Preventing Vitamin C Photooxidation in Beverage Model System by Virgin Coconut Oil-Rice Bran Oil Nanoemulsion

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Keywords: Vitamin C, Photooxidation, Nanoemulsion, Model Beverage.

Abstract: Vitamin C (L-ascorbic acid) is a water-soluble vitamin and frequently added in a beverage. This research aimed to investigate the effect of virgin coconut oil-rice bran oil (VCO-RBO) nanoemulsion on vitamin C photooxidation in beverage model system. The oil phase was VCO: RBO (3:7, v/v), surfactant (Tween 80) to oil ratio was 2.5:1 and distilled water was used as the aqueous phase by emulsion phase inversion method. One and 5% (v/v) of VCO-RBO nanoemulsion were added to a system containing vitamin C (450 and 1800 ppm), erythrosine (0-120 ppm) and citric acid (to adjust pH 2.3 and 3.2) in distilled water. The presence of light and erythrosine can degrade vitamin C in beverage model system at 1 ppm vitamin C/min, effectively. The increase in erythrosine concentration increased the vitamin C degradation in a dose-dependent manner. By using VCO-RBO nanoemulsion (1 and 5% v/v), the degradation of vitamin C in beverage model system can be inhibited. At pH 2.3, the addition of 5% (v/v) of VCO-RBO nanoemulsion in the beverage model system was more effective in preventing vitamin C photooxidation than that at pH 3.2. It suggests that VCO-RBO nanoemulsion can be added in beverage model system to protect the vitamin C photooxidation.

1 INTRODUCTION

Vitamin C or L-ascorbic acid is one of the water-soluble vitamins. It is commonly added in beverage, especially in isotonic water, for its health benefit and to meet the consumer demand. Vitamin C is known as a potent antioxidant. However, it can easily be degraded under high pH, high temperature and by photooxidation (Huang et al., 2004; Jeney-nagymate and Fodor, 2008; Yang and Min, 2009; Sheraz et al., 2015). Photooxidation is one of the main problems in food and beverage. It was induced by the presence of sensitizer such as food colorant (FD&C Red number 3) or erythrosine, riboflavin, chlorophyll, etc. (Lee et al., 1997; Yettela and Min, 2008; Yang and Min, 2009). These compounds naturally present or deliberately added to improve the appearance and functional value of products. The reaction rate of photooxidation is a lot faster than autooxidation. It can produce oxidation products that contribute to off-flavor or degradation of beneficial components like vitamin C, vitamin D, amino acid, etc. Photooxidation can be prevented by singlet oxygen quencher or antioxidant. Unfortunately, many antioxidants are lipid-soluble like β-carotene, α-tocopherol, γ-oryzanol etc. It is very challenging to use it in beverage product.

Solid lipid nanoparticle, nanostructured lipid carrier and nanoemulsion are nano-lipid based delivery systems. Some studies reported that nanoemulsion can be incorporated into beverages to increase the value of products. Zhang et al. (2020) used docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) nanoemulsion in apple juice. This nanoemulsion didn't affect basic properties such as pH, soluble solids, titratable acid and reducing sugar of apple juice. Even though it influenced the transparency of product, the addition of DHA/EPA nanoemulsion in apple juice was still acceptable by sensory test (Zhang et al., 2020). Buriti *(Mauritia flexuosa* L.) oil nanoemulsion was also potential as natural colorant replacer in isotonic sport drink (Bovi et al., 2017). Fish oil and rice bran oil was also used to be part of oil-in-water nanoemulsion before incorporating with yoghurt as reported by Zhong et al. (2018). This nanoemulsion gave some significant impacts on reduction in acidity, syneresis and peroxide value with maximum retention of EPA and DHA.

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Rice bran oil is rich in a specific antioxidant named γ-oryzanol up to 2. 6g/100 g of oil (Pokkanta et al., 2019). These compounds have been attractive because it was only found in rice bran products, especially in rice bran oil. Virgin coconut oil is known as oil which rich in lauric acid. The medium-chain fatty acid is the main ingredient to produce nanoemulsion by a low-energy method.

