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Modelling the interactions between free phenols, L-ascorbic acid, apple polyphenoloxidase and oxygen during a thermal treatment

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ABSTRACT

The kinetics of degradation of chlorogenic acid (CG), (−) epicatechin (EPI), L-ascorbic acid (AA) and polyphenoloxidase (PPO) activity from *Marie-Ménard* apple in pH 3.8 solutions at 20 and 50 °C were investigated to provide information on the impact of the presence of CG, EPI and/or AA on PPO thermostability. The effect of the heat treatment on their degradation by enzymatic and/or nonenzymatic ways was also studied. Stoechiokinetic reactions on the basis of experimental data and literature and determination of the kinetic constants (k) at 20 and 50 °C were elaborated before modelling the interaction among reactants, by fitting the reaction curves to predictive model. Apple PPO was thermolabile, denaturing after 10 min at 70 °C. Losses of PPO activity were favoured by the presence of EPI in model solutions, compared with CG, due to the formation of *o*-quinones of EPI (QEPI) lowering PPO stability. Temperature quickened both enzymatic phenol oxidations before PPO deteriorated and the whole set of the chemical reactions, including the production of secondary oxidation products and CG or EPI regeneration. Results also confirmed that AA in excess induced a fast regeneration of CG and EPI from the corresponding *o*-quinones formed enzymatically via redox chemical reactions.

1. Introduction

Discolouration and browning are well-known expressions of fruit-derived products deterioration, which may notably occur during apple juice, puree and slicing processing via the enzymatic browning reactions, when active polyphenoloxidase (PPO) has got in touch with phenolic substrates, in the presence of oxygen. These oxidative reactions generally modify the nutritional and organoleptic properties of the products and diminish storage life and consumer acceptance (Vamos-Vigyazo, 1981). Among the various classes of phenolic compounds located in the apple flesh and affecting browning reactions, cinnamic acids, mainly chlorogenic acid (CG) and flavan-3-ols among which (+) catechin and (−) epicatechin (EPI) are the most abundant phenolics. The average concentration of endogenous CG and monomeric EPI highly depends on the apple variety, ranging from 4.01–30.6 to 5.2–18.4 mg per 100 g fresh weight respectively, (Vrhovsek, Rigo, Tonon, & Mattivi, 2004). CG and EPI are the most prevalent free substrates in apples for PPO, the former phenol being more readily oxidised than the latter due to its better affinity towards the enzyme (Janovitz-Klapp, Richard, Goupy, & Nicolas, 1990b; Richard-Forget, Amiot, Goupy, & Nicolas, 1995, Chap. 11).

Pathways for the PPO oxidation of catechins and CG at room temperature and in a pH range of 3.5–8.0 have already been proposed (Jimenez-Atienzar, Cabanes, Gandia-Herrero, & Garcia-Carmona, 2004; Oszmianski & Lee, 1990), emphasising that quinonic compounds were first produced, before leading to dimers and further polymers products, and finally giving rise to browning. Although the characterization of oxidised phenol derivatives structures has ever been obtained after auto-oxidation, enzymatic and/or chemical oxidation, only few schemes of sequential reactions and kinetic studies of catechins and CG enzymatic oxidation, alone or mixed in model solutions have been carried out at 20 °C with PPO or peroxidase extracted from fruits or mushroom (Cheynier, Basire, & Rigaud, 1989; Jimenez-Atienzar et al., 2004; Lopez-Serrano & Ros-Barcelo, 2002; Oszmianski & Lee, 1990; Richard-Forget, Rouet-Mayer, Goupy, Philippon, & Nicolas, 1992).

In apple technology, inactivation of PPO activity by heat treatment such as blanching, cooking and/or the application of anti-browning chemical agents are generally used to prevent or lessen enzymatic browning in processed products. Among them, ascorbic acid (AA) is a reducing compound widely used as an additive at the beginning of the fruit products industrial processes, owing to its ability to scavenge molecular oxygen. However, its stability is greatly influenced by temperature, oxygen and metal ion content

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(Rojas & Gerschenson, 1997; Yuan & Chen, 1998) and discolouration is delayed as long as ascorbic acid is present in the medium. As soon as enzymatic phenol oxidation occurs in the presence of oxygen, AA is readily oxidised to give rise to dehydroascorbic acid (DHA) and further degradation products. Meanwhile, quinonic compounds enzymically formed are reduced back to their original phenol form (Amaki, Saito, Taniguchi, Joshita, & Murata, 2011; Isaacs & van Eldik, 1997). With higher AA levels present in the medium and provided all the oxygen present has been consumed, no enzymatic browning can occur. Conversely, in the presence of low levels of AA in the product (less than phenol compounds concentration), the ingress of oxygen into model products allows the total oxidation of AA into DHA which then results in the phenolics oxidation by oxidative enzymes and subsequent formation of quinonic products. These primary oxidation products are very unstable and reactive chemicals, highly prone to react further with either phenolic or non-phenolic compounds including AA (Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994). Moreover, these phenolic oxidation products are susceptible to inhibit apple PPO activity (Le Bourvellec, Le Quéré, Sanoner, Drilleau, & Guyot, 2004). Rates of these chemical redox reactions are highly pH-dependent, increasing in a pH range of 2–4.5.

