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Maillard reaction products as "natural antibrowning" agents in fruit and vegetable technology

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The effects of Maillard reaction products (MRPs), synthesized from a sugar (pentose, hexose, or disaccharide) and either a cysteine-related compound, an amino acid, or a sulfur compound, were investigated on polyphenoloxidase (PPO) activity from apple, mushroom, and eggplant. The optimal conditions for the production of inhibitory MRPs were performed using two-factor and five-level central experimental designs. It resulted that thiol-derived MRPs were highly prone to give rise to inhibitory compounds of PPO activity. Technological assays were also performed to test the efficiency of selected MRPs in the prevention of enzymatic browning in raw and minimally processed fruits and vegetables.

Keywords: Enzymatic browning / Maillard reaction products / Polyphenoloxidase / Thiol compound

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1 Introduction

Oxidoreduction reactions which occur in food products are either enzymatic or chemical and they take place during their storage, packaging, or processing. These reactions act on endogenous polyunsaturated lipids and phenolic compounds and are generally recognized as deleterious to the organoleptic quality of the foodstuffs. Thus, the search for active molecules and preferably natural inhibitors in the prevention of such oxidative reactions is of great concern. Among them, some Maillard reaction products (MRPs) are known to exhibit various antioxidant properties [1–3] which highly depend on the reaction conditions and the methods of measurement. Although MRPs are commonly formed in foodstuffs either during thermal processing or storage, they are often too complex to be directly extracted from food matrices and used as inhibitors. Thus, as a first approach, the study of their antioxidant role is more easily performed with model (synthetic) MRPs.

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Abbreviations: CSH, cysteine; **GSH**, glutathione; **MRP**, Maillard reaction product; **PPO**, polyphenoloxidase

In many edible plant products, such as fruits and vegetables, during post-harvest handling and processing, endogenous phenolic compound oxidation is catalyzed by oxidoreductases, notably polyphenoloxidases (PPOs) and tyrosinases, in the presence of O_2 (Fig. 1). *O*-quinones, the primary oxidation products formed, are highly reactive and rapidly condense and polymerize to dark-colored pigments referred to as enzymatic browning products. This brown pigmentation is generally considered to be detrimental to food quality as it impairs the nutritional, functional, and organoleptic properties of the product.

A wide range of chemical compounds has already been proposed to avoid or at least minimize this discoloration [4, 5]. Some act on the phenolic compounds (Fig. 1, target A), while others bind with the quinonic compounds, leading to colorless addition compounds (Fig. 1, target B), but in both cases they are either too expensive to be used in food technology or their action is only temporary, as with ascorbic acid. Sulfites and their derivatives are by far the cheapest and the most effective antibrowning agents. They act both on the *o*-quinonic compounds or directly inhibit the enzyme activity (Fig. 1, target C). However, their utilization is discontinued, owing to their adverse effects on sensitized (asthmatic) subjects.

In the present study, products generated during the Maillard reaction were tested for their ability to produce inhibitors of

