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ORIGINAL PAPER

Biocatalytic properties of a peroxidase-active cell-free extract from onion solid wastes: caffeic acid oxidation

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Abstract The exploitation of food residual sources consists of a major factor in reducing the polluting load of food industry wastes and developing novel added-value products. Plant food residues including trimmings and peels might contain a range of enzymes capable of transforming bio-organic molecules with potential phytotoxicity, including hydrolases, peroxidases and polyphenoloxidases. Although the use of bacterial and fungal enzymes has gained interest in studies pertaining to bioremediation applications, plant enzymes have been given less attention or even disregarded. In this view, this study aimed at the investigating the use of a crude peroxidase preparation from onion solid by-products for oxidising caffeic acid, a widespread *o*-diphenol, whose various derivatives may occur in food industry wastes, such as olive mill waste waters. Increased enzyme activity was observed at a pH value of 5, but considerable activity was also retained for pH up to 7. Favourable temperatures for increased activity varied between 20°C and 40°C, 30°C being the optimal. Liquid chromatography-mass spectrometry analysis of a homogenate/H_2O_2-treated caffeic acid solution revealed the existence of a tetramer as major oxidation product. Based on the data generated, a putative pathway for the formation of the peroxidase-mediated caffeic acid tetramer was proposed.

Keywords Bioremediation · Peroxidase · Onion · Caffeic acid · OMWW

Abbreviations

4-AAP	4-Aminoantipyrine
BGP	Bitter gourd peroxidase
CA	Caffeic acid
CA-OP	Caffeic acid oxidation product
CouA	*p*-Coumaric acid
FA	Ferulic acid
HRP	Horseradish peroxidase
LC-MS	Liquid chromatography-mass spectrometry
OMWW	olive mill waste water
OSWH	Onion solid waste homogenate
SA	Sinapic acid
SBP	Soybean peroxidase
S.D.	Standard deviation
TMP	Tomato peroxidase
TNP	Turnip peroxidase

Introduction

As the demand of olive oil is rapidly increasing worldwide, environmental pollution posed by olive mill wastes becomes a growing concern especially in

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the Mediterranean region. A very large amount of phenol-polluted waters are formed from the production of olive oil (olive mill waste water-OMWW), and the main problem associated with their disposal is a viable means of effective treatment. Biochemical processes used for treating OMWW are generally considered to be of high capital and operating costs with limited efficiency. This is mainly due to particularly high levels of phenolic compounds, which are considered as major contributors to the toxicity and the antibacterial activity of OMWW, and limit their microbial treatment and/or use as fertilizers (Azbar et al. 2004).

Significant research has been carried out in recent years to investigate the new possibilities offered by enzymes in waste treatment. This tendency is mainly owed to (i) the increased introduction of pollutants and recalcitrant chemicals in the environment, and the difficulty to achieve the desired degree of their removal, using conventional physico–chemical and biological means; (ii) the specificity of enzymes which can be used for more targeted treatments; and (iii) the financially viable ways that can be achieved through biotechnology for the production of various biocatalysts (Karam and Nicell 1997). Furthermore, enzymes are easier to handle, exhibit activity over wider pH, salinity and temperature ranges, and there is not need for acclimation period and increased sludge volume, two problems that are usually encountered in the implementation of microbiological processes.

Few plant tissues have been tested as sources of peroxidases that can be used for bioremediation processes. Horseradish peroxidase is the most studied enzyme in this regard, and has been used mainly for the treatment of aqueous phenols and chlorophenols (Tatsumi et al. 1996; Wu et al. 1997; Tong et al. 1998; Wagner and Nicell 2002; Cheng et al. 2006; Dalal and Gupta 2007), but also other pollutants such as polychlorinated biphenyls (Singh et al. 2000) and bisphenol A (Huang and Weber 2005). Crude and partly purified soybean peroxidase has also been used in the treatment of phenols and chlorophenols (Caza et al. 1999; Flock et al. 1999; Wilberg et al. 2002; Kennedy et al. 2002), and bitter gourd (*Momordica charantia*) peroxidase for the treatment of textile dyes (Akhtar et al. 2005).

To the best of our knowledge, onion peroxidase has never been examined with regard to its use in bioremediation studies. The investigation described herein aimed at providing some basic information on the biocatalytic properties of a crude homogenate obtained from onion solid wastes, and to clarify to some extent the oxidation pathways of caffeic acid, a model compound whose derivatives and structurally similar phenolics are abundant in OMWW (Mantzavinos and Kalogerakis 2005).

Materials and methods

Chemicals

All solvents used for chromatography were HPLC grade. Caffeic acid and 4-aminoantipyrine (4-AAP) were from Sigma Chemical Co (St. Louis, MO, USA). Hydrogen peroxide (30%) and trichloroacetic acid were from Merck (Germany).

Preparation of the onion solid waste homogenate (OSWH)

The onion solid waste used in this study was obtained from a local catering facility (Chania, Crete) after processing of brown-skin onion bulbs. The waste consisted of the apical trimmings of the bulbs, as well as the outer dry and semi-dry layers. The material was transferred to the laboratory immediately after processing, and ground in a domestic blender. An aliquot of 2 g of the ground tissue was suspended in 15 ml buffer solution under stirring, and the suspension was centrifuged at 3,000 *g* for 20 min and filtered through paper filter to remove cell debris. The clear supernatant obtained was treated with activated charcoal for decolourisation, and filtered through celite under vacuum. The clear filtrate was used as the crude enzyme source.

