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Effect of ozone processing on the colour, rheological properties and phenolic content of apple juice

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ABSTRACT

Apple juice samples were ozonated with processing variables of ozone concentration (1–4.8% w/w) and processing time (0–10 min). Effects of processing variables on colour values (*L*, *a* and *b*), rheological properties and phenolic content were studied. Significant reductions in these parameters were observed during ozonation. Second order polynomial regression modelling was used to investigate the main effects of ozone concentration and processing time on the changes in the selected quality parameters of ozonated apple juice. Predicted models were found to be significant ($p < 0.05$) with low standard error and high coefficients of determination (R^2).

1. Introduction

Apples are of interest for their nutritional composition. Fruit juice obtained from apples is increasingly promoted and consumed due to its reported beneficial effects on degenerative diseases, protective effects against cardiovascular diseases and cancer (Hollman, 2001; Kahle, Kraus, & Richling, 2005; Rice-Evans, 2001; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Sadik, Sies, & Schewe, 2003). Apples are an excellent source of several phenolic compounds and the presence of polyphenols in apples contributes towards their health promoting antioxidant properties (Khanizadeh, Tsao, Rekika, Yang, & DeEll, 2008; Robards et al., 1999; Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999; Van der Sluis, Dekker, Skrede, & Jongen, 2002). Polyphenols have attracted much attention due to their antioxidant properties (Proteggente et al., 2002). In fact, the potential health benefits of plant foods are commonly linked to their polyphenol content (Kahle et al., 2005). Apart from their reported health benefits, polyphenols contribute towards the formation of hazes and sediments, the development of characteristic flavours and prevent colour changes during processing (Oszmianski & Wojdylo, 2007).

Several incidents of food borne disease have been associated with apple juice. In 1991, an outbreak of *Escherichia coli* O157:H7 and haemolytic uremic syndrome was linked to traditionally pressed apple cider. *E. coli* O157:H7 is an enteric pathogen with a low infectious dose, which usually causes haemorrhagic colitis, but has also the potential to cause haemolytic uremic syndrome in young children, which leads to them being immunocompromised. These outbreaks led the United States Food and Drug Administration (FDA) to issue hazard analysis and critical control point (HACCP) regulations for safe and sanitary processing of juice (United States Food, 2001). Their primary performance standard is a minimum 5-log reduction of the pathogens of concern in the juice being processed (USFDA, 2001). The FDA's approval of ozone as a direct food additive in 2001 triggered interest in ozone applications. A number of commercial fruit juice processors in the US and Europe began employing ozone for pasteurisation resulting in the issuing of industry guidelines. However, these guidelines (FDA, 2004) highlighted gaps in the research literature with respect to the critical control parameters of ozone during microbial inactivation in liquid systems.

Conventional thermal processing of fruit juices remains the most widely adopted technology for shelf-life extension and preservation of apple juice. However, consumer demand for nutritious foods, which are minimally and naturally processed, has led to interest in non thermal technologies (Schilling et al., 2008). Ozone has been investigated for fruit juice processing applications including apple cider (Choi & Nielsen, 2005; Steenstrup & Floros, 2004). Willams, Sumner, and Golden (2005) studied the effect of ozone in combination with dimethyl dicarbonate and hydrogen peroxide

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for orange juice preservation. They reported that a 5-log reduction of *E. coli* O157:H7 could be achieved using ozone in combination with dimethyl dicarbonate. Similarly, Patil, Bourke, Frias, Tiwari, & Cullen, 2009 reported a 5-log reduction of *E. coli* NCTC 12900, in <7 min in orange juice. Steenstrup Dock and Floros (2004) reported that the overall inactivation of *E. coli* O157:H7 by ozone is fast enough for practical application in apple juice production. However, to date the effect of ozonation on the quality of apple juice has not been reported. The objective of this work was to investigate the effect of ozonation on the colour, rheological properties and phenolic content of ozonated apple juice as a function of ozone concentration and processing time.