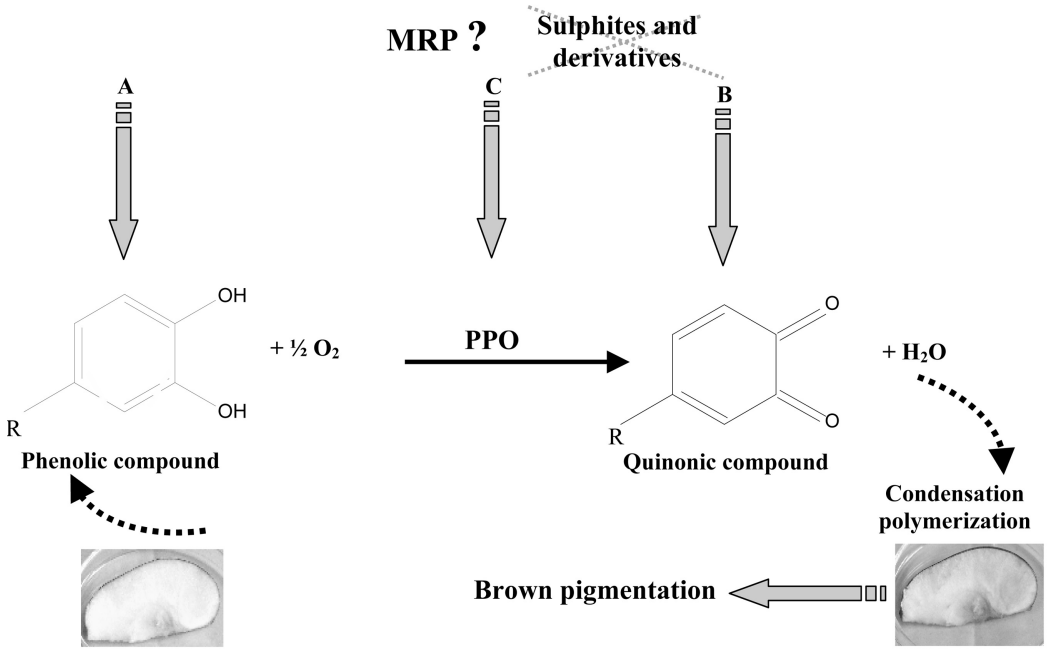

Figure 1. Occurrence of enzymatic browning and methods of prevention.

PPO activity. Optimum conditions of preparation were set up, applying the response surface methodology to investigate the influence of heating time and temperature, concentration of reactants, and pH of the mixtures on their synthesis, using aqueous mixtures of monosaccharide, (or disaccharide,) and either an amino compound, a cysteine (CSH)-related compound or other sulfur compound. Technological assays were also performed to test their ability to prevent enzymatic browning in raw or minimally processed fruits and vegetables.

2 Materials and methods

2.1 Materials

Apples (var. Red Delicious) and eggplants were picked at commercial maturity and used as PPO source. The enzyme was purified from the apple cortex according to [6]. PPO from eggplant flesh was purified in three steps, *i. e.*, extraction in the presence of ascorbic acid, L-cysteine, and polyvinylpolypyrrolidone, fractional precipitation by ammonium sulphate (20 – 70% saturation), and hydrophobic chromatography (unpublished). Pooled active fractions, stored at –20°C, were highly stable and constituted the enzymatic extracts used for subsequent experiments. PPO from mushroom was obtained from Sigma-Aldrich Chemical (St. Quentin Fallavier, France) and used without further purification. All chemicals used were the purest available and obtained either from Sigma-Aldrich Chemical (St. Louis, MO, USA) or from VWR International, Merck-eurolab (Fontenay, Bois, France).

2.2 Preparation of MRPs

Sugar (0.125 or 0.25 M) and either a CSH-related compound, an amino acid, or a sulfur compound (0.083, 0.125, or 0.25 M) were used to prepare different model aqueous MRPs (Fig. 2). According to the experiment, the initial pH of the model systems was uncorrected or adjusted from 2 to 12 using either concentrated H_3PO_4 or NaOH, to avoid their dilution. Aliquots of these model solutions were placed in Pyrex vials, sealed with silicone-Teflon septa and metallic caps. Solutions were heated at different temperatures ranging from 80 to 120°C in an air convection oven for predetermined time periods between 0.5 to 48 h. Afterwards, vials were cooled in ice and the soluble part of the mixtures directly used to determine their inhibitory effect on PPO activity. The color intensity of MRPs was also measured at 350 or 420 nm with a photodiode-array spectrophotometer (HP, model 8453A; Waldbronn, Germany).

2.3 Enzyme activity determination

PPO activity was determined by polarography at 30°C in a 1.5 mL reaction cell. The phenolic substrate consisted of 4-methylcatechol (4-MC), 20 mM (apple or eggplant PPO) or