Peroxidase activity

The assay mixture contained 0.25 ml 4-AAP (10 mM in water), 0.1 ml substrate (100 mM in DMF), 0.1 ml H_2O_2 (2 mM), 0.5 ml buffer and 0.1 ml enzyme extract. Absorbance was monitored at 510 nm for over than 2 min against suitable blank. One enzyme unit was defined as $\Delta A_{510}\ s^{-1}$. Control reactions by omitting H_2O_2 or using heat-inactivated homogenate were also carried out. In assays performed at different

temperatures, all constituents of the reaction mixture were pre-incubated either in a freezer (5°C) or in a thermostated water bath (30–60°C). For pH 2 and 8, a potassium chloride/HCl and a boric acid/NaOH buffer were used, respectively. For the pH range 3–7, a phosphate/citrate buffer was used. For all determinations, a computer-controlled HP 8452A diode array spectrophotometer was used.

Protein determination

Protein content was determined according to Bradford 1976, using bovine serum albumin as standard.

Caffeic acid oxidation

A solution of CA (10 mM) was oxidized with OSWH (total protein content 15 μg) and H_2O_2 (5 mM) for 10 min at room temperature (24 ± 2°C) and pH 4. Following this, 0.1 ml of a 10% trichloroacetic acid solution in EtOH was added, and the mixture was centrifuged at 5,000 *g* for 10 min. The clear supernatant was filtered trough 0.45 μm syringe filters, and the filtrate was used for chromatographic analyses.

HPLC-DAD analysis

The equipment utilized was an HP 1090, series II liquid chromatograph, coupled with an HP 1090 diode array detector and controlled by Agilent ChemStation software. The column was a LiChrosphere RP18, 5 μm, 250 × 4 mm (Merck), protected by a guard volume packed with the same material. Both columns were maintained at 40°C. Eluent (A) and eluent (B) were 1% formic acid and acetonitrile, respectively. The flow rate was 1 ml min^{-1}, and the elution programme used was as follows: 0–5 min, 5% B, 5–45 min, 100% B, 45–55 min, 100% B. Monitoring of the eluate was performed at 320 nm.

Liquid chromatography-mass spectrometry (LC-MS)

A Finnigan MAT Spectra System P4000 pump was used coupled with a UV6000LP diode array detector and a Finnigan AQA mass spectrometer. Analyses were carried out on a Superspher RP-18, 125 × 2 mm, 4 μm, column (Macherey-Nagel, Germany), protected by a guard column packed with the same material, and maintained at 40°C. Analyses were carried out employing electrospray ionization (ESI) at the positive ion mode, with acquisition set at 12 eV and 50 eV, capillary voltage 4 kV, source voltage 4.9 kV, detector voltage 650 V and probe temperature 400°C. Eluent (A) and eluent (B) were 2.5% acetic acid and methanol, respectively. The flow rate was 0.33 ml min^{-1}, and the elution programme used was as follows: 0–5 min, 0% B; 5–30, 100% B; 30–35, 100% B.

Statistical analyses

All determinations were carried out at least in triplicate and values were averaged and given along the standard deviation (±S.D.). For all statistics, Microsoft Excel™ 2000 was used.

Results

Biocatalytic properties

Preliminary experiments using quercetin, which is a physiological substrate for onion POD (Takahama and Hirota 2000), showed that maximum activity was attained at pH 4 (unpublished data). For this reason, initial investigation pertaining to the effect of CA and H_2O_2 concentration on the enzyme activity were performed at this pH. Increasing concentrations of CA were shown to provoke a proportional effect on the enzyme activity, up to 1 mM (Fig. 1). Thereafter, a gradual limited decline in activity was observed.

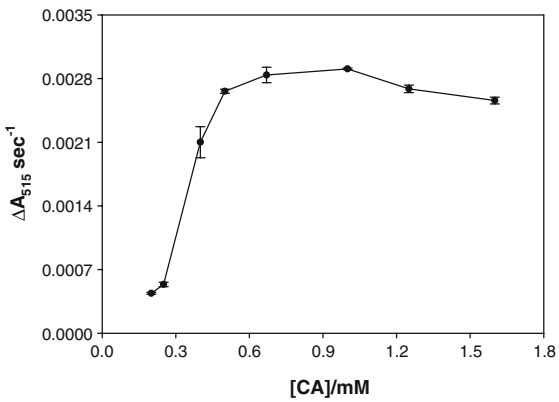

Fig. 1 Activity variation in response to caffeic acid concentration. Reaction conditions: $[H_2O_2] = 0.4$ mM; [Total protein] = 17.6 μg ml^{-1}; pH = 4; T = 24 ± 2°C

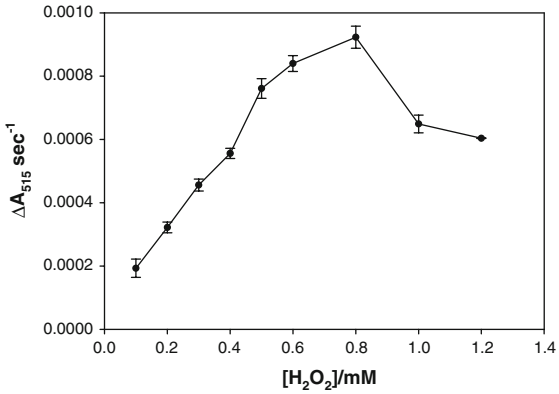

Fig. 2 Activity variation in response to H_2O_2 concentration. Reaction conditions: [CA] = 1 mM; [Total protein] = 10.2 μg ml^{-1}; pH = 4; T = 24 ± 2°C

