Effect of ozone processing on anthocyanins and ascorbic acid degradation of strawberry juice

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Article info

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1. Introduction

Fruit juices are increasingly promoted and consumed due to their reported nutritional claims. Strawberries are known for their high levels of micronutrients and phytochemical compounds (Tulipani et al., 2008). Recent studies have shown the potential benefits of anthocyanins from edible berries in human health and disease prevention. These include cardiovascular disorders, advancing age-induced oxidative stress, inflammatory responses and diverse degenerative diseases (Zafra-Stone et al., 2008). Berries such as cranberries, blueberries, strawberries and raspberries contain significant amounts of non-nutritive phytochemicals including polyphenols which are reported to reduce cancer risk (Duthie, Duthie, & Kyle, 2000). Pelargonidin-3-glucoside (P3G) is the major anthocyanin found in strawberries and is responsible for the bright red colour of fresh strawberries (Francis, 1989).

Anthocyanins may degrade due to various factors including pH, light, oxygen, enzymes, ascorbic acid and thermal treatment (Cemeroglu, Velioglu, & Isik, 1994; Jackman, Yada, Tung, & Speers, 1987; Wang & Xu, 2007). Thermal degradation of anthocyanins has been studied for blackberry (Wang & Xu, 2007), sour cherry (Cemeroglu et al., 1994), raspberry (Ochoa, Kesseler, Vullioud, & Lozano, 1992), pomegranate (Martí, Pérez-Vicente, & García-Viguera, 2001) and strawberry (Skrede, Wrolstad, Lea, & Enersen, 1992; Garzon & Wrolstad, 2002). Anthocyanin stability may also be influenced by other fruit components, especially the interaction with ascorbic acid, resulting in mutual degradation as reported in strawberry and blackcurrant products (Skrede et al., 1992). The interaction of ascorbic acid with anthocyanin pigments results in the degradation of both compounds and a decrease in product colour and nutritional quality through oxidation or condensation of ascorbic acid directly with anthocyanin pigments (Markakis, Livingston, & Fellers, 1957).

Degradation of anthocyanins or ascorbic acid in the presence of ozone could be due either to direct reaction with ozone or indirect reaction because of secondary oxidants. The direct reaction is described by the Criegee mechanism (Criegee, 1975) where ozone molecules undergo 1,3-dipolar cycloaddition with double bonds present, leading to the formation of ozonides (1,2,4-trioxolanes) from alkenes and ozone with aldehyde or ketone oxides as decisive intermediates, all of which have finite lifetimes (Criegee, 1975). This leads to the oxidative disintegration of the ozonide and formation of carbonyl compounds, while oxidative work-up leads to carboxylic acids or ketones.

To facilitate the preservation of unstable nutrients many juice processors have investigated alternatives to thermal pasteurisation, including unpasteurised short shelf life juices with high retail value. This trend has continued within the European Union. However, within the US, recent regulations by the FDA have required processors to achieve a five-log reduction in the numbers of the most resistant pathogens in their finished products. This rule comes after a rise in the number of food borne illness outbreaks and consumer illnesses associated with consumption of untreated...
obtained was immediately frozen at 2.1. Preparation of strawberry juice samples

The study also proposes a mechanism of degradation and models the degradation kinetics.

2. Materials and methods

2.1. Preparation of strawberry juice samples

2.2. Ozone treatment

Experiments were carried out in a 250 ml bubble column with an inbuilt diffuser (Fig. 1). Ozone was generated using an ozone generator (Model OL80, Ozoneservices, Canada). Extrinsic parameters of ozone concentration (0–7.8% w/w of oxygen) and treatment time (0–10 min) were varied. Ozone concentration was recorded using an ozone gas analyzer (Model OLA-DLS, Ozoneservices, Canada) at constant oxygen flow rate of 0.0625 l/min. The experimental design for this work was based upon a parallel inactivation study for E. coli O157:H7 in juice, using the same control conditions. A six-log reduction was achieved in less than 6 min at an optimum flow rate of 0.0625 l/min and a maximum ozone concentration obtainable (7.8%) at this flow rate. Experiments were conducted in triplicate.

