

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

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Received: 20 August 2015 / Revised: 27 November 2015 / Accepted: 14 December 2015 / Published online: 7 January 2016  
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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 (Moe-lants 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-Martinez 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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**Electronic supplementary material** The online version of this article (doi:10.1007/s13762-015-0930-y) contains supplementary material, which is available to authorized users.

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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 ( <i>Malus domestica</i> )	A1	MTSLSPVVTPTVPNPATKPLSPFSQNNQVSLLTTPKRSFARKVSKCATNNDQ NDAQSKLDRRNVLGLGLYGVAGMGTDPFPAFAKPIAPPDVSCKGADLPQ GAVPTNCCPPSTKIIDFKLPAPAKLRIRPPAHAVDQAYRDKYYKAMELMKAL PDDDRSFKQAAVHCAYCDGAYDQVGFPELELQHNSWLFPPHRYLYLFFFE KILGLINDPTFALPFWNWDSFAGMPLPAIYADPKSPLYDKLRSANHQPPTLVD LDYNGTEDNVSKETTINANLKIMYRQMVSNKNAKLFNGNYPYRAGDEPDGPG GSIEGTPHAPVHLWTGDNTPNFEDMGNFYSAGRDPIFFAHHSNVDRMWSIW KTLGGKRTDLTDSWLDGFLFYNENAEVAVKFDVYVNDVDDLPSGPKDT VSRPKQKRSKAKEDDEEILVIEGIEFDRDVAVKFDVYVNDVDDLPSGPKDT EFAGSFVSVPHSHKHKMKMNTILRLGLTDLLEEIEAEDDSDVVVTLVPKFGAV KIGGKIEFAS	AAA69902.1
	A2	MTSLSPVVTPTAPNPTKPLSPFSQNNQVSLLTTPKRSILGREVSCNATNND QFDQAQSKLDRRNVLGLGLYGVAGMVTDRPFGKSIAPPDVSKCGPGD LPQGAVTNCPPSTKIIDFKLPAPANLRPPAHAVDQAYRDKYYKAMEL MKALPDDDSRSFKQGAHVHCAYCDGAYDQVGFPELELQHNSWLFPPHFR YLYFCEKILGNLINDPTFALPFWNWDSFAGMPLPAIYADPKSPLYDKLRS KHQPTLVLDLYNGTEDNVSKETTINANLKIMYRQMVSNKNAKLFNGNYPY RAGDEPDGPGGSIETPHAPVHLWTGDNTPNFEDMGNFYSAGRDPIFFAH HSNVDRMWSIWKTLGGKRALDTSWLDGFLFYNENAEVAVKFDVYVNDVDDL ETKHLGYVYQDVDPWLSKPTPRRAEVALSPIAKKLGVAHPVAVASSKVV AGTEFPINLGSKISTVVKRQKRSKAKEDDEEILVIEGIEFDRDVAVKFDVYVNDVDDLPSGPKDT AEDDSDVVVTLVPKFGAVKIGKIEFAS	BAA21676.1
	A3	MASMSAPLVTSATSIPTTFLSPFSQYHRISFNGNPRHSNLQAVSCKATNNSD QNKNPSTSSNDHDEPNPVLNDRNVLGLGLYGGVAGLSDPFAVAKP VSPDLAKCGAADFPFGAVTNCPPSTQKIVDFKFPSTKLRVVRPAHTVD KAYIEKYSKAIELMKALPDDDRSFTQADLHCAVCDGAYDQVGFNLELQ IHQSWLFFPFRYYLYFHERILAKLIDDPFALPFWNWDSFAGMQLPALFAN PDSPLYDELRAVSHQPTLIDLDFNGTDETMKDAQIDANLKIMYRQMVSN KPLFFGSPYRAGTEPDGAGSIEETPHGPVHTWTDNTQNFEDMGNFY AARDPIFFSHSNIDRMWNIWKSIGTKNKINDTDWLDGFLFYDKNAELVR VTVRDLDNKKLGYTYEDVEIPWLSRPTPRRTKLARKAKAAGVAKAAGV AKAAETSSGKVVAGKDFPINLETISTVSRPKKRSKKEKEDEEILVIQ GIELDKDVAVKFDVYVNDVDEDAAPSGPKSEFAGSFVSVPHKQKESK SCLRGLTDLLEDLGAEDDESVVVTLVPRYGAQAVKIGSIIKIEFLA	AGU01537.1

