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Short Communication

Valorization of onion extracts as anti-browning agents

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Abstract

The enzymatic browning, whose main responsible is polyphenol oxidase (PPO, EC 1.14.18.1), is involved in the phenolic oxidation and colour alteration of minimally-processed fruits and vegetables. Currently, the research of new strategies to inactivate PPO is moving towards replacing synthetic additives such as organic acids and sulphites with natural inhibitors. The present study is focused on investigating the anti-browning performance of juices and distillates obtained from three onion varieties (white, yellow, and red) and Borettana onion wastes (inner layers). Their inhibitory activity on a commercial mushroom tyrosinase and some plant PPOs has been evaluated by spectrophotometric and electrophoretic analysis. The *in vivo* trials has been also carried out by monitoring over time at room temperature the colour change on potato slices under accelerated browning conditions. The effectiveness of onion samples in limiting enzymatic browning was affected by not only the enzyme source but also inhibitor type. Although distillates had higher anti-PPO capacity as confirmed by *in vitro* assays, juices showed better *in vivo* effectiveness. Hence, onions and their wastes can be valorised as a natural source of anti-browning agents to control PPO activity thus preserving sensory, antioxidant and nutritional properties of agro-food products.

Keywords: enzymatic browning, polyphenol oxidase, anti-browning, onion, agricultural wastes

Abbreviations:

PPO – Polyphenol oxidase
TYR – Commercial mushroom tyrosinase
C – Control without inhibitors
AA – Ascorbic acid (0.05% w/v)
CB – White onion juice
CG – Yellow onion juice
CR – Red onion juice
CBo – Borettana waste juice
DB – White onion distillate
DG – Yellow onion distillate
DR – Red onion distillate
Dbo – Borettana waste distillate.

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Introduction

The enzymatic browning is associated with the most of qualitative and economic losses in agro-food industry. The main responsible is polyphenol oxidase (PPO, EC 1.14.18.1), a copper-containing oxidoreductase that catalyses the oxidation of phenolic compounds in dark pigments thus leading to the antioxidant degradation and colour alteration of fruits and vegetables after post-harvest operations (Tinello and Lante 2018). Currently, the research of new anti-browning strategies is moving towards replacing synthetic additives (e.g. organic acids and sulphites) as well as thermal treatments with non-thermal technologies (Lante et al. 2013; Lanteet al. 2016) and/or natural extracts derived from plants (Zocca et al., 2011), agricultural by-products (Lante and Tinello 2015) and wastes (Zocca et al. 2010; Tinello and Lante 2017; Tinello et al. 2018; Tinello and Lante 2019). In this regard, the species belonging to the *Allium* family are recognized as a good source of healthy phenolic and organosulfur compounds (Corzo-Martínez et al. 2007; Santas et al. 2008; Mihaylova et al. 2014; Putnik et al. 2019). Moreover, Yuniarti et al. (2018) identified several phenolic PPO inhibitors such as quercetin, kaempferol, and cyanidin in onion by using metabolomics approach. Even onion by-products and wastes are rich in bioactive compounds with antioxidant and anti-browning properties (Roldán et al. 2008; Singh et al. 2009; Benítez et al. 2011; Pal and Jadeja 2019; Sidhu et al. 2019). In fact, not only onion bulbs but also peels have been previously used for the development of functional foods (Tinello et al. 2017; Piechowiak et al. 2020). Hence, the aim of this preliminary study is to *in vitro* and *in vivo* evaluate the anti-PPO activity and anti-browning effect of juices and distillates obtained from onion bulbs and wastes.

Materials and Methods

Among the enzyme sources, commercial mushroom tyrosinase (TYR; 3,130U/mL) was obtained from Sigma-Aldrich (St Louis, MO, USA), while plant PPOs were extracted from potato tubers (*Solanum tuberosum* cv. Bintje), fennels (*Foeniculum vulgare*), and eggplants (*Solanum melongena*), and freeze-dried as reported by Zocca et al. (2010). Their protein concentration was determined by the Bradford assay using bovine serum albumin as a protein standard (Bradford 1976).

