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Inhibition of Ascorbic Acid Photooxidation in Beverage Model System by Rice Bran Oil Nanoemulsion Containing γ–Oryzanol

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Abstract: Photooxidation is one of the oxidation in food and beverage. To inhibit photooxidation, the singlet oxygen quencher is needed. Since many antioxidants are lipophilic, they should be delivered by lipid-based delivery systems, i.e., nanoemulsion. γ–Oryzanol is a well-known antioxidant naturally found in rice bran oil. This study aimed to evaluate the capability of rice bran oil nanoemulsion against ascorbic acid photooxidation in the beverage. The ingredients in nanoemulsions were rice bran oil, virgin coconut oil, palm oil, Tween 80, and distilled water. Low-energy methods could fabricate nanoemulsion via emulsion phase inversion. The formula nanoemulsion could be stable for up to 30 minutes of heating in an oven at 105 °C by turbidity parameter. During storage at both 28 and 37 °C, nanoemulsions could also be stable for up to 28 days in the dark. They had narrow particle distributions (<0.15) and nano-sizes (<200 nm). Phototoxidation by singlet oxygen could induce ascorbic acid degradation. The best formulas were 6A and 7A. Adding 1 and 5% of nanoemulsions could retain ascorbic acid during a photooxidation test for up to 2 hours. The initial concentration of ascorbic acid in the beverage product, packaging, and storage place should be considered for future applications.

Keywords: nanoemulsion; ascorbic acid; singlet oxygen; beverages.

1. Introduction

Photooxidation is one of the oxidation types in food or beverages displayed under the light. Photooxidation happens through the interaction of triplet oxygen, sensitizer, and light to produce singlet oxygen. Sensitizer, as one of the main parts of photooxidation, was naturally found in food or beverage products, such as riboflavin or another natural pigment [1]. Synthetic food coloring, such as erythrosine, also induced photooxidation in beverages [2]. The singlet oxygen could react with electron-rich compounds, like vitamin A, vitamin C, linoleic acid, amino acid, phytosterol, γ-oryzanol, etc [3,4]. Thus, photooxidation could affect the formation of oxidation products and losses of nutritious compounds. Therefore, strategies to prevent photooxidation are essential.

Wang *et al*. (2020) found that reducing the transmission of specific light wavelengths by packaging type could inhibit photooxidation in milk. Applying yellow pigment-high-density polyethylene (Y-HDPE) packaging could block the violet-blue region under each LED color temperature in milk [5]. The second one is absolutely to store the food or beverages in the dark to inhibit excitation of the sensitizer due to the existence of light [1,4]. Meanwhile, using some packaging or placed in the dark could affect the appearance and sales market of the product. Therefore, the third strategy is incorporating a singlet oxygen quencher or antioxidant into the product. Many natural and synthetic antioxidants proved capable of producing singlet oxygen quenchers with various singlet oxygen quenching rates. They were carotenoids, tocopherols, butyl-hydroxy anisole (BHA), and tert-butylhydroquinone (TBHQ) [4,6,7]. Recently, the trend of consumers buying products with natural ingredients has increased.

Vitamin C can be degraded by thermal processing [8,9], storage temperature [10], aerobic oxidation [11], and photooxidation [12]. Some strategies used to protect vitamin C from degradation there were mid-pressure mercury lamps [11], encapsulation in casein gel [13] , and oil-in-water microemulsion [14]. The beverage model system is a simple model to imitate the beverage commercial. Usually, it contains sugar, citric acid, vitamin C, food coloring (FD&C Red No.3), orange flavor, and water. [15]. Food coloring or sensitizers such as FD&C Red No.3 or erythrosine were used as well, as they are commonly found in food and beverage [16].