2. Materials and methods

2.1. Chemicals for polyphenol analysis

Caffeic acid, chlorogenic acid, Folin–Ciocalteu Reagent (FCR), gallic acid and trans-4-hydroxy-3-methoxy cinnamic acid were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of apple juice samples

Unpasteurised fresh apple juice was squeezed using a Nutritaster juice extractor (Model N450, Raw Juice & Smoothies Ltd., Dublin, Ireland). Samples were placed in 500 ml plastic bottles, and stored at −25 °C prior to processing. Frozen juice samples were processed within one month of juice preparation.

2.3. Ozone processing

Experiments were carried out in a 250 ml bubble column with a built-in diffuser. Ozone was generated using an ozone generator (Model OL80, Ozoneservices, Canada). Oxygen flow rate was controlled using a gas flow regulator. The experimental design for this work was based upon a parallel inactivation study for *E. coli* O157:H7, using the same control conditions. A 5-log reduction was achieved in under 5 min at an optimum flow rate of 0.125 l min^{-1} and a maximum ozone concentration obtainable (4.8% w/w) at this flow rate (Patil et al., 2009). Ozone concentration in the gas supply was varied (1–4.8% w/w of oxygen) and recorded using an ozone gas analyser (Model OLA-DLS, Ozoneservices). Ozone treatments were performed at 20 ± 0.5 °C.

2.4. Colour determination

Juice colour was measured using a Minolta colorimeter (Model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) based on three colour co-ordinates, namely L, a, b. The colour values were expressed as L (whiteness or brightness/darkness), a (redness/greenness) and b (yellowness/ blueness). 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. Total colour difference (TCD) which indicates the magnitude of colour change after treatment was calculated using Eq. (1).

$$TCD = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad (1)$$

Colour values L, a and b values were recorded as the mean of triplicate readings.

2.5. Rheological analysis

A controlled stress rheometer (Carrimed CSL^2 – 100, TA Instruments, UK) was used for rheological analysis. Samples of 3.5 ml were measured using a concentric cylinder geometry (length = 40 mm, diameter = 26.66 mm, gap width = 4000 μm) at 25 ± 0.1 °C. Flow curves were obtained for a shear rate sweep between 0.1 and 99 s^{-1}. Shear stress–shear rate data was fitted to a power law model (Eq. (2)).

$$\eta_a = k\dot{\gamma}^{(n-1)} \quad (2)$$

where η_a is apparent viscosity (Pa s), k is consistency index, $\dot{\gamma}$ is the shear rate (s^{-1}) and n is the flow behaviour index.

2.6. Polyphenol analysis

2.6.1. FCR assay

Total phenolic content of apple juice was assessed using a modified version of the Folin–Ciocalteu assay (Singleton, Orthofer, & Lamuela, 1999). Gallic acid was used as a standard and the aqueous gallic acid solution (200 mg l^{-1}) was diluted with distilled water to give appropriate concentrations for a standard curve. For the analysis, 100 μl of methanolic fruit extract or gallic acid standard, 100 μl of methanol, 100 μl of Folin–Ciocalteu reagent and 700 μl of Na_2CO_3 were added into 1.5 ml micro-centrifuge tubes. The samples were vortexed immediately and the tubes were incubated in the dark for 20 min at room temperature. After incubation all samples were centrifuged at 13,000 rpm for 3 min. The absorbance of the supernatant was then measured at 735 nm in 1 ml plastic cuvettes using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Japan). The results were expressed in mg gallic acid equivalent/100 ml (mg GAE 100 ml^{-1} juice).