Regarding samples to be tested as PPO inhibitors, three onion varieties (*Allium cepa* L. cv. white, yellow, and red) were purchased from a local market, while the inner layers of Borettane onions were collected at the Naturello company (Pojana Maggiore, Vicenza, Italia). Juices and distillates were obtained from the peeled onions of white (CB and DB,), yellow (CG and DG) and red (CR and DR) varieties, and from Borettana onion wastes (CBo and DBo) by following the procedures described by Tinello and Lante (2017) and Lante and Tinello (2015), respectively. Onion samples were filtered through a Millipore 0.45μm filter membrane (Merck Millipore, Billerica, MA, USA) and stored at –20 °C in the dark until use. The pH values were in the range of was approximately of 5.7±0.1 and 6.2±0.2 for onion juices and distillates, respectively.

The inhibitory effect of onion juices and distillates as well as 0.05% w/v ascorbic acid (AA, reference inhibitor) on TYR and plant PPOs has been *in vitro* evaluated according to the spectrophotometric assay of Tinello and Lante (2017). Before measuring the enzyme activity, the freeze-dried enzymes were dissolved in 0.1 M sodium citrate buffer at pH 6.0 for TYR (9,390 U/mL), while at pH 6.0, 6.3, 5.5 for respectively potato (10 mg/mL), fennel (100 mg/mL) and eggplant (50 mg/mL) PPOs. The PPO activity in the absence (C, control) and in the presence of inhibitors has been detected using a Varian Cary 50 Bio UV/Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at 400 nm and 25 °C with 10 mM catechol as the phenolic substrate.

The inhibitory effect of onion samples and AA was also assessed on the isoforms isolated from TYR and plant PPOs. Non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and zymographic techniques with the L-DOPA/MBTH complex were performed in a Mini Protean II (Bio-Rad-Laboratories, Milano, Italy) at room temperature following the procedure of Lante et al. (2016). Before electrophoresis, TYR (1 mg) was solubilised with 700 μL of distilled water and 300 μL of Laemmli buffer (1.33 mol/L Tris, pH 7.4, 40% v/v glycerol, 8% w/v SDS), and centrifuged at 14,000 rpm for 2 min. An amount equal to 5 μL of TYR solution was loaded in the electrophoretic gel.

The images of zymograms were acquired by a scanner.

The anti-browning effect was *in vivo* evaluated by monitoring over time (5, 10, 20, 30, 60 min) at 25 °C the colour change on the surface of potato slices treated with distilled water (C, control), onion samples and AA after 10 mM catechol application according to Tinello and Lante (2017).

All of the data obtained from three replicates were analyzed by a one-way analysis of variance (ANOVA) using R software (3.1.2 version) Significant differences among means were determined by Tukey's multiple range test ($P \leq 0.05$).

Results and Discussion

Since the development of new inhibitors for controlling enzymatic browning requires a multidisciplinary approach, the inhibition of PPO by onion juices and distillates was widely evaluated through *in vitro* and *in vivo* trials.

The inhibitory effect of the onion samples on a commercial TYR and some vegetable PPOs was spectrophotometrically determined in comparison with that of 0.05% w/v AA as the reference anti-browning compound, using 10 mM catechol as the phenolic substrate (Table 1). Although AA exhibited the best inhibitory results, juices and distillates effectively decreased the activity of all tested enzymes, whose units were significantly ($P \leq 0.001$) lower than those of control (C) and varied depending on the PPO source and onion sample. In details, onion distillates were more effective than juices in strongly reducing the activity of potato, fennel, and eggplant PPOs whose percentage inhibition was grater than 50%. The best inhibitory effect of onion distillates was mainly observed towards potato PPO with inhibition values (86±3%) similar to that obtained after applying the reference AA (86%). Among onion distillates, DB and DBo had respectively the highest and the lowest inhibition on TYR and vegetable PPOs. Among onion juices, CR and CG showed the greatest inhibitory results. Moreover, the samples DB, CR and CG achieved the best anti-TYR activity with inhibition values (41%, 37% and 37%, respectively) similar to that of the reference AA (39%) and higher than those of pomegranate extract (27%; Zocca et al. 2011) and Brassicacaea processing water (23%; Zocca et al. 2010), at the same analytical conditions. However, onion juices and distillates did not show a lag phase, which was a typical characteristic of reducing agents such as AA (20 s for TYR; 200, 150, 92 s for respectively potato, fennel, and eggplant PPOs) that indirectly inhibited PPO by reducing *o*-quinones to colourless *o*-diphenols (Tinello and Lante 2018).