**Table 1** continued

Sample	Sample sequence	Polyphenol oxidase protein sequences	GenBank
	A4	<p>MTSLSPPVVTTPTVPNPDTKPLSPFSQNSQVSLLTTPKRSFGRKVSCKDTNNDEID                      QAQSKLERRNVLLGLGYGVGGMDTDPRGWGKAIPDVSCKGFPADLPQG                      GVPJTCCPPRSTKIIDFKLPAPAKLRIRPPAHAGDQAYRDYKHYKAMELMAKALP                      DDDPRSFKQQGAVHCAVCDGAYDQVGFPELELQIHNSWLFPLHRYLYL YFFE                      KILGLINDPTFAGPFWNWSPAGMPLPAIYADPKSPLYDKLRS AQHPPTLV                      DLDYNGTEDNVSKETTINANLKIMYRQMVSNKNAKLFNGPNYRAGDEPDFG                      GGSIEGTHAPVHLWTGDNTQNFEDMGNFYSAGRDPIFFAHHSNVDRMWSI                      WKTLGGKRADLTDSDWLD SGFLFYNAEALVRVKVRDCLKTNLGYVYQD                      VDPWLSKPTPRRAKVALSKIAKLGVAHAAV ASSKVVAGTEFFINLGSKIS                      TVV'KRPKQKRSKKAKEDEEILVIEGIEFDRDVAVKFDVYVNDVDDLPSGP                      DKTEFAGSFVSPHSHKHKMMNTILRLGLTDLLEEIEAEDDDSVVVVTLV PKF                      GAVKIGGKIEFAS</p>	BAA21677.1
Banana ( <i>Musa acuminata</i> AAA Group)	B1	<p>MVSLPKATLPLSSLPSSNSNSNSFACAFHFSYDPRRRRHAHPKISCKASDEHE                      MTANAKLDRRDVLYLGLGCGAAAGLGDISKALGNPIQAPDLTKCGPADLP                      TGATPTNCCPPYFPDKIIDFKRPPNSSPLVRPAHLVDSYLDKYKKA VEL                      MRALPADDPNFMQANVHCAYCDGAYDQIGFNLQVHNSWLFPPWHR                      FYLYFHERILGKLGDDTFALPFWNWDAPGGMKLPSIYADPSSLYDKPRDA                      KHQPPVLDLDYNGTSPSTDAEQIDQNLKIMYRQVINSNGKTPLLELSAYR                      AGDNPNGAGSLENIPHPGVHGTGDRSQPNLEDMGNFYSAGRDPIFFAHH                      SNVDRMWYLWKKLGGKHQDFNDKDWLNTTFLFYDENADLVRVTLKDCL                      QPEWLRDYDQDVEIPWLKTRPTPKALKAQKTA AKTLKATAETPPVTLQSA                      VSTTTRRPKVSRSGEKEEEEEVLIEGIEFDRDYFIKFDVFNATEGEGITP                      GASFA GSFVNVPHKHKHSKKEKKLMTRLCLGHTDLDLEDIGAEDDDDSVLVT                      IVPKAGKGVSVAGLRIDFPN</p>	AHH92831.1
Banana Predicted: polyphenol oxidase I, chloroplastic-like ( <i>Musa acuminata</i> subsp. <i>Malaccensis</i> )	B2	<p>MEGKRWL SLLLLVLVIGISMDLPREAPAASSNILKSSSARIPVNPQGGEQRDG                      SKSKGIPLKANLSVCHASFSDARPVYCCPAWKDADQTLDFEFPDPSSPVRIR                      RPAHLVDEEFVAKYERAVAIMKQIPDPHPNFWRQANMHCLYCTGAYDQM                      NSSALFKIHRSWLFFPWHRAFIFYFHERILGKFMGDDTTFALPYWSWDTPEGMW                      FPDYIRK GALNETERAIHLREAAVDDFDYVDHDLASDVQIADNLAFMYHQ                      MISGAKKTELFMGCKLRSVGEWCDGPGTIEAAPHTLHWSVGNRYNPERE                      NMGAFYSAARDEVFAHHSNIDRMWTVWKKLHGDKPEFVDQEWLESEFTF                      YDENVRLRIKVRDVLNIDKLR YR YEDIDMPWLAARPKPSVHPKIARDILKK                      RNEGVL RMPGETDRSQLSEYGSWTLDKTITVVRVDRPRINRKTQGEKEEEEI                      LLVYGIDTKRSRFVKFDVFINVDET VLSPKSREFAGTFVNLHHVSRTKSHD                      DGGMDSKMKSHLKLGISELLEDELEADEDSDSIWVTLVPRGGTGVNTTVDGV                      RIDYMK</p>	NCBI reference sequence: XP_009380367.1

