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# EFFECTIVENESS OF SOME CROWN COMPOUNDS ON INHIBITION OF POLYPHENOLOXIDASE IN MODEL SYSTEMS AND IN APPLE

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Enzymatic browning is (in most cases) an undesirable reaction which usually impairs the sensory properties and chemical changes in raw fruits and vegetables after mechanical operations (such as peeling, coring or slicing).

A great emphasis is put on research to develop new methods to prevent enzymatic browning especially in fresh-cut (minimally processed) fruits and vegetables.

The inhibition effect of crown compounds, macrocyclic ethers, benzo-18-crown-6 with sorbic acid and benzo-18-crown-6 with potassium sorbate, on polyphenoloxidase (PPO) activity was studied. The effectiveness of these compounds was evaluated by using 3,4-dihydroxy phenylalanine (L-DOPA), and chlorogenic acid (3-*o*-caffeoyl-D-quinic acid), the most widespread natural PPO substrates in fruits and vegetables, as well as browning inhibition substances on the cut surface of apples.

Results showed that crown compounds used in this study were effective, both as inhibitors of the oxidation of phenolic compounds (PPO substrates) in model solutions and as inhibitors of enzyme discolorations of real systems (fresh-cut apples).

In the earlier published papers (VUKOVIĆ et al., 1999) the synthesis of crown compound used in this study was presented.

**Keywords:** crown compounds, enzymatic browning, inhibition, polyphenoloxidase

The colour of foods is one of the most important properties which can be crucial for acceptance of some products. During fruits and vegetables processing and storage of their products numerous reactions occur. Such reactions can have influence on food colour and in general on food quality. Among them, browning discolourations, both, enzymatic and nonenzymatic, are of great importance in food industry.

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*Enzymatic browning*

The half of the world's fruit and vegetable crops is lost due to postharvest deteriorative reactions (MARTINEZ & WHITAKER, 1995). Among reactions of deterioration of fruits and vegetables and their products is enzymatic browning (SAPERS, 1993). In most cases enzymatic browning is undesirable. Enzymatic browning is a reaction that results when phenolic compounds of plants, in the presence of polyphenol oxidase (PPO) and molecular oxygen, are oxidized to o-quinones (MARQUES et al., 1995; SAPERS, 1993).

Browning occurs rapidly when the cellular integrity of plant tissues is disrupted by either physical injury during peeling, coring and slicing or physiological disorders such as scald, bitter pit and watercore (LUO & BARBOSA-CANOVAS, 1996). When this happens the phenolic compounds interact with PPO and atmospheric oxygen.

Since the sulfites are limited in their use to certain categories of food products in some countries, because they have been associated with severe allergy-like reactions in some asthmatics (TAYLOR & BUSH, 1986), food processors have turned to a number of sulfite alternatives as new enzymatic browning inhibitors (MOLNÁR-PERL & FRIEDMAN, 1990; SAPERS, 1993). At least three different modes of action for inhibitors can be considered (proposed): (1) the direct inhibition of the enzyme PPO, (2) the chemical reduction of o-quinones back into o-diphenolic compounds, and (3) the manipulation or removal of the phenolic substrates of PPO (VÁMOS-VIGYÁZÓ, 1981).

The objective of this research was to study the effectiveness of new crown compounds, macrocyclic ethers, benzo-18-crown-6 with sorbic acid (BSA), and benzo-18-crown-6 with potassium sorbate (BPS) as potential inhibitors of enzyme discolouration in model solutions with PPO substrates, L-DOPA and chlorogenic acid, as well as inhibitors of enzymatic browning of fresh-cut apples (Idared variety).

## 1. Materials and methods

*1.1. Chemicals*

For this study crown compounds have been prepared following the method described in the earlier published paper by VUKOVIĆ and co-workers (1999). The macrocyclic ether benzo-18-crown-6 is a part of compounds used in this study. This macrocyclic ether with sorbic acid (BSA) and potassium sorbate (BPS) forms stable complexes which showed ability (possible practical use) as inhibitors of PPO activity.

Mushroom polyphenol oxidase (PPO; EC 1.14.18.1) product T-7755, with activity of 3000 U $mg^{-1}$ of solid (Sigma Chemical Co., St. Louis, MO, USA) as a dry powder was dissolved in phosphate buffer (pH 6.5, 47 mmol).

PPO substrates, L-DOPA (Sigma), and chlorogenic acid (Sigma) were prepared as 2.5 mmol solutions by disolving in phosphate buffer (pH 6.5).

