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Evaluation of seaweed extracts functionality as post-harvest treatment for minimally processed Fuji apples

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ABSTRACT

The potential application of *Fucus spiralis*, *Bifurcaria bifurcata*, *Codium tomentosum* and *Codium vermilara* extracts as post-harvest treatments in minimally processed (MP) Fuji apple was investigated. 0.5% solutions of each extract were applied to MP Fuji apple and its effect on product quality was assessed over 20 days of storage at 4 ± 2 °C in terms of moisture content, soluble solids concentration (SSC), firmness, browning index (BI) and POD and PPO activities. Application of *Codium tomentosum* extract resulted in a significant reduction in BI in comparison to all other tested treatments. POD and PPO activities were also significantly lower in MP apple treated with *Codium tomentosum* extract. These results identify *Codium tomentosum* extract functionality of reducing enzymatic browning, suggesting the potential application of this extract as a natural additive in MP fruit.

Industrial relevance: This study shows the advantages of using a novel post-harvest treatment based in edible seaweed extracts to preserve fresh-cut fruits, in particular Fuji apple. The results show clearly that this type of dipping treatment decreases browning index and inhibits enzymatic activities when compared with citric acid and control treatment (water). *Codium tomentosum* extract solution showed the highest efficacy, suggesting the potential application of this extract as a natural additive to be a substitute of chemicals used in food industry and its use has been restricted. This process is efficient, versatile and of simple implementation at the industrial level once the only change in the industrial process consists in the type of post-harvest solution treatment. Associated to the easy adaptation in the industrial process, the exponential development in aquaculture sector allows the obtention of this raw material in a sustained way. Extract functionality was attributed to its demarked inhibitory action on enzymatic activities of polyphenol oxidase and peroxidase, two major enzymes involved in enzymatic browning processes.

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1. Introduction

Minimally processed products (MP) are a rapidly growing segment of the retail and food service horticultural industry due their fresh-like character, convenience, and health benefits (Qi, Hu, Jiang, Tian, & Li, 2011). The main steps of MP products production are harvesting, washing, disinfection, cutting and packaging (Daza, Alzamora, Chanes, & Gould, 1996; Shewfelt, 1987).

The physiology of MP is essentially that of the wounded tissues leading to excessive tissue softening and superficial browning, which represent the greatest hurdle to MP development and distribution. The intensity of the wounded response is affected by factors such as species, variety and initial maturity state. Studies show that the more advanced the stage of ripeness, the more susceptible the fruit is to wounds, hence to minimal processing (Bulens et al., 2012). Raw produce are expected to have a shelf-life of several weeks or months, while MP have a storage life of 4 to 7 days. Even though storage lives up to 21 days would be desirable for wide product distribution, ethylene production, respiration activity, enzymatic and non-enzymatic browning make this goal difficult (Ramos, Miller, Brandão, Teixeira, & Silva, 2013).

The growing demand for new ready to eat products is increasing, and fresh-cut apples are a new approach to encourage children to eat fruit. However, such as other fruits and vegetables, the visual appeal is one of the main concerns, i.e. no visible browning in apples surface, which is a determinant quality index for consumer acceptance. Cut apples browning is due to enzymatic browning processes in which the major enzymes involved are polyphenoloxidase (PPO) and peroxidase (POD) (Jang & Moon, 2011; Qi et al., 2011). Browning occurs almost instantly when the cell structure is destroyed, promoting contact between enzyme and substract. PPO catalyzes the hydroxylation of monophenols and oxidation of *o*-diphenols to *o*-quinones, followed by a non-enzymatic formation of melanin responsible for the black, brown or red color characteristic of enzymatic browning (Oms-Oliu et al., 2010; Tomás-Barberán & Espín, 2001).

Additionally fresh-cut fruits' and vegetables' shelf-life can also be enhanced by one of the following strategies: dipping in a solution containing antibrowning agents, exposure to active volatile substances or the application of edible coatings. Substances such as citric acid, ascorbic acid and the now forbidden sulfites have been applied as a

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surface treatment to fresh-cut fruit with the objective to reduce its visual quality deterioration (Oms-Oliu et al., 2010). Interest in the use of natural products to replace synthetic additives has been increasing, and seaweeds are a good source of compounds with potential application in food industry. Edible seaweeds are known for their high polysaccharide, protein, mineral and vitamin contents, low lipid content and for the presence of bioactive compounds, such as antioxidants (Gómez-Ordóñez, Jiménez-Escrig, & Rupérez, 2010; Haddar et al., 2012). In fact, during the last decades seaweeds or their extracts have been intensively studied as potential natural antioxidants. These properties suggest the potential application of edible seaweeds or its extracted compounds as post-harvest treatment and in the formulation of edible coatings. Edible seaweeds are widespread around the world, and could be a sustainable and cost effective source of natural antioxidants (Andrade et al., 2013).

No references were found in the literature regarding the application of edible seaweeds extracts as post-harvest treatment in MP fruit. Given the evidence of the antioxidants' presence in these organisms and the high availability of this resource, this study aims to investigate the functionality of four edible seaweed extracts, *Fucus spiralis*, *Bifurcaria bifurcata*, *Codium tomentosum* and *Codium vermilara* in reducing quality loss in MP Fuji apples.

2. Methods and materials

2.1. Materials

Fuji apples were directly obtained from a local producer in Torres Vedras, Portugal (Campotec S.A.) and stored at room temperature (25 ± 3 °C) until use.