Table 1 continued

Sample	Sample sequence	Polyphenol oxidase protein sequences	GenBank
B3		MSLLNSSLTGASSACLLRREKRRRRGRGHVHGVTCHQGGNDDRRREARQQR SRLLLDRRDMLLGGLGYGTAGPKVLAEPIPPDLKCHDANAPALDN HCCPPYSGSETILEYDFPATPLRVRPPAHLVKDDQEQYMDKYKEAVRRMKNL PAEHPWNYQQANIHCQYCNDAYYQNTDDVPVQHFSWIFLPHWRYYL HFYERILGKLIDDDTFTPFNWDTKDGMTFAIFQDAASPLYDPKRDQRH VKDGAIDLKYAYTENTASDSEIIRENLCHQKTFKHSLSLAELFMGDPVRA GEKEIQEANGQLEVIHNAHMWVGEPDGYKENMGDFSTAAARDSVFFCHH SNVDRMWDIYRNLRGNVSEFNKDWLHSTFLFDENEQLVKYKIQDCLNP TKLRYTFEQVPLPWLNINCCQTAETKSKSTAEKSLKRVGEFGTTPKALDA SNPLRVVARPKNRKKKEKQEVVLQIKDIKVTTNETARFDVYVAVPYG DLAGPDYGEFVGSFVRLAHRKKGSDGTEEQPKKKGLKLGITALLEDDA EDADKLVVTLVLRVTSVTVGGVSIKLLQTDTPAVI	NCBI reference sequence: XP_009388329.1
B4		MALQLNSSFTGASSACLLHRRSRRLNPVVVTCROGNNDDRSDAARQOKSPS LLDRRDMLLGGLGYGLTAGPKVLAKPIMPPDLKCHDAKAPALDNHCCP PYNSETISEYGFATPLRVRPPAHLVKDDQEQYLDKYKEAVRRMKNLPAH PWNYQQANVHCQYCNAYYQNTDDVPVQHFSWIFLPHWRYYLHFY ERILGLIDDDTFTPFNWDTKDGMTFAIFHEESSPLSDTKRDQRHVKDG KIVDLKYAYTENPASNSEIIRENLCHQKTFKHSLSLAELFMGDPVRAKEKEI QEANGQLEVIHNAVHMWVGEPCGYKENMGDFSTAAARDSVFFSHSNVDRL WEIYRNLRGNRIEFEDNDWLDSTFLFYDENEKLVKVKMGDCLNPTKLY TFEQVPLPWLGKINCQKTTETKSKSTTEMSLTVGEFGTTPKALDASNPLR VIVARPKKNRKKKEKQEVVLQINDIKVTTNETARFDVYVTVPYGDL	NCBI reference sequence: XP_009388330.1
Mushroom tyrosinase ( <i>Agaricus bisporus</i> )	M1	MSLIATVGPVGGVKNRLNIVDFVKNKFFTLVYRSLELLQAKEQHDYSSFFQL AGIHGLPFTWAKERPSMNLKAGYCTHGQVLFPTWHRTYLSVLEQILQG AAIEVAKKFTSNQTDVQAAQDLRQPYWDWGFELMPPDEVIKNEEVNIT NYDGKISVKNPILRYHFHPIDPSFKPYGDFATWRTTVRNPDRNRREDIPG LJKMRLEEGQIREKTYNMLKFNDAWERFSNHGISDDQHANSLESVHDD IHMVGYGKIEGHMDHPFAAFDPFVWLHHTNVDRLLSLWKAINPDVWV TSGRNRDGTMIAPNAQINSETPLEPFYQSGDKVWTSASLADTARLGSY PDFDKLVGGTKELIRDAIDDLIDERYGSKPSSGARNTAFDLADFKGITKE HKEDLKMWDWTHVAFKKFELKESFSLFYASDGGDYDQENCFVGSINA FRGTAPETCANCQDNENLIQEGFHLNHYLARDLESFEPQDVHKFLKFKGL SYKLYSRGDKPLTSLVKIEGRPLHLPPEHRPKYDHTQARVVFDDVAHV VIN	CAA11562.1

