Evaluation of natural extracts as potential enzymatic browning inhibitors

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ABSTRACT

Enzymatic browning in fruits represents a difficult problem for Food Industry, especially with recent restrictions in the use of synthetic antioxidants. It is often associated with undesirable off-flavors and negative effects on taste and nutritional value. This physiological disorder is mainly due to the oxidation of natural phenolic compounds into quinones that are polymerized to brown pigments by polyphenol oxidase (PPO). Peroxidase (POX) is also alleged to be related to browning development, by inducing H_2O_2 oxidation of phenolic compounds. Prevention of browning reactions, catalyzed by these enzymes, has traditionally been accomplished by various chemicals, as ascorbic and citric acids. Partial control of this disorder can be obtained with the application of antioxidants, which compete with common substracts for the enzymatic activity. This study assessed the potential of natural extracts, with antioxidant properties (such as soy protein concentrate, extracts of apple byproduct and olive leaves) as novel enzymatic browning inhibitors. The antioxidant capacity was assessed by the radical scavenging assay using $ABTS^+$ radical. The natural extracts inhibitory effect on PPO and POX activity was evaluated using a spectrophotometric assay by reaction of the natural extracts with the pure enzymes, using catechol and guaiacol as substrates, respectively. The present study demonstrated that olive leaves had the highest antioxidant capacity (2.688 ± 0.006 mg/mL TEAC equivalent), however, apple byproduct was the most effective on PPO and POX activity inhibition. The study has practical implications in generating novel natural extracts with potential application as anti-browning agents.

1. INTRODUCTION

It is of outmost importance the preservation of quality during fruit postharvest storage, especially for its competitiveness, allowing extension of marketing and maintenance of high selling price periods. However, there are several diseases affecting organoleptic and physiologic properties of horticultural commodities [1]. Enzymatic browning is a widespread reaction occurring in most fruits and vegetables, leading to faster quality

deterioration and shorter shelf-life. In fact, this oxidative reaction is the second most important cause of food deterioration and represents a difficult problem for Food Industry, especially with recent restrictions in the use of synthetic antioxidants [2]. It is often associated with undesirable off-flavors and negative effects on taste and nutritional value and it is mainly due to the enzymatic oxidation of natural phenolic compounds, namely monophenols, into *o*-diphenols and *o*-diphenols into quinones, which undergo further non-enzymatic polymerization, resulting in the formation of brown pigments [3,4]. The main oxidative enzymes responsible for browning are polyphenol oxidase (PPO, EC 1.10.3.1) and peroxidase (POX, EC 1.11.1.7). Therefore, the attempt to control these enzymes activity is crucial for the preservation of postharvest fruits and vegetables. However, this inhibition has traditionally been accomplished by use of various chemical compounds. Antioxidant solutions have been traditionally employed in the Food Industry, mainly as enzymes inhibitors, competing with common subtracts for the enzymatic activity. Ascorbic acid and its derivatives and sulfites has been found to be the most effective in controlling browning [3]. Natural alternatives to these costly inhibitors would be desirable. Moreover, there is a rising interest in natural antioxidants as bioactive components of foods.

Phenolic compounds are naturally present in all plant material, including food products of plant origin, and they have been reported to have multiple biological effects, including antioxidant activity and potential as oxidative enzyme inhibitors. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers [5].

In this study, the potential antioxidant and enzyme inhibitory activities of natural extracts soy protein concentrate, apple byproduct and olive leaves, rich not only in phenolic compounds but also in triterpenic acids and amine groups, were investigated.

2. MATERIALS AND METHODS

2.1 Materials

Chemicals and extracts

The commercial digestive-enzymes, Peroxidase from Horseradish and Tyrosinase from mushroom, ABTS and potassium persulfate were purchased from Sigma-Aldrich. The soy protein was purchased from market place, olive leaves collected from nature and apple byproduct provided by INDUMAP- Fruit´s industrialization (Portugal).

2.2 Methods

Extracts preparation

Olive leaves extract was prepared by reflux during 1 h 30 min with H_2SO_4 at 10% (m/v), followed by filtration, neutralization with NaOH and extraction with $C_4H_8O_2$. Apple byproduct extract was obtained by MeOH:CH_2O_2 (80:20).

Antioxidant activity

The antioxidant capacity was assessed by the radical scavenging assay using $ABTS^+$

radical [6]. Briefly, ABTS radical cation was produced from the reaction of 7 mM 2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM potassium persulfate, after incubation, at room temperature in dark, for 16 h. The ABTS solution was diluted with water to an absorbance of 0.70 ± 0.02 at 734 nm. After the addition of 1.0 mL ABTS solution to 10 µL of sample, the mixture absorbance reading was performed after 6 min. Ascorbic acid was used as standard (TEAC equivalent).

