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

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A B S T R A C T

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.

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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 azoxyostrobin, fludioxonil 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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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 C2H4 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-xyllosidase (Martinez 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; Mirdelghan 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 (Dauce-Sierra et al., 2012). Oxygen 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 thermal heat dose (temperature–time) were also determined.

2. Materials and methods

2.1. Vegetable material

Freshly harvested broccoli (Brassica oleracea L. var. Italica ‘Diplomat’) heads were obtained from a commercial farm (Lle 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 m³ 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 (C1) and 60 min (C60) of incubation at room temperature using a conductivity instrument (Model 3100, YSI, Yellow Springs, USA). The total conductivity (C7) of the solution was determined on samples after autoclaving them at 121 °C for
30 min (Fan and Sokorai, 2005). Electrolyte leakage (L) was calculated as:

$$ L = \frac{C_{00} - C_1}{C_t} \quad (1) $$

2.4. Color measurement

Color was determined by measuring parameters $L^*, a^*$, and $b^*$ with a colorimeter (Minolta CR200, Osaka, Japan) on the same 6 florets from each treatment daily during 10 days of storage at 10°C/95% RH. The total color change ($\Delta E$) was determined from $L^*$, $a^*$, and $b^*$ values: $\sqrt{(L_{0}^*-L_{1}^*)^2+(a_{0}^*-a_{1}^*)^2+(b_{0}^*-b_{1}^*)^2}$, where $L^*$, $a^*$, and $b^*$ represent the color coordinates, lightness of the color, its position between red/magenta and green and its position between yellow and blue respectively, at the start of the storage ($t_0$), and storage time ($t_1$).

2.5. Volatile analysis

Analysis of volatiles was performed on broccoli samples stored at 10°C/95% RH for 0 and 24 h. Samples consisted of about 1 g of stem tissue introduced into a 10 mL vial containing 30 µL of 0.5% 2-propanol as internal standard. A Hewlett-Packard 6890 Network GC system equipped with a Headspace Analyzer (G1888 Network Headspace Sampler, Agilent, Wilmington, DE) and coupled to a HP 5973 mass spectrometer was used to confirm the identification of volatiles present in the standards and samples. Helium was used as carrier gas at a flow rate of 1.8 mL/min. The oven temperature was programmed as follows: hold at 90°C for 1 min, rise to 140°C (15°C/min), increase to 320°C (35°C/min), and finally, hold for 1 min at this temperature. Three replicates were made for each treatment temperature with four replicates for each analysis.

3. Computational

The physical, chemical or microbiological changes in foods with time can be expressed in a general form (Taoukis and Labuza, 1989):

$$ \frac{d[A]}{dt} = k[A]^n \quad (2) $$

where A represents a physical property, chemical entity or microbial growth, n is the order of the reaction and k is the reaction rate constant. With A representing electrolyte leakage (L) of tissue, which increases with the heating time, the rate equation can be expressed as:

$$ \frac{dL}{dt} = kd^n \quad (3) $$

For a zero order reaction (i.e., electrolyte leakage increases with exposure time at a constant rate at a given temperature):

$$ \frac{dL}{dt} = k \quad (4) $$

Upon integration of equation (4):

$$ L = kt \quad (5) $$

The rate of electrolyte leakage (k) is expected to increase with temperature, which can be expressed by Arrhenius equation:

$$ k = Be^{\frac{-E_a}{RT}} \quad (6) $$

where $B$ is a pre-exponential constant; $E_a$ is the activation energy (J mol$^{-1}$); $R$ is the gas constant (8.31 J mol$^{-1}$ K$^{-1}$); and $T$ is the temperature (°K). Combining equations (4) and (5), the electrolyte leakage can be expressed as:

$$ L = tBe^{\frac{-E_a}{RT}} \quad (7) $$

Since the electrolyte leakage changes with temperature and time, the principle of time–temperature superposition can be attempted to elucidate the times at different temperatures or vice versa in order to obtain a specific electrolyte leakage response. The time–temperature shift factor, $\alpha_t$, is given by dividing the heating time, $t_h$, at a reference temperature, $T_{ref}$, by the heating time required at the test temperature ($T_t$), $t_T$, to obtain the same leakage response at the test temperature (Ding and Wang, 2007):