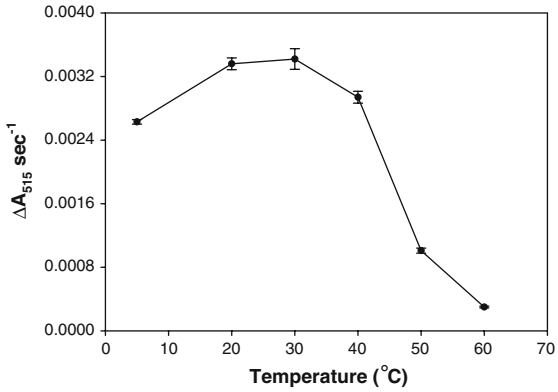

Fig. 4 Dependence of activity on temperature. Reaction conditions: [CA] = 1 mM; $[H_2O_2]$ = 0.8 mM; [Total protein] = 20 μg ml^{-1}; pH = 5

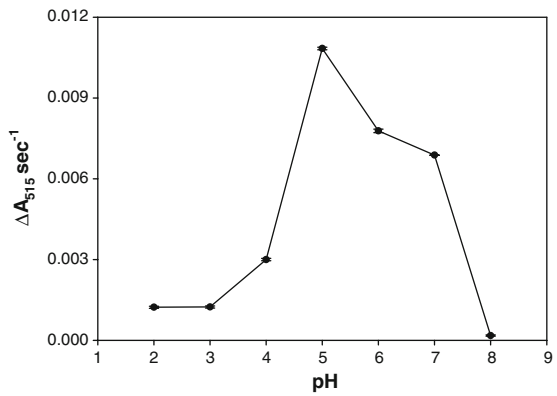

Fig. 3 Dependence of activity on pH. Reaction conditions: [CA] = 1 mM; $[H_2O_2]$ = 0.8 mM; [Total protein] = 8 μg ml^{-1}; T = 24 ± 2°C

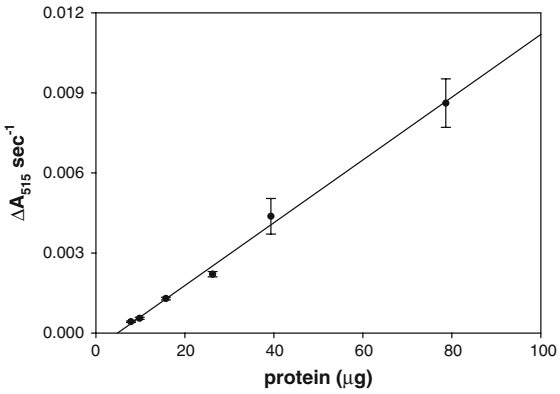

Fig. 5 Activity as a function of total protein content. Reaction conditions: [CA] = 1 mM; $[H_2O_2]$ = 0.8 mM; pH = 5; T = 24 ± 2°C

Likewise, increases in H_2O_2 concentration were shown to promote enzyme activity up to 0.8 mM. Higher concentrations were proven inhibitory in this regard (Fig. 2), a fact manifested by a decline in enzyme activity, which was more pronounced than that provoked by CA.

The examination of the pH effect over a range varying from 2 to 8 revealed that high enzyme activity was expressed between 4 and 6, whereas significant decline was seen when reactions were carried out at pH 2, 3 and 8 (Fig. 3). Maximum activity was recorded at pH 5. In a similar fashion, temperatures ranging from 20 to 40°C were favourable, the maximal activity being found at 30°C (Fig. 4). By contrast, enzyme activity was virtually trivial at 60°C.

By implementing optimal conditions with regard to substrate, activator, pH and temperature, different dilutions of the homogenate were assayed (Fig. 5). Increasing amounts of total protein in the reaction mixture provoked proportionally higher enzyme activities, suggesting that the rate of CA oxidation is directly proportional to the total enzyme concentration.

Tentative identification of the CA oxidation product

In the view of obtaining an insight into the mechanism of CA oxidation, a CA solution (1 mM) was incubated with OSWH in the presence of 0.8 mM H_2O_2 for 10 min. The mixture was then analyzed by HPLC. In the trace obtained at 320 nm (Fig. 6), one major peak

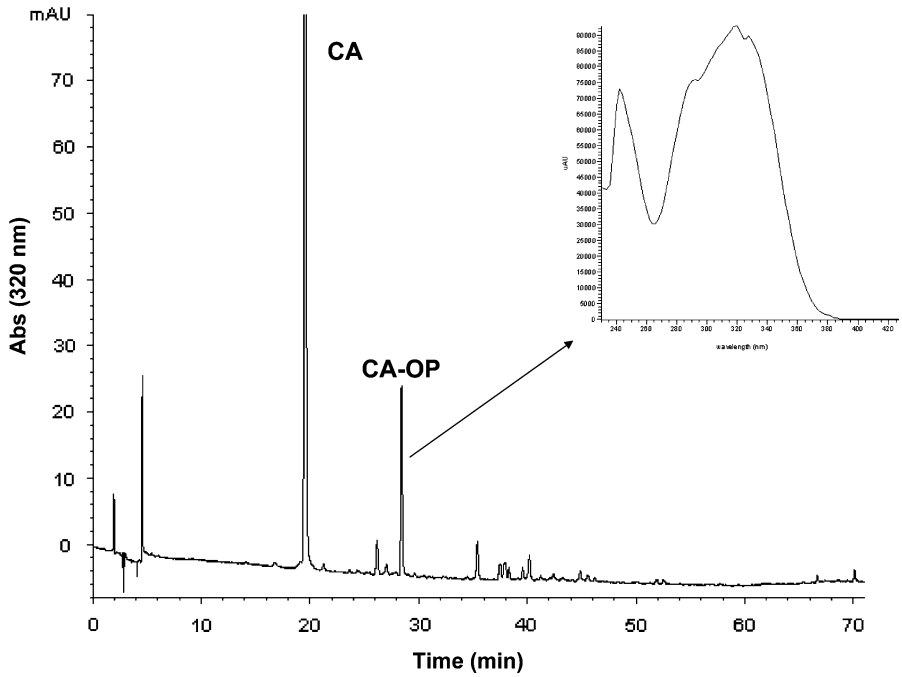

Fig. 6 Chromatographic profile of a caffeic acid solution treated with OSWH for 10 min. Reaction conditions: [CA] = 1 mM; $[H_2O_2]$ = 0.8 mM; pH = 5; T = 24 ± 2°C. Assignments: CA, caffeic acid; CA–OP, caffeic acid oxidation product. The insert picture shows the UV spectrum of CA–OP (λ_{max} = 320 nm)