AA reactivity and degradation pathways have been studied in various food matrices such as in wine (Bradshaw, Barril, Clark, Prenzler, & Scollary, 2011) or in orange juice (Manso, Oliveira, Oliveira, & Frias, 2001). According to the medium parameters (pH, dissolved oxygen level, temperature, traces of metallic ions, nature of the organic acids,...), various AA degradation mechanistic models (zero to second-order kinetics) have been proposed to fit well to the experimental data (Hsieh & Harris, 1993; Manso et al., 2001; Van Bree et al., 2012).

Therefore, apple puree can be regarded as a multi-reactant system in which components interact with each other during post-harvest processing operations enclosing heating steps. Both their evolution and interaction affect the quality of processed products. To our knowledge, studies on kinetic reaction rates of phenolics, PPO and AA alone or in combination have never been reported during heating treatment of aerobic model systems. Chow, Louarme, Bonazzi, Nicolas, and Billaud (2011) only investigated the effect of heating at 45 and 60 °C in the presence of CG and AA on apple PPO stability, showing that AA losses resulting from redox reactions were accelerated by temperature and dependent on CG and PPO levels.

Accordingly, the objectives of the present work were (1) to experimentally determine the effect of temperature at 20 and 50 °C, on the rate of AA, CG, EPI and PPO degradation in model solutions at pH 3.8, in order (2) to propose a stoechiokinetic model of the coupled reactions between the components potentially present in an apple puree matrix and (3) to determine the kinetic rates of component interactions as a function of temperature. A better knowledge of these enzymatic and/or chemical degradation reactions would help to better conceive the impact of a heat treatment on the composition and the colour of the apple end products.

2. Materials and methods

2.1. Solvents and reagents

Water was purified through a Milli-Q (Millipore, Bedford, MA) water system and used for all solutions preparations and dilutions. CG, EPI, 4-methylcatechol (4-MC), and AA were purchased from Sigma–Aldrich (St. Louis, MO). Malic acid, sodium fluoride, glacial acetic acid, metaphosphoric acid, and reagents used for the extraction and determination of apple PPO activity as well as the preparation of HPLC elution mediums were purchased from either VWR International (Fontenay-sous-bois, France) or from Sigma–Aldrich (St. Louis, MO) and were analytical grade. Chromatographic grade acetonitrile was from Acros Organics.

2.2. Fruit material

Cider apple fruits (*Marie-Ménard* var., harvest 2010) were provided by the French Institute for Cider production (Sées, France). Fresh plant material was used for the preparation of PPO extract. Apples var. *Golden Delicious*, purchased at a local market were also used to determine the concentration of endogenous free phenols used in model solutions.

2.3. PPO extraction from apple flesh

Apple PPO was extracted according to Chow et al. (2011), using 0.1 M cold phosphate buffer (pH 7.2) containing 20 mM AA, 12% (w/v) hydrated polyvinylpolypyrrolidone (PVPP) and 0.5% (w/v) Triton X100. After homogenisation and centrifugation, filtrated sample was further fractionated through a Sephadex™ G-25 M minicolumn (PD 10, GE healthcare, UK) to remove almost entirely free phenolic compounds and AA present in the extract. The filtrate solution was stored frozen at −30 °C until use in experiments.

2.4. Reaction media

Experiments were conducted using model systems filled in 1.5 ml Eppendorf™ vials and consisting of 1.4 ml malate buffer (20 mM, pH 3.8) containing CG, EPI, AA, alone or in combination and 0.1 ml PPO extract introduced last. Initial CG and EPI concentrations selected in this work (0.56 and 0.34 mM respectively) correspond to the levels determined in the flesh of var. *Golden Delicious* apple (personal data). Incubations assays were performed either at 20 °C or solutions were submitted to a thermal treatment at 50 °C, in a digital dry block heater (VWR International, Fontenay-sous-bois, France). During the isothermal heat treatments, the temperature of samples was monitored by means of a thermo probe with an accuracy of ±1 °C. To insure that initial data were accurately obtained at 50 °C, all reactants solutions (except PPO extract) were preheated for 10 min, a time required for the model solutions to equilibrate to the heater temperature. The experimental time was then set to zero (t_0) and after holding for various lengths of time (15–180 min), samples were immediately cooled in an iced water bath for 2 min. PPO activity was assayed by polarography on aliquots (50–100 µl) of the different model systems, according to the method of Chow et al. (2011). Activity at t_0 was selected as 100% and the percentage of activity remaining after each heating treatment was calculated from the initial activity. Residual CG, EPI and AA concentrations were determined from 0.6 ml extracts regularly taken from each reaction medium. They were then mixed with 4–5 volumes of either a stopping solution consisting of 20% cold acetonitrile, pH 2.5 and containing 5 mM NaF, to inhibit any residual PPO activity (CG, EPI) or 20 mM ammonium phosphate buffer, pH 2.5 (AA). In all model solutions containing the enzyme, 0.5×10^{-12} mol of PPO was added in 0.1 ml of enzymatic extract, this value corresponding to the number of moles of PPO calculated from activity measurements.

2.5. Thermal PPO degradation

Thermal inactivation of apple PPO was studied in a separate experiment performed at varying temperatures in the range 30–80 °C with exposure times ranging from 1 to 180 min.

The rate constant (k) at each temperature and the activation energy were estimated directly from experimental data of the curve giving the residual PPO activity versus time.