In this research, we used a combination of virgin coconut oil and rice bran oil, which were incorporated with Tween 80 and distilled water to make an oil-in-water nanoemulsion. This study aimed to investigate the effect of virgin coconut oil-rice bran oil (VCO-RBO) nanoemulsion on photooxidation of vitamin C in a beverage model system.

2 MATERIALS AND METHODS

2.1 Materials

Vitamin C or L-ascorbic acid for analytical grade (J.T. Baker); Tween 80, potassium iodide and iodine were obtained from Merck (Germany). Virgin coconut oil, rice bran oil, sucrose, erythrosine and citric acid were food grade that obtained from local market.

2.2 Methods

2.2.1 Preparation of Virgin Coconut Oil-Rice Bran Oil (VCO-RBO) Nanoemulsion

The VCO-RBO nanoemulsion formula were VCO: RBO (3:7, v/v), surfactant (Tween 80) to oil ratio was 2.5:1 and distilled water was used as the aqueous phase. This nanoemulsion was produced by emulsion phase inversion according to Sari et al. (2020).

2.2.2 Preparation of Beverage Model Systems

The beverage model systems was prepared according to (Ariviani et al., 2011) with slight modification. The beverage model system containing vitamin C (450-1800 ppm, w/v), erythrosine (0-120 ppm, w/v), citric acid (to adjust pH 2.3 and 3.2) in distilled water. VCO-RBO nanoemulsion (0, 1 and 5%, v/v) were added into the systems. A 10 mL of the beverages model systems was prepared into 30-mL vial with rubber cap and sealed with parafilm. The samples were illuminated at $\pm$ 3200 lux or stored in the dark up to 2 hours. Vitamin C content were analyzed in every 30 minutes.

2.2.3 Physicochemical Analysis

Vitamin C in each samples were analyzed by iodine titrimetric according to Sudarmadji et al. (1997) method. pH of samples were measured by Hanna Instrument. The color of samples were determined using a Konica Minolta Colorimeter with L*, a* and b* parameters. The erythrosine concentration were analyzed using spectrophotometer by (Yang and Min, 2009) with some modifications. Curva calibrations were constructed in each sample formula. The wavelength detection analysis according to each maximum wavelength absorption by scanning method (200-700 nm). Turbidity of samples were determined at 600 nm (Zhong et al., 2017; Sari et al., 2020).

2.2.4 Statistical Analysis

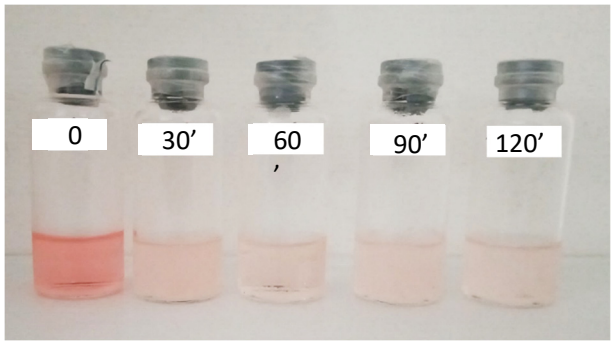
The experiment were done in duplicate. The samples were analyzed at least duplicate in each experiment. Data were analyzed by regression analysis with Microsoft Excel 2013 and IBM SPSS Statistic 24.

3 RESULTS AND DISCUSSION

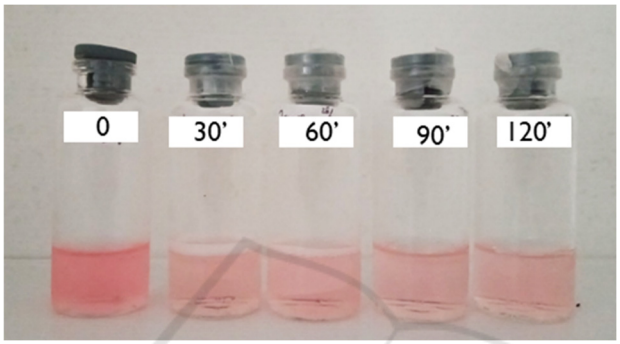
3.1 Characterization of VCO-RBO Nanoemulsion

In this study, VCO:RBO (3:7, w/w) were used as the oil phase. The surfactant (Tween 80) to oil ratio was 2.5:1. Distilled water as the aqueous phase in 80% (v/v) of the total system. Based on the previous study, this formula was selected because it gave the smallest particle size of nanoemulsion (65.64 nm) with zeta-potential was -12.16 mV (Sari et al., 2020). This formula had slight transparency, therefore only slightly affected the beverage model system's appearance.