was detected, assigned as CA–OP. Liquid chromatography-mass spectrometry analyses in negative ion mode of CA–OP gave a molecular ion at *m/z* 715 $[M–H]^-$ (Fig. 7, upper mass spectrum). A fragment indicating the loss of CO_2 (*m/z* = 671) was also detected. At 15 eV collision energy (upper mass spectrum) a daughter ion at *m/z* = 417 was prevalent, indicating cleavage of the tetramer as shown in Fig. 7. Subsequent removal of two CO_2 units from this ion would afford the fragment with *m/z* = 329, whose formation was more pronounced at increased collision energy (50 eV, lower mass spectrum). At this collision energy, the ion with *m/z* = 269 was predominant. This fragment was though to derive from further decarboxylation of the decarboxylated dimer (*m/z* = 313). The formation of this ion (*m/z* = 313) could be attributed to cleavage of the decarboxylated tetramer (*m/z* = 671). These observations were consistent with a structure of a CA tetramer, as shown in Fig. 7.

Discussion

Recent studies pertaining to the analytical composition of the polyphenolic fraction of OMWW revealed that the major substances are those possessing an *o*-diphenol feature, including various catecholic glycosides and other conjugates (DellaGreca et al. 2004), hydroxytyrosol and its glucoside, verbascoside (a ceffeic acid conjugate) and oleuropein derivatives (Obied et al. 2005), as well as caffeic acid and luteolin (De Marco et al. 2007). In this view, the examination of the oxidizability of those components by OSWH should be carried out using a substrate that would incorporate similar key structural characteristics. For this reason caffeic acid was chosen, which is a commonly and widely occurring *o*-diphenol possessing suitable structure.

The effect of H_2O_2, pH and temperature

The examination on the effect of H_2O_2 concentration on the rate of CA oxidation (Fig. 2) showed that beyond a certain point H_2O_2 may act in an inhibitory manner, decreasing enzyme activity. This phenomenon has been observed in many cases of peroxidase-catalyzed oxidation of various phenolics, including tomato peroxidase-TMP (González et al. 2006), soybean peroxidase-SBP (Flock et al. 1999; Caza et al. 1999; Wright and Nicell 1999), horseradish peroxidase-HRP (Wu et al. 1997; Tong et al. 1998; Singh et al. 2000), immobilized horseradish peroxidase (Cheng et al. 2006), and turnip peroxidase-TNP (Duarte-Vázquez et al. 2002). The inhibitory effect is considered to be attributed to factors such as attack