Table 1 continued

Sample	Sample sequence	Polyphenol oxidase protein sequences	GenBank
Polyphenol oxidase ( <i>Agaricus bisporus</i> var. <i>bisporus</i> H97)	M2	MSHLLVSPGLGGVQPRLEINNFVNDRQFSLYVQALDRMYATPQNET ASYFQVAGVHGYPILPFNDAVGPTFSPFDQWGTGYCTHSGSTLFTW HRPVVLIJLEQILSGHAQIADITYVNKSEWKKAATEFRHPYWDWA SNSVPPPEVISLPKVTTITPQKTSVANPLMRYTFNPVNDGGFYGP YNQWDTTLRQPDSTGVNAKDNVRLTSLVKNQAASLTRATYDM FNRVTTWPHFSSHTPASGGSTNSIEAIHDNIHVLVGGNGHMSDPS VAADFPIFLHHANVDRLLALWSAIRYDVWVWTPGDAQFGTYTLRY KQSVDESTDLAPWWTQNEYWKSNELRSTESLGYTYPEFVGLDM YNKDAVNKTIIRKVAQLYGPGQGGQKSLVEDLSNSHARRSQRLLAK RSRLGQLLKGLFSDWSAQIKFNRHEVGGQSFVCLFLGNVPEDPREWL VSPNLVGARHAFVRSVKTDHVAAEIGFIPINQWIAEHTGLPSFAVDLV KPLLAQGLQWRVLLADGTPAELDSLEVTILEVPSSELTDDPEPNRSRPP RYHKDITHKRGCCREA	NCBI reference sequence: XP_006463026.1
Tyrosinase ( <i>Agaricus bisporus</i> var. <i>bisporus</i> H97)	M3	MSLIAVGTGGVKNRNLNIVDFVKNKFFTLVYRSLELLQAKEQHDYS SFFQLAGHGLPFTWEAKERFSMNLKAGYCTHGQVLFPTWHRTYL SVFEQILQGAIEVANKFTSNQTDWQAAQDLRQPYWDWGFELMPP DEVIKNEEVNITNYDGKIKSVKNPILRYHFHPIDPSFKPYGDEFTWRT TVRNPDRNRREDIPGLKMRLEEGQIREKTYNMLKFNDAWERFNS HGISDDQHANSLESVHDDIHVMVG YKIEGHMDHPFAAFDPFVWL HHTNVDRLLSLWKAINPDVWVTSGRNRDGTMTGIAPNAQINDETPLE PFYQSEDKVWTSASLADTARLGYSPDFDKLVGGTKELIRDAIDDL IDERYGSKPSSGARNTAFDLLADFKGITEKHEKEDLKMVDWTHVAF KKHELKESFLLFYFASDGGDYDQENCFVGSINAFRGTTPETCANCQ DNENLIQEGFHLNHYLARDLESFEPQDVHKFLKEKGLSYKLYSRED KSLTSLSVKIEGRPLHLPPGEHRPKYDHTQDRVVFDDVAVHVIN	NCBI reference sequence: XP_006459626.1
Tyrosinase ( <i>Agaricus bisporus</i> )	M4	MSDKKSLMPLVGIPGEIKNRLNILDFVKNDKFFTLVYRALQVQLQAR DQSDYSFFQLGGIHLGPLYTEWAKAQQLHLKANYCTHGTGLF PTWHRAYESTWEQTLCEAGTVAQRFTTSDQAEWQAAKDLR QPFWDWGYWPNPDPDFIGLPDQVIRDKQVEITDYNCTKIEVENPIL HYKHFPIEPTFEGFAQWQTTMRYPDVQKQENIEGMIAGIKAAAP GFREWTFNMLTKNYTWELFSNHGAVVGAHANSELMVHNTVHFLI GRDPTLDPLVPGHMGSVPHAAFDPIFWMHHCNVDRLLALWQTMN YDVYVSEGMNREATMGLIPGOVLTEDSPLPEPFTKNQDPWQSDDD LEDWETLGFSPDFDPVKGKSKEEKSVYINDWVHKHYGFVTTQT ENPALRLSSFRQAKSDHETQYALYDWWIHAIFRYELNNSFSIIF YFDEGEGETLESIGTVDAFRGTTSSENCANCARSQDLAEFGVHLN YYIGCDIGQHADHEDDAVPLYEPRVKEYLKKRKGKGVVSAEGE LTSLVVEIKGAPYYLPVGEARPKLDHEKPIVILDDIHRVN	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