The visual sample product can be seen in Fig 1. Samples containing 5% nanoemulsion in 450 ppm vitamin C were more slightly pink than models with 1800 ppm vitamin C at the same concentration of VCO-RBO nanoemulsion and pH system (2.3). VCO-RBO nanoemulsion up to 5% (v/v) in beverage model systems didn't make samples to be turbid. The turbidities of all samples containing 1800 ppm vitamin C were relatively small (<0.1 cm^{-1}) It might be due to small size of nanoemulsion and a small portion of it to be incorporated in beverage model systems (Fig.2).



(a)



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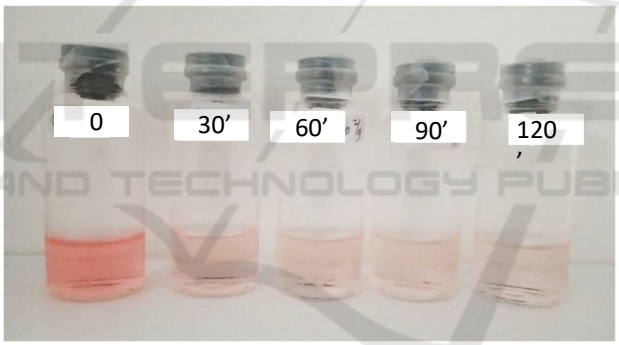
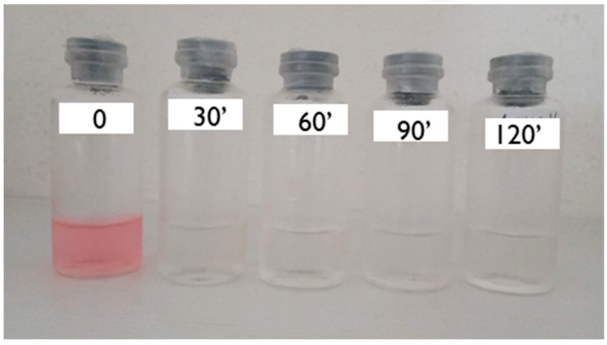


(c)



(d)

Figure 1: Visual apperarance of beverage model systems containing 450 ppm vitamin C with 0 and 5% of VCO-RBO nanoemulsion (a) and (b), respectively; 1800 ppm vitamin C with 0 and 5% of VCO-RBO nanoemulsion (c) and (d), respectively. All pH systems were 2.3.

