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Effectiveness of ascorbic acid and sodium metabisulfite as anti-browning agent and antioxidant on green coconut water *(Cocos nucifera)* subjected to elevated thermal processing

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Abstract

Thermal processing of green coconut water (GCW) caused non-enzymic browning and development of rancidity. Effect of the addition of several combinations of ascorbic acid (AA) (0 to 100 ppm) and sodium metabisulfite (SMB) (0 to 30 ppm) on brown discolouration and rancidity of GCW during elevated thermal processing (121°C for 5 min at 15 psi) was investigated. Addition of AA and/or SMB significantly (P<0.05) reduced brown discolouration of processed GCW, with SMB being approximately 7 times more effective than AA. Rancidity in GCW was significantly (P<0.05) reduced with the addition of SMB and/or AA, with SMB being the most effective; approximately 35 times more effective than AA, respectively. SMB can be recommended as an effective controller of browning and rancidity in thermally processed GCW.

Introduction

Consumers living in tropic countries can easily enjoy fresh coconut water straight from the coconuts. However, for those living outside of the tropics, whole coconuts can be hard to find. Coconut water has been dubbed "miracle water" for its numerous health-giving benefits. Besides being highly nutritious due to the presence of sugars, vitamins, minerals and proteins (Santoso *et al*., 1996; Yong *et al*., 2009; Tan *et al*., 2014), coconut water is believed to be useful in preventing and relieving many health problems, including dehydration, constipation, digestive problems, fatigue, heatstroke, diarrhea, kidney stones and urinary tract infections (Campbell-Falck *et al.*, 2000). Presence of natural cytokinin nucleotides (kinetin, kinetin riboside and trans-zeatin riboside-5′-monophosphate) in coconut water boosts the benefits of drinking coconut water for its anti-ageing effect (Ge *et al.*, 2006). Mandal *et al.* (2009) had isolated three antimicrobial peptides from coconut water, hence indicating the potential of coconut water as a natural source for antibiotics. Recently, Chang and Wu (2011) have detected the presence of (+)-catechin and (−)-epicatechin, which have anti-microbial, antioxidant and anti-cancer activities, in coconut water. Study done by Ismail *et al.* (2007) has proven that coconut water has the potential to be a natural alternative to carbohydrate-electrolyte sports drinks for its rehydration ability. Knowing the greatness of coconut water, ready-to-drink coconut water posed to have great potential in beverage market.

To commercialise coconut water as a ready-to-drink beverage, adequate heat treatment is important because coconut water is susceptible to microbial and oxidative enzymatic spoilage (Campos *et al*., 1996; Tan *et al*., 2014). Thermal processing is one of the most widely used preservation method in food industries because high temperature could provide inactivation of both microbial and enzymatic activities. However, thermal processing could also initiate various types of chemical reactions, with some causing deterioration on the quality and sensorial attributes (Aguiar *et al.*, 2012; Igual *et al*., 2014). Non-enzymic browning, which could be caused by phenols oxidation, ascorbic acid (AA) degradation and Maillard reaction (Li *et al*., 2008; Lima *et al*., 2009), and rancidity (Fan, 2002), during thermal processing are two main reasons for rejections by consumers. Phenolic compounds in coconut water could be oxidised in the similar way as tea catechins during pasteurisation, which leads to brown discolouration (Kim *et al*., 2007). Apart from that, brown discolouration during thermal treatment could also be due to the occurrence of Maillard reaction e.g. during pasteurisation, since protein and reducing sugars are available in coconut water (Jayalekshmy and Mathew, 1990; Tan *et al*., 2014). Hence, antioxidant and anti-browning agents could be added to control the occurrence of non-enzymic browning (Damasceno *et al*., 2008) and formation of

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free fatty acids (Das Purkayastha *et al*., 2012) during thermal processing.

