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Some kinetic properties of polyphenol oxidase obtained from dill (*Anethum graveolens*)

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Abstract
Polyphenol oxidase (PPO) was partially purified from dill by $(NH_4)_2SO_4$ precipitation followed by dialysis and gel filtration chromatography. Polyphenol oxidase activity was measured spectrophotometrically at 420 nm using catechol, dopamine and chlorogenic acid as substrates. Optimum pH, temperature, and ionic strength were determined with three substrates. The best substrate of dill PPO was found to be chlorogenic acid. Some kinetic properties of the enzyme such as V_{max}, K_M and V_{max}/K_M were determined for all three substrates. The effects of various inhibitors on the reaction catalysed by the enzyme were tested and I_{50} values calculated. The most effective inhibitor was L-cysteine. Activation energies, E_a, were determined from the Arrhenius equation. In addition, activation enthalpy, ΔH_a, and Q_{10} values of the enzyme were also calculated.

Keywords: *Polyphenol oxidase, dill,* Anethum graveolens*, kinetic properties, activation energy, inhibition*

Introduction

Polyphenol oxidase (PPO) enzyme is a major enzyme which is responsible for the browning reaction in damaged plant tissues and fruits [1,2,3]. This enzyme catalyzes two distinct reactions involving molecular oxygen [4,5]: (i) the o-hydroxylation of monophenol (monopheolase activity) and (ii) the oxidation of *o*-diphenol to *o*-quinones (diphenolase activity). The kinetic reaction mechanism of polyphenol oxidase has been elucidated [6,7,8]. When plant tissues are damaged, enzymatic browning, which is catalyzed by PPO, occurs. This is an economic problem for producers, processors and consumers. The main step in enzymatic browning is the oxidation of phenolic compounds to corresponding quinones by PPO in the presence of oxygen. The quinones then condense to form darkened pigments [9,10]. These reactions known as enzymatic browning are not generally desirable for the food industry, but can be used for preparation of dark tea. Dill has been used increasingly as a seasoning vegetable [11]. An important food product in the east Anatolian part of Turkey, Northeastern Europe and Russia is herb cheese. A number of herbs, such as *Thymus* sp., *Allium* sp., *Ferula* sp. and dill (*Anethum graveolens*) are used in making herb cheese. Especially, dill is used as a digestive, gas expectorant, degasifier, spasm remover, urine disposing, milk increasing and general regulator. In addition to these applications, dill is a rich source of antioxidative material [12,13,14]. There are many works related to PPO from different plants such as wheat [15], Allium sp. [19], *sorghum* [17], Beta vulgaris L. [18], tea leaf [19], lettuce [20], dog-rose [21], F*erula* sp. [22], A*nethum graveolens L.* [23], peaches [24], apples [25,26,27,28], grapes [29,30,31], kiwis [32], *Salvia species* [33] and *Thymus* [34].

To the best of our knowledge, the kinetic properties of PPO obtained from dill have not studied. Our purpose was to study the activation energy (E_a), activation enthalpy(ΔH_a), and Q_{10} of PPO with catechol, dopamine, and chlorogenic acid as substrates under optimum conditions.

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Materials and methods

Plant material

Dill (*Anethum graveolens*) used in this study was obtained fresh in local markets in Turkey, and kept for 2 days in the refrigerator at 4°C before PPO extraction. All chemicals used in this study were of analytical grade and used without further purification.

Enzyme extraction and purification

12 g of dill leaves was cleaned and prepared for extraction. The leaf sample was immersed in liquid nitrogen, in a Dewar flask to disrupt cell membranes, then ground to a powder using mortar and pestle. The frozen powdered material was added to 25 mL of 0.5 M phosphate buffer containing 0.5% polyvinylpyrrolidone (PVP) at pH 6.5 and 10 mM ascorbic acid and mixed with a magnetic stirrer for 4 min at 4°C. The crude extract was filtered through cotton gauze and the filtrate was centrifuged at 13,500 × g for 30 min at 4°C. The supernatant was tested between 0–20%, 20–40%, 40–60% and 60–80% to find the required saturation point with solid $(NH_4)_2SO_4$. The precipitated PPO in the 20–60% $(NH_4)_2SO_4$ fraction was separated by centrifuging at 13,500 × g for 30 min. The pellet was dissolved in 6.0 mL of 0.2 M phosphate buffer pH 6.5 and dialysed at 4°C in the same buffer for 24 h with three changes of buffer during dialysis. The dialysed sample was used as the PPO enzyme source in the following experiments [35].