Rice bran oil has been widely used as a cooking oil and a good source of natural antioxidants. It contains 29.6-38.6 mg of α-tocopherol/100 g of oil [17–19], 0.7 mg of β-carotene/100 g of oil [19], and 29-1386 mg of γ–oryzanol/100 g of oil [4,17,18,20,21]. The presence and positive interaction effect of γ–oryzanol and vitamin E promote the stability of rice bran oil in system thermal oxidation tested [22,23]. Adding γ–oryzanol also increased sunflower oil's oxidative and thermal stability[24]. Tao *et al*. (2022) also found that γ-oryzanol might have a potential application as a natural antioxidant in foods, depending on their concentration and storage temperature [25]. Since γ–oryzanol is an attractive antioxidant, many researchers have tried to apply γ–oryzanol to food or beverage products. Because it is lipophilic, γ–oryzanol needs a carrier system before incorporating into an aqueous product such as a beverage. The types of nanoscale delivery systems are solid lipid nanocarrier, nanostructured lipid, and nanoemulsion [26,27]. Nanoemulsion is an emulsion with a small particle size (20-200 nm), unstable in thermodynamic stability as well as emulsion, metastable, a surface-to-mass ratio of 70-330 m2/g, transparent to translucent appearance. It can be a potential carrier to deliver lipophilic nutraceuticals. Since γ–oryzanol is an attractive antioxidant, many researchers have tried to apply γ–oryzanol to food or beverage products. Because it is lipophilic, γ–oryzanol needs a carrier system before incorporating into an aqueous product such as a beverage. The types of nanoscale delivery systems are solid lipid nanocarrier, nanostructured lipid, and nanoemulsion. [27]. Nanoemulsions can be fabricated using two energy methods: high- or low-energy. Producing nanoemulsions by high-pressure homogenizers, microfluidizers, or ultrasonicators is a high-energy method. The advantages of this method are that nanoemulsions can be more easily controlled and can be used in diverse oils or emulsifier types or materials with different viscosities [27]. Meanwhile, the low-energy methods are emulsion phase inversion, spontaneous emulsification, and phase inversion temperature. This method used simple mixing to produce emulsion on the nanoscale [28,29].

Some researchers have successfully applied oil-in-water nanoemulsion in beverage model systems to protect vitamin C from degradation or enrich beverages with antioxidants such as lycopene or β-carotene [14,15]. Nanoemulsion is also essential for delivering non-polar nutrients into a polar beverage. Zhang *et al*. (2020) have applied docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in apple juice. Even though apple juice-fortified nanoemulsion changes the color characteristics, it was still acceptable [30]. In 2020, Xu *et al*. also applied cinnamon essential oil nanoemulsion and ascorbic acid into cloudy apple juice to prevent browning oxidation [31]. Yoghurt could also be fortified by sweet almond and sesame oil nanoemulsion [32]. This study also used isotonic water commercial containing 1000mg/500 mL of vitamin C as the subject test for further application tests.

By far, the Application of rice bran oil nanoemulsion to inhibit photooxidation in beverages is still rare. This study produced oil-in-water nanoemulsions by emulsion phase inversion. Then, this study applied rice bran oil nanoemulsion in beverage model systems and isotonic water commercials containing vitamin C. Therefore, this research aimed to investigate the addition of rice bran oil-in-water nanoemulsion in either beverage model systems or isotonic water commercials containing vitamin C for inhibiting vitamin C losses and to explore nanoemulsions' stability.

2. Materials and Methods

2.1. Materials.

Rice bran oil, palm oil, and virgin coconut oil from the local markets. Tween 80, potassium iodide, iodine (Merck, Germany), distilled water, ascorbic acid (J.T. Baker), and isotonic orange water commercial. The isotonic water commercial consists of Vitamin C 1000 mg, 100% fresh orange juice (7.2 gr), sodium chloride, calcium lactate, magnesium chloride, potassium phosphate, fructose, sugar, orange flavor, acidity regulator, and water up to 500 mL.

2.2. Methods.

2.2.1. Fabrication of rice bran oil-in-water (o/w) nanoemulsion.

In this study, the four best formulas from the previous research were used [35]. These formulas are given in Table 1. The palm oil : rice bran oil (4:6, w/w) or virgin coconut oil : rice bran oil (3:7, w/w) were separately prepared as an oil phase. The surfactant (Tween 80) to mixed-oil ratio was 2.5:1 and 3:1, and aquadest was 80% (w/w) as an aqueous phase. The fabrication of nanoemulsion by emulsion phase inversion as a low-energy method [35].

Table 1. The rice bran oil nanoemulsion formulas.

Formula	**Mixed-oil (w/w)**	**Surfactant to oil ratio (w/w)**	**Aquadest (w/w)**
6A	Palm oil : rice bran oil (4:6)	2.5:1	80%
6B		3:1	
7A	Virgin coconut oil : rice bran oil (3:7)	2.5:1	
7B		3:1	

2.2.2. O/W nanoemulsion stability tests.

2.2.2.1. Heating stability at 105 °C.

All of the nanoemulsion formulas were separately prepared and poured into some 10 mL screw tubes. Then, the tubes were placed in the oven at 105 °C for 45 minutes. Turbidity analysis [36] was carried out every 5 minutes in triplicate.