Various approaches to control the development of browning and rancidity have been carried out, e.g. thermal inactivation of enzymes and addition of chemical additives. Nowadays, antioxidants are one of the common methods to preserve foodstuffs by retarding deterioration, rancidity and/or discolouration (Arogundade and Mu 2012). Among the wide variety of additives, sulphites and AA are the popular additives used in food industry. Browning inhibition by sulphites is caused by the reaction between sulphite ions and quinines (Danilewicz e*t al*., 2008). Despite its effectiveness, usage of sulphites as antioxidants is governed by restrictions set by World Health Organisation (WHO) due to health-related issues incurred by sulphite-sensitive individuals (Li *et al*., 2008). Increased suspicious of toxic and carcinogenic effects of synthetic antioxidants resulted in demand for healthier and natural alternatives, such as AA. The effectiveness of AA as anti-browning agent is reflected by its ability to reduce phenoxyl radicals and quinone forms of phenolics back to colourless diphenols in a coupled oxido-reduction reaction (Louarme and Billaud, 2012). Despite the efficiency of AA, its use is limited by the organoleptic consideration. Campos *et al*. (1996) reported that the flavour quality of coconut water was altered when AA exceeded 200 ppm.

To our knowledge, very little studies have been performed on coconut water processing, with majority of studies on coconut water were related to enzyme inactivation, physicochemical properties of coconut water and health benefits of coconut water. The aim of this paper was to provide information on the effect of AA and sodium metabisulfite (SMB) in brown discolouration and rancidity of green coconut water (GCW) during elevated thermal processing (121°C for 5 min at 15 psi). In addition, the effectiveness between these additives in reducing the development of brown discolouration and rancidity of GCW during elevated thermal processing will also be compared.

Materials and Methods

Materials

Ascorbic acid (AA), trichloroacetic acid (TCA), butylated hydroxytoluene (BHT) and 1,1,3,3-tetraethoxypropane (TEP) used in this study were purchased from Sigma-Aldrich Co., St. Louis, USA. Sodium metabisulfite (SMB) and 2-thiobarbituric acid (TBA) were purchased from Merck KGaA, Darmstadt, Germany and Alfa Aesar, Lancashire, UK, respectively. All chemicals used in this study were of analytical grade, except for AA and SMB, which are of food grade.

Coconut water

Green coconuts (aged 6 months old from coconut variety of Malayan Tall) were purchased from Anba Coconuts located at Abu Siti Lane, Penang. Surface of the coconut husks was cleaned with distilled water and 1% bleach (Clorox) before a stainless steel knife was used to perforate the fruit mesocarp. GCW was manually extracted from the coconut fruit and filtered through muslin cloth. The filtered GCW from several fruits (3 to 5 coconuts with similar maturity age) was pooled in a 2 L Schott bottle and kept temporary in an icebox with ice packs. All GCW was prepared on the same day of purchase and extraction. Sample preparation was triplicated.

Thermal treatment of GCW

The effect of AA and SMB on brown discolouration of GCW was investigated by adding AA (0, 50 and 100 ppm) and SMB (0, 15 and 30 ppm) to the GCW samples. GCW samples (80 mL) with added AA and/or SMB were transferred into individual 100 mL Schott bottle. The GCW samples were subjected to elevated thermal processing (121°C for 5 min at 15 psi) using a SX-700 high-pressure steam steriliser (Tomy, Japan). After the thermal treatment, all GCW samples were cooled in ice water prior to browning index and malonaldehyde measurements.

Browning index

Brown discolouration of GCW samples was determined using browning index measurement and was performed using a CM-3500d spectrophotometer (Konica Minolta, Japan) at wavelength of 420 nm according to method as described by Tan *et al*. (2012). GCW was transferred into a 10 mm optical quartz cell (Konica Minolta, Japan) to measure absorbance. The absorbance at wavelength 550 nm was also recorded to correct for any turbidity in the GCW (Tan *et al*., 2012). Browning index was defined based on the following equation:

$$\text{Browning index} = Abs420 - Abs550 \qquad \text{(Eq. 1)}$$

where, *Abs*420 is the absorbance at 420 nm and *Abs*550 is the absorbance at 550 nm.