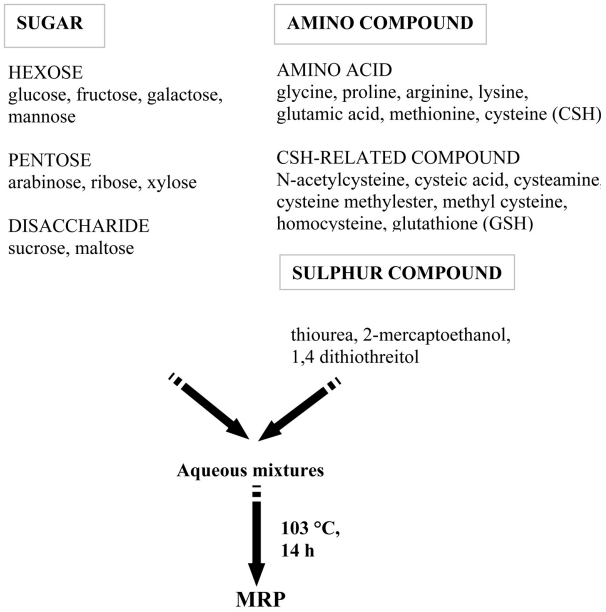

Figure 2. Nature of the reactants used in the preparation of model MRPs.

2 mM (mushroom PPO) in 0.1 M air-saturated citrate-phosphate buffer solution at pH 4.5 (apple and mushroom PPO) or 5.0 (eggplant PPO). The reaction rate was calculated from the initial slope of the progress curve giving oxygen uptake *versus* time using a Clark oxygen microprobe. Activity was expressed as nmoles of oxygen consumed per second (nkatals) under the assay conditions. For inhibition studies, MRPs (1–10 μL) were added to the phenolic substrate before the enzyme extract. PPO activity, in the presence of MRP, was expressed as a percentage maximum activity measured without MRP in the reaction medium.

2.4 Experimental designs

The method described [7] allowed to study the effects of different couples of independent variables, namely pH and heating temperature, heating time and temperature, concentration of each of the initial reactants, on the formation of MRP inhibitors of PPO activity, prepared from various model systems. The dependent variable (experimental response) investigated in this study was the residual PPO activity. A 2-factor and 5-level experimental design was used to allow estimation of all coefficients of first-order and second-order interactions. Within the limits of the experimental domain, a second-order polynomial model was fitted to the dependent variables, using the following equation:

$$Y = b_0 + \sum_i b_i X_i + \sum_{ij} b_{ij} X_i X_j + \sum_{ii} b_{ii} X_i^2 \qquad (1)$$

where Y is the estimated response, b_0, b_i, b_{ij}, b_{ii} are the parameter estimates corresponding to the constant, linear, interactive, and quadratic effects, respectively, using the least squares method, and X_i, X_j are the independent variables in coded values. The Student's *t*-test was used to check the reliability of the polynomial and the significance of parameters. The level of statistical significance for the process variables was defined at $p = 0.05$. Results reported are the average of at least three measurements and the averages of data are considered.

2.5 Technological trials

Slices of apple, eggplant, or mushroom were dipped for 10–15 min at 25°C in a citrate-phosphate buffer solution (0.1 M, pH 4.5 or 5.0) alone (control) or containing either $Na_2S_2O_5$ added as sulfite (1.25–4.0 mM) or MRPs (equivalent to 0.75–3.75 mM initial thiol concentration) prepared under the optimum conditions determined by the response surface methodology. Apple purée was prepared at 25°C by mixing cut-up apple in a citrate-phosphate buffer solution (0.1 M, pH 4.5) alone (control) or containing either $Na_2S_2O_5$ added as sulfite (2.5 mM) or MRPs (equivalent to 3.75 mM initial thiol concentration). Thereafter, all samples were stored at 25°C for 0.5–7 h and examined for the possible occurrence of browning.

3 Results and discussion

It is well-known that the production of antioxidant MRPs highly depends on the nature of the reactants, their concentrations, the ratio sugar to amino compound, the combination of heating time and temperature, and also the initial pH of the model systems.