2.2.2.2. Storage stability at 37 °C and ambient temperature.

The two formulas, 7A, and 7B were prepared and placed into some 10 mL vials. The vials were closed and placed in an incubator at 37 °C or ambient temperature in the dark container for 4 weeks. Particle size, polydispersity index, and zeta-potential were analyzed every 2 weeks.

2.2.2.3. Application of o/w nanoemulsion into beverage model system and isotonic orange water commercial.

The sample formulas and treatments are given in Table 3. All samples were stored under light (± 3200 lux) or in a dark container for 2 hours at room temperature. Vitamin C analysis [37] and color parameter (a*) by chroma-meter (Konica Minolta) were done every 30 minutes. The experiments were done in duplicate.

Table 3. The sample formulas contain isotonic water samples with or without erythrosine 120 ppm and nanoemulsion.

No.	Isotonic water commercial	Erythrosine 120 ppm	Nanoemulsion (v/v)	Light
1.	√	√	-	√
2.	√	-	-	√
3.	√	√	-	-
4.	√	√	6A (1%)	√
5.	√	√	6A (5%)	√
6.	√	√	7A (1%)	√
7.	√	√	7A (5%)	√

2.2.2.4. Particle size measurements.

Particle diameter, polydispersity index (PDI), and zeta-potential of nanoemulsions were measured by particle size analyzer (Microtrac, USA) at 25 °C. The samples were diluted 10 times with distilled water before analysis. The analysis was done at least in triplicate.

2.2.2.5. Statistical analysis.

Regression analysis by Microsoft Excel 2013 was used to analyze the data.

3. Results and Discussion

3.1. O/W nanoemulsion stability.

In this research, four nanoemulsion formulas based on our previous study [35] were selected. The formulas are given in Table 1. Figure 1 shows the turbidity changes of four nanoemulsion formulas during storage in an oven at a temperature of 105 °C as an accelerated stability test. The nanoemulsion formula containing VCO and rice bran oil (3:7, w/w) as an oil phase (7A and 7B) was more stable than 6A and 6B. The 7A and 7B formulas were relatively stable during the heating test until 30 minutes.

Meanwhile, the 6A and 6B formulas had turbidity stability for up to 25 minutes. The longer the heating time, the more it will increase the turbidity of the nanoemulsion or phase separation of each nanoemulsion formula. It might be due to dehydration in the head group of Tween 80, leading to changes in its capability as a non-ionic surfactant [38].

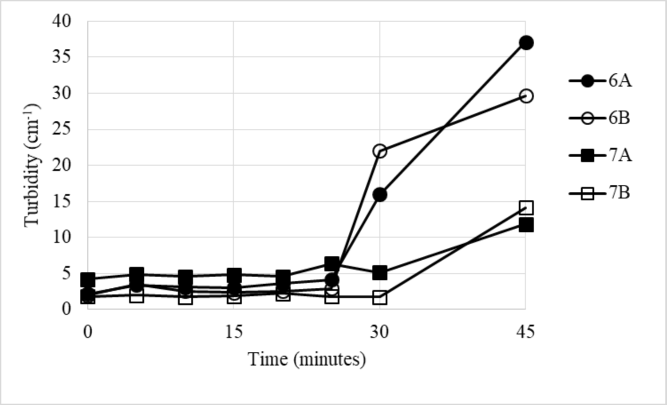
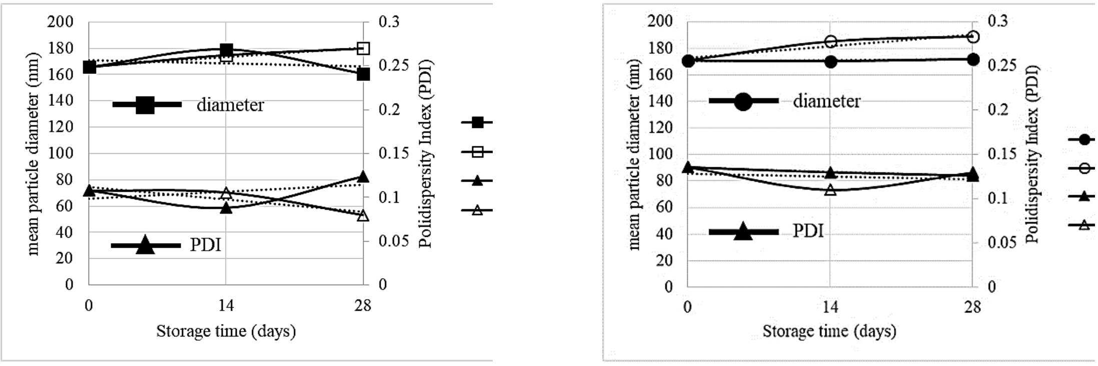