For further purification of PPO, the dialyzed enzyme was fractionated by gel filtration chromatography. A column with 100 mL bed volume was prepared using Sephadex G-100 and equilibrated with 0.05 M phosphate buffer pH 6.5. The dialyzed enzyme solution was applied to the column and eluted with the equilibration buffer at an elution rate of 15–20 mL/h.

The column eluate was collected in test tubes as 4 mL volume and elution continued until zero absorbance was obtained at 280 nm. Each eluate fraction that showed absorbance at 280 nm was assayed for PPO activity (see Figure 1). A_{280} and PPO activity were plotted against the tube number. The fractions having PPO activity were collected and degree of purification was determined by measuring specific activity before and after purification. Specific activity was determined from PPO activity and quantitative protein determination using the Bradford protein dye-binding method [36].

Determination of PPO activity

PPO activity was determined by measuring the increase in absorbance at 420 nm with a spectrophotometer (LKB Biochrom Ultrospec II). The sample cuvette contained 100 μL of the enzyme solution and 2.90 mL of substrate solution in various concentrations.

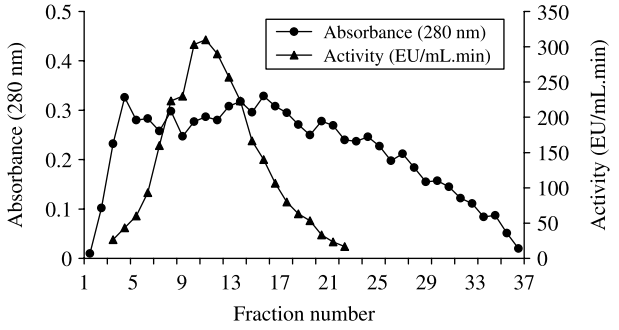

Figure 1. Purification of polyphenol oxidase from Dill by gel filtration chromatography on Sephadex G-100.

The blank sample contained only 3 mL of substrate solution. The reaction was carried out at various temperatures and pH values with the substrates mentioned below. PPO activity was calculated from the linear part of the curve giving absorbance values at 420 nm versus time [37,38,39,40]. One unit of PPO activity (EU/mL.min) was defined as the amount of enzyme that caused an increase in absorbance of 0.001/min [41,42].

Enzyme kinetics

Effect of substrate concentration. PPO activities were measured with three different substrates at varying concentrations (0.33, 0.83, 1.67, 2.50, 3.33, and 4.17 mM for catechol and dopamine; 0.11, 0.28, 0.55, 0.83, 1.11, and 1.39 mM for chlorogenic acid) under optimum conditions of pH, ionic strength, and temperature. For the determination of Michaelis constant (K_M) and maximum velocity (V_{max}) values and V_{max}/K_M ratio of the enzyme, K_M and V_{max} values of PPO for each substrate were calculated from a plot of 1/v against 1/[S] by the method of Lineweaver and Burk [43]. The catalytic power of the enzyme was determined from the relationship V_{max}/K_M [33,44].

Effect of pH. PPO activity was determined with three different substrates (catechol, dopamine, and chlorogenic acid) at a concentration of 10 mM. Appropriate buffers (0.1 M citrate/0.2 M phosphate for pH 4.0–5.5, 0.2 M phosphate for pH 5.5–7.0, and Tris–HCl for pH 7.0–9.0) were used for the determination of optimum pH of PPO. The optimum pH values obtained from this assay were used in all subsequent experiments.

The stability of the enzyme was determined by measuring activity in the various buffers [0.1 M citrate/0.2 M phosphate for pH 3.5–5.0, 0.2 M phosphate for pH 5.5–7.5] every 3 days using catechol as substrate under optimum conditions (pH, temperature and ionic strength).

Effect of temperature. PPO activity was measured at different temperatures in the range from 0 to 85°C using the 3 different substrates to determine the optimum temperature of the enzyme. The desired conditions were provided by using a Polyscience bath (Model 9105).

Effect of ionic strength. Ionic strength effect on the enzyme was studied with 10 mM concentration of substrates using 0.062, 0.125, 0.500, and 1.00 M concentrations of K-phosphate buffer at optimum pH of the substrates.