Measurement of malonaldehyde using TBA-based assay

Measurement of malonaldehyde in GCW was based on the method as described by and Fan (2002) with slight modification. GCW (5 g) was added to

a test tube containing 5 mL of either Solution A (containing 20% TCA and 0.01% BHT) or Solution B (containing 20% TCA, 0.01% BHT and 0.65% TBA). Samples were heated at 95°C for 35 min in a WB22 water bath (Memmert, Germany). After heating, samples were cooled in ice water for 15 min and absorbance at 440, 532 and 600 nm were recorded using UV-1650 PC spectrophotometer (Shimadzu, Japan).

Standard solutions of TEP were prepared according to the method as described by Pegg (2001) to give final concentrations of 0.001, 0.004, 0.006, 0.008 and 0.01 mM in distilled water. All the standard solutions were added with 5 mL of 0.65% TBA. Standards were heated at 95°C for 35 min in a water bath. After heating, standards were cooled in ice water for 15 min and absorbance 532 was recorded. Standard curve with absorbance value against standard concentration (in M) was plotted.

TBA value, expressed as mg malonaldehyde equivalents/kg sample, was calculated according to the following equations:

$$A = (Abs532_{sol.B} - Abs600_{Sol.B}) - (Abs532_{Sol.A} - Abs600_{Sol.A)} \quad \text{(Eq. 2)}$$

$$B = (Abs440_{Sol.B} - Abs600_{Sol.B}) \times 0.0571 \quad \text{(Eq. 3)}$$

$$\text{TBA value} = [S^{-1} \times MW \times 10^6 \times (A - B)] / m \quad \text{(Eq. 4)}$$

where, *Abs*440 is the absorbance at wavelength 440 nm, *Abs*532 is the absorbance at wavelength 532 nm, Abs600 is the absorbance at wavelength 600 nm, S^{-1} is the gradient of the standard curve, MW is the molecular weight of malonaldehyde (72.03), 10^6 converts the units so that results can be expressed as mg malonaldehyde equivalents/kg sample and m is the sample mass (in g).

Statistical analysis

A 3×3 factorial design was used to study the effects of two factors; AA concentration (X_1) and SMB concentration (X_2), on browning index and TBA value of GCW samples. Three AA concentrations (0, 50 and 100 ppm) and three SMB concentrations (0, 15 and 30 ppm) were tested. Three replicates were carried out for a total of 27 runs. Analysis of variance (ANOVA), Tukey's test for multiple comparisons, interaction plots and regression equations were used for analysing the data. SPSS version 20 (SPSS, USA) was used to complete these statistical analyses.

Relative effectiveness of SMB as anti-browning agent (measured by the ability to reduce browning index) or as antioxidant (measured by the ability to reduce TBA value) was calculated using the coefficients from the regression equations generated. Relative effectiveness of SMB was calculated in comparison to AA using the following equation:

$$\text{Relative effectiveness} = C_{X2} / C_{X1} \quad \text{(Eq. 5)}$$

where, CX_2 is the coefficient of X_2, which represents SMB, and CX_1 is the coefficient of X_1, which represents AA.

Results and Discussion

Effect of AA and SMB on brown discolouration

All GCW samples were subjected to elevated thermal processing (121°C for 5 min at 15 psi) using an autoclave. All processed GCW showed brown discolouration with different extent of browning intensity. The amount of AA and SMB added had an influence on the magnitude of brown discolouration of GCW, with higher AA and SMB concentrations yielded GCW with lower browning index (Table 1).