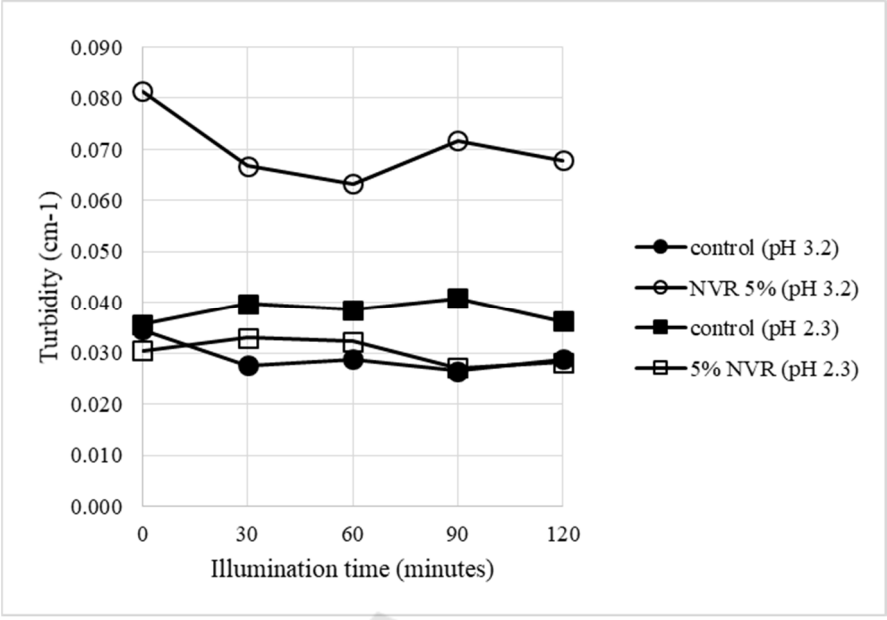


Figure 2: The changes of turbidity in photooxidation of beverage model systems (vitamin C 1800 ppm) (NVR=VCO-RBO Nanoemulsion).

3.2 The Effect of Light and Sensitizer on Vitamin C

To investigate the cause of vitamin C degradation, we used some sets of samples that were illuminated or stored in the dark, with and without of erythrosine as a sensitizer. According to Fig 3, the samples containing 120 ppm or erythrosine and held in a lightbox at ± 3200 lux up to 2 hours gave a vitamin C degradation almost 1 ppm of vitamin C/min ($y = -0.9724x + 1863.1$; $R^2 = 0.8732$; $p<0.05$). Meanwhile, the relatively stable vitamin C content was performed by samples stored in the dark ($y = -0.2182x + 1926.9$; $R^2 = 0.6749$; $p>0.05$) or without sensitizer and stored under light ($y = -0.6682x + 1833.4$; $R^2 = 0.3489$; $p>0.05$). By hypothesis null analysis in regression statistic, these two latter slopes were almost 0. It means that photooxidation can only occur by a combination of sensitizer and light. This study was similar to previous studies. At pH 4, 5.6 and 7, the 50 and 100 ppm of ascorbic acid were declined in the photooxidation in the presence of food colorant red nr 3. Meanwhile, ascorbic acid content was relatively stable under dark for one hour (Yang and Min, 2009). The degradation of riboflavin was also faster under the light than in the dark (Huang et al., 2004). It suggests that singlet oxygen was involved in these has sensitizer, light and triplet oxygen can produce singlet oxygen that can degrade vitamin C. The reaction between ascorbic acid with singlet oxygen produces unstable hydroperoxide of ascorbic acid (Choe and Min, 2005).

The effect of the initial concentration of vitamin C before photooxidation was also studied. From Fig.4 showed that the 450 and 1800 ppm of ascorbic acid content gives a relatively same of vitamin C degradation rate for about 1 ppm/min ($p>0.05$). At different pH (2.3 and 3.2), the degradation rate of vitamin C with 1800 ppm as initial content was also relatively same (0.97 and 1.02 ppm vitamin C/min, respectively, $p>0.05$).

Comparatively, the increasing of erythrosine concentration gave a significant effect on decreasing of vitamin C in a dose-dependent manner (Fig.5). At 40 and 80 ppm, the degradation rate of vitamin C were 0.62 and 0.69 ppm/min, respectively. Meanwhile, by using 120 ppm in the reaction system, the degradation rate of vitamin C up to 1.04 ppm/min. From these results, it was concluded that the difference of initial concentration or pH (2.3 and 3.2) didn't give any significant difference in the declining rate of vitamin C. Erythrosine concentration gave significant effect on the degradation rate of vitamin C in photooxidation system. The higher erythrosine concentration induced more singlet oxygen. Therefore, vitamin C will be degraded more frequently.

