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Inactivation of pectic lyases by light exposure in model systems and fresh-cut apple

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ABSTRACT

UV-C light exposure caused the inactivation of pectin lyases from *Aspergillus japonicus* and pectate lyase from apple under non-thermal conditions. Samples exposed to 20 W m^{-2} UV-C light showed D_L values, defined as the time needed for 90% enzyme activity reduction, around 20 min. However, an initial activation phase was observed for fungin pectin lyase, while UV-C light resistant forms of pectate lyase were identified in apple. Lyase inactivation occurred as a consequence of enzyme cleavage into fragments without catalytic activity having MW around 5 kDa. Fresh-cut apple slices exposed for a few minutes to UV-C light resulted significantly firmer than the untreated ones during 4 days of refrigerated storage, reasonably due to the decrease in activity of both endogenous and microbial lyases on the surface of the wound apple tissue.

Industrial relevance: UV-C light blanching allows to non-thermally increase the enzymatic stability of the surface of fresh-cut fruit and vegetables.

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1. Introduction

Fresh-cut fruits and vegetables are tremendously growing segments in retail establishments due to increasing consumer demand for fresh, healthy and convenient foods. The shelf life of these products is strictly related to water loss, enzymatic browning, microbial growth and texture deterioration, among others. Modifications of cell wall structure and composition are considered the major factors of texture deterioration of fresh-cut fruit and vegetables (Rose & Bennett, 1999). Although the mechanisms of this process remain unclear and are still subjected to much speculation, they can be, at least in part, ascribed to the activity of the same cell wall-modifying enzymes involved in fruit growth and development (Goulao, Santos, de Sousa, & Oliveira, 2007). The major enzymes affecting cell wall integrity and thus fruit firmness are polygalacturonase, pectin methyl esterase, cellulase and pectic lyase (Prasanna, Prabha, & Tharanathan, 2007; Tucker & Grierson, 1987). These endogenous enzymes are generally released upon tissue wounding thus accelerating the physiological disassembly of the pectin matrix. However, a contribution to texture deterioration may also derive from microbial enzymes associated to hygienic spoilage (Hamdy, 2005; Jayani, Saxena & Gupta, 2005).

At the moment, only the application of calcium and other cations, either by dipping, spraying or edible coating, has been demonstrated to produce beneficial effects on the texture of fresh-cut plant derivatives (Beirão-da-Costa, Cardoso, Martins, Empis, & Moldão-Martins, 2008; Saftner, Bai, Abbott, & Lee, 2003; Soliva-Fortuny, & Martín-Belloso, 2003). However, these chemical substances strengthen plant cell walls by cross-linking with carboxyl groups of pectin chains found in the middle lamella but do not inactivate enzymes.

The inactivation of cell wall degrading enzymes would be very important in the food industry because of their influence on the final quality of fresh-cut fruit and vegetables. Enzyme inactivation in plant tissues is usually performed by conventional technologies mainly based on heat treatments. These processing operations, however, are well known to exert strong negative consequences on the "fresh-like" appearance, which is a basic quality requirement for these products. Different non-thermal physical strategies, including the exploitation of high pressure, electric fields and different electromagnetic waves, have also been proposed to reach food enzymatic stability (Barbosa-Canovas, Pothakamury, Palou, & Swanson, 1998; Espachs-Barroso, Van Loey, Hendrickx, & Martín-Belloso, 2006; Liu, Gao, Peng, Yang, Xu, & Zhao, 2008; Lopez, Vercet, Sanchez, & Burgos, 1998; Sun & Song, 2003). Despite being expensive, these treatments present the ultimate criticisms of being hardly applicable in the manufacture of fresh-cut fruit and vegetables.

Ultraviolet-C (UV-C) treatment, which exploits the germicidal effect of the radiation from the electromagnetic spectrum from 200 to 280 nm, is a powerful non-thermal germicidal method which has raised a large attention in the last years due to easy use and favourable costs of equipment, energy and maintenance as compared to other non-thermal antimicrobial technologies (Koller, 1965; Miller, Jeffrey, Mitchell & Elasri, 1999; Smith & Hanawalt, 1969). Moreover, such technology has not been reported to form known toxic or significant non-toxic by products (Keyser, Műller, Cilliers, Nel, & Gouws, 2008).

Nowadays, ultraviolet light is widely used for sterilisation purposes of equipment, devices and food surface (Bintsis, Litopoulou-Tzanetaki & Robinson, 2000; Guerrero-Beltràn & Barbosa-Cànovas, 2004). In

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addition, it has also been proposed to prolong the shelf life of sugar syrups, apple cider and fruit juices (Choi & Nielsen, 2005; Huang & Toledo, 1982; Kaess & Weidermann, 1973; Nakayama & Shinya, 1981; Stother, 1999; Tran & Farid, 2004; Wallner-Pendleton, Sumner, Froning & Stetson, 1994). As recently suggested, these positive effects could be attributed to the influence of UV-C light on the activity of the enzymes present in food.

UV-C light exposure has actually been demonstrated to exert contradictory effects on the activity of different enzymes in fresh-cut Cantaloupe melon (Lamikanra, Kueneman, Ukuku & Bett-Garber, 2005). The enzyme pectin methyl esterase in orange juice was indicated to be not affected by UV-C treatment, whilst polyphenoloxidase in mango nectar and alkaline phosphatase were reported to lose their activity (Guerrero-Beltràn & Barbosa-Cànovas, 2006; Keyser et al., 2008; Tran, & Farid, 2004). More recently, UV-C light was shown to inactivate mushroom polyphenoloxidase by inducing protein aggregation (Manzocco, Quarta & Dri, 2009).