3.1 Nature of the reactants

MRPs coming from heated solutions of glucose with CSH were used as reference in these comparative studies. Under our conditions, neither the nonreacted starting materials nor the thermal degradation products generated from sugars alone elicited an inhibitory effect on enzyme activity. Conversely, high concentrations of thermal degradation products from CSH and GSH were inhibitors of mushroom PPO only. Figure 3 illustrates the contribution of MRPs prepared from mixtures of CSH with either an hexose (Fig. 3A), a pentose (Fig. 3B), or a disaccharide (Fig. 3C) to the inhibitory potency *versus* PPO from apple. Among the hexoses, MRPs derived from galactose were the most efficient. Although to a lesser extent, mannose and glucose also significantly reduced PPO activity, whereas with fructose, MRPs slightly inhibited the activity of the enzyme. With

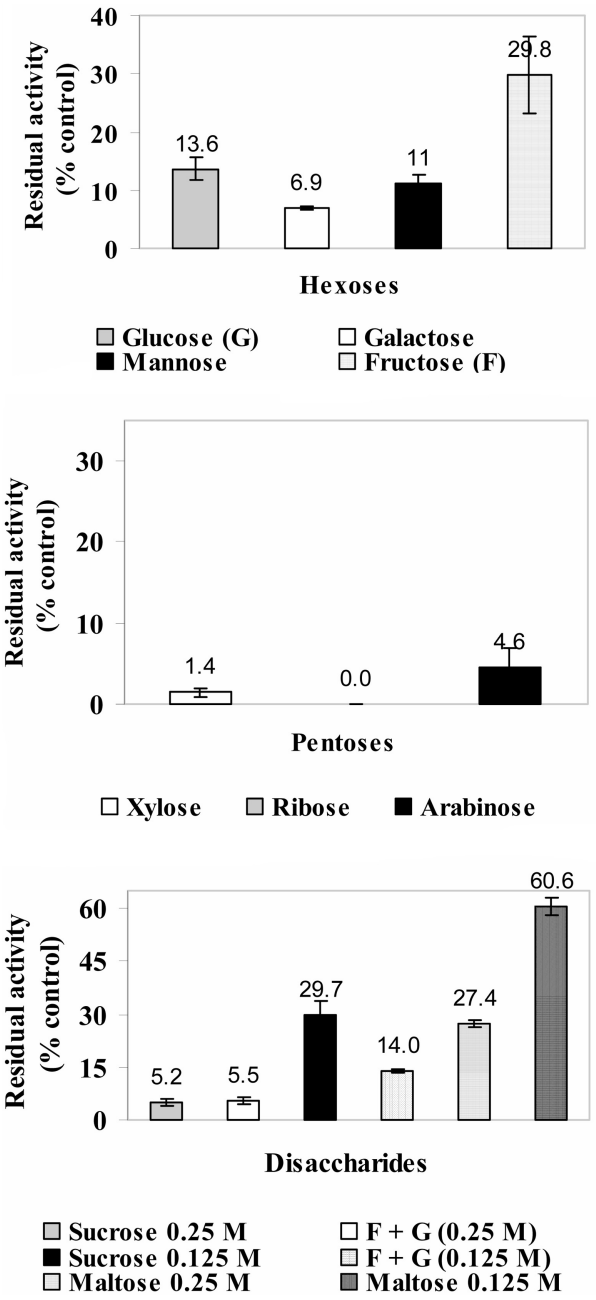

Figure 3. Inhibitory potency of MRPs on apple PPO activity, in relation with the nature of (A) hexoses, (B) pentoses, and (C) disaccharides. Activity was measured in the presence of MRPs (10 μL) prepared by reacting CSH (0.25 M) with a sugar (0.25 or 0.125 M) at 103°C for 14 h.

regards to the pentoses-derived MRPs, the order of effectiveness was ribose > xylose > arabinose, the residual PPO activity being from 3.2 (arabinose) to nearly 16 times lower (ribose) than the glucose counterpart. This confirms the fact largely demonstrated that in the Maillard reaction the reactivity of pentoses is higher than that of hexoses. In mixtures prepared with disaccharides, using the same concentration of reactants, it was observed that those containing sucrose presented an inhibitory potency nearly 5 times higher than when maltose was the sugar selected to produce MRPs. This could look quite surprising considering the reducing character of the latter. Actually, heating conditions seemed enough to allow hydrolysis of the glycosidic linkages. Thus, the bond between the two reducing functions (α-1,2) in sucrose should be more labile than the bond (α-1,4) in maltose. Hence, when comparing the formation of inhibitory compounds derived from equimolar (0.125 or 0.25 M) invert sugar with CSH, a similar response was obtained for residual PPO activity, confirming that hydrolysis of sucrose was complete under the assay conditions. Conversely, the same reaction conditions did not lead to the complete hydrolysis of the maltose.