Figure 1. The turbidity changes of nanoemulsions placed in the oven at 105 °C for up to 45 minutes.

In the storage test, the characteristics of the 7A and 7B nanoemulsion formulas were monitored. These nanoemulsion formulas were relatively stable during storage in the dark conditions, either at ambient temperature (28 °C) or 37 °C in the incubator. The mean particle size diameter of nanoemulsions was 160-180 nm. There was no significant difference in mean particle size diameter during the test ($p>0.05$). In addition, these nanoemulsions had <0.15 polydispersity index, so they were monodisperse (Figure 2) or close to uniform size. Figure 3 shows the zeta potential of nanoemulsions during treatments. As long as Tween 80 was used as a non-ionic surfactant, the zeta-potentials were low, <-20 mV. It might be due to the adsorption of the OH- group from H_2O on the droplet surface [39].

In these findings, zeta potential did not contribute to nanoemulsion stability. The main factors for nanoemulsion stability on the high surfactant-to-oil ratios (SOR) were 2.5:1 and 3:1 (w/w). The more high surfactant, the interfacial tension will be decreased. Therefore, the smaller droplet will be formed and stable. [40]. By the small-size particles, the nanoemulsion is stable against flocculation, sedimentation, creaming, and phase separation [41]. Because Tween 80 had low zeta potential, the nanoemulsion stability mechanism of Tween 80 was also steric repulsion; therefore, the coalescence or agglomeration between droplets could be prevented [39,42].



(a) **(b)**

Figure 2. Mean particle diameter and polydispersity index (PDI) of nanoemulsions prepared by (**a**) 7A formula (VCO:RBO 7:3 (w/w), SOR 2.5:1 (w/w), aquadest 80% (w/w)); (**b**) 7B formula (VCO:RBO 7:3 (w/w), SOR 3:1 (w/w), aquadest 80% (w/w)). Both of them were stored in the dark, either at ambient temperature or at 37 °C up to 28 days.

Virgin coconut oil (VCO) and palm oil were also added to the nanoemulsion formula to assist the process of nanoemulsion formation. Mohammed *et al*. (2021) found that VCO can be extracted from coconut by four techniques: fermentation, dry, enzymatic, and chilling and thawing techniques. VCO was reported rich in lauric acid, accounting for 47.95-48.83%. The total of medium chain fatty acid (MCFA) also varies, ranging from 61.77-63.11% [33]. Meanwhile, palm oil was classified as an oil that is rich in polyunsaturated fatty acid (PUFA). Consequently, they have different viscosity. The viscosity of VCO and palm oil amounted to 48-51 cP [33]and 90 cP, respectively. Surh *et al.* (2017) found that the oil viscosity was an important matter. The lower the cP value, the faster the surfactant can move from the oil to the aqueous phase. Therefore, the formation of nanoemulsions with smaller sizes can be achieved quickly [34].

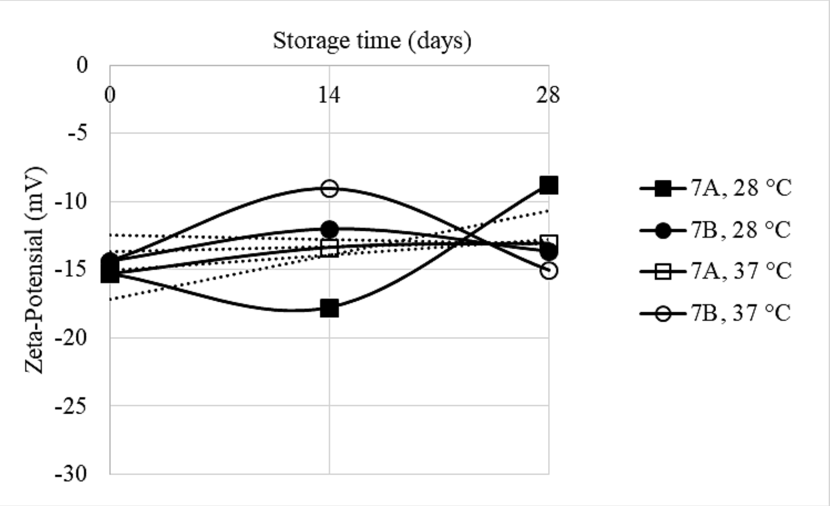


Figure 3. Zeta-potential of 7A and 7B formula during storage in the dark, either at ambient temperature or at 37 °C up to 28 days.