2.3. Colour determination

The colour of ozonated juice samples was measured using a HunterLab colorimeter (ColorFlex, ModelA60-1010-615, Hunter Associates Laboratory Inc., Reston, Virginia, USA). The instrument (65°/0° geometry, D25 optical sensor, 10° observer) was calibrated using white (L = 92.8; a = –0.8, b = 0.1) and black reference tiles. The colour values were expressed as L* (whiteness or brightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). Total colour difference (TCD) (Tiwari, Muthukumarappan, O’Donnell, and Cullen, 2008a) (Eq. (1)) indicates the magnitude of colour change after treatment (Minolta, 1994). Colour measurements were taken in triplicate for each experiment conducted with the mean values reported.

\[
\text{TCD} = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}
\] (1)

where \(L_0, a_0\) and \(b_0\) are the colour values of untreated juice.

2.4. Anthocyanins content

Anthocyanins were determined following the HPLC (Simadzu Model no: SPD - M10A VP, Simadzu Co., Japan) analytical procedure outlined by Zabetakis, Leclerc, and Kajda (2000) which involves extraction of 5 ml of sample with the mixture of methanol, acetic acid and water in ratios 25:1:24. Samples were centrifuged (Sanyo MSE Mistral 3000i, UK) for 10 min at 2000g and 4 °C. 5 ml of the supernatant was filtered through 0.45 μm PTFE syringe filters (Phenomenex, U.K) and placed in an autosampler vial. The mobile phase was a solution comprising of a mixture of acetonitrile (83 ml), methanol (33 ml) and acetic acid (170 ml) which were mixed with trichloroacetic acid (0.65 g) which was previously dispersed in water and made to a final volume of 1 l with distilled water. Separations were conducted on a Zorbax SB C18, 5 μm, 150 x 4.6 mm column (Agilent Technologies, Dublin, Ireland). The sample loop was 20 μl with an isotropic flow rate of 1ml/min and the total run time was less than 8 min. Detection was carried out at 520 nm. For quantification, external calibration curves for pelargonidin-3-glucoside (P3G) was prepared at concentrations from 25 μg/ml to 100 μg/ml. Results are expressed as mean values of three assays for each replicated experiment.
2.5. Ascorbic acid determination

Ascorbic acid content was determined following the HPLC (Simadzu Model no: SPD - M10AVP, Simadzu Co., Japan) analytical procedure outlined by Lee and Coates (Lee & Coates, 1999). 10 µl aliquot of samples were injected onto a Simadzu C18 (15 cm × 4.6 cm, pore size 5 µ) coupled with HyperODS guard column. 25 ml juice samples were pipetted into 50 ml centrifuge tubes containing 5 ml of 2.5% metaphosphoric acid. Samples were centrifuged for 10 min at 2000 rpm and 4 °C (Sanyo MSE Mistral 3000ii, UK). 5 ml of the supernatant was filtered through 0.45 PTFE syringe filters (Phenomenex, U.K) and placed in an autosampler vial. The mobile phase was 25 mM KH2PO4 (adjusted to pH 3.0 with phosphoric acid) with a flow rate of 1 ml/min. Eluate was monitored by UV detection at 245 nm. Chromatograms were recorded and processed with EZStart Chromatography Software V.7.2.1. The results were reported as the mean of three assays for each experiment conducted.

2.6. Degradation kinetics

The degradation of anthocyanin or ascorbic acid due to ozone and oxygen gas can be described by Eq. (2).

\[ \frac{d[A]}{dt} = K[A]^n [O_3]^m \]  
(2)

where \([A]\) is concentration of anthocyanin (P3G) or ascorbic acid; \([O_2]\) is dissolved ozone concentration during treatment; \(K\) (min\(^{-1}\)) is reaction rate constant; \(n\) is the reaction order; \(m\) is reaction order with respect to ozone (\(O_3\)); and \(t\) is time (min). In Eq. (2) when dissolved ozone concentration is in excess, compared to P3G concentration, \(m = 0\). Zimeri and Tong (1999) reported that if ozone concentration remains constant during the course of the kinetic study Eq. (2) reduces to Eq. (3)

\[ \frac{d[A]}{dt} = K[A]^n \]  
(3)

where,

\(K = K'[O_3]^m\)  
(4)

If anthocyanin or ascorbic acid degradation follows first order kinetics with respect to concentration \(n = 1\), Eq. (3) becomes

\[ \frac{d[A]}{dt} = K[A] \]  
(5)

Rearranging and integrating Eq. (5) would yield

\[ - \int \frac{d[A]}{A} = \int Kdt \]  
(6)

\[ \log_e \left( \frac{A_0 - A}{A_0 - A_n} \right) = -Kt \]  
(7)