2.6.2. Polyphenolic profile (HPLC–DAD)

HPLC-analysis was performed on a Varian Pro Star (Varian Inc., Walnut Creek, USA) chromatography system, equipped with a module 210 solvent delivery system, a module 510 column thermostat, a module 410 autosampler and a module 335 diode array detector (DAD) with an absorbance detection range between 190 and 950 nm. Separations were conducted on a Zorbax SB C18, 5 μm, 150 × 4.6 mm column (Agilent Technologies, Dublin, Ireland). The gradient profile was based on the method of Tsao and Yang (2003). Acetic acid in 2 mM sodium acetate (final pH 2.55, v/v) was used as eluent A and 100% acetonitrile was used as eluent B. The column temperature was set at 37 °C and the flow rate was 1 ml min^{-1}. The solvent gradient programme was set as follows: initial conditions 100% A, 0% B; 0–45 min, 0–15% B; 45–60 min, 15–30% B; 60–65 min, 30–50% B; 65–70 min, 50–100% B. Prior to injection sample extracts were filtered using a PTFE syringe with 0.22 μm filters. The injection volume was 10 μl. Hydroxybenzoic acids, dihydrochalcones, flavanones and flavanols were monitored at a wavelength of 280 nm, hydroxycinnamic acid derivatives at 320 nm and flavonols at 360 nm. For quantification and identification purposes standard curves of analytes of interest were prepared using methanolic solutions of the standards listed above.

2.7. Experimental design

Degradation of colour values (L, a and b), total colour difference (TCD) and phenolic content was analysed using second-order polynomial models. Polynomial regression equations were developed to describe the effects of ozone concentration (% w/w) and processing time (min) on apple juice. The general form of the quadratic polynomial model regression equation employed in this study is shown in Eq. (3).

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_i \sum_{j=i+1} \beta_{ij} X_i X_j, \quad (3)$$

where Y is the predicted response, β_0 the constant coefficient, β_i the linear coefficient, β_{ii} the quadratic coefficient, β_{ij} is the cross-product

coefficients. This equation contains linear terms X_1, X_2 quadratic or square terms (X_1^2,X_2^2) and interaction (X_1X_2) terms are also included in the equation. By using this equation linear, quadratic and interactive effects of independent variables X_i (0–4.8% w/w) and X_j (0–10 min) on dependent variable (Y) were determined. Three dimensional curves of the response surface were developed using Minitab (V.15.0) software while holding the variables constant in the second-order polynomial model. Analysis of variance (ANOVA) was carried out using the PROC NLIN procedure (SAS V.9.1, SAS Institute, NC, USA). All trials were conducted in triplicate.

3. Results and discussion

3.1. General

Table 1 lists the characteristics of the apple juice prior to ozone treatment. Total phenol content expressed as gallic acid equivalent (GAE, mg/100 ml) was 638 ± 123 mg/100 ml of juice. Three major phenolic compounds namely chlorogenic acid (234 μg/ml), caffeic acid (18.5 μg/ml) and cinnamic acid (130 μg/ml) were detected in fresh apple juice. Kahle et al. (2005) reported that polyphenol content ranges from 154 to 970 mg/l in dessert and cider apple juices. The distribution of many of the phenolics in apple juice is influenced by various factors including genetic or cultivar, extraction method, processing, and enzyme treatment (Khanizadeh, Tsao, Rekika, Yang, & DeEll, 2007; Khanizadeh et al., 2008).

Table 1
Characteristics of fresh and ozonated apple juice.

Parameters	Fresh apple juice	Ozonated apple juice (4.8% w/w for 10 min)
L value	32.6 ± 0.79[b]	56.0 ± 2.81[a]
a value	22.2 ± 0.18[a]	8.89 ± 1.59[b]
b value	35.9 ± 0.71[b]	45.8 ± 1.10[a]
Chlorogenic acid	226 ± 53.98[a]	1.85 ± 0.10[b]
Caffeic acid	18.5 ± 2.40[a]	0.62 ± 0.10[b]
Cinnamic acid	130 ± 22.8[a]	0.25 ± 0.04[b]
Total phenol (GAE, mg/100 ml)	638 ± 123[a]	321 ± 18.6[b]
pH	3.81 ± 0.23[a]	3.79 ± 0.41[a]
Consistency index (k)	9.11 ± 0.03[a]	1.30 ± 0.05[b]
Flow behaviour index (n)	0.37 ± 0.01[b]	0.65 ± 0.02[a]

[ab] Values followed by the same letter are not significantly different ($p < 0.05$).