3.2. Application of o/w nanoemulsion into beverage model system and isotonic water commercial.

The four formulas of nanoemulsions employed had viscosity in the range of 5.9-6.3 cP. Nanoemulsion viscosity was smaller than nanostructured lipid carriers (NLC) (18-26 cP) [43]. The different ingredients used in these formulas contributed to their viscosity. NLC used a matrix combination of solid-lipid (such as palm stearin) and liquid oil as an oil phase. Meanwhile, the nanoemulsion formula only contains oil (single or mixed oil) as an oil phase. Both nanoemulsion and water had relatively similar viscosity. The water viscosity was 2 cP. Therefore, if nanoemulsion was applied to a beverage, it will not affect beverage viscosity. Since aquadest was used as an aqueous phase, the nanoemulsion had a slightly neutral pH (5.95-6.24). Rice bran oil nanoemulsion was added as a beverage model system or isotonic water commercial as a lipid-based delivery system.

The sample containing erythrosine 120 ppm and stored under light had lower vitamin C retention (<70%) than the sample without erythrosine and or stored in the dark (>90%) (Figure 4). It showed the combination of erythrosine and light leading to photooxidation. The erythrosine was not only known as a food colorant, but it had the capability to be an effective photosensitizer. Singlet oxygen produced from the photooxidation process reacts with vitamin C or ascorbic acid; consequently, some of vitamin C is degraded. Yang *et al.* (2009) also found that vitamin C containing Red Nr 3 could degrade when placed under light for 1 hour because of vitamin C's reaction with single oxygen. The reaction of singlet oxygen with ascorbic acid could form ascorbic acid hydroperoxides [12,44]. Photodegradation could not only degrade ascorbic acid but also carotenoids.

Adding γ-oryzanol in beverage model systems did not increase the vitamin C retention since solubility matter. Nanoemulsion with or without γ-oryzanol didn't significantly affect vitamin C retention. It might be because the singlet oxygen quenching rate of γ-oryzanol was lower than ascorbic acid. The singlet oxygen quenching rate of γ-oryzanol was 3.04 x 10^6/M/s [4]. Meanwhile, the ascorbic acid had a singlet oxygen quenching rate of 1.10-15.3 x 10^7/M/s [12,45]. For further analysis, this study only used nanoemulsion without γ-oryzanol synthetic standard.