Lowering the concentration ratio of sugar : amino compound to 1 : 2 (glucose + fructose, both 0.125 M or maltose 0.125 M), substantially decreased the MRP inhibitory potency on PPO activity. This shows that the initial concentration of the sugar must be equal or higher than the initial concentration of CSH to generate inhibitory compounds during the development of the Maillard reaction. Finally, if the inhibitory potency of MRPs produced from an equimolar combination of glucose + fructose (both 0.125 M) was compared to that obtained with glucose (0.25 M), it could be noticed that partly substituting glucose for fructose did not modify the overall inhibitory potency of the compounds produced. Thus, in the presence of CSH, pentoses were the most prone to generate highly inhibitory compounds, followed by sucrose and hexoses, except fructose and maltose which were less efficient in producing active MRPs.

Considering the nature of the amino acids, it resulted that the inhibitory effect of MRPs prepared with glucose (0.25 M) and glycine, proline, arginine, or lysine (each 0.25 M) did not behave as efficient inhibitors of PPO activity, more than 86% residual activity remaining with high amounts (100 μL) of MRP solutions in the reaction medium. Conversely, those derived from glucose with CSH and GSH were very potent, even at low concentrations (< 5 μL) in the reaction mixture [3]. Therefore, to highlight the structural requirements for the thiol reactant to exhibit a strong antioxidant effect, others studies were carried out, selecting glucose as the sugar reactant. Equimolar (0.25 M) solutions were prepared with an amino acid, a CSH-related compound, or another sulfur derivative. As a result, among CSH-related compounds, it was first confirmed that the participation of an amino group was essential to produce inhibitory MRPs. Moreover, results showed that, in addition to the thiol group, the presence of a carboxylic group in the structure of the initial CSH-derived reactant was a determining factor for the antioxidant activity of the MRPs [8].

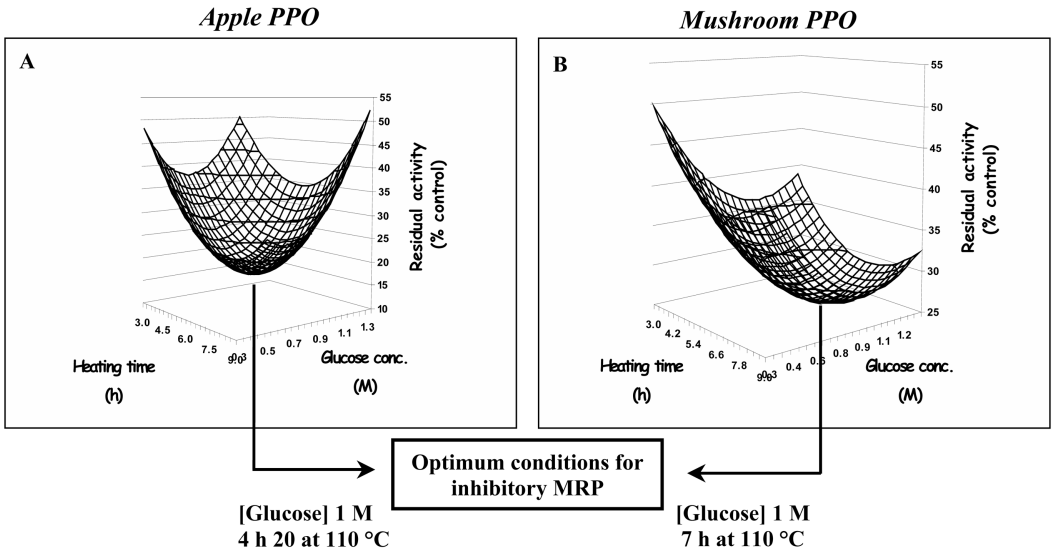

Figure 4. Response surface plots for the changes of (A) apple and (B) mushroom PPO activity, varying the heating time (3–9 h) and initial glucose concentration (0.3–1.3 M) in a glucose-GSH model system where the GSH concentration (0.15 M) was kept constant. Solutions were adjusted to pH 3 before heating at 110°C. Enzyme activity was measured in the presence of (A) 1 or (B) 5 μL MRPs.

