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Biopreservation of 'Birgah' Eggplant from Polyphenol Oxidase Activity Assayed *In Vitro* with Onion (*Allium Cepa* L.) by-products

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Eggplants are vegetables largely consumed in many recipes as fresh and whole product or, more recently, as minimally processed vegetable (MPV). The main issue in diced eggplant consists in the oxidation due to polyphenol oxidase (PPO, EC 1.14.18.1), an enzyme producing pigments responsible for the undesirable dark color which spoiled the product. The aim of this work was to study fresh or heat-treated (100 °C for 15 min) onion by-products (paste, juice and bagasse) in order to evaluate in vitro the PPO enzyme inhibition capacity in minimally processed eggplant cv. 'Birgah'. The heated onion juice was the most suitable anti-browning extract among all by-products tested, followed by pasteurised paste and fresh juice (54.2, 41.2 and 37.3 % reduction in PPO activity, respectively). Data suggested that the thermal treatment enhances the effects of the extracts probably due to a synergic action between some endogenous active compounds present in onion and Maillard reaction products obtained during heating.

1. Introduction

Eggplant (*Solanum melongena* L.) is a solanacea widely cultivated in Sicily in open field, greenhouse or cold tunnel. Its fruits are largely consumed as fresh product and, more recently, as minimally processed vegetable (MPV), a rising commercial area in the fresh-cut industry. Unfortunately, their quality tends to decline by endogenous enzymatic activities whose reactions are promoted under certain technological conditions of production.
The various processes of chopping or slicing cut through cells and release cell contents at the sites of wounding. Subcellular compartmentalization is disrupted at the cut surfaces, and the mixing of substrates and enzymes (mainly, oxidases and pectinases), which are normally separated can initiate reactions that do not occur otherwise.
A serious shelf-life constraint for diced eggplant consists in the oxidation due to polyphenol oxidase (PPO, EC 1.14.18.1), an enzyme producing pigments responsible for the browning which spoiled vegetables and fruits (Castaner et al., 1999; Dincer et al., 2002).
PPO is located in cell organelles such as chloroplasts, mitochondria and peroxisomes where it is firmly bound to the membrane and may even be found in the soluble fraction of the cell. In young and unripe fruits, it is mostly present under conjugated form, while in the ripe fruits (Conforti et al., 2007). Van et et al. (1984) found increased PPO activity in response to mechanical shock.

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PPO catalyzes the hydroxylation of monophenols to *o*-diphenols (cresolasic activity) and the oxidation of *o*-diphenols to the corresponding *o*-quinones (catecolasic activity) which represent the main reaction products. The *o*-quinones can react with phenols or otherwise, copolymerizing, thus producing molecules with high molecular weight (melanoidins) responsible for the undesirable dark colour (Barbagallo et al., 2009). The specific reaction sequence which results in brown or black-coloured products depends on the specific structure of the polyphenolic substrate.
Usually in food preservation sulfites and their derivatives were used as they prevent the polymerization of quinones, combining irreversibly with them and forming colorless compounds, but their use may cause allergic reactions in asthmatics (Sapers, 1993; Martinez and Whitaker, 1995). Consequently, the current tendency is using natural inhibitors such as phenols, organic acids and ascorbic acid, to replace sulfating agents to prevent or minimize browning in vegetables (Sapers et al., 1989; Kwak and Lim, 2005; Raju and Bawa, 2006; Jeong et al., 2008).
Recent studies have shown that sulfhydryl (SH or thiol) compounds are good inhibitors of the enzyme PPO (Ding et al., 2002). In particular, onions are rich in S-alk(en)yl-L-cysteine sulfoxides (ACSO), thiosulfinates and propene disulfide (Davidson et al., 1983; Griffiths et al., 2002). Therefore, it is generally assumed that these natural sulfur compounds of low molecular weight contained in onions, may be usefully adopted to inhibit PPO.
The aim of this innovative work was to study *in vitro* the effect of fresh or pasteurised onion by-products (paste, juice and bagasse) as PPO inhibitors of minimally processed eggplants cv. 'Birgah', in order to use these natural and alternative substances to extending the shelf life of this vegetable.

2. Material and Methods

The eggplants were grown in cold greenhouse in the Eastern coast of Sicily (Pachino, Italy), whereas the anti-browning extracts were obtained in DISPA laboratory by processing onion wastes (residues and surpluses of bulbs provided by CNR) from Giarratana area (Ragusa, Italy), where this vegetable is traditionally cultivated.