*1.2. Activity assay*

The enzyme activity was determined by spectrophotometer CECIL 2000 (CECIL INSTRUMENTS, England). Reaction mixture, in a total volume of 3 ml, included L-DOPA (2.5 mmol), enzyme (16 μg $ml^{-1}$) dissolved in phosphate buffer (pH 6.5, 47 mmol) and certain concentration of solution of complex compounds, BSA (0.34 to 7.5 mmol) or BPS (0.5 to 5.0 mmol). As reference ("blank") a solution without inhibitors addition was used.

Before measurements, reaction solution (PPO substrate and crown compound in phosphate buffer) were thermostated for 20 min at 25 °C. The enzyme solution was added to treatment solution before measurement.

The appearance of the brown colour, in the case of the L-DOPA was measured at 475 nm as a function of time (for 10 min), and in the case of the chlorogenic acid at 420 nm. The enzyme activity was calculated from the initial part of the curve of the absorption vs reaction time (ΔA/min) (WEEMAES et al., 1997). Measured values were transformed into % inhibition.

*1.3. Apple preparation for browning measurements*

Apples (Idared variety) were purchased from the local growers and kept at 4 °C until needed. Before sample preparation, apples were held at least one hour at room temperature. To prepare samples, apples were washed with water, peeled and cut into dices (1 cm×1 cm×1 cm) with a sharp knife. One hundred g samples were immediately immersed for 20 s in treatment solution, 0.5% w/v BSA and 0.5% w/v BPS. The excess solution was then blotted with adsorbent tissue and samples were packed in plastic boxes, covered with plastic film to prevent evaporation and stored at 4 °C.

*1.4. Treatment evaluation*

*1.4.1. Inhibition of PPO substrates oxidation.* The inhibition of oxidation of PPO substrates (L-DOPA and chlorogenic acid prepared as model solutions) with crown compounds, that was used in this study, was based on a large number of studies which have established that copper ions ($Cu^{2+}$) can be captured in the cavity of a crown compounds (PEDERSEN & FRENDSDORFF, 1972; HIRAOKA, 1982). The ionic diameter of $Cu^{2+}$ has a value 1.44 Å and is therefore able to form stable complexes with BSA and BPS, which have the cavity diameter between 1.7 and 2.2 Å (PEDERSEN & FRENDSDORFF, 1972). The crown compounds that we used in this study showed their inhibitory effect on mushroom PPO.

To compare the effectiveness of these crown compounds in inhibition of PPO activity, sorbic acid and potassium sorbate, as a part of molecule of crown compounds, were also used.

Inhibition of PPO in model solutions was determined spectrophotometrically (spectrophotometer CECIL 2000, England) at 475 nm for L-DOPA for 10 min, and at 420 nm for chlorogenic acid for 10 min.

*1.4.2. Browning assessment.* The degree of browning of apple samples was monitored by reflectance measurements immediately after cutting, treatment with crown compounds solutions ("0" time), and during storage of apple samples (untreated and treated) on day 4, 7, and 11. L*, a*, and b* values were measured by the tristimulus colorimeter Minolta CR-300 (Minolta Camera Co., Osaka, Japan) using the averaging mode with fifteen replications. Based on the measured data the calculation of effectiveness of each inhibitor was performed by equation:

$$\Delta E_{ab} = ((\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2)^{1/2}$$

## 2. Results and discussion

The effectiveness of crown compounds BSA and BPS on inhibition of oxidation of substrates L-DOPA and chlorogenic acid is shown as the concentration of the inhibitors ($I_{50}$) that causes 50% inhibition of PPO activity (Tables 1 and 2).

The results showed that BSA (5.0 mmol) had an inhibitory effect of 89.7% on oxidation of L-DOPA (2.5 mmol) in the presence of PPO (16 μg $ml^{-1}$) (Fig. 1). When chlorogenic acid (2.5 mmol) was used as a PPO substrate, the BSA (5.0 mmol) had 79.3% reducing effect on its oxidation (Table 2).