Activation energy (E_a), activation enthalpy (ΔH_a), and Q_{10} determination. The activation energy is calculated from experimental results obtained for enzyme reactions by using the Arrhenius equation, i.e.

$$\ln k = \ln A - E_a/RT$$

where k is the enzyme activity value (EU/mL.min), A is the frequency factor or pre-exponential factor, Ea is the activation energy (joule/mol), T is the temperature (K) and R is the gas constant ($8.314\,J\,K^{-1}\,mol^{-1}$). The graph of lnk versus 1/T gave a straight line. The parameter A was obtained from the intercept at $1/T = 0$ and the activation energies of reactions was calculated from the slopes of the lines [33,45,46].

PPO activities of dill were measured at different temperatures in the range 5–85°C and the activation energy (E_a), activation enthalpy (ΔH_a), and Q_{10} (increase in reaction rate per 10°C increase in temperature) values of the enzyme were determined. The desired temperatures were provided by using a constant-temperature circulator for temperatures above 20°C. In this experiment, except for PPO, all the substances of the activity assay medium, were brought to the desired temperature (5–85°C), and the reaction was initiated by the addition of enzyme. Then, a graph of lnk versus 1/T was drawn for each substrate and E_a values were calculated from these graphs. ΔH_a was calculated at 25°C (T = 298,15 K) from the formula $\Delta H_a = E_a - RT$ [47,48]. Q_{10} values of the enzyme are obtained by dividing higher activity values by lower activity values of measurements performed at two different temperatures that were 10 degrees apart [49,50].

Effect of inhibitors. Inhibitor effects on PPO activity were studied by using the following inhibitors: L-cysteine, glutathione, and ascorbic acid at five different concentrations of inhibitors with 10 mM catechol substrate at pH 6.5. Percent activity graphs were drawn from these results to find I_{50} values. Later, using 5 different concentrations of the substrates, PPO activities were measured at 3 different constant inhibitor concentrations with the inhibitors indicated above. Lineweaver–Burk graphs of this data were used to determine K_i (dissociation constant) for each inhibitor.

Results and discussion

Extraction and purification of PPO

The extraction of PPO was carried out in 0.5 M phoshpate buffer pH 6.5, containing 0.5% polyvinylpyrrolidone and 10 mM ascorbic acid and preciptated by $(NH_4)_2SO_4$ method. PPO activity was found to be the highest in the precipitate of the 20–60% $(NH_4)_2SO_4$ fraction and this saturation point was used for all the extraction processes. Polyvinylpyrrolidone was used during extractions to bind the phenols which could inactivate the PPO. It is well documented that oxidation of phenolic compounds by PPO produces quinones which would inhibit PPO. Therefore, ascorbic acid was used to reduce quinonic compounds back to the phenolic compounds during extraction.

Results for the purification of PPO are given in Table I. When the purification steps were compared, there was 2.18 fold purification after ammonium sulfate precipitation, and 2.84 fold purification after gel filtration chromatography. Plots of absorbance at 280 nm and PPO enzyme activity of eluate fractions from gel filtration chromatography are shown in Figure 1. Fractions 5–16 were pooled for the determination of purification degree.

Table I. Purification of polyphenol oxidase from dill

Purification steps	Total volume (mL)	Activity (EU/mL)	Total activity (EU)	Protein (mg/mL)	Total protein (mg)	Specific Activity (EU/mg of protein)	Yield (%)	Purification n-fold
Crude extract	67.00	747	50049	0.16	10.52	4757.96	100.00	1.00
$(NH_4)_2SO_4$ precipitation and dialysis	22.33	1680	37514	0.16	3.62	10370.37	74.95	2.18
Gel filtration chromatography	120.30	230	27669	0.02	2.05	13530.07	55.28	2.84

Table II. Optimum pH and temperature, and K_M and V_{max} and V_{max}/K_M values of PPO dill

Substrate	Optimum pH	Optimum temperature (°C)	K_M (M)	V_{max} (EU/mL.min)	V_{max}/K_M (EU/mmol.s)
Catechol	6.5	10	2.17×10^{-3}	774.30	5947
Dopamine	8.5	55	1.67×10^{-3}	66.90	668
Chlorogenic acid	5.5	10	8.35×10^{-4}	464.30	9267

Effect of pH on activity

Activity of purified enzyme was measured with 3 different substrates to determine optimum pH for each substrate (Table II). Optimum pH with catechol substrate was found to be phosphate buffer pH 6.5, Tris–HCl buffer pH 8.5 with (+) dopamine, and phosphate buffer pH 5.5 with chlorogenic acid.