$$ \alpha_t = \frac{t_h}{t_T} \quad (8) $$

Rewriting equation (7) for electrolyte leakage at the reference temperature ($L_{ref}$):

$$ L_{ref} = t_{ref}Be^{\frac{-E_a}{RT_{ref}}} \quad (9) $$

And the leakage at temperature, $T$ ($L_T$):

$$ L_T = t_{T}Be^{\frac{-E_a}{RT_T}} \quad (10) $$

Combining equations (9) and (10):

$$ \frac{L_{ref}}{L_T} = \left(\frac{t_{ref}}{t_T}\right)^{\frac{-E_a}{R}} \quad (11) $$

With the leakage response to heat at both the temperatures being the same, equation (11) becomes:

$$ \log a_t = \frac{E_a}{2.303RT_{ref}} \left[ \frac{1}{T_{ref}} - \frac{1}{T_T} \right] \quad (12) $$

or

$$ \log a_t = \frac{E_a}{2.303RT_{ref}[T_T - T_{ref}]} \quad (13) $$

Also, the sensitivity of electrolyte leakage to temperature can be expressed by the activation energy ($E_a$):

$$ E_a = \frac{2.303RT_{ref}[T_T - T_{ref}]}{(T_T - T_{ref})} \log a_t \quad (14) $$

4. Results and discussion

4.1. Time–temperature relationships: superposition method

Because of lack of a defined heat doses for treatment of commodities, we attempted to determine time equivalence for different treatment temperatures for a specific tissue response as electrolyte leakage based on time–temperature superposition concept. The establishment of time equivalence at different temperatures to obtain 5% electrolyte leakage response to heat allows the application of heat at higher temperatures for short times and vice versa. This approach is much like the commercial sterilization of low-acid foods using lower temperatures for longer times and higher temperatures for shorter times, where the response is the inactivation of Clostridium botulinum spores (Ramesh, 2003).

Electrolyte leakage of broccoli tissue increased with both heating temperature and time (Fig. 1), and the leakage rate increased with increase in temperature, where the increase in leakage with exposure time at a specified temperature was of zero order, i.e., long times at lower temperatures and short times at higher temperatures to bring about a certain level of leakage, suggesting that the concept of time-temperature superposition is well applicable.
The threshold electrolyte leakage that does not affect the preservation of broccoli florets was evaluated. Broccoli florets were exposed to heat at an arbitrary temperature of 43 °C based on the leakage pattern (Fig. 1) for 0, 3, 130, 257 and 386 min to achieve five levels of electrolyte leakage (0.0, 2.5, 5.0, 7.5 and 10.0%), stored for up to 10 days at 10 °C, and the color and visual appearance were monitored daily. It was observed that at 2.5% leakage level was not significantly different from that of non-heat treated florets (2.3%), whereas at the leakage level of 10%, the florets exhibited fungal infection and off-odors at the end of storage. The presence of infection was not visually detected in florets at either 5% or 7.5% leakage level, but the color retention was superior at the leakage level of 5% at the end of storage, and therefore, the heat dose required to cause a leakage level of 5% was considered appropriate for heat treatment of broccoli florets without causing any irreversible damage to the tissue (data not shown). Time–temperature superposition was carried out to determine the equivalent heating times at various temperatures to obtain the heat dose for 5% level of electrolyte leakage.

The time–temperature superimposed master curve seen in Fig. 2 clearly shows that electrolyte leakage response to heat dose using different time–temperature combinations with the reference temperature of 37 °C is effectively superimposable and unified. Effectively, the electrolyte leakage–heat exposure time curves in Fig. 1 are shifted horizontally along the time axis to the right. The magnitude of shifting, the shift factor, to superpose on the master curve depended on the heating temperature as seen in the inset of Fig. 3. The electrolyte leakage rate or the shift factor was not linear with temperature, but was exponential (Fig. 3). From the master curve, the equivalent heating times can be derived for any temperature in the range of 37–52 °C for broccoli florets which can be estimated by basically reversing the procedure (Equation (11)). Furthermore, it would be interesting to see whether the temperature shift factors to electrolyte leakage in broccoli could be applicable to time–temperature behavior of other physiological responses such as respiration rate.