In conclusion, model systems prepared from glucose, fructose, or a pentose with a thiol compound, namely CSH or GSH or a combination of glutamic acid with CSH (two of the three amino acids constitutive of the tripeptide GSH) were the most prone to give rise to MRP highly inhibitors of PPO activity [9, 10].

3.2 Other physicochemical reaction conditions

To set up the other optimal conditions for MRP synthesis, various central experimental designs of the second order were performed. In the study shown in Fig. 4, MRPs were prepared from mixtures containing glucose and GSH. The GSH concentration (0.15 M) was kept constant, whereas the glucose concentration ranged from 0.3 to 1.3 M. Solutions were heated at 110°C for times ranging from 3 to 9 h. Figure 4 shows the response surface plots for the effects of the heating time and the glucose concentration on residual apple or mushroom PPO activity. When the response obtained is minimal, MRPs prepared under these conditions are the most efficient in inhibiting PPO activity. Thus, to most inhibit apple PPO (Fig. 4A), optimal conditions for MRP synthesis are obtained with a glucose concentration of 1 M and heating of the mixtures at 110°C for about 4 h. To most inhibit mushroom PPO (Fig. 4B), optimal conditions are similar concerning the glucose concentration, but the heating time at 110 °C has to be extended up to 7 h.

From the results of additional experimental designs [11, 12], it appears that with mixtures composed of glucose with CSH or GSH, optimal reaction conditions for the production of inhibitory MRPs are achieved with a concentration of the sugar higher than that of the thiol reactant, respectively, in the range of 0.8–1 M for the glucose and of 0.15–0.5 M for the thiol compound. Moreover, the initial pH of the solutions must be close to 3 before heating, as with mixtures containing cysteine [11]. Under these conditions, heating either at 103°C for 14 h or at 110°C for times ranging from 4 to 7 h are optimal to generate MRPs highly active against enzymatic browning of apple and mushroom.

3.3 Technological aspects

From a technological point of view, it was important to assess that MRPs were also prone to prevent browning in raw and minimally processed fruits and vegetables. For this, some MRPs were selected according to two criteria: a strong inhibitory potency *versus* PPO activity and minimal color intensity measured by absorbance values at 350 or 420 nm of the model systems. They were tested on sliced apple, mushroom, or eggplant and on apple purée, with steeping and mixing conditions allowing the comparison of the efficiency of metabisulfite and MRPs on the browning control. After storage at room temperature for 6–7 h, slices and apple purée were examined for visual appearance (Figs. 5 and 6).

With apple slices (Fig. 5A) it was observed that, compared to the control, metabisulfite was highly efficient in its ability to avoid browning and even led to a whitening aspect of the fruit

