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Methodology for the determination of hormetic heat treatment of broccoli florets using hot humidified air: Temperature–time relationships

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ABSTRACT

Broccoli (*Brassica oleracea*) is one of the most consumed produce among *Brassica* crops because of its content in bioactive compounds such as glucosinolates and flavonoids. Preservation of this vegetable is a challenging task because of its rapid senescence, manifested as floret yellowing. In order to delay this undesirable aspect, several postharvest treatments have been explored including heat treatment, to extend its marketable life. Although heat treatments using arbitrary combinations of temperature and time have been found effective in slowing down the yellowing of broccoli florets, there is no clear methodology for the selection of temperature–time variables for heat application. The objective of this work was to establish a temperature–time relationship using membrane electrolyte leakage response as an indicator of heat severity. Broccoli florets were treated with hot humidified air at temperatures from 32 to 52 °C for periods ranging from 5 to 1440 min. Electrolyte leakage was determined by measuring the conductivity of cell eluate from broccoli stems in 0.4 M mannitol solution. The percentage of electrolyte leakage increased with exposure time at each temperature test following zero order kinetics. The electrolyte leakage rate increased with temperature, but the Arrhenius plot showed a clear broken linear pattern with a break with a transition or critical temperature zone of 42–45 °C. Although equivalent times for heating at different temperatures can be estimated from the kinetics of electrolyte leakage, the selection of treatment temperature needed to be below 42 °C, where the florets stored at 10 °C/95% RH for 10 days, showed changes in color with the progress of senescence without causing excessive anaerobic conditions and/or tissue damage. Heat treatment of florets at temperatures in the critical zone led to excessive accumulation of ethanol as a result of anaerobic respiration, while treatments with temperatures above the critical zone (>45 °C) led to severe anaerobic conditions as well as tissue damage, despite enhanced color retention of broccoli florets treated with temperatures above 42 °C. Heat treatment at 41 °C for 180 min as hormetic heat dose for broccoli florets is suggested. The results of this work suggest that the selection of treatment temperature is of primary consideration for heat treatment of fresh produce.

1. Introduction

Fruits and vegetables are an important part of a healthy diet because they are sources of vital nutrients such as vitamin C, thiamine, niacin, pyridoxine, folic acid and minerals, including zinc, calcium, potassium, phosphorus and other phytochemicals which decreases the risk of chronic diseases (Oguntibeju et al., 2013). However, fruits and vegetables are highly perishable, susceptible to physiological disorders such as chilling injury and internal discoloration as well as to microbial attack throughout storage. In order to reduce fungal and bacterial diseases; chemical methods are employed, including sulfur dioxide (Rivera et al., 2013), chemical fungicides such as azoxystrobin, fludioxinil and pyrimethanil (Kanetis et al., 2007). Nonetheless, the use of chemicals to extend the postharvest life of fruits and vegetables has become less and less acceptable by consumers, as they may contribute to environmental pollution and/or may be harmful to human health.

Recently, new physical treatments are gaining interest and increasingly being considered for prolonging the postharvest life of fruits and vegetables. Among them, heat treatment has received much interest as a pre-storage treatment for the postharvest preservation of fruits and vegetables (Klein and Lurie, 1992; Lurie,

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1998; Lurie and Pedreschi, 2014). Heat treatment can be performed using hot water (>40 °C), forced hot air, high-temperature controlled atmospheres using steam, solar energy, infrared, microwave and radio frequency (Hansen and Johnson, 2007). Among the beneficial effects, heat treatment has been shown to delay the ripening of fruits by reducing ethylene production and hydrolytic cell wall enzymes. Likewise, chilling injury and diseases in commodities are reduced by heat treatments. Ethylene production is reduced at temperatures between 30 and 40 °C as the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to C_2H_4 is vulnerable to heat treatment (Yu et al., 1980). Strawberries exposed to hot-air at 45 °C for 3 h soften more slowly than the non-heated fruits, presumably due to the inactivation of hydrolytic cell wall enzymes such as polygalacturonase, β-galactosidase, *endo*-1,4-β-D-glucanase and β-xylosidase (Martínez and Civello, 2008). Heat treatment has also been shown to delay the yellowing of broccoli; attributed, due in part, to reduced chlorophyllase activity (Funamoto et al., 2002).

The prevention of chilling injury by heat treatment has been associated with the presence of heat shock proteins (HSPs) and polyamines stimulated (Lurie and Pedreschi, 2014; Mirdehghan et al., 2007). These responses can condition or protect plants against subsequent exposure to low temperature, preventing chilling injury. Furthermore, heat treatment controls postharvest diseases either by direct inhibition of pathogens or by the induction of host-defenses such as pathogenesis-related (PR) proteins (Lurie, 1998; Pavoncello et al., 2001).

