3$

Results and discussion

Tables 2 and 3 show the homology between different PPOs, used in this study. As seen, the highest homology was seen between apples, and the minimum homology was observed between banana. Table 4 shows the quantity of HH, FF, HF, FH, H1–20H, F1–20F, H1–20F, and F1–20H sequences. Table 5 demonstrates the selected sequences, containing H and F. Based on this result, the best sequence was histidine–leucine–phenylalanine–histidine (HLFH). The average of intermolecular energy between HLFH and some phenolic and benzoic compounds is shown in Table 6. As seen, DHPC had less intermolecular energy.

Figures 1, 2, and 3 show the inhibition of enzymatic browning at 4, 25, and 37 °C, respectively. As seen, the inhibitor had the highest efficacy at 4 °C. The decrease in inhibition of enzymatic browning was seen with the increase in temperature. Figure 4a, b shows the inhibition of enzymatic browning at pH 5 and 9, respectively. As seen, the decrease in pH led to increase in inhibition of enzymatic browning.

Here, four different sequences of apple, banana, and mushroom were used. Although the GenBank number of these sequences is different, further sequences must be studied in future. The homology study showed that mushroom had less homology among others. Since mushroom is a kind of herb, its sequence has less similar to apple and banana. Since H and F are in different places of protein sequences, it cannot be certainly declared which they are in the actual place. It was found that H1H–H2H–H3H–H4H–H5H–H8H–FF–F3F was similar. Remarkably, this similarity was only in number and not in sequence. Note, HLFH was the best sequence and it had the most similarity among the sequences. It must be mentioned that further studies are needed to show whether HLFH sequences are in active site or not. MD simulation showed that DHPC was the best candidate for this study. Dirks–Hofmeister et al. (2012) compared the characteristics of PPO to find the structure–function correlation within the plant PPOs. They showed differences in enzyme–substrate interactions. Also, they found that one amino acid side chain, position HB2 + 1, was the best. Nokthai et al. (2010) analyzed the active site of PPO by molecular modeling. They found that epicatechin and catechin had high affinity with the enzyme. Based on their results, trihydroxybenzoic acid had high affinity and specificity. A homology modeling for PPO was done by Mallick et al. They showed 224 hydrogen bonds, 15 helices, and 50 turns (Mukherjee et al. 2011). Koval et al. (2006) modeled the active site of PPO. Saeidian (2013) showed the inhibition of PPO by L-glycine. The agent inhibited PPO activity at 0.4 mM in pH 6.7. Klabunde et al. (1998) showed that the catalytic copper center was accommodated in a central four-helix bundle. Also, metal-binding sites were composed of three H ligands.

Conclusion

It could be concluded that DHPC can interact with PPOs. Moreover, DHPC had more efficacies at low temperature and pH. It seems that this compound can be used for different fruits and vegetables to inhibit enzymatic browning. It must be mentioned that some additional experiments, such as toxicity test, must be done in future studies.

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