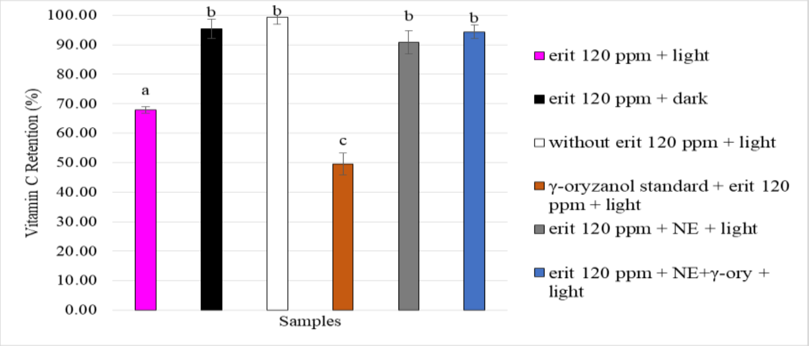


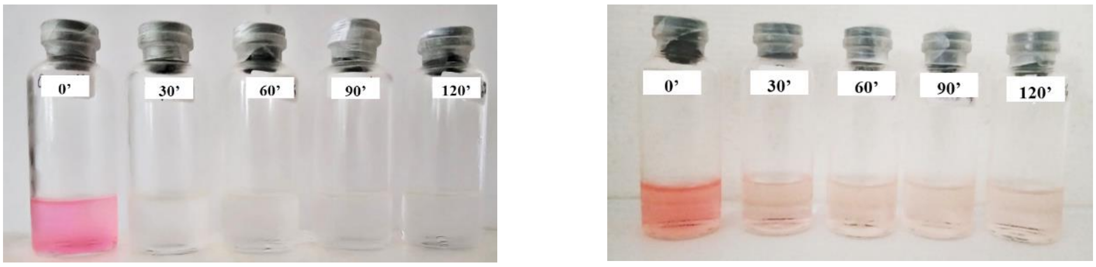
Figure 4. Effect of light, erythrosine, nanoemulsion, and γ-oryzanol on vitamin C retention during photooxidation of beverage model systems containing vitamin C 1800 ppm. Note: erit refers to erythrosine, and NE is nanoemulsion.

The vitamin C degradation rate in the beverage model system containing 1800 ppm of vitamin C was 1.03 ppm/min (Table 4). Adding 1% and 5% (v/v) of 6A nanoemulsion formula could protect vitamin C from degradation. However, adding nanoemulsion, even only 1% (v/v), affected the appearance significantly (Figure 5). Before photooxidation, the sample was pink, but after being stored under light, the pink was totally lost. This plausible mechanism was that the oxidation product of vitamin C could degrade erythrosine quickly. The three main components for photooxidation are sensitizer (erythrosine), light, and triplet oxygen. If there was no erythrosine, the photooxidation didn't occur. Therefore, vitamin C is relatively stable because there is no longer a photooxidation process.

Table 4. The effect of 6A nanoemulsion formula on photooxidation of beverage model system containing vitamin C.

Amount (%, v/v)	**Regression equation**	**R^2**	**P-value[1]**	**Vitamin C degradation rate (ppm/min)**
- (control)	Y = -1.0258x + 1933.8	0.8138	0.0362	1.0258
1	Y = -0.5055x + 1731.2	0.7633	0.0528	0.5055 ≈ 0
5	Y = -0.3753x + 1770.9	0.5827	0.1332	0.3753 ≈ 0

[1] With α < 5%, p-value < 0.05 showed that the slope was significantly different with 0



(a) **(b)**

Figure 5. The visual appearance of beverage model systems samples containing vitamin C 1800 ppm and erythrosine 120 ppm with 1% (v/v) of rice bran oil nanoemulsion (6A formula) **(a)** and without 1% (v/v) of rice bran oil nanoemulsion (6A formula) **(b)** during several periods of time (minutes).

Besides, this study also tested one beverage commercial product, isotonic water containing vitamin C 1000 mg/500 mL or 2000 ppm. Table 5 showed that vitamin C was also relatively stable without erythrosine added or stored in the dark. This trend was similar to the previous section. It means that the interaction between erythrosine, light, and triplet oxygen leads to the effective degradation of vitamin C in commercial isotonic water by photooxidation. About 1 and 5% of 6A and 7A nanoemulsion formulas were applied to it before being stored under light for up to 2 hours. These nanoemulsions could prevent vitamin C from degradation in the beverage model system as well (Table 6). But adding rice bran oil nanoemulsion in this product also affects the isotonic water commercial's color by a* parameter.

Table 5. The effect of light and or sensitizer presence on photooxidation of isotonic water commercial.

Light	Sensitizer 120 ppm	Regression equation	R^2	P-value[1]	Vitamin C degradation rate (ppm/min)
√	√	Y = -0.8394x + 2055.3	0.9442	0.0057	0.8394
√	-	Y = 0.6078x + 2042	0.7685	0.0510	0
-	√	Y = 0.292x + 2042	0.3146	0.3253	0

[1] With $\alpha < 5\%$, p-value < 0.05 showed that the slope was significantly different with 0

Table 6. The effect of rice bran oil nanoemulsion on photooxidation of isotonic water commercial.