In general, most plants, vegetables and fruits show maximum activity at or near neutral pH values [37,51,52]. Different optimum pHs for PPO obtained from various sources are reported in the literature. For example, it is reported that optimum pH values are 5.5 for strawberry [35], 6.0 for DeChaunac grape [53], 7.0 for Amasya apple [28], aubergine [45], Yali pear [54], and *Ferula sp.* [22], 7.5 for *Allium* sp. [16] and 8.5 for dog-rose [21], using catechol as a substrate. However, when using 4-methylcatechol as a substrate the pH optimum is 4.5 for strawberry [35], 6.0 for aubergine [45], 8.5 for dog-rose [21] and 9.0 for Amasya apple [28] and 8.5 for dog-rose [21] using dopamine as a substrate and 7.0 for dog-rose [21] and 8.6 for Amasya apple [28] using pyrogallol as a substrate. PPO activity varies with the source of enzyme and substrate within a relatively wide range of pH. Although, in most cases, pH optima have been reported between 4.0 and 7.0, it should be noted that the optimum pH can also be affected by the type of buffer and the purity of enzyme [45].

Stability of PPO activity was studied when pH value was ranging from 4.0–7.0 over a period of 35 days by using catechol as substrate. It was found that PPO activity of dill was highest at pH 6.5, but decreased at similar rates at each of the pH values studied (Figure 2).

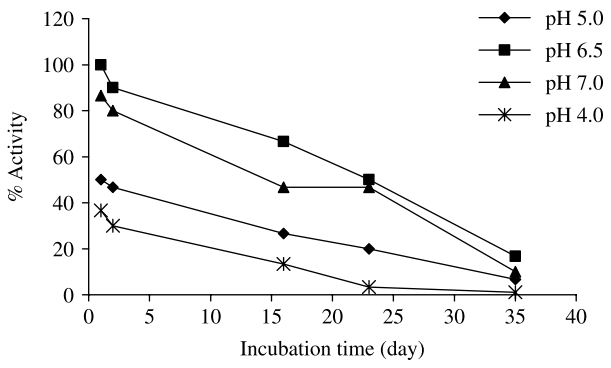

Figure 2. Stability of PPO activity at various pH values using catechol as a substrate.

The optimum ionic strength of the enzyme was estimated to be 0.5 M for catechol and dopamine, and 0.25 M for chlorogenic acid substrates. Each measurement was performed at optimum pH of the substrates with different concentrations of K-phosphate buffer.

Effect of temperature on activity

The temperature effects on PPO activity of dill were studied betwen 5°C and 85°C with each of the three substrates used in the experiments (Table II). As seen in the table, optimum temperatures are substrate-dependent. It is found that the optimum temperature is 10°C for catechol and chlorogenic acid, and 55°C for dopamine. It is reported that optimum temperature for PPO is 15°C for Amasya apple [28], 20°C for DeChaunac grape [53], 25°C for dog-rose [21], 30°C for aubergine [45], 12°C for *Ferula* sp. [22], and 40°C for Chinese cabbage [55], using catechol as a substrate, 20°C for dog-rose [21], 30°C for aubergine [45], 25°C for *Ferula* sp. [22], and 56°C for Amasya apple [29], using 4-methylcatechol as a substrate, and 15°C for dog-rose [21] and 70°C for Amasya apple [28] using pyrogallol as a substrate.

Enzyme kinetics and substrate specificity

In this study, we selected three widely used substrates (catechol, dopamine and chlorogenic acid) for kinetics studies. K_M and V_{max} values were calculated from the Lineweaver–Burk graphs for substrates and are shown in Table II. As seen in Table II, the PPO of dill has a great affinity towards chlorogenic acid ($K_M = 8.35 \times 10^{-4}$ M) of the three catechol derivatives tested [56], as seen from the high V_{max}/K_M ratio (9267 EU/mmol.s). When the V_{max} values for the three substrates are compared, it was found that the V_{max} for catechol was higher than for the other substrates. Consequently, catechol was used as substrate in the other kinetics studies [57]. At the short assay times used, these substrates do not originate significant suicide inactivation of polyphenol oxidase [58,59].