Heat treatment can have negative impacts as well, when high temperatures or long exposure times are used, including changes in membrane fluidity, lipid rafts and the activation of ion channels (Mittler et al., 2012). High respiration rates can lead to anaerobic conditions due to depleted tissue oxygen as well as membrane disruption and progressive leakage (Duarte-Sierra et al., 2012). Off-odor development and increased production of ethanol were observed in broccoli after, presumably severe hot water treatment at 52 °C for 3 min that could create anaerobic conditions in the tissue (Forney and Jordan, 1998). Moderate water loss observed in heat-treated commodities is likely due to a higher respiration and transpiration rates (Lydakis and Aked, 2003; Schirra et al., 2000).

The severity of heat treatment involves two factors: temperature (heat intensity) and heating time. Heat processing of foods is a well-known operation and is designed to inactivate targeted microbial spores, cells or enzymes (Karel and Lund, 2003). The microbial resistance to heat is expressed by the time required to obtain a 1 log reduction in the microbial count (*D value*) at a specific temperature, and it decreases with increase in temperature. The effect of temperature on microbial resistance is expressed by *z* value, characteristic of the organisms; where *z* value is the change in temperature required to affect a response of 1 log change in *D value*. For commercial sterilization of low-acid foods, the assigned heat or sterilization value is 3 min at the reference temperature of 121 °C (Rees and Bettison, 1991). The relationship between *D* and *z* values permits the determination of the sterilization value at temperatures other than 121 °C. Extension of this approach to heat treatment of fresh produce could allow the establishment of equivalent heating times at different temperatures to obtain a specific tissue response.

Current investigations on heat treatment of fresh produce arbitrarily select temperature–time variables, and evaluate the impact of heat treatment on targeted-responses that relate to the preservation of fresh produce. Furthermore, there is little information available with respect to temperature that may cause irreversible damages to fresh fruits and vegetables. It is imperative to acquire information with respect to temperature thresholds, above which heat treatment becomes harmful to the produce rather than imparting beneficial effects.

Thus the objective of this work was to establish a methodology to define heat doses (heating temperature and time) by hot humidified air relative to a reference heat dose (reference temperature and time) using electrolyte leakage response in broccoli. The temperature threshold for broccoli that causes negligible physiological damage, and the hormetic heat dose (temperature–time) were also determined.

2. Materials and methods

2.1. Vegetable material

Freshly harvested broccoli (*Brassica oleraceae* L. var. Italica 'Diplomat') heads were obtained from a commercial farm (Ile d'Orléans, Québec, Canada). Florets (300 g) of uniform size (approximately, 7 cm) were separated from heads and randomly arranged in 500 mL plastic punnets. The punnets were placed in 5 L plastic containers provided with ventilation and a layer of water at the bottom to maintain high humidity (>95%), and the containers were stored in a controlled chamber overnight at 4 °C before heat exposure.

2.2. Heat treatment

Heat treatment was performed on the florets stored overnight at 4 °C in a closed 2 m^3 chamber with continuous air circulation equipped with controlled air heating system and a humidity control system using steam injection for saturation of air. Seven temperatures, 37, 42, 43, 44, 45, 47 and 52 °C were chosen based on previous studies reviewed by Lurie (1998), with heating time ranging from 5 to 1440 min. Florets were heat treated at 37 °C for 0, 40, 120, 180, 360 and 1440 min; at 42–44 °C for 0, 30, 60, 120, 180, 360 and 1440 min; at 45 °C; 47 °C; and 52 °C for up to 360 min, 120 min and 60 min, respectively. The temperature of the floret stem (about 1 cm in diameter) was monitored with temperature probes (5 mm in length) placed at three locations along the length of the floret stem (bottom, middle and the top location near the buds). The come-up time to the desired temperature varied between 6 min for 52 °C and 12 min for 37 °C. The heat exposure time was logged once the desired temperature was reached as monitored by the temperature probes. Five florets were used for each treatment. After heat treatment, the heated florets were submerged up to the stem immediately into 0.9% NaCl isotonic solution (to prevent osmotic flow) for 1 min at room temperature. Florets were then immediately surface dried and cooled down to temperature below 10 °C inside a disinfected controlled chamber with constant airflow at 1 °C for 10 min, and subsequently stored at 10 °C/95% RH until further analysis. Electrolyte leakage was performed within 1 h after heat treatment, and volatile analysis was carried out at two intervals: after treatment (0 h) and 24 h after treatment.

2.3. Electrolyte leakage

Electrolyte leakage was assayed from stem cubes (5 mm in length and approx. 0.7 g) obtained from six randomly chosen floret stem discs for each time–temperature combination (*i.e.*, at 37 °C for 0, 40, 120, 180, 360 min). After surface drying and cooling the florets, 2 to 3 stem discs *ca.* 7 mm were sectioned from the middle of the floret stem using a scalpel. Broccoli stem cubes were placed into 50 mL falcon tubes with 25 mL of 0.4 M mannitol solution and mixed under agitation for 1 h. Electrical conductivity of the solution was measured at 1 min (C_1) and 60 min (C_{60}) of incubation at room temperature using a conductivity instrument (Model 3100, YSI, Yellow Springs, USA). The total conductivity (C_T) of the solution was determined on samples after autoclaving them at 121 °C for