2.1 Minimally processed eggplant preparation

Eggplant fruits uniformly sized, round shaped and light purple coloured were selected. In DISPA laboratory, the fruits were dipped into a cold 100 ppm chlorine solution for 30 sec, left to dry, sliced as cubes (2.5 x 2.5 x 2.5 cm) and packaged under room atmosphere in double barrier polyethylene terephthalate (PET) film bags and refrigerated for 3 h at 4.0 ± 0.5 °C (95 % UR). PPO inhibition assays were carried out after homogenizing fruits into an iced bath for 5 min, minimizing light and oxygen exposition by wrapping the samples in aluminum film.

2.2 Onion by-products preparation

In DISPA laboratory these bulbs were peeled, diced manually with a sharp knife and dipped in a cold 100 ppm chlorine solution for 30 s. The diced bulbs were homogenized into an iced bath for 5 min, minimizing the direct exposition to light and oxygen by wrapping samples with aluminum film.
A sample of the homogenate (fresh paste) was further processed through a domestic friction screw press to obtain two different onion by-products: ‘fresh juice’ (liquid fractions) and ‘fresh bagasse’ (solid fraction). Each of the three by-products were further separated into two sub-samples, the first one was immediately frozen at -18 °C, the second one was pasteurised (at 100 °C for 15 min) into a conventional autoclave and then stored at -18 °C until analysis, thus obtaining an onion ‘stabilised paste’, ‘stabilised juice’ and ‘stabilised bagasse’.
For the enzymatic assays, each of the 6 different onion by-product fractions was centrifuged at 10,000 g for 20 min at 4 °C and the supernatants were vacuum filtered through a 0.45 µm membrane filter (Whatman). These products were the eggplant PPO inhibitors used for the enzymatic assays.

2.3 Polyphenol oxidase (PPO) determination

Ten grams of eggplant homogenate were added of 25 mL cold acetone (-20 °C) and continuously stirred for 10 min. The homogenate was vacuum filtered through Whatman No. 42 paper and the acetone powder collected and suspended in 15 mL 0.1 M sodium phosphate buffer at pH 5.8, kept overnight at 4 °C, and then filtered under vacuum. Clear solution was ultrafiltered through a 50 kDa

membrane (Millipore, Milan, Italy) and utilized as enzymatic extract assayed spectrophotometrically (Cary IE-100 UV-VIS, Varian, USA) at 505 nm. The PPO activity was assayed using catechol as phenolic substrate, N,N-Dimethylformamide (DMF), sodium phosphate buffer and MBTH (3-metil-2-benzotiazolinone idrazone) as chromophore agent, according to a modified version of the method proposed by Spagna et al. (2005). Between PPO eggplant extract and the corresponding onion by-products 1:2 ratio was always applied. A sodium phosphate buffer added to eggplant extract was applied as control treatment. The reaction was stopped with H_2SO_4 1M. Blank was prepared adding H_2SO_4 before the enzymatic mixture addition.
One unit of PPO activity was defined as the amount of enzyme which produces 1 µmol of adduct per min at 25 °C under the conditions above described. The results were expressed as Ug^{-1} FW and were also calculated the values of percentage REA (relative enzymatic activity).

2.4 Browning index
Browning index was measured from the homogenized product into an iced bath, after centrifugation at 10,000 g per 20 min at 2 °C, filtration through Whatman No. 2 under vacuum on Buchner funnel and following spectrophotometric reading at 420 nm (Jeong et al., 2008). High absorbance values correspond to a greater tissue browning. Results are reported as percentage of variation respect to the untreated control (Relative Browning Index = 100).

2.5 Statistical analysis
Data were statistically analysed by analysis of variance (ANOVA) using CoStat version 6.003 (CoHort Software), assuming onion matrix (paste, juice and bagasse) and thermal treatment (fresh and pasteurised) as experimental factors. Differences among means were evaluated for significance using the Tukey-HSO test at $p \leq 0.05$. Different letters above bars represent statistical differences.