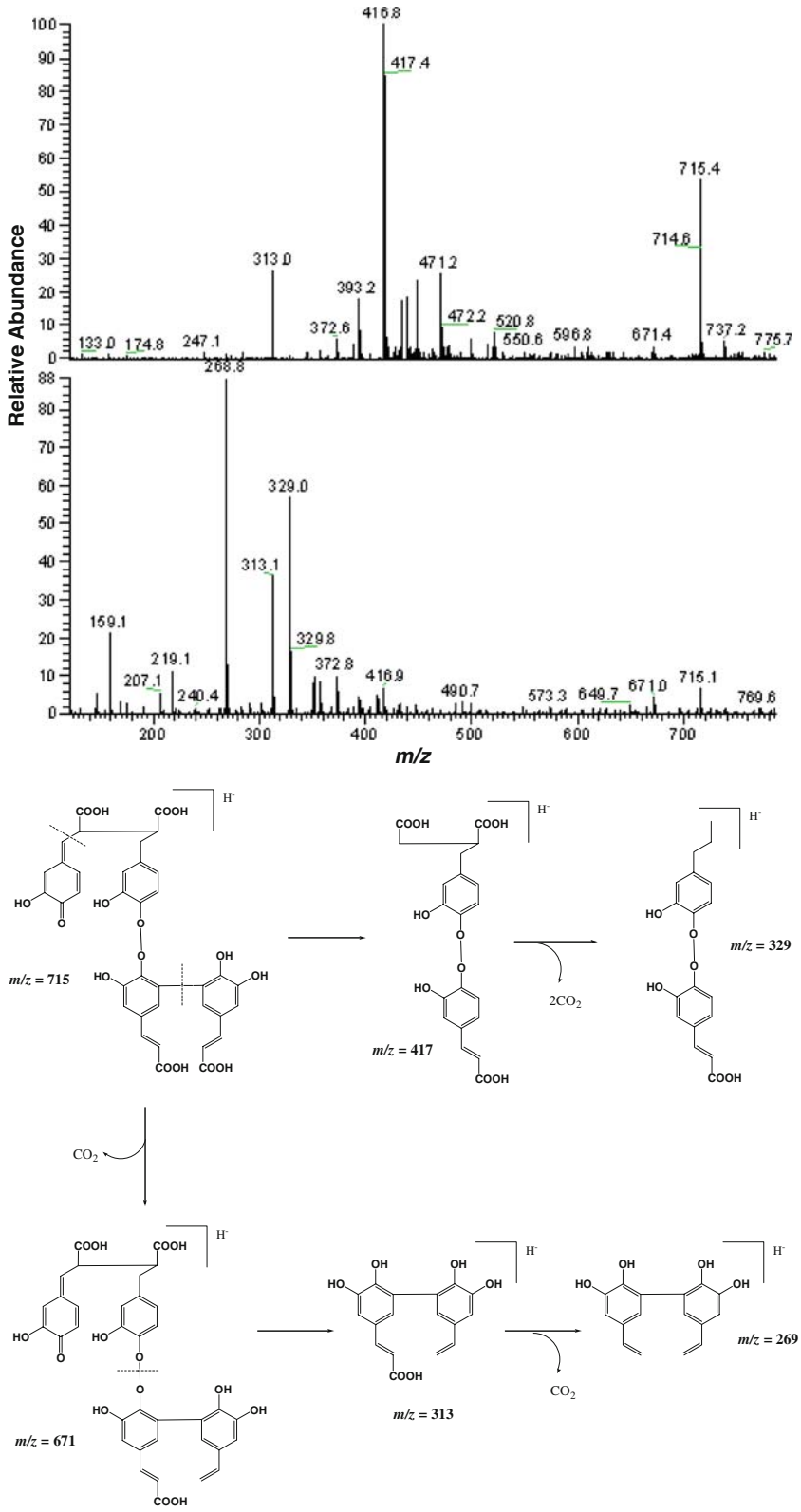

Fig. 7 Mass spectrum (upper figure) and the putative fragmentation pathway proposed (lower figure) for the CA–OP

by phenoxy radicals produced, side-effects caused by excess of H_2O_2 and sorption/occlusion of the enzyme by precipitated products formed from coupling reactions (Huang and Weber 2004). The H_2O_2/CA ratio at which maximum activity was recorded was 0.8, which is equal to those found in HRP-mediated oxidation of 2-chlorophenol and 4-chlorophenol and very close to those of *o*-cresol (0.9), *p*-cresol (0.9) and 2,4-dichlorophenol (0.7) (Caza et al. 1999).

The OSWH exhibited a pH optimum between 5 and 6 (Fig. 4), and it is also noteworthy that it retained significant activity within a pH range from 4 to 7. This outcome contrasts previous results, which indicated that quercetin oxidation, the physiological substrate of the enzyme, by a crude onion peroxidase extract was favoured at pH 8 (Takahama and Hirota 2000). Peroxidases from other plant residual sources that have been used in studies pertaining to phenol removal, such as HRP (Tong et al. 1997), SBP (Wright and Nicell 1999; Geng et al. 2001), TNP (Duarte-Vázquez et al. 2002) and bitter gourd peroxidase-BGP (Akhtar and Husain 2006), showed optimal pH around 7, 6, 7 and 6, respectively. In these cases too it was seen that pH optimum may differ significantly with different substrates.

Using HRP, phenol and 4-chlorophenol removal was more efficient at pH values 8–9 rather than 7 (the pH optimum for HRP) (Tong et al. 1997). Polychlorinated biphenyl removal using HRP was also more pronounced at a pH range 4–7 (Singh et al. 2000). Similar results were reported for SBP, which showed pH optima between 5.5 and 8 when used for the removal of a variety of chlorophenols (Caza et al. 1999). By contrast, other investigations indicated that SBP was more effective in removing certain phenols from artificial waste water at pH 9 (Wright and Nicell 1999), 8.2 (Kennedy et al. 2002) and 7 (Bassi et al. 2004). Removal of chlorophenols from model media using TNP and BGP was shown to be optimal within a pH range 5–7 (Duarte-Vázquez et al. 2002) and 5–6 (Akhtar and Husain 2006), respectively. Removal of textile dyes by the use of BGP was nevertheless favoured within a pH range of 3 to 4 (Akhtar et al. 2005).

Concerning the effect of temperature on the rate of the catalytic activity, the OSWH exhibited significant activity over a large range of temperatures, varying from 5 to 40°C, but thereafter the activity declined to considerably lower levels. The temperature at which optimum activity was attained (30°C) is close to that found for BGP and HRP (Akhtar et al. 2005; Bódalo et al. 2006; Yamada et al. 2007), which lied between 30°C and 40°C, and somewhat lower than TMP that showed an optimum between 40°C and 50°C (González et al. 2006). By contrast, SBP has been reported to be much more active at 22 than at 4°C (Kennedy et al. 2002), while other studies demonstrated that SBP is even more active at 80°C than at 20°C (Geng et al. 2001).

Oxidation mechanism

Peroxidases catalyze the oxidation of a wide variety of substrates, using H_2O_2 or other peroxides (Veitch 2004). In general terms, the majority of reactions catalyzed by the classic plant peroxidases can be represented as follows, where RH and $R^{\bullet}$ represent a reducing substrate and its oxidized radical product, respectively,

$$H_2O_2 + 2RH \rightarrow 2H_2O + 2R^{\bullet}$$

Such reactions are believed to be implicated in the oxidation of *p*-hydroxycinnamates and other analogues and subsequent cell wall cross-linking reactions, which can be brought about through radical coupling of hydroxycinnamates and their dehydrodimers (Ralph et al. 2004). Similar products were also generated after oxidizing mixtures of CA, ferulic acid (FA), *p*-coumaric acid (CouA) and sinapic acid (SA) with potato and horseradish peroxidase (Arrieta-Baez and Stark 2006) and mixtures of FA and resveratrol with *M. charantia* peroxidase (Yu et al. 2007). The latter enzyme source was used to produce SA tetramers as well (Liu et al. 2007). In the same fashion, the first step in the cascade of reactions that could lead to a CA tetramer is the formation of CA radicals, as claimed for peroxidase-catalyzed oxidation of ferulic acid (Oudgenoeg et al. 2001; Derat and Shaik 2006), (Fig. 8). Coupling of radicals could then produce various dimers. On the other hand, simple radical coupling could not substantiate the formation of a tetramer with molecular weight 716 Da, as the one detected in the CA solution oxidised with OSWH/H_2O_2. It appears therefore that a disproportionation step should intervene. This assumption is adequately corroborated by experimental data on the non-enzymic oxidation of CA. In particular, studies on the electrochemical oxidation of FA and CouA showed that both compounds are oxidized through electron transfer from the corresponding phenolate ion even at pH values lower than pK_a (Hapiot et al.