3.2. Colour degradation

Fig. 1(i–iv) shows the 3D response surface plots for *L*, *a*, *b* and TCD values. Colour values for untreated apple juice were 32.6 ± 0.79, 22.2 ± 0.18, 35.9 ± 0.71 for *L*, *a* and *b* value respectively. During ozonation juice samples were observed to be lighter in colour i.e. increased *L* and *b* value, whereas *a* values of apple juice samples were found to decrease with increase in processing time and ozone concentration. Mean *L* and *b* values significantly increased ($p < 0.05$) to 56.0 ± 2.81 and to 45.8 ± 1.10 respectively, while the mean *a* value decreased to 8.89 ± 1.59 (Table 1) and the mean total colour difference (TCD) value was 30.84 at the highest processing conditions employed i.e. at ozone concentration of 4.8% w/w and processing time of 10 min.

L, *b* and TCD values were significantly influenced by both ozone concentration and processing time ($p < 0.0001$). Whereas, *a* value significantly increased with ozone concentration ($p = 0.0056$) and processing time ($p = 0.0016$) as shown in Table 2. Table 3 shows the regression coefficient for the predicted models for colour values. It is indicated that the predicted response models for colour parameters were found to fit well with the experimental data. The models presented showed high correlation coefficients (R^2) of 0.97, 0.89 and 0.92 for *L*, *b* and TCD, however the R^2 value for *a* was 0.62. Predicted models were highly significant for all colour values ($p < 0.0001$) except *a* ($p = 0.005$).

Canonical analysis and ANOVA of second order quadratic models revealed that linear models were significant with $p < 0.0001$ for

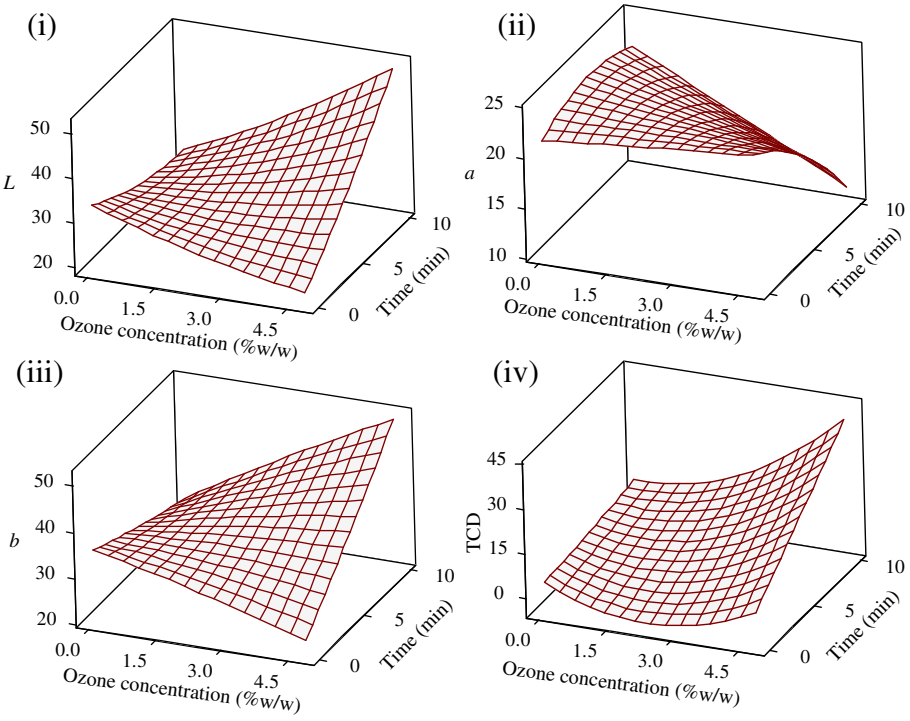

Fig. 1. Effect of ozone concentration (% w/w) and processing time (min) on (i) *L*, (ii) *a*, (iii) *b* and (iv) TCD values of apple juice.