This observation was similar to that of the work on PPO from dog-rose [21], *ferula* sp. [22], and Amasya apple [28]. There are a number of compounds such as dopamine [21,60], catechol [21,28,35,45], chlorogenic acid, L-dopa [21,28,35,60], pyrogallol [21,28,35,60], caffeic acid [35,53], *p*-cresol [21,35,60], tyrosine [21,53], and 4-methylcatechol [21,28,35,45] used as substrates for polyphenol oxidase in the literature.

Table III. Activation Energy (E_a), Activation Enthalpy (ΔH_a), and Q_{10} values calculated for PPO dill

Substrate	Slope E_a/R	Activation Energy (E_a) (kJ/mol)	Activation Enthalpy (ΔH_a) (kJ/mol)	Q_{10}	R^2
Catechol	1739.60	−14.46	−16.94	1.22	0.9839
Dopamine	−1472.90	12.25	9.77	1.21	0.9858
Chlorogenic acid	905.40	−7.53	−10.01	1.10	0.9817

Table IV. I_{50} and K_i values and inhibition modes for 3 inhibitors for PPO from dill

Inhibitors	I_{50} (M)	[I] (M)	K_i (M)	Average values of K_i (M)	Type of inhibition
Ascorbic acid	4.26×10^{-5}	3.33×10^{-5}	6.47×10^{-5}	6.04×10^{-5}	Noncompetitive
		6.66×10^{-5}	6.92×10^{-5}		
		1.00×10^{-4}	4.73×10^{-5}		
Glutathion	3.25×10^{-5}	5.00×10^{-5}	8.40×10^{-5}	8.49×10^{-5}	Noncompetitive
		6.66×10^{-5}	8.97×10^{-5}		
		8.33×10^{-5}	8.09×10^{-5}		
L-Cysteine	2.04×10^{-5}	2.22×10^{-5}	2.92×10^{-5}	2.67×10^{-5}	Noncompetitive
		3.33×10^{-5}	2.89×10^{-5}		
		4.44×10^{-5}	2.20×10^{-5}		

Activation energy (E_a), activation enthalpy(ΔH_a), and Q_{10} determination

The activation energies were calculated in a temperature range from 10°C to 75°C for catechol, dopamine and chlorogenic acid. For each substrate, Arrhenius plots were constructed by using temperature-activity values. Activation energies were calculated from slopes (Table III). In view of the fact that optimum temperature was 10°C for catechol and chlorogenic acid and 55°C for dopamine, it is reasonable that the E_a for dopamine would be positive and E_a negative for the other two substrates. The activation enthalpies were calculated and are shown in Table III. Q_{10} values were also calculated in the temperature range 10–75°C for catechol, dopamine and chlorogenic acid, and are given in Table III. These results are consistent with the literature finding [32,33,61].

Effect of inhibitors

I_{50}, K_i values and inhibition modes for three inhibitors are given in Table IV. From the Lineweaver-Burk plots, it was concluded that the inhibition modes for all three inhibitors are noncompetitive. The strongest inhibitor was found to be L-cysteine [62]. As seen from the I_{50} values, L-cysteine was the most effective inhibitor for dill, followed by ascorbic acid and glutathione, respectively.

There are a number of inhibitors, such as sodium metabisulphite [21,53,63], ascorbic acid [21,53,63,64], glutathione [32,45,53], sodium diethyldithiocarbamate [32,53,54], L-cysteine, sodium azide, tannic acid, benzoic acid and β-mercaptoethanol [21] used by researchers to prevent enzymatic browning.

L-cysteine can easily form complexes with quinones and, thereby, inhibiting secondary oxidation and polymerisation reactions [65]. L-cysteine can also act as a reducing agent [35]. Ascorbic acid reduces quinones to hydroquinones and does not directly inhibit PPO [66]. It will prevent enzymatic browning only as long as it is present in the reduced form. This prevents the formation of key intermediates and inhibits the activity of the oxidase [67].

Conclusion

The characterization of PPO is important because of the browing reactions, which occur in fruits and vegetables. In this respect, PPO enzyme of dill was partially purified and PPO activity was measured spectrophotometrically using selected substrates and inhibitors. The best substrate of PPO enzyme was found to be chlorogenic acid (high V_{max}/K_M ratio) while L-cysteine is the most effective inhibitor. The three inhibitors used in this study caused inhibition effects at a concentration of 10^{-5} M and can be used safely in making herb cheese. In addition, activation energies (E_a), activation enthalpy (ΔH_a), and Q_{10} values of the enzyme were calculated.