The non-linearity of leakage rate response or shift factor with temperature suggests the existence of a threshold temperature; below which leakage rate is linear with temperature, and above which the rate of leakage increases exponentially (Fig. 3). A plot of Equation (13) (Fig. 4) and Arrhenius plot (Fig. 5) shows that the relationship between leakage rate or shift factor and temperature is broken linear, and that there is a critical temperature zone between 42–45 °C, and the mid-point in the critical zone being the critical temperature (Tc = 43 °C).

Interestingly, after reviewing the literature on the beneficial and adverse effects of heat treatment on ripening fruits, Paull and Chen (2000) identified two types of tissue responses to heat: a normal cellular response when the produce are heat treated at temperatures below 42 °C with beneficial effects such as delayed ripening; but cellular damage when produce are heat treated at temperatures above 45 °C. However, the critical temperature zone may not be universal for all types of fresh fruits and vegetables and this aspect may need further attention.

The existence of a critical temperature or critical temperature zone for membrane leakage rate suggests that the membrane itself undergoes thermal transition, presumably due to the melting of membrane lipids, and that the leakage behavior of the membrane...
Fig. 4. Determination of critical temperature zone for broccoli florets from the relationship between shift factor \(a_T\) and temperature difference \((T_T-T_{ref})\) (Equation (13)).

Fig. 5. Arrhenius plot of electrolyte leakage rate against treatment temperature. The activation energy \(-E_a/R\) was calculated from the slope of each temperature zone: Super-critical (52–45 °C), Critical (43–45 °C) and sub-critical (42–37 °C).

is altered as a consequence. In the light of the presence of critical zone for broccoli tissue, we may class the temperatures below 42 °C as sub-critical zone, the temperature range from 42–45 °C as critical zone, and the temperatures above 45 °C as super-critical zone (Fig. 5). The super-critical temperatures must be avoided as they can harm the tissue; and the temperatures below the critical temperature could be considered for heat treatment. It also follows that the time equivalence concept cannot be appropriate for the entire temperature range of 37–52 °C for broccoli tissue, but can well be useful for the selection of time–temperature for temperatures below the critical temperature, recognizing that the fundamental prerequisite in the selection of heat treatment is the identification of the critical temperature. The experimental evidence presented here for the presence of critical zone temperature is largely in agreement with the literature assessment of Paull and Chen (2000).

The activation energy for membrane leakage from Arrhenius plot for the three temperature zones is shown in Table 1. The critical zone exhibited the highest \(E_a\) (234.9 kJ mol\(^{-1}\)), followed by the super-critical zone (151.0 kJ mol\(^{-1}\)) and the sub-critical zone (27.9 kJ mol\(^{-1}\)). The high \(E_a\) (the threshold energy) for electrolyte leakage in the critical temperature zone suggests that membrane integrity is improved by some mechanism in response to heat treatment in this range of temperatures, while it also becomes sensitive to heat that membrane could be easily perturbed by small changes in temperature, as seen by the coefficient of thermal leakage rate \(\alpha\), i.e., the increase in leakage rate with an increase in temperature of 1 °C (Table 1). The \(\alpha\) values were 1.92, 1.50 and 1.08 for the critical zone, super-critical zone and sub-critical zone, respectively.