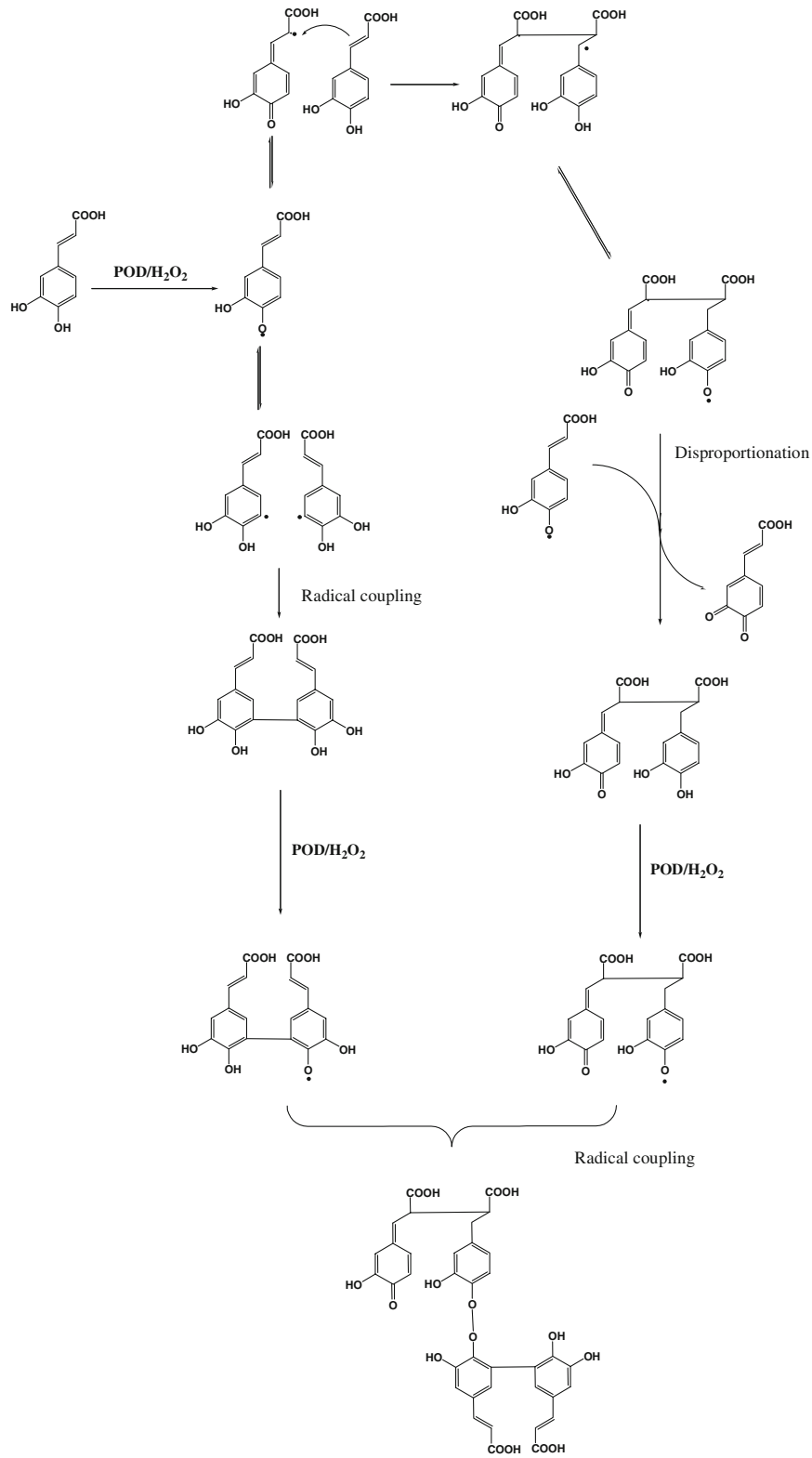

Fig. 8 Putative, generalised oxidation pathway of caffeic acid leading to the formation of the tetramer

1996). The phenolate is then oxidised to the phenoxyl radical, which then dimerises by radical-radical coupling, according to the general pattern:

$$PhOH \leftrightarrows PhO^- + H^+$$

$$PhO^- \leftrightarrows PhO^\bullet + e^-$$

$$PhO^\bullet + PhO^\bullet \rightarrow \text{dimer}$$

Nevertheless, the behaviour of CA was very different. In neutral or acidic pH the *o*-quinone could be rapidly formed by disproportionation of the *o*-semiquinone. During electrolysis experiments the formation of coupling products was observed, which indicated that *o*-quinone generation preceded and was indeed involved in their formation. These findings were also supported by the outcome on the spectroelectrochemical and chemical oxidation of hydroxycinnamic acids in aprotic medium (Petrucci et al. 2007). The characterization of products of tyrosinase-mediated CA oxidation revealed that formation of dimers primarily occurred, but there was also evidence for trimer formation (Pati et al. 2006).

In general, the stoichiometry between H_2O_2 and phenolic substrates in peroxidase-catalyzed reactions has been reported to be one-to-one (Hewson and Dunford 1976; Zhang and Nicell 2000). The deviation between measured and theoretical stoichiometries has been postulated to be the result of several mechanisms, in which products of the catalytic process are polymers larger than dimers. In this view, the tentative identification of the caffeic acid tetramer might explain the observed H_2O_2/CA ratio for optimal activity.