Table 1

*The effectiveness of different inhibitors on PPO activity in solutions of L-DOPA*

| Inhibitors | $I_{50}$ (mmol)[a] |
|---|---|
| Complex of benzo-18-crown with potassium sorbate | 0.720 |
| Complex of benzo-18-crown with sorbic acid | 0.870 |
| Sorbic acid | 1.350 |
| Potassium sorbate | 2.530 |

[a] $I_{50}$: the concentration of the inhibitors that causes 50% inhibition of PPO activity

Table 2

*The effectiveness of different inhibitors on PPO activity in solutions of chlorogenic acid*

| Inhibitors | $I_{50}$ (mmol)[a] |
|---|---|
| Complex of benzo-18-crown with potassium sorbate | 1.520 |
| Complex of benzo-18-crown with sorbic acid | 1.760 |
| Sorbic acid | 2.650 |
| Potassium sorbate | 2.530 |

[a] $I_{50}$: the concentration of the inhibitors that causes 50% inhibition of PPO activity

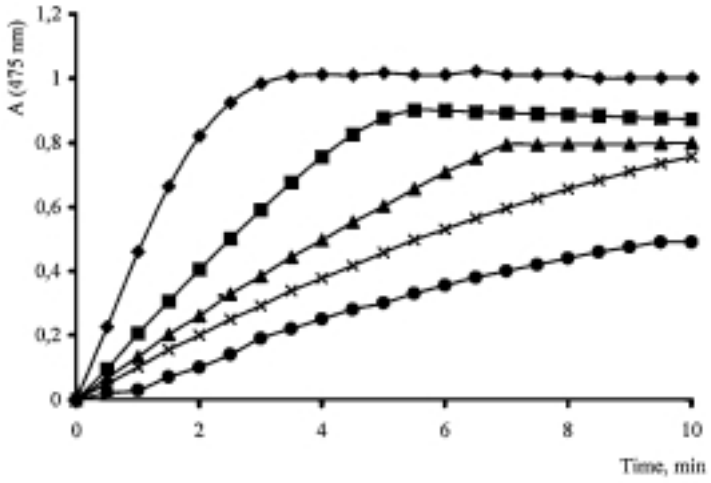


Fig. 1. Effect of different concentrations (0.5 mmol to 5.0 mmol) of complex crown compound benzo-18-crown-6 with potassium sorbate on PPO activity in solution of L-DOPA. ◆: Control; ■: 0.5 mmol BPS; ▲: 1.0 mmol BPS; x: 3.0 mmol BPS; ●: 5.0 mmol BPS

When the solution of BPS (5.0 mmol) was used as an inhibitor of oxidation of L-DOPA (2.5 mmol) it had 90.3% reducing effect on PPO activity. When chlorogenic acid was used as PPO substrate, BPS reduced PPO activity by 80.5%.

The effect of crown compounds (BSA and BPS), as potential browning inhibitors on apple dices is presented in Table 3.

Table 3

*Effect of crown compounds, as potential browning inhibitors, applied to apple dices*

| | | Colour parameters | | |
|---|---|---|---|---|
| Treatment | Day | L value[a] | a value[a] | ΔE |
| Control | 0 | 84.59 (1.84) | –4.21 (0.60) | |
| | 4 | 80.01 (3.46) | –1.40 (1.19) | 9.161 |
| | 7 | 77.88 (3.47) | –1.31 (1.10) | 10.566 |
| | 11 | 77.53 (1.53) | –0.13 (0.75) | 11.614 |
| 0.5% complex of benzo-18-crown with potassium sorbate | 0 | 82.53 (2.10) | –4.33 (0.32) | |
| | 4 | 81.34 (1.74) | –3.24 (0.31) | 3.089 |
| | 7 | 81.41 (1.48) | –2.50 (0.40) | 3.731 |
| | 11 | 79.50 (2.28) | –1.67 (0.58) | 6.388 |
| 0.5% complex of benzo-18-crown with sorbic acid | 0 | 83.40 (2.65) | –3.82 (0.85) | |
| | 4 | 80.63 (2.90) | –1.68 (1.70) | 4.482 |
| | 7 | 80.27 (2.71) | –1.50 (1.08) | 4.954 |
| | 11 | 80.14 (3.14) | 0.01 (1.02) | 6.104 |

[a] L and a values (means of 15 replicates)
Values in parentheses are standard deviation (SD)

The browning reaction in apple samples was delayed after treatment with 0.5% (w/v) solutions of BSA and BPS significantly. Total colour change ($\Delta E_{ab}$) in treated apples with both crown compounds was significantly lower than in untreated apples (control) during storage.

## 3. Conclusions

Novel crown compounds, benzo-18-crown-6 with sorbic acid, and benzo-18-crown-6 with potassium sorbate had significant inhibitory effect on mushroom PPO as a catalyst of oxidation of phenolic substrates L-DOPA and chlorogenic acid. Both compounds (BSA and BPS) had a slightly higher effect of enzyme inhibition in case of L-DOPA.

The results obtained in this study showed that the storage life of cut apples can be extended, by applying crown compounds (benzo-18-crown-6 with sorbic acid, and benzo-18-crown-6 with potassium sorbate) as browning inhibitors, for 11 days of storage at 4 °C.

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