Eq. (7) assumes complete degradation of anthocyanin or ascorbic acid content after prolonged treatment. A special case of first-order model is the fractional-conversion model (Van den Broeck, Ludikhuyze, Loey, & Hendrickx, 2000) which may be used when a fraction of the compound under study remains after the kinetic study (Levenspiel, 1972). A similar use of a fraction conversion model was used by Zimeri and Tong (1999) for degradation kinetics of epigallocatechin gallate as a function of pH and dissolved oxygen in a model liquid solution. In this study treatment time was 10 min which was based on preliminary trials conducted on microbial inactivation. When a fraction of compound with non-zero equilibrium concentration is related to time as follows:

\[ \log_e \left( \frac{A_0 - A}{A_0 - A_n} \right) = -Kst \]  
(8)

\[ f = \frac{A_0 - A_t}{A_0 - A_n} \]  
(9)

A plot of the logarithm of \((1-f)\) against time is linear and the rate constant \((K_s)\) is the negative of the slope (Levenspiel, 1972).

\[ \log_e (1-f) = \log_e \left( \frac{A_0 - A_t}{A_0 - A_n} \right) = -Kst \]  
(10)

Rearranging Eq. (8) and Eq. (10) results in following expression:

\[ At = A_n + (A_0 - A_n) \times e^{-Kst} \]  
(11)

From the above equation it may be observed that when \(A \rightarrow 0\), the equation tends to a first-order kinetic model (Eq. (7)).

The estimation of the kinetic parameters was done by a classical two step regression method. First the reaction rate constants were determined by fitting the experimental data to zero and first order kinetic models (Eq. (12)) and (Eq. (7)), respectively

\[ C = C_0 + Kt \]  
(12)

where, \(C\) is the studied parameter \((L^*, a^*, b^*, TCD, P3G and ascorbic acid)\) at any given reaction time, \(C_0\) are initial values of untreated samples and \(K\) are rate constants. In the second step the rate constants were modelled as a function of ozone concentration. Data fitting was considered significant at a probability level of 95%. Parameters of this model \((K, K_p, A_n)\) were estimated using proc nlin program of nonlinear regression (SAS Version 9.1, SAS Institute, Cary, NC). A Gauss-Newton algorithm was used to determine the parameters of the mathematical equations adjusted by non-linear regression. The goodness of fit was assessed by the adjusted coefficient of determination \(R^2\) along with an analysis of residuals. A nonlinear estimation procedure was used for minimising the sum of squared error (SSE) between experimental and predicted values using Eq. (13).

\[ SSE = \sum_{i=1}^{n} [Log_e(A)_\text{exp} - Log_e(A)_\text{pred}]^2 \]  
(13)

where \(n\) is the number of experimental runs.

3. Results and discussion

Table 1 shows the characteristics of the strawberry juice prior to ozone treatment. Anthocyanins content as pelargonidin-3-glucoside (P3G) strawberry juice was 41.4 ± 1.10 mg/100 ml of juice. The anthocyanins content of strawberry strongly depends upon the cultivar as well as on agronomic practices such as harvesting stage. The anthocyanins content for strawberry juice is reported to vary from 14.8 mg/100 g (Wrolstad, Putnam, & Varseveld, a As pelargonidin-3-glucoside.

Table 1 Characteristics of the strawberry juice before ozonation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Juice characteristics before ozonation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total soluble solids (Brix)</td>
<td>9.82 ± 0.412</td>
</tr>
<tr>
<td>pH</td>
<td>3.11 ± 0.031</td>
</tr>
<tr>
<td>Titratable acidity (g/100 ml)</td>
<td>0.75 ± 0.019</td>
</tr>
<tr>
<td>Anthocyanins (mg/100ml)</td>
<td>41.4 ± 1.10</td>
</tr>
<tr>
<td>Ascorbic acid (mg/100ml)</td>
<td>53.3 ± 0.432</td>
</tr>
<tr>
<td>Lightness (L*)</td>
<td>28.0 ± 1.21</td>
</tr>
<tr>
<td>Redness (a*)</td>
<td>44.8 ± 1.17</td>
</tr>
<tr>
<td>Yellowness (b*)</td>
<td>33.7 ± 2.34</td>
</tr>
</tbody>
</table>

a As citric acid.
b As pelargonidin-3-glucoside.
1970) to values as high as 50.3 mg/100 g (Pilando, Wrolstad, & Heatherbell, 1985). The ascorbic acid content of strawberry juice was 53.3 ± 0.43 mg/100 ml. Total soluble solids (°Brix), pH, and titratable acidity as citric acid was 9.82, 3.11 and 0.75 g/100 g citric acid, respectively.