Table 1. Browning index and TBA value obtained from GCW samples added with AA and SMB prior to elevated thermal processing (121°C for 5 min at 15 psi)

Sample ID	Concentrations (ppm)[2]		Browning Index	TBA Value[3]
	AA	SMB		
Fresh[1]	0	0	0.0085 ± 0.0006^{e}	10.73 ± 0.23^{e}
1	0	0	0.0689 ± 0.0020^{a}	50.26 ± 0.58^{a}
2	0	15	0.0470 ± 0.0013^{b}	35.90 ± 0.61^{c}
3	0	30	0.0387 ± 0.0011^{c}	33.88 ± 1.00^{d}
4	50	0	0.0536 ± 0.0025^{b}	49.95 ± 0.32^{ab}
5	50	15	0.0466 ± 0.0018^{c}	39.23 ± 0.68^{c}
6	50	30	0.0376 ± 0.0016^{d}	31.73 ± 0.93^{d}
7	100	0	0.0534 ± 0.0032^{b}	47.74 ± 0.60^{b}
8	100	15	0.0449 ± 0.0020^{c}	36.07 ± 0.61^{c}
9	100	30	0.0375 ± 0.0004^{d}	29.95 ± 1.07^{d}

[1]Control represents fresh GCW without any thermal processing.
[2]AA: ascorbic acid; SMB: sodium metabisulfite.
[3]TBA (thiobarbituric acid) value expressed as mg malonaldehyde equivalents/kg sample.
Comparisons within the same column (a-e) are shown in the table with the data written as mean ± standard deviation of 3 replicates. Means followed by different letter are significantly different at $P<0.05$ level of significance according to Tukey's Multiple-Range Test.

The brown discolouration of GCW could be a consequence of enzymatic browning reactions and non-enzymatic browning reaction, e.g. Maillard reaction and phenol oxidation (Li *et al.*, 2008). However, since heat treatment at 90°C for 550 s was sufficient to inactivate both polyphenol oxidase and