The improved membrane integrity in the critical zone in response to heat may be attributable to heat induced factors acting in tandem such as heat shock proteins (Civello et al., 1997; Lurie and Pedreschi, 2014; Pavoncello et al., 2001; Woolf and Ferguson, 2000), and metabolites such as ethanol, a primary metabolite of anaerobic respiration, at low concentrations, but the latter can be detrimental at higher concentrations (Pesis, 2005). The above-mentioned induced factors do not appear to be highly manifest at temperatures below 42 °C (sub-critical zone). The intermediate \(E_a\) value in the super-critical zone suggests that the protective effect of the heat-induced factors is mitigated, likely by the counteraction of above two factors.

4.2. Ethanol production by high temperatures

Depletion of oxygen from the plant tissue at a high treatment temperature can be expected because of reduction in the solubility of gases with increases in temperature (degassing), and also due to faster depletion of oxygen in the tissue because of high respiration rates at the elevated temperatures. Consequently, anaerobic conditions may prevail leading to increased anaerobic respiration and the conversion of pyruvate to acetaldehyde, ethanol and other volatiles (Forney and Jordan, 1998). It would seem then that the ethanol present in the heated florets at temperatures in the critical zone is optimal in contributing to the integrity of the membrane; whereas the higher levels of ethanol are produced at temperatures in the super-critical range, counteract the effect of HSPs produced in response to heat, leading to weakened membrane integrity.

Accumulation of ethanol in florets was evident after 24h of storage with increasing exposure temperature (Fig. 6), where the

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Class</th>
<th>Shift factor (a_T)</th>
<th>Activation energy, (E_a) (kJ mol(^{-1}))</th>
<th>Coefficient of thermal leakage rate (\alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>Sub-critical</td>
<td>1.00</td>
<td>27.9</td>
<td>1.50</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td>1.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>43</td>
<td></td>
<td>2.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>Critical</td>
<td>7.94</td>
<td>234.9</td>
<td>1.92</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>11.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>Super-critical</td>
<td>28.57</td>
<td>151.0</td>
<td>1.08</td>
</tr>
<tr>
<td>52</td>
<td></td>
<td>178.57</td>
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</tbody>
</table>
presence of ethanol was not detectable in zero-time samples. The increase of ethanol followed an exponential trend similar to the electrolyte leakage rate and $a_i$ (Fig. 3). Increase of ethanol in vegetable tissues is a sign of anoxia caused by depletion of oxygen from the tissue by the applied heat since oxygen solubility in water decreases with increases in temperature leading to increased anaerobic respiration. In addition to ethanol production in florets, off-odor was perceived on florets treated at 47°C and 52°C. The latter has also been previously reported in florets treated with hot water at 52°C by Forney and Jordan (1998). They attributed the development of off-odor to cis-3-hexen-1-ol, dimethyl trisulfide (DMTS) and dimethyl sulfide (DMS). The biosynthesis of off-odor compounds, particularly the sulfur compounds, has been related to the increased levels of free sulfur amino acids under anaerobic conditions (Derbal et al., 1998). Furthermore, protein degradation in plant cells increases with temperature (Ferguson et al., 1994), leading to the production of free sulfur containing amino acids which can act as substrate for the generation of off-odor volatiles.

4.3. Hormetic heat dose determination

The yellowing of broccoli florets was delayed with heat treatment above 39°C for equivalent exposure times (Fig. 7). At this temperature, the yellowing was similar to the non-heated broccoli. Color change was the lowest at temperatures between 41°C and 43°C. Although the color retention at 45°C and 47°C was not significantly different from that of the florets treated at either 41°C or 43°C for equivalent exposure times, off-odor was evident in florets treated at 45°C, and more so, at 47°C. In addition, the incidence of diseases was evident in florets treated at 47°C at end of storage of 10 days at 10°C/95% RH (data not shown), indicating that the florets had cellular damage. Thus, the heat treatment at temperatures below 43°C for equivalent times can be considered for heat treatment of broccoli florets from the standpoint of color retention. However, the attendant increased anaerobic respiration associated with critical zone temperatures would prescribe temperatures below 42°C for equivalent times for treatment, which provide optimal color retention. In addition, heat treatment of florets below this temperature showed normal physiology, i.e., color change with the progress of senescence without causing either excessive anaerobic respiration or heat damage. Hence, a treatment temperature of 41°C for equivalent time of 180 min is put forth as hormetic heat dose for broccoli florets not only from the point of color retention and the associated delay in senescence, but also because this temperature does not cause excessive anaerobic condition in the tissue nor cellular damage.