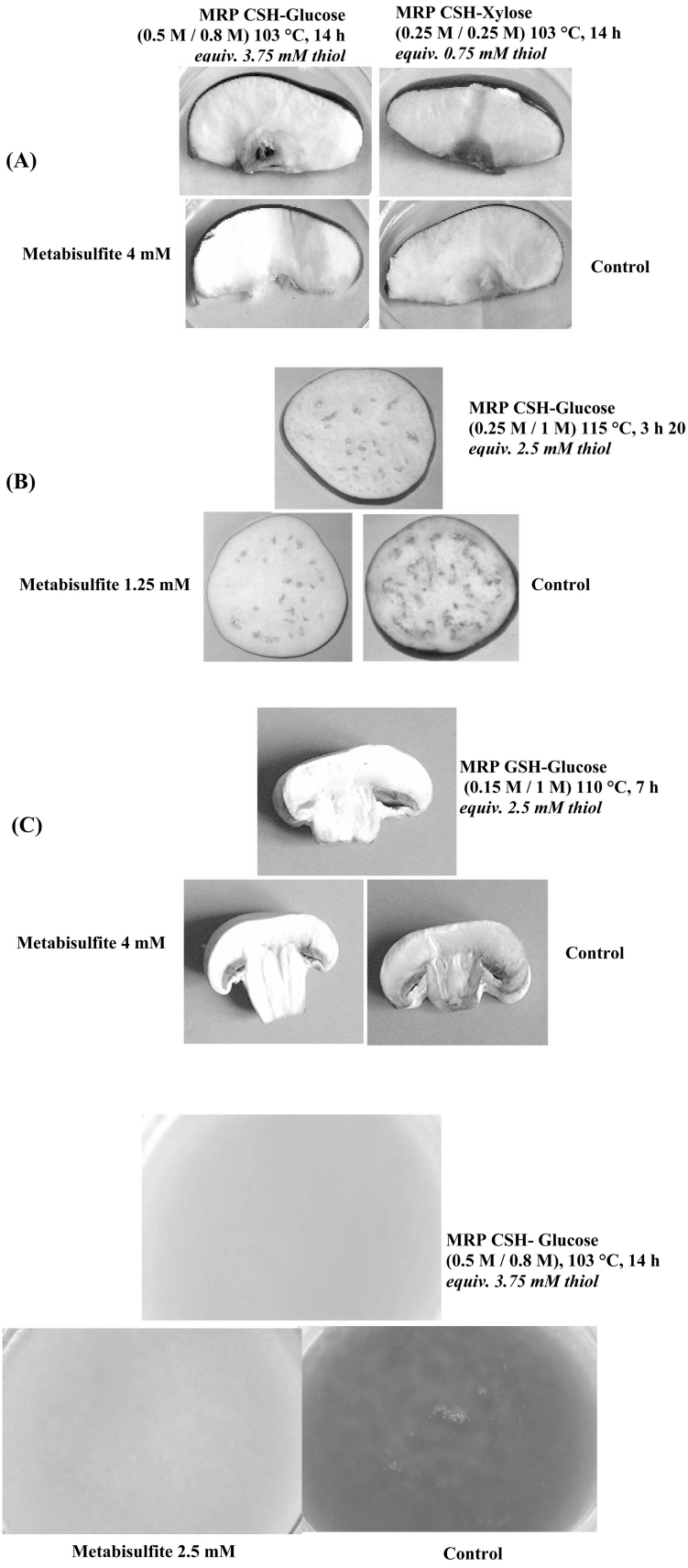

Figure 5. Visual appearance of (A) apple, (B) eggplant and (C) mushroom slices after steeping for 10–15 min and storage at room temperature for (A, B) 6 or (C) 7 h.

Figure 6. Visual appearance of apple purée after storage at room temperature for 3 h.

surface. MRPs prepared from two different mixtures were also quite efficient, notably those prepared with CSH and xylose. With eggplant slices (Fig. 5B), very prone to browning, it resulted that control slices were highly pigmented. Conversely, in the presence of either metabisulfite or MRPs prepared with CSH, no browning occurred. The same trend was noticed with mushroom slices (Fig. 5C), attesting an efficiency of selected MRPs (in this case prepared with glucose and GSH) similar to that of metabisulfite. Again, with apple purée (Fig. 6), CSH-derived MRPs were as efficient in preventing browning as metabisulfite. These few examples confirm that, from a technological point of view, thiol-derived MRPs presenting a low $abs._{350\text{ or }420\text{ nm}}$ value and a high inhibitory potency could potentially replace sulfites as anti-browning agents in fruit and vegetable technology.

4 Concluding remarks

This is the first study on the comparative efficiency of soluble MRPs derived from monosaccharide with thiol compounds able to inhibit enzymatic browning at very low concentrations in raw or minimally processed fruits and vegetables. Moreover, MRPs can be prepared very easily under thermal process flavoring conditions. *In vitro*, they are able to inactivate PPOs from various origins, partly by chelating the copper ions of the active site of the protein [13, 14].