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References

[1] Mathew AG, Parpia HAB. Adv Food Res 1971;19:75–145.
[2] Richard-Forget C, Gauillard A. J Agric Food Chem 1997;45: 2472–2476.
[3] Singh HP, Ravindanath SD. J Sci Food Agric 1994;64(1): 117–120.

[4] Halaouli S, Asther M, Sigoillot JC, Hamdi M, Lomascolo A. J Appl Microbiol 2006;100(2):219–232.
[5] Mayer AM. Phytochemistry 2006;67(21):2318–2331.
[6] Espin JC, Garcia-Ruiz PA, Tudela J, Varon R, Garcia-Canovas F. J Agric Food Chem 1998;46(8):2968–2975.
[7] Fenoll LG, Penalver MJ, Rodriguez-Lopez JN, Varon R, Garcia-Canovas F, Tudela J. Int J Biochem Cell Biol 2004; 36(2):235–246.
[8] Penalver MJ, Fenoll LG, Rodriguez-Lopez JN, Garcia-Ruiz PA, Garcia-Molina F, Varon R, Garcia-Canovas F, Tudela J. J Mol Catal B-Enz 2005;33(1–2):35–42.
[9] Matheis G. Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung 1983;176:454–462.
[10] Gowda LR, Paul B. J Agric Food Chem 2002;50:1608–1614.
[11] Slaon AE. Food Technol 2004;58:16.
[12] Lisiewska Z, Kmiecik W, Korus A. J Food Comp Anal 2006; 19:134–140.
[13] Zheng GQ, Kenney PM, Lam LKT. Planta Medica 1992; 58(4):338–341.
[14] Al-Ismail KM, Aburjai T. J Sci Food Agric 2004;84(2): 173–178.
[15] Okot-Kotber M, Liavoga A, Yong KJ, Bagorogoza K. J Agric Food Chem 2002;50(8):2410–2417.
[16] Arslan O, Temur A, Tozlu I. J Agric Food Chem 1997;45: 2861–2863.
[17] Dicko MH, Hilhorst R, Gruppen H, Traore AS, Laane C, Van Berkel WJH, Voragen AGJ. J Agric Food Chem 2002; 50(13):3780–3788.
[18] Escribano J, Gandia-Herrero F, Caballero N, Pedreno MA. J Agric Food Chem 2002;50:6123–6129.
[19] Hadler J, Tamuli P, Bhaduri AN. J Nutr Biochem 1998;9: 75–80.
[20] Chazarra S, Cabanes J, Escribano J, Garcia-Carmona F. J Agric Food Chem 1996;44:984–988.
[21] Sakiroglu H, Küfrevioglu IÖ, Kocacaliskan I, Oktay M, Onganer Y. J Agric Food Chem 1996;44:2982–2986.
[22] Erat M, Sakiroglu H, Kufrevioglu OI. Food Chem 2006;95: 503–508.
[23] Arslan O, Tozlu I. Italian J Food Sci 1997;9(3):249–253.
[24] Flurkey WH, Jen JJ. J Food Biochem 1980;4:29–41.
[25] Murata M, Kurokami C, Homma S. Biosci Biotechnol Biochem 1992;56:1705–1710.
[26] Murata M, Kurokami C, Homma S, Matsuhashi C. J Agric Food Chem 1993;41:1385–1390.
[27] Murata M, Tsurutani M, Tomita M, Homma S, Kaneko K. J Agric Food Chem 1995;43:1115–1121.
[28] Oktay M, Kufrevioglu I, Kocacaliskan I, Sakiroglu H. J Food Sci 1995;60:495–499.
[29] Lamikanra O, Sharon DK, Mitwe NM. J Food Sci 1992;57: 686–689.
[30] Wissemann KW, Lee CY. J Food Sci 1981;46:506–514.
[31] Yılmaz H, Sakiroglu H, Küfrevioglu I. Agrochimica 2003; XLVII-N.1–2:21–27.
[32] Park EY, Luh BS. J Food Sci 1985;50:679–684.
[33] Gundoğmaz G, Doğan S, Arslan O. Food Sci Technol Int 2003;9(4):309–316.
[34] Dogan S, Dogan M. Food Chemistry 2004;88(1):69–77.