Fig. 8 illustrates the evolution of color retention of heat-treated broccoli florets at 37°C (reference temperature) for equivalent time of 1440 min; 41°C for 180 min (hormetic heat dose); and 47°C (super-critical zone temperature) for 12 min during storage at 10°C for 10 days. The color change was acceptable ($\Delta E \approx 5.0$) in broccoli florets treated at 37°C for 5 days, but it remained nearly unchanged in the florets treated either at 41°C or 47°C, but the latter also exhibited off-odors and incidence of disease.

Reduction of yellowing of broccoli has been reported to be a consequence of significant reduction in the activities of chlorophyll catabolic enzymes such as chlorophyll peroxidase, chlorophyll oxidase and ACC oxidase by heat (Funamoto et al., 2002; Funamoto et al., 2003; Terai et al., 1999). The retention of color with increasing temperature may be also related to the alteration of

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Fig. 6. Accumulation of ethanol in broccoli florets after heating at different temperatures for equivalent times. Abundance of ethanol was detected in heat treated broccoli florets at different temperatures after 24h of storage at 10°C/95% RH. Data point is the mean of 3 observations, and the vertical bar represents standard deviation of the mean.

Fig. 7. Determination of hormetic heat dose (temperature-equivalent time) for color retention of broccoli florets. Color changes, expressed as total color difference ($\Delta E$), after 10 days of storage at 10°C/95% RH. Data point is the mean of 6 observations, and the vertical bar represents standard deviation of the mean. (*) denotes heat damage.

Fig. 8. Color change ($\Delta E$) in heat heated broccoli florets during storage at 10°C/95% RH for 10 days. Broccoli florets were heated at 37°C (equivalent time, 1440 min); at 41°C (equivalent time, 180 min) and at 47°C (equivalent time, 12 min). The heat treatment at 41°C/180 min represents hormetic heat dose. Data point is the mean of 6 observations, and the vertical bar represents standard deviation of the mean.
light reflecting characteristics of vegetable surface due to air removal from the tissue (Tijsskens et al., 2001b).

Excessively heated green vegetables turn olive green and then brown due, in part, to the formation of phloeythin by exchange of Mg⁺ and H⁺ in part, by the liberation of plant acids during heating and the lowering of tissue pH (Tijsskens et al., 2001a). However, if the heating is performed in an adiabatic system as was in this study, the plant acids such as acetic acid would escape, favoring alcalization of the cells and reducing the formation of phloeythin (Gross, 1991; Lowe, 1937).

The transition temperature zone from 42 °C to 45 °C demarcates the beneficial and detrimental temperature zones for heat treatment of broccoli florets. Even though the temperatures in the critical zone (42–45 °C) or above 45 °C preserve the color of broccoli (Fig. 7), they present either moderate or severe harmful effects, respectively. Excessive anaerobic respiration and moderate production of ethanol are the marks of the critical zone, while elevated levels of ethanol production and tissue damage are the marks of the super-critical temperature zone. Thus, the temperature of 41 °C for 180 min can be considered a horneric heat dose for treating broccoli florets.

5. Conclusions

This study establishes a methodology to determine the application of horneric dose of heat to broccoli florets to improve their shelf life and maintain their quality during storage, and it can be extended to other fresh crops. It also allows the establishment of equivalent heat exposure time at various treatment temperatures to obtain a specific level of electrolyte leakage response, which is 5% for broccoli. The heat treatment at 41 °C for 180 min is considered horneric heat dose for broccoli florets from the stand point of color retention without creating excessive anaerobic conditions as observed in the critical temperature zone, nor creating severe anaerobic conditions along with cellular damage as observed in the super-critical temperature zone. This work also shows the importance of suitable temperature selection for heat treatment designed to maintain quality of fresh produce.

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