3.1. Colour degradation kinetics

Ozonated strawberry juice samples were observed to be lighter in colour i.e. increased L* value, whereas a* and b* values were found to decrease with increase in ozone concentration (% w/w).

![Graphs showing changes in a) lightness value (L*−L0), b) red-green value (a*/a0), c) blue – green value (b*/b0) and d) total colour difference (TCD) of strawberry juice during ozonation at a gas flow rate of 0.625 l min⁻¹ with varying concentration of (•) 1.6% w/w, (□) 3.2% w/w, (△) 4.8% w/w, (■) 6.4% w/w and (▲) 7.8% w/w, respectively.

![Graphs showing changes in reaction rate constants for (a) lightness value (K_EL), (b) red-green value (K_Ea), (c) blue – green value (K_Eb) and (d) total colour difference (K_ETCD) of strawberry juice with respect to ozone concentration at a gas flow rate of 0.625 l min⁻¹.

Fig. 2. Changes in a) lightness value (L−L0), b) red-green value (a/a0), c) blue – green value (b/b0) and d) total colour difference (TCD) of strawberry juice during ozonation at a gas flow rate of 0.625 l min⁻¹ with varying concentration of (•) 1.6% w/w, (□) 3.2% w/w, (△) 4.8% w/w, (■) 6.4% w/w and (▲) 7.8% w/w, respectively.

Fig. 3. Changes in reaction rate constants for (a) lightness value (K_EL), (b) red-green value (K_Ea), (c) blue – green value (K_Eb) and (d) total colour difference (K_ETCD) of strawberry juice with respect to ozone concentration at a gas flow rate of 0.625 l min⁻¹.
and treatment time (min). Mean \( L' \) values increased from 28.0 to 53.4, while mean \( a' \) and \( b' \) values decreased from 44.8 to 11.1 and from 33.7 to 17.2, respectively, at ozone concentration of 7.8 \% w/w and treatment time of 10 min. Relative changes in lightness (\( L' \)), red-green (\( a' \)), blue-yellow (\( b' \)) values and TCD values with reference to the control as a function of treatment time (min) for various ozone concentration levels at a gas flow rate of 0.625 l/min\(^{-1}\) are shown in Figs. 2a–d. A zero order kinetics model fitted well to \((p < 0.05)\) \( L' - L_0 \) and TCD values, whereas first order models fitted well to \( a'/a_0 \) and \( b'/b_0 \) values. Reaction rate constants (\( K_{EL}, K_{EG}, K_{EB}\) and \( K_{ECD} \)) obtained using Eqs. (7) and (12) were found to increase linearly (Fig. 3) with ozone concentration, with regression coefficients (\( R^2 \)) of 0.944, 0.993, 0.989 and 0.953 for \( K_{EL}, K_{EG}, K_{EB} \) and \( K_{ECD} \), respectively. Fig. 3 shows non-zero reaction rate constants (\( K_1, K_2, K_3, K_{ECD} \)) with no ozone in feed gas (oxygen) indicating degradation solely due to oxygen. These results demonstrate the significant effect of oxygen and ozone on the colour degradation of the samples studied. The oxygen proportion in the feed gas plays a synergistic role in colour degradation. Strawberry juice colour is a mix of red and yellow. Thus, Hunter \( a' \) and \( b' \) values or some combination of \( a' \) and \( b' \) may be considered as the physical parameters describing visual colour degradation (Rodrigo, Loey, & Hendrickx, 2007). To describe visual colour degradation a combination of \( L, a, b \) values should be considered. Ahmed, Shivhare, and Ramaswamy (2002a) reported that any change in Hunter \( a, b \) values is associated with simultaneous changes in \( L \) value. Various combinations such as \( L, a'b, L, a, b \) of tristimulus \( L, a, b \) values have been used to represent the change in visual colour of thermally processed chilli puree (Ahmed, Shivhare, & Sandhu, 2002b) and high pressure processed tomato puree and strawberry juice (Rodrigo et al., 2007). Changes in strawberry juice is associated with anthocyanin degradation since anthocyanins are responsible for appealing, bright red colour of strawberry juice. Table 2 shows the correlation matrix and relation with the parameters. Strong correlation coefficient (\( r \)) of P3G with \( a'b'/L' \) (\( r = 0.97 \)) and with
3.2. Anthocyanins and ascorbic acid degradation

Anthocyanin and ascorbic acid content were found to decrease from 41.4 to 0.76 and 53.3 to 7.57 mg/100 ml respectively after 10 min treatment time. Fig. 4a–b shows the degradation of P3G and ascorbic acid with respect to treatment time (min) at varying ozone concentration (% w/w). P3G and ascorbic acid content significantly decreased with increasing treatment time (min) and ozone concentration (% w/w). Almost 39.9% and 23.8% of P3G and ascorbic acid respectively, were degraded at treatment time of 3 min and ozone concentration of 7.8 % w/w for P3G and ascorbic acid respectively.