Based on zymograms (Fig. 1), onion distillates (Fig. 1A), especially DB, showed a greater inhibitory effect than juices (Fig. 1B) and AA on the activity of the one isoform of TYR by completely reducing the colour intensity of the corresponding band.

Altough electrophoretic results (Fig. 1) confirmed the spectrophotometric ones (Table 1), the *in vivo* trials showed that onion juices were more effective than distillates in limiting over time the colour change on the surface of potato slices. This could be due to the high volatility of potential inhibitors contained in the onion distillates.

Table 1. The inhibitory effect* on commercial TYR and plant PPOs.

PPO source	Inhibitors									
	C	**AA**	**CB**	**CG**	**CR**	**CBo**	**DB**	**DG**	**DR**	**DBo**
TYR										
activity, U/min	469^{d}	286^{a}	357^{d}	295^{a}	294^{a}	396^{c}	276^{a}	369^{c}	312^{ab}	389^{c}
inhibition, %		39	24	37	37	16	41	21	33	17
Potato										
activity, U/min	232^{d}	32^{a}	164^{bc}	146^{b}	146^{b}	177^{c}	30^{a}	40^{a}	28^{a}	37^{a}
inhibition, %		86	30	37	37	24	87	83	88	84
Fennel										
activity, U/min	266^{f}	37^{a}	138^{e}	136^{d}	123^{d}	166^{d}	67^{b}	76^{b}	75^{b}	102^{c}
inhibition, %		86	48	49	54	38	75	71	72	62
Eggplant										
activity, U/min	211^{f}	52^{a}	195^{ef}	169de	141^{cd}	179^{e}	92^{b}	112^{bc}	103^{b}	115^{bc}
inhibition, %		75	7	20	33	15	57	47	51	45

*The inhibitory eeffect was calculated as follows: % inhibition = $[(\Delta A_{control} - \Delta A_{inhibitor})/\Delta A_{control}]$ x 100%, where $\Delta A_{control}$ is the absorbance variation per minute at 400 nm without inhibitor, and $\Delta A_{inhibitor}$ is the absorbance variation per minute at 40 nm with inhibitor.

C: control without inhibitors; AA: 0.05% w/v ascorbic acid; CB: white onion juice, CG: yellow onion juice, CR: red onion juice; CBo: Borettana waste juice; DB: white onion distillate; DG: yellow onion distillate; DR: red onion distillate; DBo: Borettana waste distillate.

[a-f] Values with different letters are statistically different ($P \leq 0.05$) as determined by Tukey's multiple range test.