(3S)-2-(3,4-dihydroxyphenyl)-3,5,7-chromanetriol (DHPC) was purchased from Merck, Germany. Apple, banana, and

**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

mushroom were provided from different shops of Yazd, Iran.

### 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 HSHKH	HNSWLFFPFH	HNSWLF	HRYLYF	FAH	FPFH FFAH FAHH	FFPFH FFAHH	FALPF FAGSF
A2	HAPVH HSHKH	HNSWLFFPFH	HNSWLF	HRYLYF	FAH	FPFH FFAH FAHH	FFPFH FFAHH	FALPF FAGSF
A3	HGPVH	HQSWLFFPFH	HRISSF HQSWLF	HRYLYF	FSH	FSHH FPFH FFSH	FFPFH FFSHH	FLSPF FALPF FAGSF
A4	HSHKH HAPVH	HNSWLFFPLH	HNSWLF	HRYLYF	FAH	FAHH FPLH FFAH	FFAHH FFPLH	FAGP FAGSF
B1	HGPVH HKHKKH	HFSYPDRRRH HNSWLFFPWH	HNSWLF	HNSWLFF	FAH	FAHH FPWH FFAH	FFPWH FFAHH	FACAF FAGSF FYLYF FALPF
B2	HNTLH	HRSWLFFPWH HNFWRQANMH	HRSWLF	HRSWLFF HGDKPEF	FAH	FKIH FPWH FMYH FAHH FFAH	FFPWH FIYFH FFAHH FVNLH	FAGTF





**Table 5** continued

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

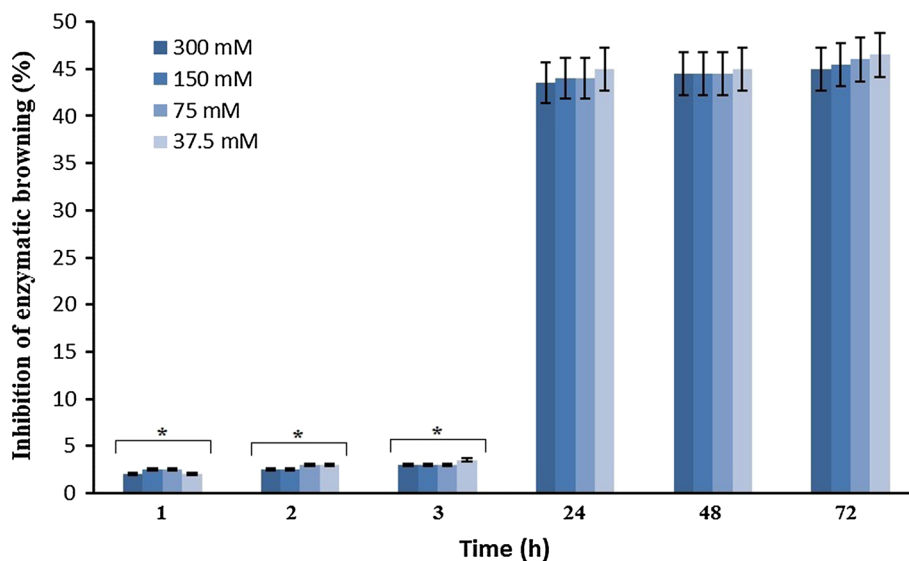
**Table 6** The average of intermolecular energy and number of hydrogen bonding between HLFH and some phenolic and benzoic compounds

	Intermolecular energy	No. of hydrogen bonding
(+)-Epicatechin	-0.05	3
(3S)-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