2.2. Chemicals

Galic acid and 2,2-Diphenyl-1-picrylhydrazyl (DPPH·) were purchased from Sigma-Aldrich Co. (Steinhem, Germany). Citric acid, polyvinylpyrrolidine (PVP), guayacol, hydrogen peroxide (H_2O_2) and cathecol were obtained from Scharlab, S.L. (Sentmenat, Spain). Folin–Ciocaulteu reagent and sodium carbonate were purchased from Panreac Química (Barcelona, Spain) and Merck (Darmstadt, Germany), respectively. Ethanol was reagent grade and obtained from Aga (Lisboa, Portugal) and distilled water was used.

2.3. Seaweed material

Fresh *Fucus spiralis*, *Bifurcaria bifurcata*, *Codium tomentosum* and *Codium vermilara* samples were collected from Peniche coast, Portugal in September 2012, immediately placed on ice after collection and transported in insulated, sealed ice boxes. Seaweeds were washed with distilled water at room temperature, without exposure to direct light, and kept at −80 °C, prior to freeze drying. The dried material was milled and kept at −80 °C, until it was subjected to extraction.

2.4. Preparation of seaweed extracts

Ethanolic seaweed extracts (SE) were prepared by stirring 2.0 g of dried material, 22.5 ml of water and 7.5 ml of ethanol, protected from light for 6 h. After centrifugation at 2000 *g* for 10 min to remove undissolved debris the supernatant was collected and filtered through a Büchner funnel. Extraction solutions were dried by vacuum-evaporator at 40 °C, frozen at −80 °C and freeze dried (24 h). The dried extracts were stored at −80 °C until further analysis.

2.5. Preparation of seaweed extracts solutions

Solutions were prepared by dissolving extracts or citric acid (0.5 g/100 ml water) in distilled water. Two control conditions were used: distilled water (A) and 0.5% citric acid (B). The treatment formulations tested were: (C) 0.5% *Fucus spiralis*, (D) 0.5% *Bifurcaria bifurcata*, (E) 0.5% *Codium tomentosum*, and (F) 0.5% *Codium vermilara*.

2.5.1. Solutions characterization

2.5.1.1. Total phenolic content. Total phenolic content was determined as gallic acid equivalents (GAE) (Rodrígues-Rojo et al., 2012) with a few modifications. In a microfuge tube were added distilled water (790 μl), 10 μl of sample and 50 μl of Folin–Ciocaulteu reagent. After 2 min was added 150 μl of Na_2CO_3 to 20% (w/v). After 1 h incubation at room temperature in the dark, the absorbance was measured at 755 nm and compared to a prepared gallic acid calibration curve.

2.5.1.2. Assay of DPPH free radical-scavenging activity. The DPPH radical-scavenging activity was assayed by the method described in Al-Dabbas et al. (2007) with some modifications. DPPH solution (0.1 mM) in absolute ethanol was prepared. For the final reaction 10 μl of sample and 990 μl of DPPH solution were mixed. After 30 min of incubation at room temperature in the dark, absorbance was measured at 517 nm, distilled water being used as the blank. All samples were analyzed in triplicates. The DPPH radical-scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity}(\%) = [(\text{Absorbance of the sample} - \text{Absorbance of blank})/\text{Absorbance of blank}] \times 100 \quad (1)$$

2.5.1.3. Enzymatic activity. Enzyme extraction was carried out according to Jang and Moon (2011) with minor modifications. Apple wedges were first frozen at −80 °C in order to minimize the enzymatic activity changes. To minimize variability between sample results, each enzyme extract was obtained from three apples homogenized in a twofold amount of chilled 50 mM sodium phosphate buffer (pH 7.0) containing PVP (50 g/l) for 3 min at room temperature and protected from light. The homogenate was filtered through cheese cloth and the filtrate was centrifuged at 12,000 *g* for 30 min at 4 °C. The supernatant solution was used in experiments. Crude extract samples were divided into small aliquots and frozen at −80 °C for 24 h. After thawing at 0 °C, the samples were immediately used.

Enzymatic activity was determined according to Ponce, Roura, del Valle, and Moreira (2008) with some modifications. POD activity was determined spectrophotometrically at 470 nm using guayacol as the substrate and H_2O_2 as the hydrogen donor. The substrate mixture contained 10 ml of 1% guayacol, 10 ml of 0.3% hydrogen peroxide and 100 ml of 0.05 mM sodium phosphate buffer (pH 6.5). The reaction cuvette contained 2.90 ml substrate mixture and 0.1 ml enzyme extract and 0.03 ml of coating solution. Enzymatic activity was defined by the slope of absorvance *vs.* time with one unit of activity defined as a change in absorbance of 0.01 min^{-1} per milligram of fresh apple (U/mg_{FA}). PPO activity was measured spectrophotometrically at 400 nm. The substrate mixture contained 20 mM cathecol as substrate in 5 mM sodium phosphate buffer (pH 7). The reaction cuvette contained 2.87 ml of substrate mixture and 0.1 ml PPO extract and 0.03 ml of coating solution. Enzymatic activity was defined as described for POD.

For POD and PPO the reference cuvette contained only substrate mixture. For each enzyme source, a reagent blank was prepared with 0.1 ml distilled water instead of extract (control sample).

2.6. Apple treatment

Apple slicing and treatment application were performed according to the methods described by Rojas-Graü, Tapia, and Martín-Belloso (2008) and Wu and Chen (2013). Apples with uniform size, color, and

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