However, it should be noted that no information is available about the effects of UV-C light on cell wall degrading enzymes involved in the softening process occurring during storage of fresh-cut fruit and vegetables.

On the basis of these considerations, the aim of the present paper was to evaluate if, and at what extent, UV-C light exerting the highest germicidal effect (253.7 nm) could also represent an unconventional tool to inactivate enzymes responsible for plant tissue softening. Such information could be useful in order to develop novel approaches to non-thermally inactivate cell wall degrading enzymes in fresh-cut fruit and vegetables.

To this purpose, the research was focused on the possibility to non-thermally inactivate cell wall degrading enzymes having both microbial or endogenous origin by their UV-C light exposure. In particular, pectic lyases were chosen as representative enzymes degrading pectic substance by transelimination mechanisms yielding to unsaturated oligogalacturonates. Among pectic lyases, microbial pectin lyase and pectate lyase from plant tissues are the two most important enzymes (Gummadi & Kumar, 2006). The research was articulated in three steps. First, an aqueous solution containing pectin lyase from *Aspergillus japonicus* was exposed to increasing intensity of UV-C light. The effect of light was then assessed by evaluating the residual enzymatic activity and studying enzyme molecular changes by HPLC-gel permeation. Following, the effect of UV-C light on the activity of pectate lyase in apple juice and fresh-cut slices was considered. Finally, the possibility to apply UV-C light exposure to obtain fresh-cut apple slices with increased firmness was studied.

2. Materials and methods

2.1. Pectic lyase solution

A. japonicus pectolyase (4.5 U/mg, Sigma, St. Louis, MO, USA) containing pectin lyase and polygalacturonase was used. The solution was prepared by dilution in 0.1 M phosphate/citrate buffer at pH 6.0 (J.T. Baker, Deventer, Holland). Initial lyase activity on 0.5% (w/v) pectin from apple (Sigma, St. Louis, USA) in 0.1 M phosphate/citrate buffer at pH 6.0 was 0.004 Abs/min corresponding to 9.6 U. One unit of pectic lyase activity is the amount of enzyme which produces 1 mol of unsaturated pectin per minute under the testing conditions. The molar extinction coefficient of unsaturated pectin was assumed to be $5.5 \cdot 10^3 M^{-1}\ cm^{-1}$ (Jayani et al., 2005). Aliquots of 0.5 mL enzyme solution were introduced into 1.5 mL capacity polypropylene Eppendorf tubes and submitted to UV-C light treatment.

2.2. Fruit sample handling

Golden delicious apples were purchased at the local market and stored at 4 °C. They were washed, wiped, cored and centrifuged by using a domestic centrifuge (FP800 Kenwood electronic, Havanthants, UK). These operations were carried out at 4 °C. The juice was then clarified by centrifuging for 5 min at 5000 rpm at 4 °C (Beckman Avanti J-25 Beckman Instruments Inc., Palo Alto, CA, USA). Aliquots of 12 mL of apple juice were introduced into Petri plates with a 60 mm diameter without cover (Vetrotecnica, Padova, Italy) and submitted to UV-C light treatment. Thickness of apple juice layer was 4 mm.

Apples were washed, wiped, cored and manually cut into 1 cm thickness slices. Slices were submitted to UV-C light treatment and stored in the dark for up to 6 days at 4 °C.

Additional slices having 2 mm thickness were also prepared and submitted to UV-C light treatment.

2.3. UV-C light treatments

15 W lamps (OF, OSRAM GmbH HNS, Munich, Germany) with maximum emission at 253.7 nm were used. The lamps were positioned into a thermostated cell (Climacell 222, MMM Medcenter, Einrichtungen GmbH, Graefelfing, Germany) equipped with a system of air moisture control settled at 95 ERH % to avoid sample dehydration during light treatment. Pectic lyase solution, apple juice and apple slices were treated at 4 °C by perpendicularly placing them at different distances from the lamp. Apple slices were UV-C light treated at 4 °C for 5 min. In order to expose to the light both apple slice surfaces, apple slices were perpendicularly light treated by positioning them between two parallel lamps.

UV-C light irradiance on the solution of pectin lyase from *A. japonicus* was 0, 11.3, 13.8, 19.1 and 33.4 W m^{-2}. Irradiance on apple juice was 21.9 W m^{-2}. Irradiance on apple slice surface was 13.8 W m^{-2}.

No temperature changes were observed as a consequence of lighting in all samples.

2.4. Thermal treatment

Pectin lyase solution was heated at 70 °C for up to 5 min into a TC1 thermostatic bath (YELLOW line, IKA-Werke, Germany). After thermal treatment, samples were quickly cooled under running water.

2.5. Temperature

Sample temperature was measured by a thermocouple probe (Hanna Intruments, Tersid s.r.l., Milan, Italy).

2.6. Weight loss

Apple slices were weighted by a 2400 g capacity balance with 0.01 g divisions (TM 2000, Gibertini Elettronica, Novate, Italy).

2.7. Irradiance

Irradiance was measured using a portable luminometer (HD-2102.2 Delta Ohm, Padua, Italy) equipped with a UV light probe (LP471 UVC, Padua, Italy).

2.8. Firmness

Firmness was measured by a puncture test using an Instron 4301 (Instron LTD, High Wycombe, UK). The instrumental settings and operations were accomplished using the software Automated Materials Testing System (version 5, Series IX, Instron LTD, High Wycombe, UK). On the test day, apple slices were punctured with a 1.5 mm cylindrical probe. Crosshead speed was set at 100 mm/min. Force–distance curves were obtained from the puncture tests and firmness was taken as the force (N) required to puncture the slices 7 mm.

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