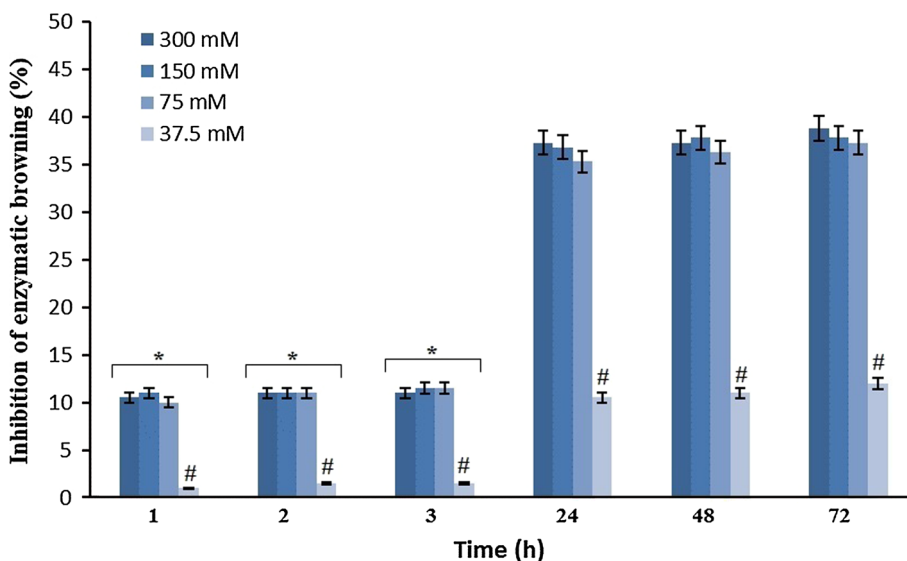
	Intermolecular energy	No. of hydrogen bonding
4-(2H3)methyl-1,2-benzenediol	−0.01	3
4-Hydroxy(2H4)benzoic acid	−1.71	3
4-Hydroxy(carboxy-11C)benzoic acid	−0.10	2
4-Hydroxy(carboxy-13C)benzoic acid	−0.53	3
4-Hydroxy(carboxy-13C)benzoic acid1	−0.02	3
4-Hydroxy(carboxy-14C)benzoic acid	−0.27	2
4-Hydroxybenzoate	−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
<i>o</i> -Hydroxybenzoic acid	−0.00	3
<i>p</i> -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.  $n = 3$ 

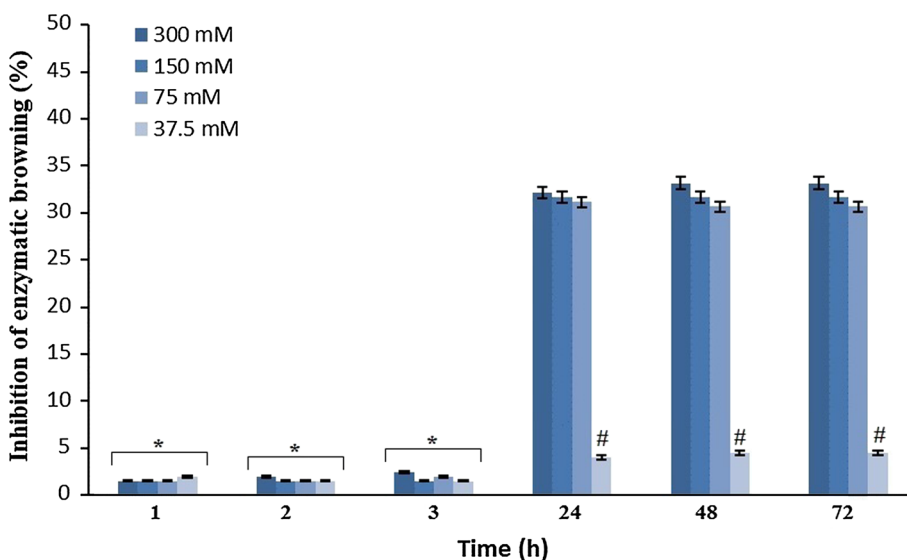
### Experimental study

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  $\mu$ L 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