5 References

[1] Nicoli, M. C., Elizalde, B. E., Pitotti, A., Lerici, C. R., Effects of sugars and Maillard reaction products on polyphenol oxidase and peroxidase activity in food. *J. Food Biochem.* 1991, *15*, 169–184.

[2] Tan, B. K., Harris, N. D., Maillard reaction products inhibit apple polyphenoloxidase. *Food Chem.* 1995, *53*, 267–273.

[3] Billaud, C., Peyrat-Maillard, M.-N., Chow, Y.-N., Berset, C., Nicolas, J., Antioxidant properties of model Maillard systems prepared from glucose with proline, glycine, lysine, arginine, cysteine or glutathione. *Proceedings of the Third International Congress "Pigments in Food: More than Colours"*, Dufossé, L. (Ed.), Quimper, France, June 14–17, 2004, pp. 223–225.

[4] Nicolas, J., Billaud, C., Philippon, J., Rouet-Mayer, A.-M., Enzymatic browning. Biochemical aspects, in: Caballero, B., Trugo, L., Finglas, P. M. (Eds.), *Encyclopedia of Food Sciences and Nutrition*, 2nd ed., Vol. 1, London: Academic Press, London 2003, pp. 678–686.

[5] Nicolas, J., Billaud, C., Rouet-Mayer, A.-M., Philippon, J., Enzymatic browning. Technical aspects and assays, in: Caballero, B., Trugo, L., Finglas, P. M. (Eds.), *Encyclopedia of Food Sciences and Nutrition*, 2nd ed., Vol. 1, London: Academic Press, London 2003, pp. 686–692.

[6] Janovitz-Klapp, A., Richard, F., Nicolas, J., Polyphenol oxidase from apple, partial purification and some properties. *Phytochemistry* 1989, *28*, 2903–2907.

[7] Delacroix, A., Porte, C., Méthodes d'optimisation en chimie analytique. *Techn. Ing.* 1987, *4*, 1–22.

[8] Billaud, C., Maraschin, C., Peyrat-Maillard, M.-N., Nicolas, J., Maillard reaction products derived from thiol compounds as inhibitors of enzymatic browning of fruits and vegetable. Structure-activity relationship. *Ann. N.Y. Acad. Sci.* 2005, in press.

[9] Billaud, C., Roux, E., Brun-Mérimée, S., Maraschin, C., Nicolas, J., Inhibitory effect of unheated and heated D-glucose, D-fructose and L-cysteine solutions and Maillard reaction products model systems on polyphenoloxidase from apple. I. Enzymatic browning and enzyme activity inhibition using spectrophotometric and polarographic methods. *Food Chem.* 2003, *81*, 35–50.

[10] Billaud, C., Brun-Mérimée, S., Louarme, L., Nicolas, J., Effect of glutathione and Maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple. I. Enzymatic browning and enzyme activity inhibition. *Food Chem.* 2004, *84*, 223–233.

[11] Billaud, C., Maraschin, C., Nicolas, J., Inhibition of polyphenoloxidase from apple by Maillard reaction products prepared from glucose or fructose with L-cysteine under various conditions of pH and temperature. *Lebensm.-Wiss.-Technol.* 2004, *37*, 69–78.

[12] Billaud, C., Chow, Y.-N., Nicolas, J., Inhibition of apple and mushroom phenoloxidase activity by Maillard reaction products prepared from a glucose-glutathione model system using a response surface methodology. French Annual Congress «Chimiométrie», Paris, France, December 3–4, 2003, pp. 139–142.

[13] Roux, E., Billaud, C., Maraschin, C., Brun-Mérimée, S., Nicolas, J., Inhibitory effect of unheated and heated D-glucose, D-fructose and L-cysteine solutions and Maillard reaction products model systems on polyphenoloxidase from apple. II. Kinetic study and mechanism of inhibition. *Food Chem.* 2003, *81*, 51–60.

[14] Brun-Mérimée, S., Billaud, C., Louarme, L., Nicolas, J., Effect of glutathione and Maillard reaction products prepared from glucose or fructose with glutathione on polyphenoloxidase from apple. II. Kinetic study and mechanism of inhibition. *Food Chem.* 2004, *84*, 235–241.