Table 2
ANOVA of the factors obtained from RIDGE analysis of the regression model for various parameters.

Parameters	Ozone concentration (% w/w)		Processing time (min)	
	F value	$p(F > f)$	*F* value	$p(F > f)$
L value	142	<0.0001	165	<0.0001
a value	5.76	0.0056	7.56	0.0016
b value	37.0	<0.0001	42.7	<0.0001
TCD	45.4	<0.0001	38.1	<0.0001
Chlorogenic acid	5.11	0.0098	30.7	<0.0001
Caffeic acid	5.02	0.0106	15.4	<0.0001
Cinnamic acid	11.6	0.0002	55.6	<0.0001
Total phenol (GAE, mg/100 ml)	0.45	0.7208	55	<0.0001

L, *b*, TCD and $p < 0.001$ for *a* value. Quadratic models were insignificant for *a* and *b* values and significant for *L* ($p < 0.01$) and TCD ($p < 0.001$) values. Statistical analysis showed that the interaction (cross-product) among parameters was significant for all colour parameters in all cases. Overall colour changes observed during ozonation were distinct, indicating major changes in the appearance of apple juice colour.

3.3. Rheological properties

Apparent viscosities as a function of shear rate for ozonated apple juice are shown in Fig. 2. Flow curves for all samples exhibit shear thinning behaviour during ozonation. Significant changes

Table 3
Regression coefficient and ANOVA of regression parameters of the predicted quadratic models.

Factors	*L*	*a*	*b*	TCD[a]	Total Phenol[b] (μg/ml)	Chlorogenic acid (μg/ml)	Caffeic acid (μg/ml)	Cinnamic acid (μg/ml)
Intercept								
β_0	33.3	21.5	35.4	4.21	686	236	17.7	126
	(0.84)[c]	(1.70)	(1.54)	(2.26)	(39.2)	(13.1)	(1.08)	(4.72)
Linear								
β_1	−3.69****	0.43^{ns}	−2.02*	−7.01***	−25.8	−33.6*	−3.80**	−23.6***
	(0.55)	(1.11)	(1.01)	(1.48)	(25.7)	(14.8)	(1.23)	(5.36)
β_2	−1.51****	0.67 ^{ns}	−1.01*	0.27	−117****	−38.8****	−3.48****	−24.4****
	(0.25)	(0.51)	(0.46)	(0.68)	(11.9)	(7.40)	(0.61)	(2.67)
Quadratic								
β_{11}	0.24***	−0.01 ^{ns}	−0.11	1.52****	3.53	3.05	0.46*	2.44*
	(0.10)	(0.20)	(0.18)	(0.27)	(4.63)	(2.43)	(0.20)	(0.88)
β_{22}	0.07**	−0.06 ^{ns}	0.02	0.02	8.07****	1.91*	0.22***	1.37****
	(0.02)	(0.05)	(0.04)	(0.06)	(1.05)	(0.62)	(0.05)	(0.23)
Cross product								
β_{12}	0.79****	−0.26**	0.76****	0.63****	0.57	1.08	0.084	0.88**
	(0.04)	(0.09)	(0.08)	(0.12)	(2.05)	(0.91)	(0.07)	(0.33)
R^2	0.97	0.62	0.89	0.92	0.90	0.93	0.92	0.97
CV (%)	4.02	12.2	6.63	36.4	15.4	33.0	48.4	29.8
Model	<0.0001	0.0013	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] Total colour difference.
[b] Gallic acid equivalent (μg/ml).
[c] Values in parenthesis () indicate standard error.
* Significant at $p < 0.05$.
** Significant at $p < 0.01$.
*** Significant at $p < 0.001$.
**** Significant at $p < 0.0001$.