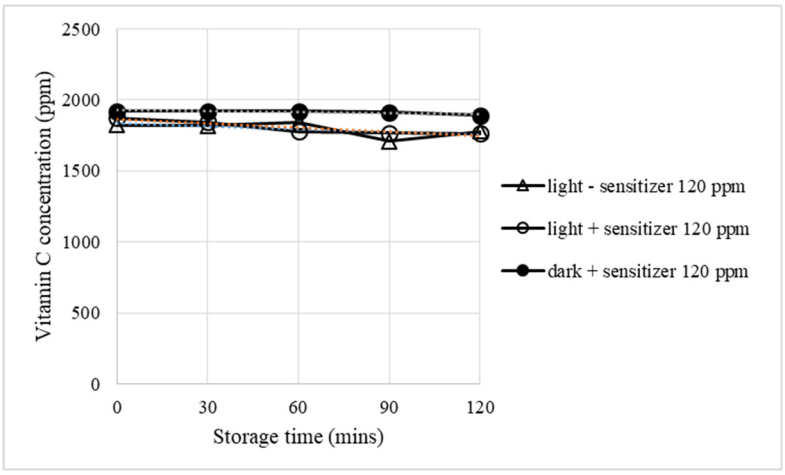


Figure 3: Effect of light (± 3200 lux) and sensitizer (120 ppm erythrosine) on vitamin C changes in beverage model system up to 120 minutes stored.

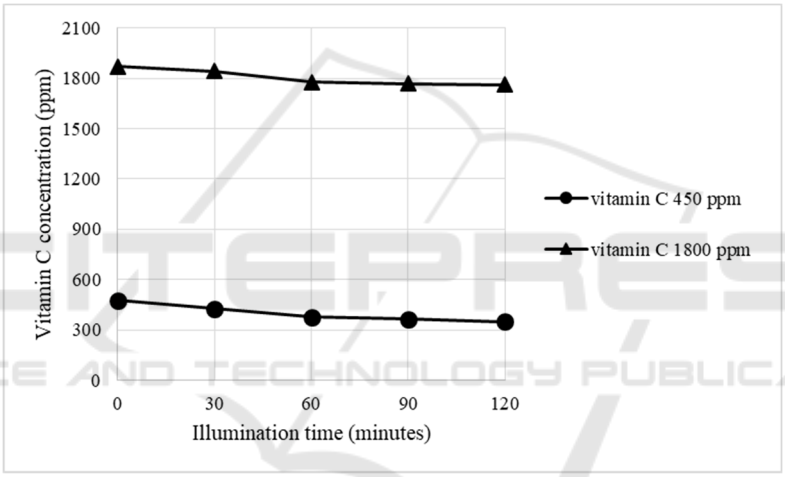


Figure 4: Effect of initial vitamin C concentration (450 and 1800 ppm) on degradation of vitamin C in photooxidation of beverage model system (light intensity ± 3200 lux at room temperature).

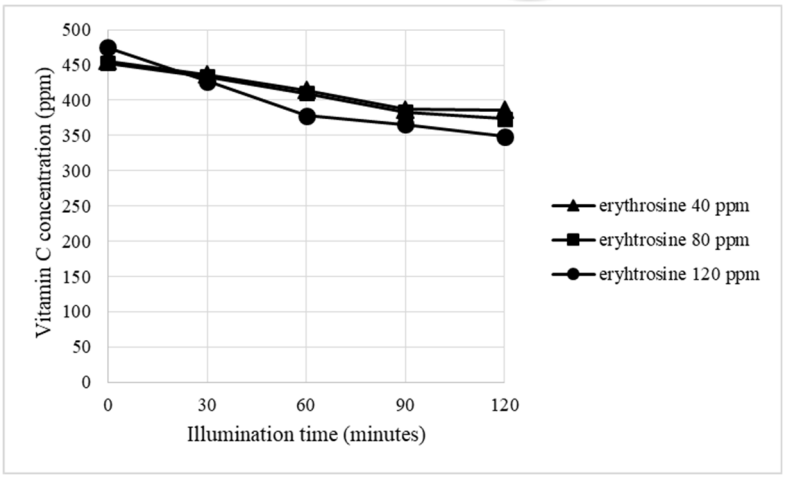


Figure 5: Effect of erythrosine concentration degradation of vitamin C in photooxidation of beverage model system (light intensity ± 3200 lux at room temperature).

