

Polyphenoloxidase (PPO) activity and osmotic dehydration in Granny Smith apple

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Abstract: Osmotic dehydration (OD) permits the preservation of foods via a decrease in water content and an increase in solute concentration. Osmotically dehydrated fruits such as apple are suitable for the manufacture of desserts, cakes, salads, yoghurts, etc. Different microstructural and engineering aspects of OD are already known, but its effects on enzymatic activity are still unknown. This study analyses the activity of polyphenoloxidase (PPO) in fresh Granny Smith apples and the effect that OD by immersion in sucrose-saturated syrup has on this activity. The low PPO activity found in the edible parenchyma of osmotically dehydrated apples is attributed to penetration by the osmotic agent and flooding of the intercellular spaces, which produces a low moisture content and a limited O₂ concentration in the immediate environment of the enzyme. These results show that OD prevents enzyme–substrate interaction. Thus the low PPO activity would reduce browning of this type of product.

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Keywords: osmotic dehydration; polyphenoloxidase; apple; enzymatic activity; edible parenchyma

INTRODUCTION

Fruits and vegetables play a very important role in our diet and nutrition, since they are a source of not only raw fibre but also essential nutrients, vitamins and minerals. The seasonal nature of the production of many fruits and vegetables, together with their high water content which makes them perishable, has led to the search for different technologies (refrigeration, drying processes, etc) to preserve them and to permit us to make these products available at any time.

Osmotic dehydration of fruits is a preservation technique that allows product availability all-year-long and product stabilisation^{1,2} through a reduction in water activity (a_w).³ Therefore this procedure can be considered as an alternative preservation method for the fruit and vegetable industry during high-production periods. Osmotically dehydrated fruits thus become a product with interesting market expectations in some food sectors, such as dairy desserts, cakes, fruit salads, etc. The Granny Smith apple is growing in importance as a consequence of its suitability for storage and preservation. In addition, it is widely accepted by consumers, so it can be considered as a 'target fruit' for osmotic dehydration preservation studies.

Enzymatic browning caused by polyphenoloxidase (PPO; EC 1.14.18.1, EC 1.10.3.1) activity in fruits and vegetables is a great problem for

the food industry and one of the main causes of spoilage during postharvesting treatment, storage and processing.^{4,5} The inhibition of browning is important in order to maintain the sensory attribute of colour⁶ in apples. PPO catalyses browning reactions because it transports the available O₂ to phenolic substrates, which are abundant in apples.^{7–9} There are no references available on the effect of osmotic dehydration on enzyme activity. The aim of this study was to analyse how osmotic dehydration of Granny Smith apple by immersion in sucrose-saturated syrup affects PPO activity.

MATERIAL AND METHODS

Material

Granny Smith apples (*Malus communis* L.) were purchased from a local market and stored at 4 °C until treatment. For each treatment, four apple rings (15 mm thick, 75 mm external diameter and 23 mm internal diameter, peeled and cored) were obtained from four different apples.

For osmotic dehydration the apple rings were submerged in a sucrose solution (65°Brix, 25 °C) and placed in a shaker for 2 or 8 h. The fresh sample was taken as reference. The ratio kg syrup kg⁻¹ fruit was higher than 50:1, so it could be assumed that

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the concentration of the solution remained constant during dehydration.

Cryo-SEM (low-temperature scanning electron microscopy)

A Jeol JSM-5410 scanning electron microscope (Izasa, Barcelona, Spain) was used together with a Cryo CT-1500C unit (Izasa). The sample was placed in the holder, fixed with slush nitrogen ($T \leq -210^\circ\text{C}$), transferred frozen to the Cryo unit, etched and gold coated (2 mbar, 2 mA). The prepared sample was then examined under the microscope at 15 kV and -130°C .

Enzymatic methods

Fresh and osmotically dehydrated samples were freeze-dried (Lioalfa-6, Telstar, Barcelona, Spain) at -40°C and 10^{-2} mbar and ground (Seward Stomacher 80, Biomaster Lab System, London, UK) in phosphate buffer (pH 6.5) containing 10 g l^{-1} polyvinylpyrrolidone (PVPP) and 2.5 g l^{-1} tensioactive Extran (1:5, g sample ml^{-1} buffer). The suspension thus obtained was centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge, Du Pont Instruments, Hanau, Germany) for 30 min at $16\,000 \times g$ and 4°C . The supernatant of the centrifuged solution was filtered. PPO enzymatic activity was then tested in the filtered extracts. A 0.033 M 4-methylcatechol solution was used as substrate^{10,11} and spectrophotometric values were recorded at 420 nm. PPO activity was expressed as $\Delta A_{420}\text{ min}^{-1}$. Four replications were performed.