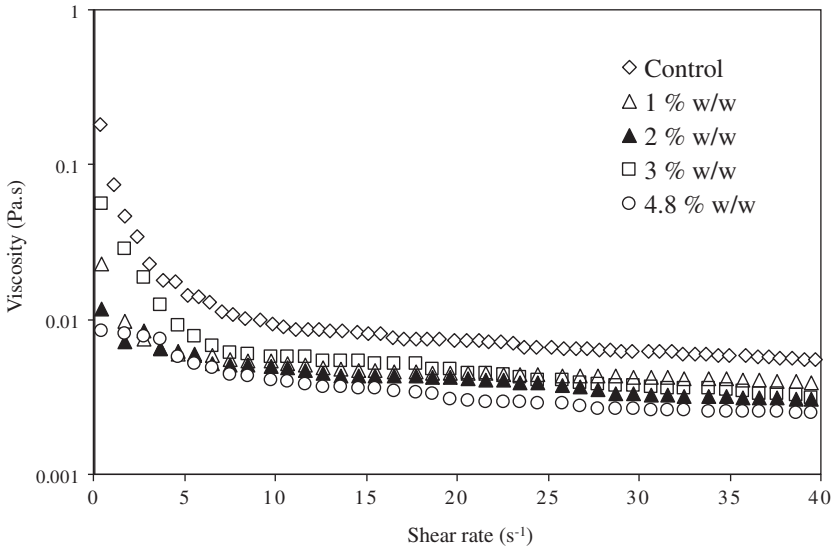

Fig. 2. Effect of ozone concentration (% w/w) on the apparent viscosity of cloudy apple juice at a fixed processing time of 10 min.

were observed in consistency (k) and flow behaviour (n) indices of apple juice after ozonation. Cloudy apple juice is a dilute colloidal dispersion of solid particles in a solution of pectins, proteins, sugars, organic acids, and salts and hence has a high consistency index (k) (Benítez, Genovese, & Lozano, 2007, 2009). The consistency index (k) decreased as a function of ozone concentration from 9.11 (control) to 1.3 (ozone concentration 4.8% w/w and processing time of 10 min) (Table 1). A trend towards increased Newtonian flow behaviour at increasing ozone concentration and processing time was observed, with ozonated apple juice (ozone concentration 4.8% w/w and processing time of 10 min) showing the highest n value of 0.65 compared to 0.37 for control (Table 1). A decrease in apparent viscosity (Pa s), k value and an increase in n value during ozonation arise from the breakdown of a colloidal suspension which may be caused by the depolymerisation of macromolecules present in the juice suspension (Tiwari, Muthukumarappan, O'Donnell, Chenchaiah, & Cullen, 2008).

3.4. Phenolic content

Fig. 3(i–iv) shows the 3D response surface plots for chlorogenic acid, caffeic acid, cinnamic acid and total phenol content of apple juice during ozone processing. During ozonation at higher processing conditions (4.8% w/w ozone concentration for 10 min processing time) a decrease of 99.1%, 96.6%, 99.8% and 49.7% was observed for chlorogenic acid, caffeic acid, cinnamic acid and total phenol content respectively. However, a processing time required to achieve 5-log reductions for *E. coli* under similar experimental conditions (Patil, Valdramidis, Cullen, Frias, & Bourke, 2010) showed a 66.5%, 73.5% and 65.0% reduction for chlorogenic acid, caffeic acid and cinnamic acid respectively. It was found that polyphenols are prone to oxidation as observed in the case of strawberry and blackberry anthocyanins (Tiwari, O'Donnell, Muthukumarappan, & Cullen, 2009; Tiwari, O'Donnell, Patras, Brunton, & Cullen, 2009). This reduction is most likely due to the strong oxidation potential (+2.07 eV) of ozone. The degradation of polyphenols during ozonation may result from a variety of possible chemical reactions. These reactions may be direct reactions of ozone with the target compound or its intermediates and radical reactions between hydroxyl radicals (produced through ozone decomposition catalysed mainly by the hydroxide ion (OH^-) (Cullen, Tiwari, O'Donnell, & Muthukumarappan, 2009).