3. Results and Discussion

The adding of fresh or heat stabilised onion by-products, produced a generally *in vitro* PPO enzyme inhibition, but the significant interaction “onion matrix x thermal treatment” clearly pointed out that the thermal treatments, with the exclusion of the bagasse matrix, were the main responsible for the eggplant polyphenol oxidase inhibition (Figure 1).
The highest effect was achieved by the ‘stabilised juice’, that decreased the PPO enzyme activity to 1.84 Ug^{-1} FW, representing a dramatic reduction when compared with control (-54 %) and ‘fresh juice’ (-27 %) respectively. In previous works it has been shown that heated onion extracts were more effective in prevention of pear and banana browning than fresh onion extracts (Kim et al., 2005; Lee, 2007). When enzymatic unit calculation in terms of REA is considered, the residual PPO activity reached after adding different onion by-products as natural inhibitors, allowed to obtain the following order: onion ‘stabilised bagasse’ (REA= 76.8 %), ‘fresh bagasse’ (REA= 73.7 %), ‘fresh paste’ (REA= 71.1 %), ‘fresh juice’ (REA= 62.7 %), ‘stabilised paste’ (REA= 58.8 %), ‘stabilised juice’ (REA= 45.8 %). These results are in agreement with those previously reported, revealing that stabilising thermal treatment enhances the inhibition effect upon PPO in eggplant. This effect may be due to a synergic action between some endogenous bioactive sulfur compounds present in onion and others products obtained during heating (Roldán et al., 2008).
Moreover, observing the differences between matrices, it should be emphasized how the effectiveness of onion by-products, progressively increases with the increase of water content, and juice resulted more effective than paste and this last more than bagasse, despite the heat-treatment. This evidence could suggest an hydrophilic nature of the inhibiting agent contained in onion matrix.
The results about the effectiveness of onion by-product extracts were confirmed ($p \leq 0.05$) also in terms of percentage of relative browning index as compared to the untreated control (Figure 2).
To a higher PPO inhibition in eggplant by onion by-products corresponded a browning index reduction. These results seem interesting for a real perspective future scale-up, although the technological and stabilisation processes applied may influence significantly the PPO inhibition capacity of onion extracts.

4. Conclusion

Polyphenol oxidase activity in minimally processed eggplant fruit was significantly reduced *in vitro* by the different onion by-products tested, with more evident results with stabilised juice. These positive results were confirmed by a reduced percentage of browning index.

The onion treatments may allow to prolong the shelf-life of minimally processed eggplant, under appropriate *in vivo* dipping conditions, suggesting combinations of different inhibitors associated with the variation of gas concentration inside packaging.

The observation on the highest effectiveness of the onion juices could also represent an interesting tool for a real perspective future scale-up for MPV treatment oriented to the application of the liquid onion by-product fractions.

Furthermore, this last could present functional advantages due to the easier application of liquid matrices when compared to the whole by-products.