The catalytic activity of the enzyme preparation was measured as a function of the substrate concentration. A graphical evaluation of the results was obtained by inserting the data into the Michaelis–Menten equation

$$V_0 = \frac{V[S]}{K_m + [S]} \quad (1)$$

where $[S]$ is the substrate concentration available at the start of the reaction ($\text{mol } 10^3\text{ m}^{-3}$), V_0 is the initial reaction rate ($\Delta A\text{ min}^{-1}$), V is the maximum rate ($\Delta A\text{ min}^{-1}$) and K_m is the Michaelis–Menten constant ($\text{mol } 10^3\text{ m}^{-3}$). In order to obtain an equation of a straight line and a more reliable determination

of V and K_m , the Michaelis–Menten equation was transformed into the double-reciprocal form (the Lineweaver–Burk plot)

$$\frac{1}{V_0} = \frac{1}{V} + \frac{K_m}{V} \frac{1}{[S]} \quad (2)$$

and the reciprocal form (the Eadie–Hofstee plot)

$$V_0 = V - K_m \frac{V_0}{[S]} \quad (3)$$

Statistical analysis

Statistical analysis (ANOVA) was performed using Statgraphics software (Manugistics, Rockville, MD, USA). Differences were regarded as significant at $P = 0.05$.

RESULTS AND DISCUSSION

In a previous study¹² it was established that during osmotic dehydration (OD) the penetration of the osmotic agent increases with the dehydration time. The penetration of the osmotic agent results in a water loss and a sugar increase in the cells in addition to the displacement of air from the intercellular spaces. In fact, the apoplast in fresh apple parenchyma is favourable for the gas and solute exchange (Fig 1A); however, the dehydrated apple parenchyma is shrunk and flooded with the osmotic solution, mainly in the parenchyma layers closer to the apple–osmotic solution interface (Fig 1B). All these events, together with the decrease in O_2 availability in the microstructural surrounding of the enzyme, will affect the PPO activity. This is the reason why the PPO activity of Granny Smith apple osmotically dehydrated by immersion in sucrose syrup is calculated in this paper.

Four replications were performed for PPO and the ANOVA showed that there were no significant differences between the values. For this reason, the average values are shown in the graphs (Figs 2–4). The PPO activity versus substrate concentration plot (Michaelis–Menten plot, Fig 2) for both fresh and OD apple parenchyma shows a first segment of order one and a second segment of order zero. It can be observed that the longer the OD treatment, the lower

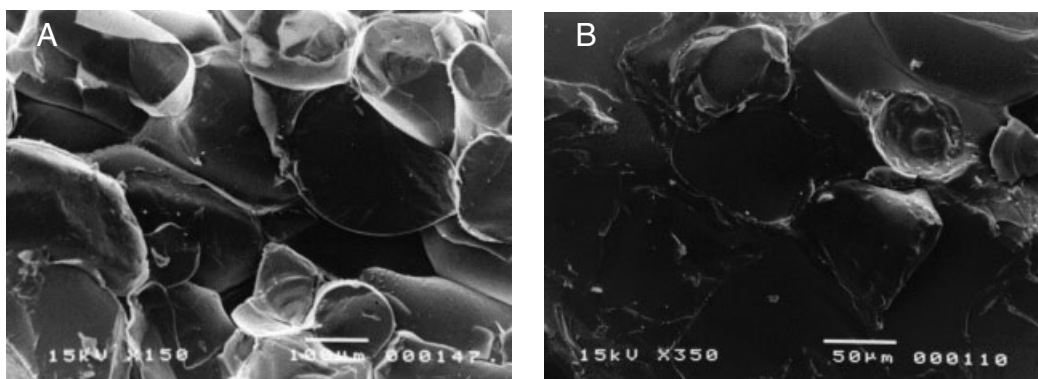


Figure 1. Cryo-SEM: A, fresh apple; B, 8 h osmotically dehydrated apple.

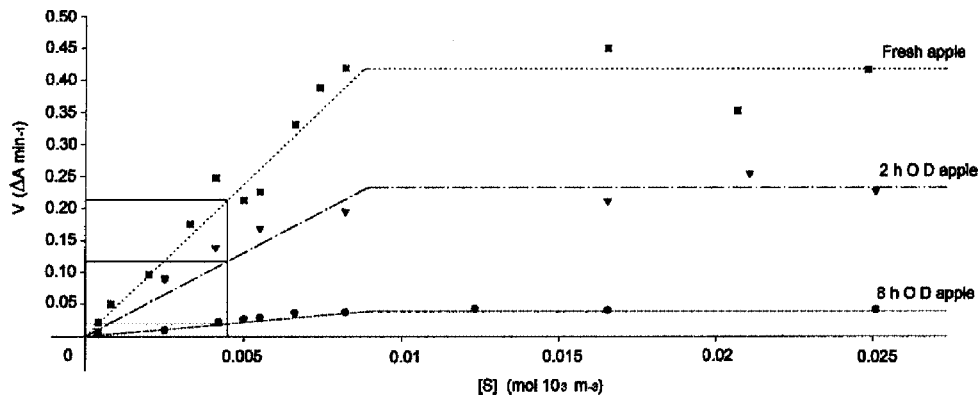


Figure 2. Polyphenoloxidase Michaelis–Menten plot of fresh and 2 and 8 h osmotically dehydrated apple.