Chlorogenic acid, caffeic acid, cinnamic acid and total phenol content of ozonated apple juice were all significantly influenced by processing time ($p < 0.0001$) while ozone concentration was not significant for total phenol (Table 2). Table 3 shows the regression coefficient for the predicted colour value models. The predicted response models for phenolic content were found to fit well with the experimental data. The models presented showed high correlation coefficients (R^2) of 0.90, 0.93, 0.92 and 0.97 for total phenol, chlorogenic acid, caffeic acid and cinnamic acid respectively. Predicted models were highly significant for phenolic content ($p < 0.0001$).

Canonical analysis and ANOVA of second order quadratic models revealed that both linear and quadratic models were significant with $p < 0.0001$ for all parameters. Whereas, the interaction (cross-product) among parameters was insignificant in every case except for cinnamic acid ($p < 0.01$) indicating that only linear and quadratic effects of the independent variables of ozone concentration and processing time caused significant effects on the response surface. According to Xue, Chen, and Wang (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 ·OH, HO^{2}·, $\cdot O_2^-$, and $\cdot O_3^-$ which facilitate degradation. A similar oxidative degradation of ascorbic acid in presence of oxygen has been reported by Kennedy, Rivera, Lloyd, Warner, and Jumel (1992). Zimeri and Tong (1999) reported a degradation mechanism of epigallocatechin gallate in the presence of dissolved oxygen in a model liquid solution.

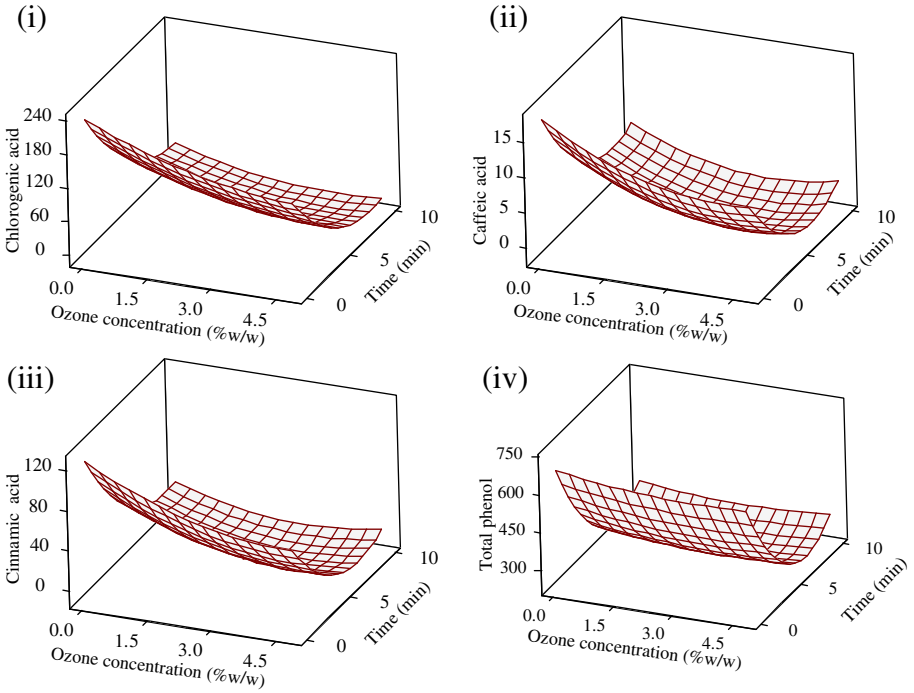

Fig. 3. Effect of ozone concentration (% w/w) and processing time (min) on (i) polyphenolic content of apple juice, (ii) chlorogenic acid, ii) caffeic acid, (iii) cinnamic acid and (iv) total phenol content.

4. Conclusion

The results presented in this study demonstrate the effect of ozone concentration and processing time on colour degradation, rheological properties and retention of polyphenols. Apple juice colour, rheological properties and phenolic content were observed to be significantly influenced by ozonation. It is concluded that while ozonation can be employed as a preservation technique for processing of apple juice, its impact on the nutritional and quality parameters of apple should be considered.

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