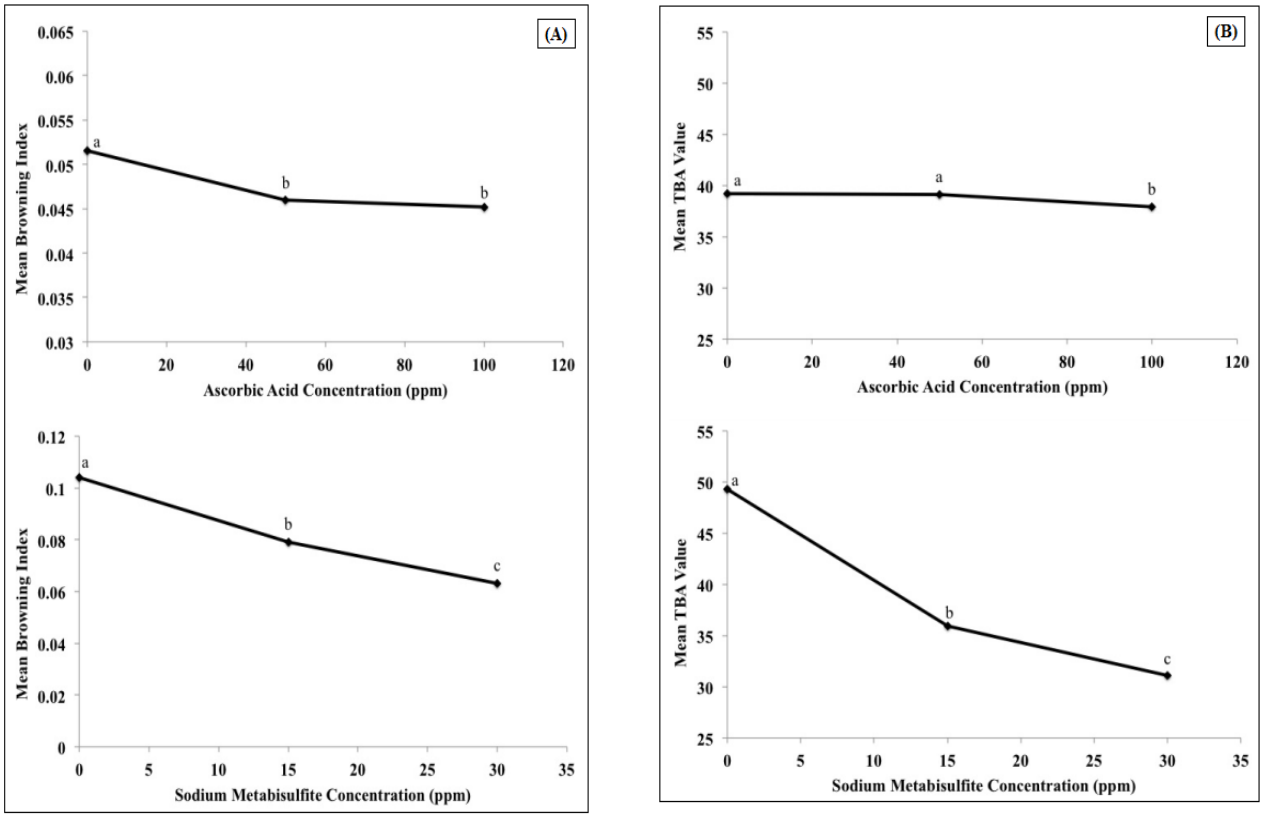

Figure 1. Interaction plots for (a) mean browning index and (b) mean TBA value as a function of AA and SMB. Means with different letter are significantly different at $P<0.05$ level of significance according to Tukey's multiple-range

peroxidase present in coconut water (Campos *et al*., 1996) the contribution of enzymatic browning was small. Owing to the elevated thermal processing condition used in this study, brown discolouration of coconut water could be caused mainly by non-enzymatic reaction, such as the Maillard reaction, degradation of AA and/or oxidation of phenolic compounds (Li *et al*., 2008; Lima *et al*., 2009), since the thermal treatment applied was sufficient to deactivate the enzymes responsible for brown discolouration (Campos *et al*., 1996; Tan *et al.*, 2014). (+)-Catechin and (−)-epicatechin found in coconut water (Chang *et al*., 2011) could be oxidised in a similar way as tea catechins during heating or pasteurisation, which causes green tea liquor to became darker and deeper yellow in colour (Kim *et al*., 2007).

Addition of AA and SMB into GCW had significant (P<0.05) effect on brown discolouration in all samples (Table 2). The effectiveness of AA in controlling browning was previously demonstrated on slices of carambola (Weller *et al*., 1997), white wines (Peng *et al*., 1998), fresh-cut cantoloupe melon (Lamikanra and Watson, 2001) and banana smoothies (Wang *et al.*, 2013), while the effectiveness of sulphites in controlling browning had also been demonstrated on slices of ivy gourd (Kulkarni and Vijayanand, 2012) and coconut husk (Mohpraman and Siriphanich, 2012).

To further understand the effect of AA and SMB concentrations on the brown discolouration of GCW, interaction plots were constructed and comparisons between the tested levels of concentration for the same additive were determined using Tukey's Multiple-Range Test. When AA and SMB were absence in GCW, the browning index was high and increased by approximately 8 times, from 0.0085 (fresh untreated CGW) to 0.0689. With the addition of AA from 0 to 50 ppm, browning index was significantly (P<0.05) reduced. However, no significant difference (P>0.05) was observed when AA concentration was increased from 50 and 100 ppm (Figure 1A). On the contrary, browning index decreased significantly (P<0.05) with an increase in SMB concentration from 0 to 30 ppm (Figure 1A).

Based on the data obtained, a regression equation (Eq. 6) was generated to estimate the degree of effectiveness of AA and SMB in reducing brown discolouration of GCW samples as a result of the elevated thermal processing. Based on the coefficients of X_1 and X_2 shown in Eq. 6, relative effectiveness of SMB in comparison to AA was calculated using Eq. 5. SMB was found to be relatively more effective (relative effectiveness of ~7×) in reducing the browning index when compared to AA.