The general shape of degradation curve for P3G and ascorbic acid by ozone treatment at any ozone concentration level appears to be exponential. The P3G and ascorbic acid content values as a function of treatment time (min) at a varying ozone concentration level were fitted to the first-order inactivation model (Eq. (7)) and fraction conversion model (Equation (11)). $K_{P3G}$ and $K_{P3F}$ values increased from 0.098 to 0.429 min$^{-1}$ and 0.101 to 0.556 min$^{-1}$ respectively and ascorbic acid degradation reaction rate constant $K_{AA}$ values increased from 0.056 to 0.229 min$^{-1}$ and 0.0452 to 0.491 min$^{-1}$ respectively as the ozone concentration increased from 0.0 to 7.8 % w/w. Rate constants for both the first order kinetic model ($K_e$ min$^{-1}$) and the fraction conversion kinetic model ($K_e$ min$^{-1}$) increased with increases in ozone concentration, following an exponential increase (Figs. 5a and b) with $R^2$ values of 0.95, 0.96, 0.99 and 0.97 for $K_{P3G}$, $K_{P3F}$, $K_{AA}$ and $K_{FAA}$ respectively. The experimental values for P3G and ascorbic acid content were plotted against the predicted values from Equation (7) and (11). Both first order and fraction conversion model showed a high correlation between observed and predicted values for ascorbic acid ($R^2 = 0.99$) (Fig. 6). However, for P3G the fraction conversion model predicted better compared to first order (Fig. 6) with $R^2$ values of 0.99 and 0.96, respectively.

The fit performance of the simple first-order model was good for ozone concentration but seemed to decrease at higher ozone concentration levels for both P3G and ascorbic acid content as can be seen from the degradation curve in Fig. 4 which displays a shoulder at higher concentration. However, when the data were fitted to a fractional conversional model (Eq. (7)), a consistently high fit irrespective of the ozone concentration was found ($R^2 > 0.98$) for both P3G and ascorbic acid. A fractional conversion model takes into account a non-zero concentration left after the end of kinetic study under experimental conditions.

3.3. Degradation mechanism

Anthocyanins or ascorbic acid might be oxidised either by direct interaction with ozone as discussed in the introduction or due to the formation of various intermediate radicals (Scheme 1). This process leads to electrophilic or nucleophilic reactions occurring with the aromatic compounds that are substituted with an electron donor (e.g. OH$^-$) having high electron density on the carbon compounds in ortho and para positions. Chemical reactions involve breakage of old bonds and formation of new bonds. According to bond dissociation energy theory, the lower the bond dissociation energy the more reactive the bond (Luo, 2005).

In the degradation of anthocyanins, the ring-opening phase due to formation of ozoneide is the crucial step of degradation (Fig. 7). In this phase, the aromatic ring is broken down and degradation efficiency rises quickly (Xue, Chen, & Wang, 2008). According to

![Scheme 1. Formation and transformations of intermediate radicals.](image-url)
Xue et al. (2008) ozone plays an important role not only in the degradation process of organic dye but also in the formation of other high-reactive species, such as $\cdot OH$, $HO^-$, $O_2^-$, and $\cdot O^-$ which facilitates degradation. A similar oxidative degradation of ascorbic acid in the presence of oxygen has been reported by Kennedy, Rivera, Lloyd, Warner, and Jumel (1992), Samaniego-Esguerra, Boag, and Robertson (1991). Zimeri and Tong (1999) reported a degradation mechanism of epigallocatechin gallate in the presence of dissolved oxygen in a model liquid solution.

4. Conclusions

Ozonation of strawberry juice with a sufficiently large dose to meet FDA regulations, will result in a significant degradation of anthocyanins, ascorbic acid and colour. The kinetics of degradation for these parameters may be modelled with zero order, first order and fraction conversion models. The degradation of anthocyanin and ascorbic acid leads to a loss of bioavailability and antioxidant capacity of these compounds present in strawberry. Thus, the effects of ozonation on the nutritional properties of strawberry juice or juice products containing strawberry juice should be considered by processors prior to its adoption as a preservation technique.

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References


