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Inhibition of riboflavin photosensitized off flavor in milk products with O/W microemulsion containing astaxanthin and α-Tocopherol

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Abstract: The objective of this research was to evaluate the ability of O/W microemulsion containing astaxanthin and α-Tocopherol to inhibit the formation of dimethyl disulfide in an aqueous model system containing methionine, skim milk, and full cream milk due to riboflavin photosensitization. In addition, the formation of hexanal and the presence of sunlight flavor were also determined in skim milk and full cream milk. Astaxanthin 200 ppm and α-Tocopherol 1000 ppm were incorporated into the O/W microemulsion. Two percent of O/W microemulsion containing astaxanthin and α-Tocopherol was added to the samples and exposed to fluorescence light at 2000 Lux in the showcase at 10^0C for up to 8 h. The O/W microemulsion containing astaxanthin and α-Tocopherol effectively inhibited the formation of dimethyl disulfide in an aqueous model system containing methionine, skim milk and full cream milk. Hexanal formation was inhibited in skim milk and full cream milk. The presence of sunlight flavor in skim milk and full cream milk could be detected by panelists after 2 and 4 h of fluorescent light exposure, respectively. However, the sunlight flavor was not detected when O/W microemulsion containing astaxanthin and α-Tocopherol was added to the skim milk and full cream milk.

Keywords: Astaxanthin, α-Tocopherol, microemulsion, riboflavin photosensitization, off flavor milk

Introduction

Milk and milk products are susceptible to riboflavin photosensitization. Naturally, milk contains riboflavin. In the presence of light, riboflavin acts as a photo sensitizer. Riboflavin plays important roles in the formation of light-induced off-flavor in milk through free radical generation (type I pathway) and/or singlet oxygen oxidation (type II pathway). More than 99% of the reaction between triplet sensitizer and triplet oxygen produce singlet oxygen (Min and Boff, 2002).

The presence of singlet oxygen in the milk stored under light was confirmed by electron spin resonance spectroscopy (Bradley and Min, 2003). Singlet oxygen has different properties from triplet oxygen. Singlet oxygen is a non radical and an electrophilic compound. It may directly react with electron-rich double bonds without the formation of free-radical intermediates (Min and Boff, 2002). The reaction temperature has little effect on the oxidation rate of singlet oxygen with foods due to the low activation energy of singlet oxygen i.e. 0 to 6 kcal/mole (Yang and Min, 1994). Moreover there is no induction period taking place in the singlet oxygen oxidation (Gordon, 2001).

Riboflavin photosensitization induces two distinctive off-flavors in milk which make it less acceptable to consumers. The first off flavor is "sunlight" flavor giving a burnt and oxidized odor in milk. Dimethyl disulfide and methional derived from oxidation of sulfur containing amino acids like methionine, are reported to be responsible for the "sunlight" flavor. The other off-flavor is "cardboard-like or metallic" flavor, which develops in milk with prolonged duration of light exposure. This "cardboard-like or metallic" flavor comes from secondary lipid oxidation products including hexanal, pentanal, ketones, alcohols, and hydrocarbons (Gaafar and Gaber, 1992; Jung and others, 1998; Skibsted, 2000; Pereda and others, 2008).

According to the Plastic Bottle Institute at Washington D.C., approximately one-half of the fluid milk products in plastic containers remained in the dairy case and were exposed to the lights for at least 8 h (Anonymous, 1997). White and Bulthaus (1982) reported that 53 out of 90 samples of milk in plastic jugs purchased at grocery stores were rated as having a moderate to strong light-induced off-flavor. Chapman and others (2005) reported that trained panelists could detect light-oxidized flavor defects in reduced fat (2%) fluid milk that had been exposed to 2000 Lux for 15 to 30 minutes. An untrained consumer panel detected light oxidized flavor defects in milk after it was exposed to light between 54 minutes and 2 h.

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Natural compounds such as astaxanthin and α-Tocopherol are known as effective singlet oxygen quenchers (Min and Boff, 2002; Kim and others, 2006). However, its poor water dispersibility limits its application in aqueous food systems. Therefore, incorporation of astaxanthin and α-Tocopherol into O/W microemulsion is expected to facilitate better dispersion in aqueous food systems. Our previous research had successfully made stable O/W microemulsion containing astaxanthin and α-Tocopherol. Due to its highly unsaturated structure, astaxanthin is sensitive to heat, oxidation, and light. Addition of α-Tocopherol 1000 ppm significantly improved the stability of astaxanthin in O/W microemulsion, by extended the time to reach loss of 50% astaxanthin in O/W microemulsion from 8.7 weeks to 58.8 weeks stored at room temperature and protected from light (Yuwanti *et al*., 2010).