3.3 Effect of VCO-RBO Nanoemulsion on Photooxidation of Vitamin C in Beverage Model Systems Containing Various Erythrosine Concentration

In this study, we added VCO-RBO nanoemulsion at 1 and 5% (v/v) in beverage model system. The 450 ppm vitamin C was relatively stable in beverage model systems containing various erythrosine concentration (40-120 ppm) and 5% of VCO-RBO nanoemulsion (Table 1). It might be due to the capability of nanoemulsion to maintain erythrosine in photooxidation. By using 5% VCO-RBO nanoemulsion, the color of the beverage model system was more slightly pink than the control (Fig. 1). It was concluded that nanoemulsion could maintain the stability of erythrosine; therefore, less singlet oxygen was produced and 450 ppm vitamin C was relatively constant for photooxidation reaction time (Table 1). Adding 1% of VCO-RBO nanoemulsion could avoid vitamin C degradation almost 33 and 60% at pH system was 3.2 and 2.3, respectively. By using regression statistical analysis, 5% of VCO-RBO nanoemulsion could protect vitamin C degradation (slope ≈ 0) at 1800 ppm of vitamin C as an initial concentration in pH 2.3 and 3.2. The natural antioxidant in oil phase such as α-tocopherol was suspected responsible to protect vitamin C avoid photooxidation. Some researchers found that rice bran oil contained 13.2-29.95 mg α-tocopherol /100 g oil (Pestana et al., 2008; Dhavamani et al., 2014; Yang et al., 2018). It was also known as effective singlet oxygen quencher with the singlet oxygen quenching rate was 4.9×10^7 up to 3.54×10^8 /M/s (Nishida et al., 2007; Kim et al., 2009; Ouchi et al., 2010).

Comparatively with photooxidation in vitamin C 450 ppm, the preventing mechanism by VCO-RBO nanoemulsion in beverage model systems containing 1800 ppm of vitamin C at pH 2.3 and 3.2 is not still clearly understood. Although 1 and 5% of VCO-RBO nanoemulsion can protect vitamin C degradation by photooxidation (Table 2), the a* values samples containing 5% VCO-RBO nanoemulsion at pH 2.3 and 3.2 were lower than the control (Fig. 6). This value was positively correlated with erythrosine concentration (Fig. 7). Therefore, VCO-RBO nanoemulsion couldn't maintain erythrosine in high concentration of vitamin C beverage model systems.

Table 1: The regression equations of beverage model systems containing VCO-RBO nanoemulsion (0 and 5% v/v) and various erythrosine concentration (40-120 ppm) in photooxidation reaction system.

Erythrosine conc. (ppm)	VCO-RBO nanoemulsion % (v/v)	Regression equation	R^2	Degradation rate (ppm vitamin C/min)	p-value*
40	0	Y = - 0.6234x + 453.24	0.9524	0.6234	0.004
40	5	Y = - 0.2875x + 504.44	0.5852	0.2875 ≈ 0	0.132
80	0	Y = - 0.69x + 452.02	0.9851	0.69	0.001
80	5	Y = - 0.0146x + 499.5	0.0021	0.0148 ≈ 0	0.941
120	0	Y = - 1.0451x + 461.37	0.9263	1.0451	0.009
120	5	Y = - 0.1651x + 453.55	0.678	0.1651 ≈ 0	0.087
*p-value<0.05 means the slope was significantly different from 0.					

Table 2: The regression equations of beverage model systems containing VCO-RBO nanoemulsion (0-5% v/v) at pH 2.3 and 3.2 in photooxidation reaction system.

pH	VCO-RBO nano-emulsion % (v/v)	Regression equation	R^2	Degradation rate (ppm vitamin C/min)	p-value*	p-value**
2.3	0	Y = -0.9724x + 1863.1	0.8138	0.9724	0.02	0.885
3.2	0	Y = -1.0258x + 1933.8	0.8732	1.0258	0.036	
2.3	1	Y = -0.401x + 1895	0.8072	0.401	0.038	0.110
3.2	1	Y = -0.6696x + 1883.3	0.9507	0.6696	0.005	
2.3	5	Y = -0.1303x + 1818.9	0.0358	0.1303 ≈ 0	0.761	1
3.2	5	Y = -0.3796x + 1821	0.5096	0.3796 ≈ 0	0.176	
* p-value<0.05 means the slope was significantly different from 0						
** p-value>0.05 means the two slopes were not significantly different at different pH and same amount of VCO-RBO nanoemulsion						