Table 2. The results of the factorial experiment for browning index and TBA value obtained from GCW samples added with AA and SMB prior to elevated thermal processing (121°C for 5 min at 15 psi)

Source[1]	Sum of Squares	df	Mean Square	F	P-value
Browning Index					
X_1	0.0002	2	0.0001	29.341	<0.0001
X_2	0.0020	2	0.0010	270.787	<0.0001
$X_1 . X_2$	0.0003	4	0.000068	18.790	<0.0001
Error	0.000065	18	0.000004		
Total	0.0025	26			
TBA Value[2]					
X_1	9.672	2	4.836	6.286	0.009
X_2	1608.851	2	804.426	1045.717	<0.0001
$X_1 . X_2$	7.655	4	1.914	2.488	0.08
Error	13.847	18	0.769		
Total	1640.025	26			

[1]X_1, ascorbic acid concentration (0, 50 and 100 ppm); X_2, sodium metabisulfite concentration (0, 15 and 30 ppm).
[2]TBA: thiobarbituric acid.

$$Y = 0.0646 - 1.34E\text{-}4X_1 - 9.29E\text{-}4X_2 + 4.77E\text{-}6X_1X_2 \quad \text{(Eq. 6)}$$

where, Y is the browning index, X_1 is the concentration of AA (in ppm) and X_2 is the concentration of SMB (in ppm).

Effect of AA and SMB on rancidity

GCW have been reported to contain a trace amount of oil, and the level of coconut oil has been shown to increase with maturity of the coconut fruit (Santoso *et al.*, 1996; Tanqueco *et al.*, 2007; Tan *et al.*, 2014). Presence of coconut oil could cause rancidity to form free fatty acids, which will lead to the deterioration of organoleptic quality of the coconut water (Das Purkayastha *et al.*, 2012). Study done by Fan (2002) showed that a thermally processed apple juice had a higher malonaldehyde concentration compared to fresh apple juice. To overcome this problem, one could either remove the oil from coconut water during processing or suppress the occurrence of rancidity during processing with the aid of antioxidants, with the latter one being of interest in this study.

Both additives showed significant (P<0.05) effect in reducing TBA value in GCW (Table 2). In the absence of AA and SMB, occurrence of rancidity (as denoted by the increased in TBA value) was detected as a result of elevated thermal processing conditions used in this study (Table 1). The amount of AA and SMB added into GCW had an influence on the level of rancidity of GCW samples; with higher AA and SMB concentrations yielded GCW with lower TBA values. The interaction plot for AA shows no significant changes on the TBA value when AA was added at concentration below 50 ppm (Figure 1B). However, significant (P<0.05) reduction in TBA value was observed at AA concentration above 50 ppm. On the other hand, an increase in SMB concentration from 0 to 30 ppm significantly (P<0.05) decreased the TBA values (Figure 1B).

Based on the data obtained, a regression equation (Eq. 7) was generated to estimate the degree of effectiveness of AA and SMB concentrations in reducing the TBA value of GCW samples as a result of elevated thermal processing applied in this study. Relative effectiveness of SMB was found to be more effective (relative effectiveness of ~35×) in reducing the TBA value when compared to AA.

$$Y = 48.77 - 1.78E\text{-}2X_1 - 6.24E\text{-}1X_2 + 3.10E\text{-}4X_1X_2 \quad \text{(Eq. 7)}$$

where, Y is the TBA value (in mg malonaldehyde equivalents/kg sample), X_1 is the concentration of AA (in ppm) and X_2 is the concentration of SMB (in ppm).

Conclusion

Significant (P<0.05) decrease in both browning index and TBA value was observed in GCW samples with AA and/or SMB subjected to elevated thermal processing (121°C for 5 min at 15 psi). SMB appeared to be approximately 7 and 35 times more effective in suppressing brown discolouration and rancidity, respectively, during elevated thermal processing when compared to AA. Hence, SMB is considered relatively more effective, when compared to AA, as an anti-browning agent as well as antioxidant for GCW subjected to elevated thermal processing.

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