Microemulsion is thermodynamically stable, transparent, and isotropic dispersion having droplet-particles size ranging from 5 to 100 nm. Microemulsion consists of water, oil, and surfactants, typically in conjunction with a co-surfactant. Microemulsion as a delivery system in food application offers several advantages. It has a clear and transparent appearance, has low viscosity; increases the solubility of hydrophobic substances, and is easily prepared and handled (Engstrom and Larsson, 1999; Flanagan and Singh, 2006; Cho and others, 2008).

The objective of this research was to evaluate the ability of O/W microemulsion containing astaxanthin and α-Tocopherol to inhibit the formation of dimethyl disulfide in an aqueous model system containing methionine, skim milk, and full cream milk due to riboflavin photosensitization. In addition, the formation of hexanal and the presence of sunlight flavor were also determined in skim milk and full cream milk.

Materials and Methods

Materials

Chemicals used in this research were astaxanthin (20%) from Oryza Oil & Fat Chemical Co., Ltd. Japan, α-Tocopherol, Span 80, riboflavin, methionine, dimethyl-disulfide, and hexanal (Sigma, Sigma-Aldrich Co, USA), Span 40 (Aldrich, Sigma-Aldrich Co, USA), and Tween 80 (Merck Chemicals, Germany). Virgin coconut oil (VCO) with peroxide value of 0.194 meq/kg oil and moisture content of 0.18% was obtained from a local VCO producer at Yogyakarta. The skim milk powder and full cream milk powder were obtained from local supermarkets.

Preparation of O/W microemulsion containing astaxanthin and α-Tocopherol

The O/W microemulsion was prepared as described by Yuwanti *et al*. (2010). Combination of nonionic surfactants, virgin coconut oil, and water were used to make O/W microemulsion with the proportion of 20:4:76. A combination of nonionic surfactants was Tween 80, Span 80 and Span 40 with the proportion of 90:3.33:6.67. Astaxanthin 200 ppm and α-Tocopherol 1000 ppm were mixed with VCO and surfactants, heated on a hot plate and stirred with magnetic bar at 70^0C for up to 10 min. Water was added by titration while the mixtures were heated and stirred for up to 20 min.

Riboflavin photosensitization of an aqueous model system containing methionine, skim milk and full cream milk.

An aqueous model system was prepared to contain 928 ppm methionine with or without 1.7 ppm riboflavin. Liquid skim milk and full cream milk were reconstituted with water from skim milk and full cream milk powder according to the suggested serving method as described on the product label. Two percent of O/W microemulsion containing astaxanthin and α-Tocopherol were added to these samples. Ten milliliters of sample was poured into 20 mL transparent serum bottle, closed tightly with rubber seal of Venoject Terrumo. A portion of samples was kept at 10^0C in the dark (wrapped in aluminum foil) and another portion was exposed under fluorescent light at 2000 Lux in the showcase at 10^0C for up to 8 h.

Trapping of dimethyl disulfide and hexanal

Trapping of dimethyl disulfide and hexanal from the headspace of bottle serum was performed using 75 μm CAR/PDMS of SPME fiber as described by Lee and Min (2009). Sample bottles were kept in a 30^0C water bath for 10 min to achieve the equilibrium of volatile compounds between headspace and liquid in the sample. The headspace volatile compounds in air-tight sealed sample bottles were isolated with 75 μm CAR/PDMS of SPME fiber. Sample bottles were put in a 30^0C water bath for 30 min while CAR/PDMS of SPME fiber was exposed to the headspace. The isolated volatile compounds by SPME fiber were injected in GCMS to determine the dimethyl disulfide and hexanal content. Standard compounds of dimethyl disulfide and hexanal were diluted in full cream and trapped as described above.

Analysis of dimethyl disulfide and hexanal in SPME with SIM-GCMS.

A GC-2010 coupled with GCMS-QP20108S Shimadzu was used for analysis of dimethyl disulfide and hexanal. The column was a 30 m Rxi-5ms (Restex) of a 0.25 mm ID and 0.50 μm film thickness. Helium was used as carrier gas with a flow rate at 3 mL min^{-1}. A splitless injection method was used. The oven temperature was held at 40^0C for 10 min and increased from 40 to 280^0C at 10^0C min^{-1} and held for 25 min. The temperatures of injector and detector were 250 and 300^0C, respectively. The isolated volatile compounds in the SPME were desorbed at 250^0C of GC injector for 2 min. The selected ions for dimethyl disulfide and hexanal in SIM (Selected Ion Monitoring) mode were m/z 94 and 72, respectively.