Enzymatic activity

Prior to the natural extracts inhibitory effect measurement on PPO and POX activity, the concentrations of enzyme, substrate and reaction time were optimized. The method was performed using a spectrophotometric assay (using a Thermo Scientific Multiskan GO) measuring at 420 and 470 nm, respectively, the reaction of the natural extracts with the pure enzymes, using catechol and guaiacol as substrates, respectively. Then, the enzyme activity in each inhibition reaction was determined using SkanIt™ Software version (4.1). The % of enzyme activity inhibition was calculated using control (i.e., substrate without inhibitor) as the highest enzyme activity (i.e., 0 % of inhibition). All the concentrations were determined and optimized based on previous works (data not shown).

3. RESULTS AND DISCUSSION

3.1 Antioxidant activity and enzymatic activity

According to Figure 1, olive leaves extract, at 25 $mg.mL^{-1}$ presented the highest antioxidant activity (2.688 ± 0.006 $mg.mL^{-1}$ TEAC equivalent), followed by soy protein, which at a higher concentration (47.62 $mg.mL^{-1}$) showed an antioxidant activity of 0.44 ± 0.02 $mg.mL^{-1}$ TEAC equivalent, and apple byproduct extract with an activity of 0.09 ± 0.02 $mg.mL^{-1}$ TEAC equivalent at 16 $mg.mL^{-1}$.

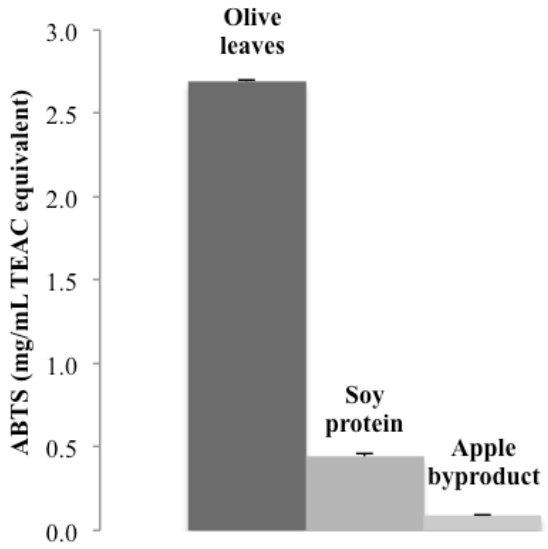

Figure 1. Antioxidant activity of natural extracts and soy protein through the $ABTS^{+}$ method. Values are expressed in $mg.mL^{-1}$ ascorbic acid and represent an average of three analytical replicates.

Validating its higher antioxidant activity, olive leaves extract demonstrated to be rich on tocopherol (data not shown) whose primary function is as an antioxidant. Regarding soy protein and according to the literature, it is not expected to show a high ABTS scavenging activity unlike apple byproduct, which is mainly constituted by chlorogenic acid, procyanidins and epicatechin, strong antioxidants. The low antioxidant activity of observed in apple byproduct extract may be attributed to the low concentration used in this study.

Table 1 shows, as expected, ascorbic acid, at a concentration of 5 mM, was effective in reducing both enzymes activity. The results also show that only apple byproduct extract was capable of reducing PPO activity, which can be explained by the presence of chelating agents, that can reduce the this enzyme activity. In agreement with the antioxidant activity results, olive leaves extract proved to be quite effective on POX inhibition activity, comparing to ascorbic acid. Concerning soy protein, it inhibited POX activity by ca. 20%. It is possible that there are components acting as inhibitors of enzyme activity which may not be measured using ABTS method. A thorough investigation of antioxidant components of the various extracts is being conducted.

Table 1. Different extracts and soy protein % of inhibition relatively to the control. Results are shown in mean of three replicates ± SD

Enzyme	Ascorbic acid	Olive leaves	Soy protein	Apple byproduct
PPO	100 ± 2.57	0	0	11.6 ± 3.12
POX	63.53 ± 0.75	44.81 ± 1.48	21.49 ± 2.34	60 ± 1.16

4. CONCLUSIONS

The present study demonstrated that leaves from olive tree showed the highest values of antioxidant activity within the natural extracts. It is important to highlight the inhibitory effect of apple byproduct on PPO, despite the low antioxidant activity. The present study has practical implications in generating novel natural extracts with potential application as anti-browning agents.

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