It seems like there is a behind mechanism by this nanoemulsion to prevent photooxidation of 1800 ppm vitamin C in beverage model systems. In another studies, vitamin C fortification (40-80 mg/100mL) can degrade anthocyanin and color loss in cranberry juice because the high concentration of vitamin C could increase oxidation products of vitamin C that degrade anthocyanin (Li et al., 2014; Roidoung et al., 2016, 2017). Meanwhile, in this study, samples containing 450 and 1800 ppm of vitamin C and 120 ppm of sensitizer without nanoemulsion gave the same trend of a* values during photooxidation (Fig. 8). Only samples containing 1800 ppm of vitamin C with VCO-RBO nanoemulsion that had transparent appearance and lower a* than the 450 ppm of vitamin C (Fig. 9).

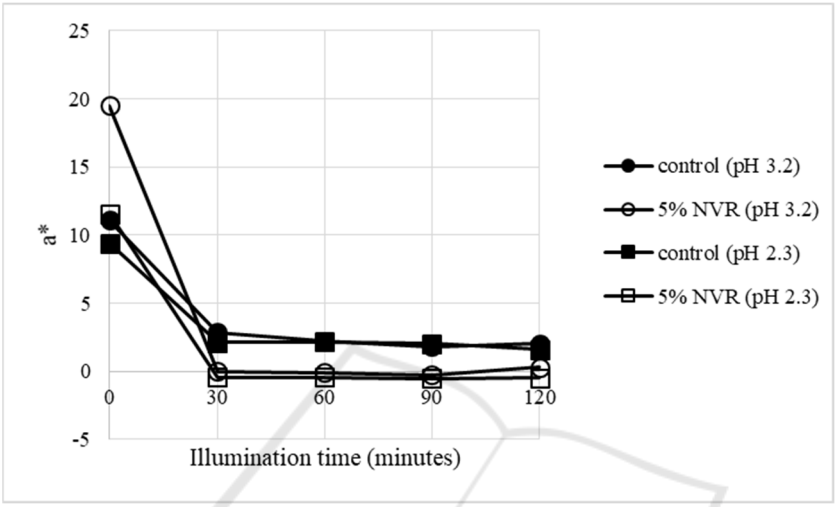


Figure 6: The changes of a* (redness) in photooxidation of beverage model systems (vitamin C 1800 ppm). (NVR=VCO-RBO Nanoemulsion).

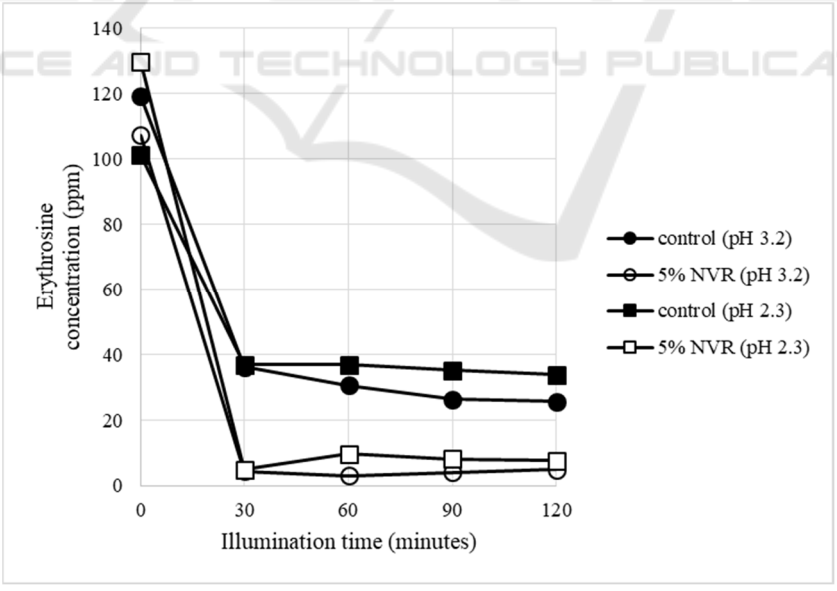


Figure 7: The changes of erythrosine concentration in photooxidation of beverage model system (vitamin C 1800 ppm). (NVR=VCO-RBO Nanoemulsion).