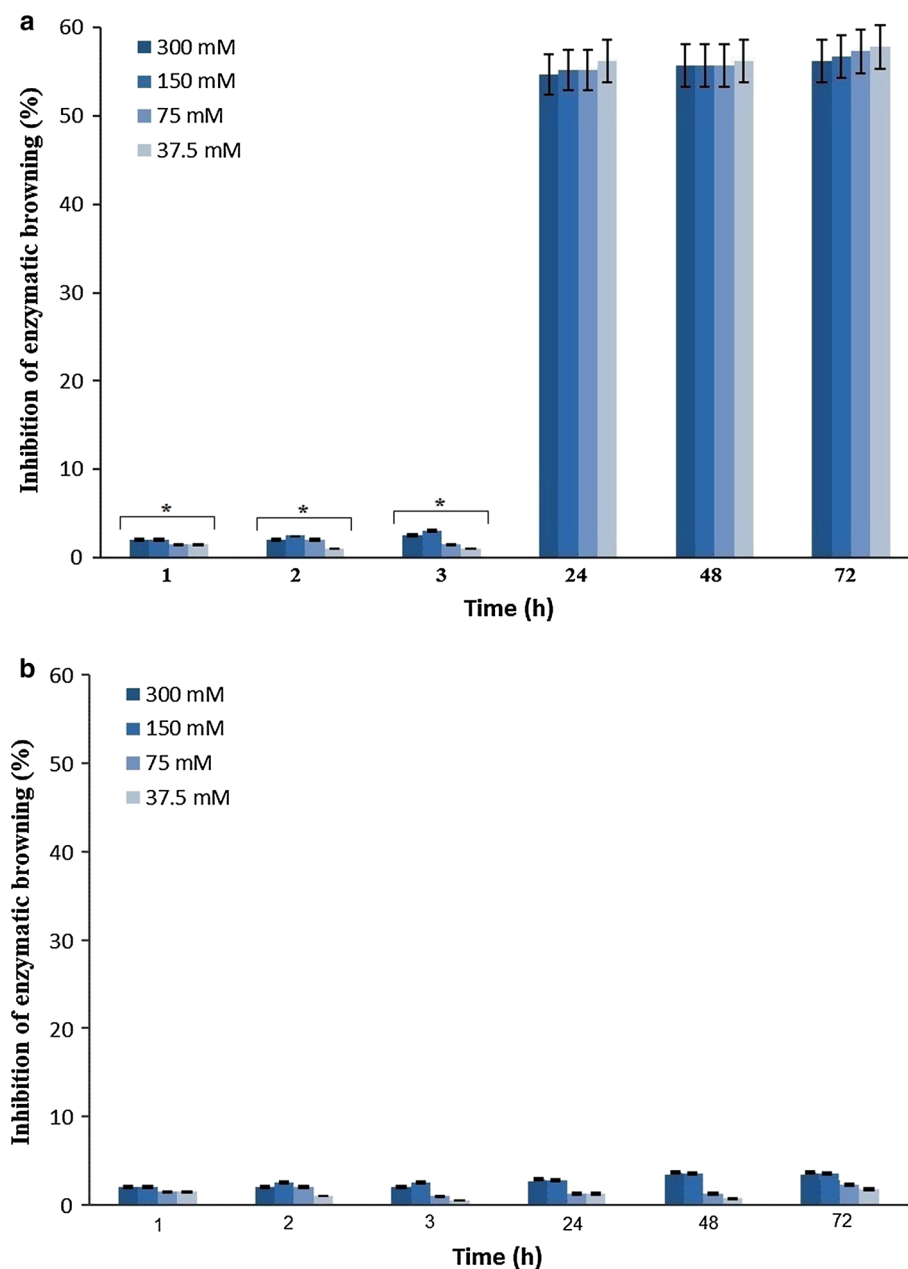
enzymatic browning was calculated, according to Formula 2 (Holderbaum et al. 2010).

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

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

$$I = \frac{TC_c - TC_t}{TC_c} \tag{2}$$

where (*I*) is the inhibition of enzymatic browning, *TC<sub>c</sub>* is the total color in control, and *TC<sub>t</sub>* 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.

**Acknowledgments** This article was supported by Pajoohesh Medical Lab (Grant Number: 2015-9). The authors thank the laboratory staff of the Yazd Pajoohesh Medical Lab.

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