Sensory evaluation of sunlight flavor in riboflavin photosensitized skim milk and full cream milk

Eighteen selected panelists were employed to evaluate the presence of sunlight flavor in riboflavin photosensitized skim milk and full cream milk. The presence of sunlight flavor in light (2000 Lux) exposed skim milk and full cream milk for up to 8 h in the showcase at 10^0C were evaluated using triangle test every 2 h. Skim milk and full cream with the addition of 2% O/W microemulsion containing astaxanthin and α-Tocopherol were exposed to fluorescent light for up to 8 h. Control samples were prepared using the same samples and wrapped in aluminum foil. One set of samples consisted of three coded samples in which 2 of them were identical, and one of them was different or odd. Those two identical samples could be the treated samples or could be the control samples. The panelists were instructed to open the bottle, bring it near the nose and sniff for 1-2 seconds. Sniffing between two samples was done at least after 5 seconds interval. Finally the panelists were instructed to identify the odd sample. However, when the panelists were unable to detect the odd sample, they could identify the samples that were not different. Before referring to the statistical table, one-third sum of no difference answers was relocated by adding them to the correct answer (Carpenter *et al*., 2000).

Results and Discussion

Riboflavin photosensitization of methionine has been reported to result in the formation of dimethyl disulfide (Gaafar and Gaber, 1992; Jung *et al*., 1998; Skibsted, 2000). In this study a SIM (Selected Ion Monitoring) chromatogram of dimethyl disulfide in an aqueous model system containing methionine was presented in Figure 1. Riboflavin photosensitization of skim milk and full cream milk was expected to result in the formation of dimethyl disulfide and hexanal. The SIM chromatogram of dimethyl disulfide and hexanal in skim milk and full cream were presented in Figure 2.

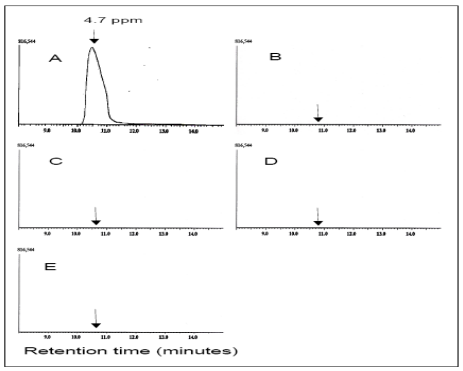

Figure 1. SIM Chromatogram of dimethyl disulfide in an aqueous model system containing methionine. A= + riboflavin, exposed to light 8 h, B = + riboflavin, 0 h, C = + riboflavin, dark, D = without riboflavin, exposed to light 8 h, E = + riboflavin, exposed to light 8 h, + 2% O/W microemulsion containing astaxanthin and α-Tocopherol

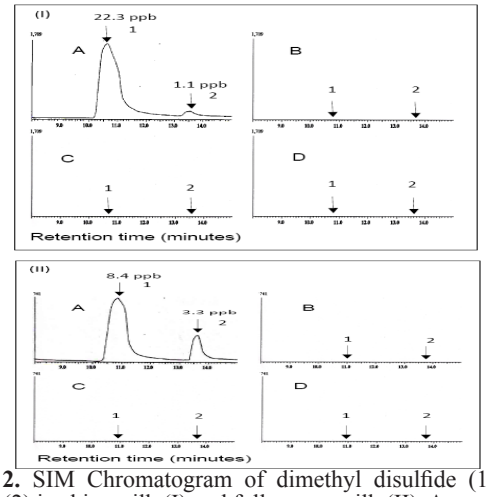

Figure 2. SIM Chromatogram of dimethyl disulfide (1) and hexanal (2) in skim milk (I) and full cream milk (II). A= exposed to light 8 h, B = 0 h, C = dark, D = exposed to light 8 h, + 2% O/W microemulsion containing astaxanthin and α-Tocopherol

Dimethyl disulfide was formed during riboflavin photosensitization of aqueous model system containing methionine, skim milk and full cream milk with the concentration of 4.7 ppm, 22.3 ppb and 8.4 ppb, respectively. In these systems there were methionine as a substrate, riboflavin as a photo-sensitizer and light as an energy source. Riboflavin could absorb energy from light and transferred it to triplet oxygen to form singlet oxygen (Min and Boff, 2002). Subsequently dimethyl disulfide was formed as a result of methionine oxidation by singlet oxygen which is a type II pathway. However, there is also a possibility dimethyl disulfide formation due to methionine reaction with free radical generated by excited riboflavin (type I).

Dimethyl disulfide was not formed in an aqueous

model system containing methionine, skim milk and full cream milk which was wrapped in aluminum foil (dark) or in samples exposed to light with the addition of 2% O/W microemulsion containing astaxanthin and α-Tocopherol. In dark condition there was no light energy for riboflavin photosensitization. Astaxanthin and α-Tocopherol are known as singlet oxygen quenchers, therefore the addition of O/W microemulsion containing astaxanthin and α-Tocopherol was expected to inhibit the formation of dimethyl disulfide.

Hexanal was found in skim milk and full cream milk which were exposed to light with the concentration of 1.1 ppb and 3.3 ppb, respectively. It was not formed in skim milk and full cream milk which were wrapped in aluminum foil (dark). Hexanal was also absent in these milk products with the addition of 2% O/W microemulsion containing astaxanthin and α-Tocopherol even when they were exposed to light.