Nanoemulsion Formula	Amount (%, v/v)	Regression equation	R^2	P-value[1]	Vitamin C degradation rate (ppm/min)
- (control)	-	Y = -0.8394x + 2055.3	0.9442	0.0057	0.8394
6A	1	Y = -0.3828x + 2108.3	0.0958	0.6123	0
6A	5	Y = -0.8899x + 1983.1	0.6784	0.0865	0
7A	1	Y = -0.7713x + 2055.3	0.747	0.0588	0
7A	5	Y = -1.0685x + 1993.8	0.315	0.3249	0

[1] With $\alpha < 5\%$, p-value < 0.05 showed that the slope was significantly different with 0

In short, there were some probable mechanisms by this rice bran oil-in-water nanoemulsion to prevent vitamin C degradation. First, triplet oxygen, one of the most important substances prone to photooxidation, had very low solubility in water. Therefore, it would be difficult for triplet oxygen to penetrate the nanoemulsion interface. Meanwhile, vitamin C was soluble at the nanoemulsion interface. Thus, it was relatively safe from photooxidation [46]. Second, the initial vitamin C concentration plays a vital role in the photooxidation reaction system on both product systems. When this study used 1800 ppm or higher concentration of vitamin C, the appearances by visual or a* parameter measured by chromameter were very different during the photooxidation process (Figures 5 and 6). This study found that singlet oxygen produced at the initial photooxidation reaction could react with erythrosine quickly (less than 5 minutes). The erythrosine was also a main substance to form singlet oxygen via photooxidation. Because erythrosine was totally lost in early treatment, further photooxidation could have been prevented. Third, α-tocopherol, which naturally occurs in rice bran oil and palm oil, was thought to have an effect on protecting ascorbic acid from degradation by singlet oxygen. Alfa-tocopherol was an effective singlet oxygen quencher at a rate of $3.54\text{x}10^8$/M/s [7].

The addition of 1% and 5% (v/v) palm oil-rice bran oil nanoemulsion, as well as VCO-rice bran oil, prevented ascorbic acid damage in beverage model systems and commercial isotonic drinks containing 120 ppm erythrosine and exposed to + 3200 lux light for up to two hours at room temperature. This is likely related to the inhibition of triplet oxygen movement in the interfacial layer, which inhibits photooxidation [46]. In addition, creating a thin film layer via microemulsion (o/w) inhibits the contact interaction between erythrosine, ascorbic acid, erythrosine,nd oxygen in the beverage model system [47].

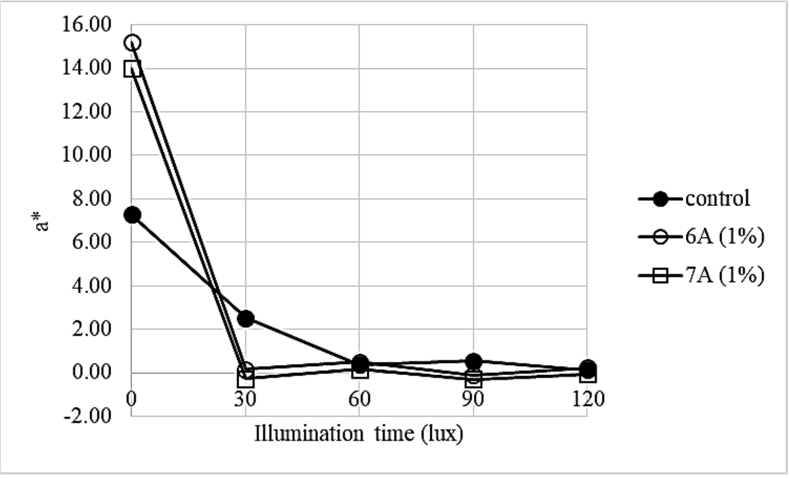


Figure 6. The a* parameter changes of isotonic water commercial with or without rice bran oil nanoemulsion (1%, v/v) during storage under light at 3300 lux for up to 2 hours.

4. Conclusions

Rice bran oil nanoemulsions could be produced by low-energy methods via emulsion phase inversion. The four nanoemulsion formulas had excellent stability when heated in the oven at 105 °C for up to 30 minutes. The 7A and 7B formulas are also stable during storage for up to 28 days at ambient temperature or 37 °C. Although these formulas had low zeta potential, they had a high surfactant-to-oil ratio (2.5:1 and 3:1), maintaining nanoemulsion stability. The best formulas were 6A (palm oil:rice bran oil was 4:6 w/w and surfactant to oil ratio was 2.5:1 w/w) and 7A (virgin coconut oil:rice bran oil 3:7 w/w and surfactant to oil ratio was 2.5:1 w/w). Adding 1 and 5% (v/v) of rice bran oil nanoemulsion into beverage model systems or isotonic water commercial could prevent vitamin C from degradation. Based on these findings, adding delivery systems like nanoemulsion should consider the initial vitamin C concentration, ingredient (the presence of sensitizer), packaging, and product storage place.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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