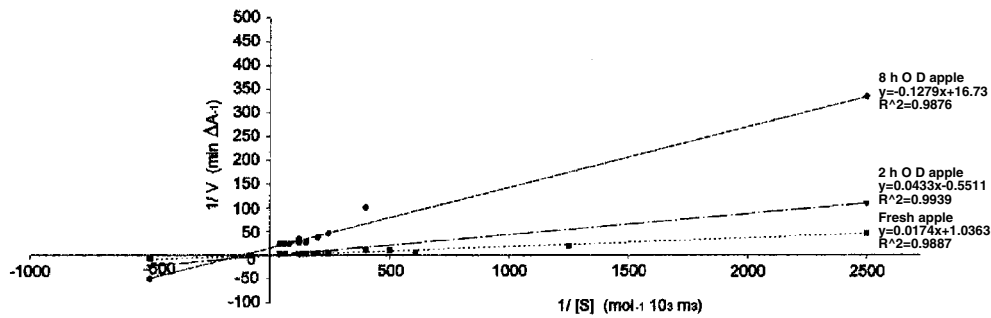


Figure 3. Polyphenoloxidase Lineweaver–Burk plot of fresh and 2 and 8 h osmotically dehydrated apple.

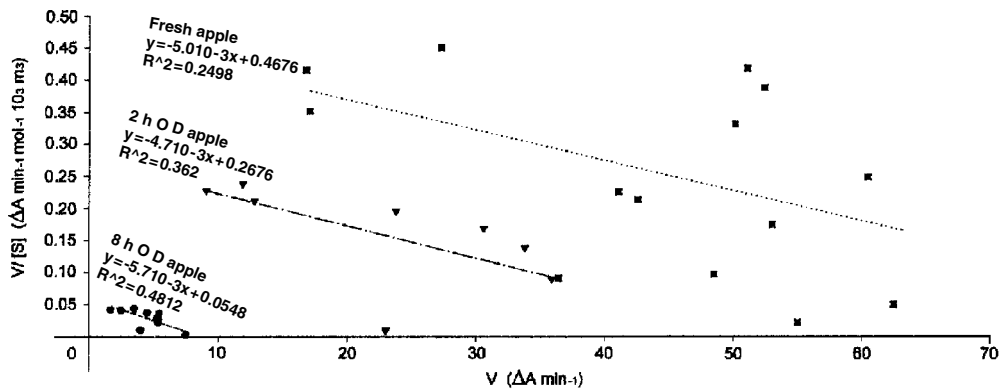


Figure 4. Polyphenoloxidase Eadie–Hofstee plot of fresh and 2 and 8 h osmotically dehydrated apple.

the enzymatic activity. The second segment provides the maximum value of PPO rate. These values are $0.43 \Delta A \text{ min}^{-1}$ for fresh apple, $0.23 \Delta A \text{ min}^{-1}$ for 2 h OD apple and $0.04 \Delta A \text{ min}^{-1}$ for 8 h OD apple. The substrate concentration at which the reaction rate is half of its maximal value is the Michaelis–Menten constant (K_m , eqn (1)). K_m shows the same value, $4.5 \times 10^{-3} \text{ mol } 10^3 \text{ m}^{-3}$, for all treatments.

The coincidence between K_m and the decrease in enzyme activity for fresh and osmotically dehydrated apple (2 and 8 h) indicates that a non-competitive inhibition is taking place.^{13,14}

The double-reciprocal plot of Lineweaver–Burk ($1/V_0$ vs $1/[S]$, Fig 3) for both 2 and 8 h osmotic treatments confirms the non-competitive inhibition, because the K_m values are very similar (7.8×10^{-3} and $7.6 \times 10^{-3} \text{ mol } 10^3 \text{ m}^{-3}$ respectively), whereas the

enzymatic activity values decrease as OD progresses. The K_m values calculated by Lineweaver–Burk are higher than the K_m values calculated by Michaelis–Menten. It has been established¹⁵ that the Lineweaver–Burk method may have inherent anomalies, as can be observed in the present study.

The reciprocal plot of Eadie–Hofstee (V_0 vs $V_0/[S]$, Fig 4) shows that the K_m values are very similar for both 2 and 8 h osmotic treatments (4.7×10^{-3} and $5.7 \times 10^{-3} \text{ mol } 10^3 \text{ m}^{-3}$ respectively), which again corroborates the non-competitive inhibition. However, it is established¹⁶ that the linearisation of the Michaelis–Menten curve as suggested by Eadie–Hofstee increases the statistical deviation of the Michaelis–Menten kinetics. As an example, when the experimental data for OD apples are fitted to the Eadie–Hofstee plot, the correlation coefficients

obtained (0.36 and 0.48 for 2 and 8 h respectively) are not suitable.

From these results it can be concluded that osmotic treatment has an inhibiting effect on PPO enzymatic activity in Granny Smith apple; the longer the dehydration time, the lower the PPO activity as a consequence of the inhibiting effect. It is expected that any variety of apple should have the same PPO enzymatic pattern (non-competitive inhibition) when it undergoes OD. However, some apple varieties tend to suffer greater enzymatic browning than others, so the initial point for the determination can vary among different varieties. The osmotic treatment produces kinetic behaviour that can be adjusted to a non-competitive inhibition model. Moreover, the inhibition is influenced by the microstructural events which take place in the apple parenchyma during OD.

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