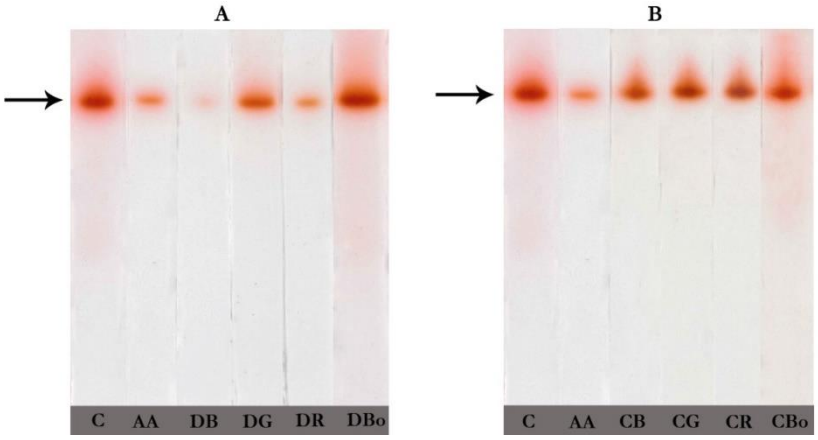

Figure 1. SDS-PAGE 12% zymograms of TYR (16 U loaded for lane). Panel A: TYR without inhibitors (C) and with 0.05% w/v ascorbic acid (AA), white onion distillate (DB), yellow onion distillate (DG), red onion distillate (DR), Borettana waste distillate (DBo). Panel B: TYR without inhibitors (C) and with 0.05% w/v ascorbic acid (AA), white onion juice (CB), yellow onion juice (CG), red onion juice (CR), Borettana waste juice (CBo). The arrows indicate the enzymatic isoforms of TYR on the zymograms.

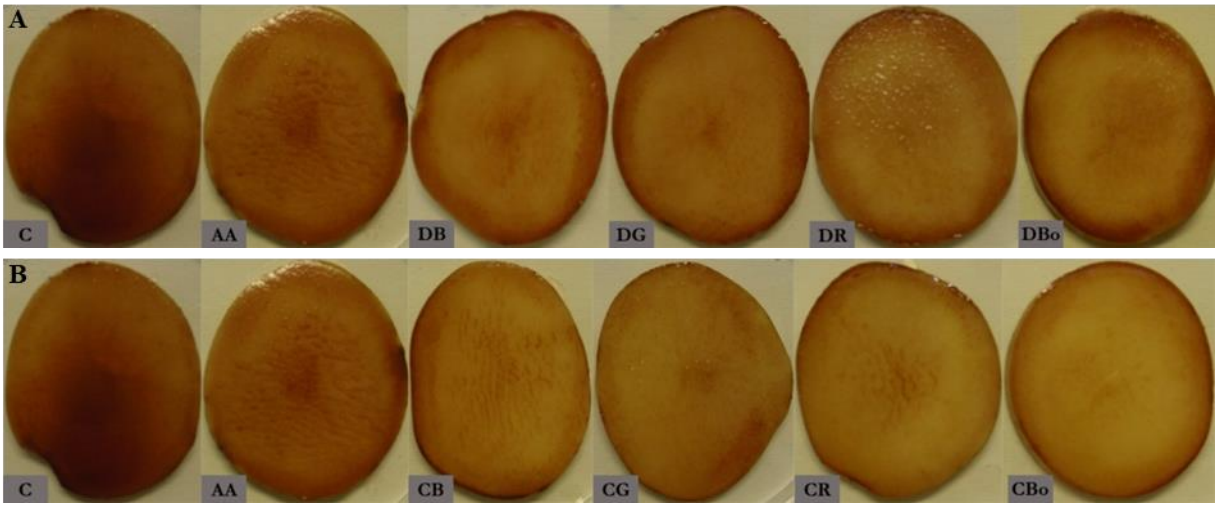

Figure 2. The anti-browning effect on potato slices. Panel A: Potato slices treated with distilled water (C), 0.05% w/v ascorbic acid (AA), white onion distillate (DB), yellow onion distillate (DG), red onion distillate (DR), Borettana waste distillate (DBo). Panel B: Potato slices treated with distilled water (C), 0.05% w/v ascorbic acid (AA), white onion juice (CB), yellow onion juice (CG), red onion juice (CR), Borettana waste juice (CBo). The browning surface has been monitored 30 min after the application of 10 mM catechol at 25 °C.

Conclusions

Onion juices and distillates, which have been demonstrated effective in controlling PPO activity and consequently limiting the colour alteration in minimally-processed vegetables, could be considered safe, cheap, and eco-friendly sources of inhibitors. Their anti-browning grade was affected by the enzyme origin, sample type, and onion variety. However, the *in vitro* results was not supported by *in vivo* trials. Therefore, further research is needed focusing particularly on the chemical characterization of onion samples to identify the bioactive compounds with anti-browning effectiveness. Moreover, the inner layers of Borettane onions could be recovered to produce natural anti-browning agents for agro-food industry thus converting these agricultural wastes into value-added products.

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