[35] Wesche-Ebeling P, Montgomery MW. J Food Sci 1990;55: 1320–1325.
[36] Bradford MA. Anal Biochem 1976;72:248–254.
[37] Wong TC, Luh BS, Whitaker JR. Plant Physiol 1971;48:19.
[38] Muchuweti M, Mupure CH, Ndhlala AR, Kasiyamhuru A. J Sci Food Agric 2006;86:328–332.
[39] Espin JC, Varon R, Tudela J, Garcia Canovas F. Biochem Mol Biol Int 1997;41(6):1265–1276.
[40] Munoz JL, Garcia-Molina F, Varon R, Rodriguez-Lopez JN, Garcia-Canovas F, Tudela J. J Anal Biochem 2006;351(1): 128–138.
[41] Benjamin ND, Montgomery MW. J Food Sci 1973;38: 799–806.
[42] Fenoll LG, Rodriguez-Lopez JN, Garcia-Molina F, Garcia-Canovas F, Tudela J. IUBMB Life 2002;54(3):137–141.
[43] Lineweaver H, Burk D. J Amer Chem Soc 1934;56:658–666.
[44] Gandia-Herrero F, Jimenez-Atienzar M, Cabanes J, Garcia-Carmona F, Escribano J. Biol Chem 2005;386:601–607.
[45] Dogan M, Arslan O, Dogan S. Int J Food Sci Technol 2002; 37:415–423.
[46] Atkins PW. Physical Chemistry. New York: W.H. Freeman Company; 1990.
[47] Kanade SR, Paul B, Appu Rao AG, Gowda LR. Biochem J 2006;395(Pt 3):551–562.
[48] Onganer Y, Sakiroglu H, Küfrevioglu OI. Chimica Acta Turcica 1998;26:41–46.
[49] Peier AM, Alison JR, David AA, Moqrich A, Earley TJ, Hergarden AC, Story GM, Colley S, Hogenesch JB, McIntyre P, Patapoutian S, Bevan AA. Science 2002;296 (5575):2046–2049.
[50] Özer N, Bilgi C, Ögüs IH. Int J Biochem Cell B 2002;34: 253–262.
[51] Siddiq M, Sinha NK, Cash JN. J Food Sci 1992;57: 1177–1179.
[52] Cash JN, Sistrunk WA, Stutte CA. J Food Sci 1976;41: 1398–1402.
[53] Lee CY, Smith NL, Penesi AP. J Sci Food Agric 1983;34: 987–991.
[54] Zhou H, Feng X. J Sci Food Agric 1991;57:307–313.
[55] Nagai T, Suzuki N. J Agric Food Chem 2001;49:3922–3926.
[56] Munoz J, Garcia-Molina F, Varon R, Rodriguez-Lopez JN, Garcia-Ruiz PA, Garcia-Canovas F, Tudela J. J Agric Food Chem 2007;55(3):920–928.
[57] Baritaux O, Amiot MJ, Richard H, Nicolas JJ. Sciences des Aliments 1991;11:49–62.
[58] Canovas FG, Tudela J, Madrid CM, Varon R, Carmona FG, Lozano JA. Biochim Biophys Acta 1987;912(3):417–423.
[59] Tudela J, Canovas FG, Varon R, Jimenez M, Garciacarmona F, Lozano JA. Biophys Chem 1988;30(3):303–310.
[60] Nagai T, Suzuki N. J Agric Food Chem 2001;49:3922–3926.
[61] Aragba SS, Ajiboye OL, Ugboko LA, Essienette SY, Afolabi PO. J Sci Food Agric 1998;77:459–462.
[62] Garcia-Molina F, Penalver MJ, Rodriguez-Lopez JN, Garcia-Canovas F, Tudela J. J Agric Food Chem 2005; 53(16): 6183–6189.
[63] Augustin MA, Ghazali HM, Hashim H. J Agric Food Chem 1985;36:1259–1265.
[64] Yang C-P, Fujita S, Kohno K, Kusaboyashi A, Ashrafuzzaman MA, Hayashi N. J Agric Food Chem 2001;49(3):1446–1449.
[65] Davis R, Pierpoint WS. Biochem Soc Trans 1975;3:671.
[66] Anderson JW. Phytochemistry 1968;7:1973.
[67] Schwartz B, Olgin AK, Klinman JP. Biochemistry 2001;40: 2954–2963.