The implementation of an enzymic process involving onion POD might depend on the factors that govern the effectiveness of the catalysis (pH, temperature), but also on the nature of the substrates to be removed. Since, however, peroxidases are capable of oxidizing a wide range of phenolics and the reactions proceed thereafter independently of enzymic intervention (radical coupling, desproportionation), then the spectrum of applicability of onion POD could be wide, embracing various agri-food wastes, such those generated by olive mill wastes, wineries, potato processing plant, etc.

Conclusions

In the study presented herein, the use of a peroxidase-active, crude preparation from onion solid wastes is proposed for the first time as an alternative source of an oxidative enzyme that could have a prospect in bioremediation applications. The preliminary investigations carried out showed that conditions for optimal enzyme activity might lie within a range of 5–6 and 20–30°C, for pH and temperature, respectively. Efforts to elucidate the oxidative behavior employing liquid chromatography-mass spectrometry, with regard to a model substrate, caffeic acid, provided strong evidence that the oxidative pathway(s) implicated may have uncommon features with those encountered in the oxidation of other hydroxycinnamates; this finding merits a profounder examination.

References

Akhtar S, Husain Q (2006) Potential applications of immobilized bitter gourd (*Momordica charantia*) peroxidase in the removal of phenols from polluted water. Chemosphere 65:1228–1235

Akhtar S, Khan AA, Husain Q (2005) Potential of immobilized bitter gourd (Momordica charantia) peroxidases in the decolorization and removal of textile dyes from polluted wastewater and dying effluent. Chemosphere 60:291–301. doi:10.1016/j.chemosphere.2004.12.017

Arrieta-Baez D, Stark RE (2006) Modeling suberization with peroxidase-catalyzed polymerisation of hydroxycinnamic acids: cross-coupling and dimerization reactions. Phytochemistry 67:743–753. doi:10.1016/j.phytochem.2006.01.026

Azbar N, Bayram A, Filibeli A, Muezzinoglu A, Sengul F, Ozer A (2004) A review of waste management options in olive oil production. Crit Rev Environ Sci Technol 34:209–247. doi:10.1080/10643380490279932

Bassi A, Geng Z, Gijzen M (2004) Enzymatic removal of phenol and chlorophenols using soybean seed hulls. Eng Life Sci 4:125–130. doi:10.1002/elsc.200420021

Bódalo A, Gómez JL, Gómez E, Bastida J, Máximo MF (2006) Comparison of commercial peroxidases for removing phenol from water solutions. Chemosphere 63:626–632. doi:10.1016/j.chemosphere.2005.08.007

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. doi:10.1016/0003-2697(76)90527-3

Caza N, Bewtra JK, Biswas N, Taylor KE (1999) Removal of phenolic compounds from synthetic wastewater using soybean peroxidase. Water Res 33:3012–3018. doi:10.1016/S0043-1354(98)00525-9

Cheng J, Yu SM, Zuo P (2006) Horseradish peroxidase immobilized on aluminium-pillared interlayered clay for the catalytic oxidation of phenolic wastewater. Water Res 40:283–290. doi:10.1016/j.watres.2005.11.017

Dalal S, Gupta MN (2007) Treatment of phenolic wastewater by horseradish peroxidase immobilized by bioaffinity

layering. Chemosphere 67:741–747. doi:10.1016/j.chemosphere.2006.10.043

De Marco E, Savarese M, Paduano A, Sacchi R (2007) Characterization and fractionation of phenolic compounds extracted from olive oil mill wastewaters. Food Chem 104:858–867. doi:10.1016/j.foodchem.2006.10.005

DellaGreca M, Previtera L, Temussi F, Zarrelli A (2004) Low-molecular-weight components of olive oil mill wastewaters. Phytochem Anal 15:184–188. doi:10.1002/pca.766

Derat E, Shaik S (2006) An efficient proton-coupled electron-transfer process during oxidation of ferulic acid by horseradish peroxidase: coming full cycle. J Am Chem Soc 128:13940–13949. doi:10.1021/ja065058d

Duarte-Vázquez MA, Ortega-Tovar MA, García-Almendarez BE, Regalado C (2002) Removal of aqueous phenolic compounds from a model system by oxidative polymerisation with turnip (*Brassica napus* L. var purple top white globe) peroxidase. J Chem Technol Biotechnol 78:42–47. doi:10.1002/jctb.740

Flock C, Bassi A, Gijzen M (1999) Removal of aqueous phenols and 2-chlorophenol with purified soybean peroxidase and raw soybean hulls. J Chem Technol Biotechnol 74:303–309. doi :10.1002/(SICI)1097-4660(199904)74:4<303::AID-JCTB38>3.0.CO;2-B

Geng Z, Rao J, Bassi AS, Gijzen M, Krishnamoorthy N (2001) Investigation of biocatalytic properties of soybean seed hull peroxidase. Catal Today 64:233–238. doi:10.1016/S0920-5861(00)00527-7

González PS, Capozucca CE, Tigier HA, Milrad SR, Agostini E (2006) Phytoremediation of phenol from wastewater, by peroxidases of tomato hairy root cultures. Enzyme Microb Technol 39:647–653. doi:10.1016/j.enzmictec.2005.11.014

Hapiot P, Neudeck A, Pinson J, Fulcrand H, Neta P, Rolando C (1996) Oxidation of caffeic acid and related hydroxycinnamic acids. J Electroanal Chem 405:169–176. doi:10.1016/0022-0728(95)04412-4

Hewson WD, Dunford HB (1976) Oxidation of *p*-cresol by horseradish peroxidase compound I. J Biol Chem 251:6036–6042

Huang Q, Weber WJ (2004) Peroxidase-catalyzed coupling of phenol in the presence of model inorganic and organic solid phases. Environ Sci Technol 38:5238–5245. doi:10.1021/es049826h