Publication on inhibition off flavor in milk products due to riboflavin photosensitization using singlet oxygen quencher is very limited. Jung *et al*. (1998) reported that ascorbic acid could decrease the formation of dimethyl disulfide and off flavor in skim milk. Ascorbic acid is a water soluble compound; therefore it could be directly added into skim milk. However, the addition of ascorbic acid alone could not protect milk from off flavor, because ascorbic acid would only give a significant effect if added along with α-Tocopherol as reported by Van Aardt *et al*. (2005a). Direct addition of α-Tocopherol to light-exposed milk could decrease off flavor (Van Aardt *et al.*, 2005b) but only off flavor from oxidation of oil fraction and in relatively longer period of storage i.e. 4-6 weeks.

Incorporation of astaxanthin and α-Tocopherol into O/W microemulsion could provide better dispersion of these compounds in milk as an aqueous system. Astaxanthin and α-Tocopherol in O/W microemulsion could disperse in the continuous phase. Therefore, it could inhibit not only riboflavin photosensitization of unsaturated fat in the dispersed phase, but also riboflavin photosensitization of methionine in continuous phase. It should be noted that riboflavin photosensitization of methionine occurred in the early period of storage under light exposure. Incorporation of astaxanthin and α-Tocopherol into O/W microemulsion was considered as a new way to inhibit riboflavin photosensitized off flavor in milk.

Sensory evaluation to detect the presence of sunlight flavor in milk samples, which resulted from riboflavin photosensitization, was carried out using triangle test. In this test, eighteen selected panelists were instructed to identify the one odd sample from three coded samples presented. However, when these panelists could not detect the distinctive sample, they could choose no difference option. All of the correct answers were added up, and one-third of the no difference answers were incorporated to the correct answers. The total correct answer was compared with values in the Table which contain the number of panelist in a triangle test required to give correct answer and to determine the significant difference (Carpenter *et al*., 2000). Results from the sensory evaluation of the presence of sunlight flavor in the skim milk and full cream milk as determined by triangle test were presented in Table 1 and Table 2.

Table 1. Results of Triangle test on the presence of detectable sunlight flavor in skim milk by 18 panelists

Duration of light exposure (hours) and treatment	Correct answer	No-difference answer	Significance
2 - without O/W microemulsion	9	4	95%
4 - without O/W microemulsion	15	-	99.9%
6 - without O/W microemulsion	16	-	99.9%
8 - without O/W microemulsion	18	-	99.9%
8 + O/W microemulsion containing astaxanthin and α-Tocopherol	3	4	< 95% (Not significant)

Table 2. Results of Triangle test on the presence of detectable sunlight flavor in full cream milk by 18 panelists

Duration of light exposure (hours) and treatment	Correct answer	No-difference answer	Significance
2 - without O/W microemulsion	5	4	< 95% (Not significant)
4 - without O/W microemulsion	10	-	95%
6 - without O/W microemulsion	16	-	99.9%
8 - without O/W microemulsion	17	-	99.9%
8 + O/W microemulsion containing astaxanthin and α-Tocopherol	4	4	< 95% (Not significant)

The panelists could detect the presence of sunlight off flavor when the skim milk was exposed to light for 2 h, or when the full cream milk was exposed to light for 4 h. However, the panelists could not detect the presence of sunlight flavor in the samples even after 8 h of light exposure when 2% of O/W microemulsion containing astaxanthin and α-Tocopherol was added to the skim milk and full cream milk. The duration of light exposure to result in the presence of detectable sunlight flavor in skim milk was significantly shorter than that of the full cream milk. This phenomenon might be due to the presence of higher concentration of dimethyl disulfide in the skim milk than that of the full cream milk, or due to the rich flavor of the full cream milk that slightly masks the perception of sunlight flavor by the panelits. However, this proposed explanation remain to be further examined.

Conclusion

The O/W microemulsion containing astaxanthin

and and α-Tocopherol was found to be able to inhibit the formation of dimethyl disulfide in an aqueous model system containing methionine, skim milk and full cream milk due to riboflavin photosensitization. It also effectively inhibited hexanal formation in skim milk and full cream milk. Skim milk exposed to fluorescent light for 2 h resulted in detectable sunlight flavor by the panelists. However, it took at least 4 h of light exposure of full cream milk to develop detectable sunlight flavor. The panelists could not detect the presence of sunlight off flavor in light exposed skim milk and full cream milk with the addition of O/W microemulsion containing astaxanthin and α-Tocopherol. Finally, it can be inferred that incorporation of astaxanthin and α-Tocopherol into O/W microemulsion was a new and an effective way to inhibit riboflavin photosensitization off flavor in milk.

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