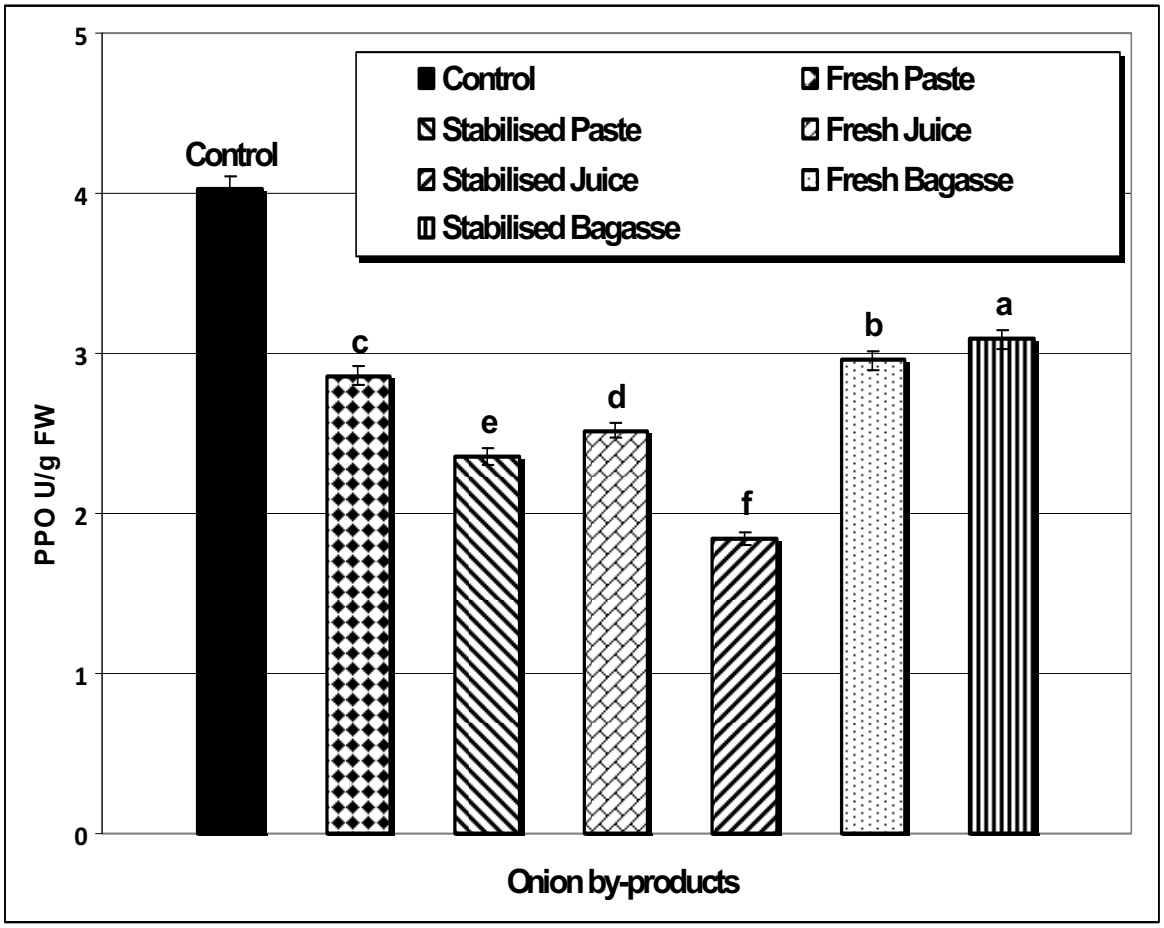

Figure 1: PPO in vitro of minimally processed eggplant treated with different fresh and thermal stabilised onion by-products extracts

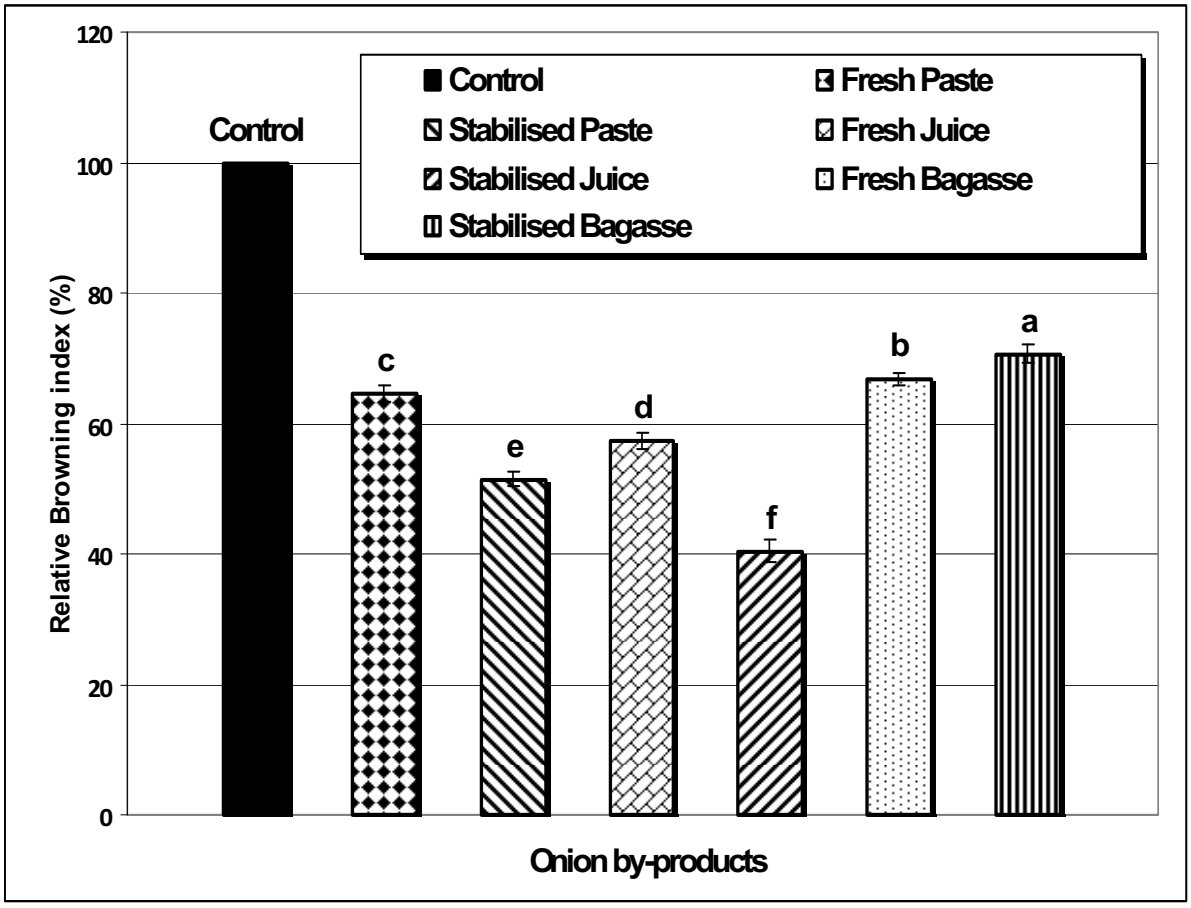

Figure 2: Relative browning index of minimally processed eggplant treated with different fresh and thermal stabilised onion by-products extracts

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