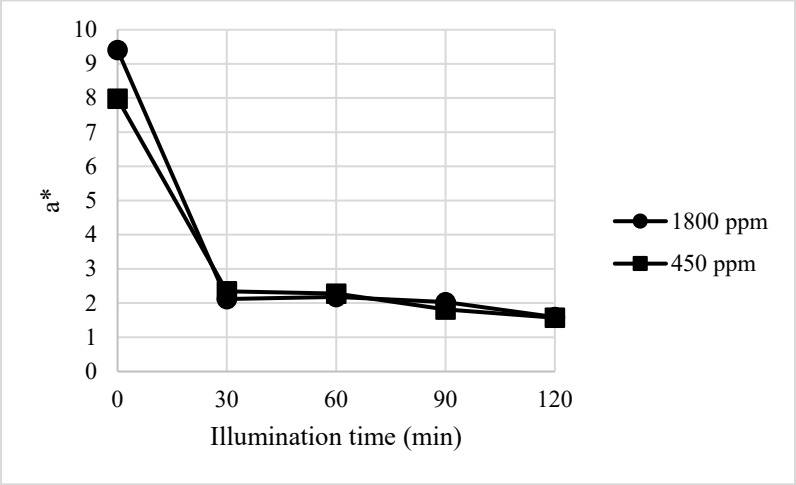


Figure 8: The changes of a* values of beverage model systems (vitamin C 450 and 1800 ppm) without nanoemulsion.

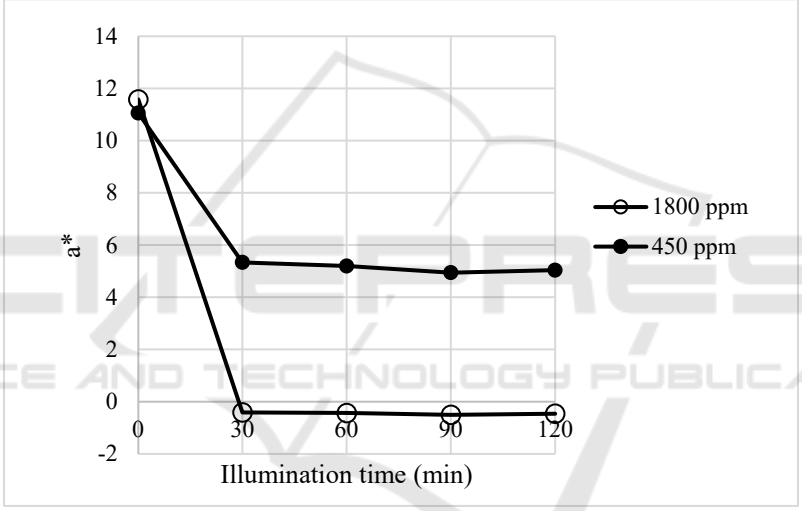


Figure 9: The changes of a* values of beverage model systems (vitamin C 450 and 1800 ppm) with 5% of VCO-RBO nanoemulsion during illumination.

4 CONCLUSIONS

The presence of light and erythrosine can degrade vitamin C in beverage model system at 1 ppm vitamin C/min, effectively. The increasing of erythrosine concentration affected on decreasing of vitamin C in a dose-dependent manner. By using VCO-RBO nanoemulsion (1 and 5% v/v), the degradation of vitamin C in beverage model system can be prevented. In pH 2.3, the 5% (v/v) of VCO-RBO nanoemulsion in beverage model system was more useful to avoid vitamin C photooxidation than in pH 3.2. It suggests that VCO-RBO nanoemulsion can be added in beverage model system to avoid the photooxidation of vitamin C.

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