Huang Q, Weber WJ (2005) Transformation and removal of bisphenol A from aqueous phase via peroxidase-mediated oxidative coupling reactions: efficacy, products, and pathways. Environ Sci Technol 39:6029–6036. doi:10.1021/es050036x

Karam J, Nicell JA (1997) Potential applications of enzymes in waste treatment. J Chem Technol Biotechnol 69:141–153. doi :10.1002/(SICI)1097-4660(199706)69:2<141::AID-JCTB694>3.0.CO;2-U

Kennedy K, Alemany K, Warith M (2002) Optimisation of soybean peroxidase treatment of 2, 4-dichlorophenol. Water SA 28:149–158

Liu H-L, Wan X, Huang X-F, Kong L-Y (2007) Biotransformation of sinapic acid catalysed by Momordica charantia peroxidase. J Agric Food Chem 55:1003–1008. doi:10.1021/jf0628072

Mantzavinos D, Kalogerakis N (2005) Treatment of olive mill effluents. Part I. Organic matter degradation by chemical and biological processes-an overview. Environ Int 31:289–295. doi:10.1016/j.envint.2004.10.005

Obied HK, Allen MS, Bedgood DR Jr, Prenzler PD, Robards K (2005) Investigation of Australian olive mill waste for recovery of biophenols. J Agric Food Chem 53:9911–9920. doi:10.1021/jf0518352

Oudgenoeg G, Hilhorst R, Piersma SR, Boeriu CG, Gruppen H, Hessing M et al (2001) Peroxidase-mediated cross-linking of a tyrosine-containing peptide with ferulic acid. J Agric Food Chem 49:2503–2510. doi:10.1021/jf000906o

Pati S, Losito I, Palmisano F, Zambonin PG (2006) Characterization of caffeic acid enzymatic oxidation by-products by liquid chromatography coupled to electrospray ionisation tandem mass spectrometry. J Chrom A 1102:184–192. doi:10.1016/j.chroma.2005.10.041

Petrucci R, Astolfi P, Greci L, Firuzi O, Saso L, Marrosu G (2007) A spectroelectrochemical and chemical study on oxidation of hydroxycinnamic acids in aprotic medium. Electrochim Acta 52:2461–2470. doi:10.1016/j.electacta.2006.08.053

Ralph J, Bunzel M, Marita JM, Hatfield RD, Lu F, Kim H et al (2004) Peroxidase-dependent cross-linking reactions of *p*-hydroxycinnamates in plant cell walls. Phytochem Rev 3:79–96. doi:10.1023/B:PHYT.0000047811.13837.fb

Singh A, Billingsley KA, Ward OP (2000) Transformation of polychlorinated biphenyls with oxidative enzymes. Bioproc Eng 23:421–425. doi:10.1007/s004499900121

Takahama U, Hirota S (2000) Deglucosidation of quercetin glucosides to the aglycone and formation of antifungal agents by peroxidase-dependent oxidation of quercetin on browning of onion scales. Plant Cell Physiol 41:1021–1029. doi:10.1093/pcp/pcd025

Tatsumi T, Wada S, Ichikawa H (1996) Removal of chlorophenols from wastewater by immobilized horseradish peroxidase. Biotechnol Bioeng 51:126–130. doi :10.1002/(SICI)1097-0290(19960705)51:1<126::AID-BIT15>3.0.CO;2-O

Tong Z, Qingxiang Z, Hui H, Qin L, Yi Z (1997) Removal of toxic phenol and 4-chlorophenol from waste water by horseradish peroxidase. Chemosphere 34:893–903. doi:10.1016/S0045-6535(97)00015-5

Tong Z, Qingxiang Z, Hui H, Qin L, Yi Z (1998) Kinetic study on the removal of toxic phenol and chlorophenol from waste water by horseradish peroxidase. Chemosphere 37:1571–1577. doi:10.1016/S0045-6535(98)00140-4

Veitch NC (2004) Structural determinants of plant peroxidase function. Phytochem Rev 3:3–18. doi:10.1023/B:PHYT.0000047799.17604.94

Wagner M, Nicell JA (2002) Detoxification of phenolic solutions with horseradish peroxidase and hydrogen peroxide. Water Res 36:4041–4052. doi:10.1016/S0043-1354(02)00133-1

Wilberg K, Assenhaimer C, Rubio J (2002) Removal of aqueous phenol catalysed by a low purity soybean peroxidase. J Chem Technol Biotechnol 77:851–857. doi:10.1002/jctb.646

Wright H, Nicell JA (1999) Characterization of soybean peroxidase for the treatment of aqueous phenols. Biores Tech 70:69–79

Wu Y, Taylor KE, Biswas N, Bewtra JK (1997) Comparison of additives in the removal of phenolic compounds by

peroxidase-catalyzed polymerisation. Water Res 31:2699–2704. doi:10.1016/S0043-1354(97)00119-X

Yamada K, Shibuya T, Noda M, Uchiyama N, Kashiwada A, Matsuda K et al (2007) Influence of position of substituent groups on removal of chlorophenols and cresols by horseradish peroxidase and determination of optimum conditions. Biosci Biotechnol Biochem 71:2503–2510. doi:10.1271/bbb.70298

Yu B-B, Han X-Z, Lou H-X (2007) Oligomers of resveratrol and ferulic acid prepared by peroxidase-catalyzed oxidation and their protective effects on cardiac injury. J Agric Food Chem 55:7753–7757. doi:10.1021/jf0711486

Zhang G, Nicell JA (2000) Treatment of aqueous pentachlorophenol by horseradish peroxidase and hydrogen peroxide. Water Res 34:1629–1637. doi:10.1016/S0043-1354(99)00326-7