STABILITY OF VIRGIN COCONUT OIL EMULSION WITH MIXED EMULSIFIERS TWEEN 80 AND SPAN 80

Lastri Wiyani[1], Andi Aladin[1], Setyawati Yani[1] and Rahmawati[2]
[1]Department of Chemical Engineering, Muslim University of Indonesia, Jl Urip Sumoharjo, Makassar Indonesia
[2]Faculty of Pharmacy, Muslim University of Indonesia, Jl Urip Sumoharjo, Makassar Indonesia
E-Mail: daffaya01@yahoo.com

ABSTRACT

Virgin coconut oil emulsion (VCOE) is an alternative VCO product which has a reduced amount of oily taste when the VCO is consumed directly. This study was aimed to obtain the formulation of VCOE and determine the physical and chemical properties and also stability of emulsion with the addition of mixed emulsifiers Tween 80 and Span 80 (T80S80). Emulsions were formulated using mixed emulsifiers with the mass ratio of T80 to S80 of 100:0; 80:20; 60:40; 40:60; 20:80 and 0:100, respectively. The best ratio of the emulsifier was used to determine the stability of VCO and water mixtures with the diversity ratio of VCO to water were 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90, respectively; the concentrations of emulsifier used were 1, 0.75 and 0.5%. VCOE was successfully formulated in the mixture of VCO-water ratio of 80:20. The VCOE products have high viscosities in each concentration of emulsifier and remained stable in room temperature. The contents of the peroxide number were 1.51 to 1.53 meq/kg sample and free fatty acid were 0.09 to 0.1%, which indicated that the emulsions were not rancid.

Keywords: VCO emulsion, emulsifier, stability.

INTRODUCTION

Virgin coconut oil (VCO) is produced from fresh and mature coconuts by mechanical or natural techniques without heating, so the nature of the oil is unchanged [1]. VCO has a higher phenolic content and antioxidant activity compared to copra-derived coconut oil. Based on the benefits of this value-added functional oil, the development of a VCO-based emulsion product as a novel nutritional food supplement will indirectly increase the consumption of VCO because most consumers dislike the oily taste of pure VCO [2], [3]. One alternative for reducing the oily taste is by formulating VCO in the emulsion. The transformation of VCO into a more palatable and stable VCO-based emulsion product will also be an advantage for the VCO-producing industry [3].

Thermodynamically, an emulsion is an unstable system. However, the emulsion is the basis of many food products. The emulsion's stability is very important for ensuring its quality. This property is a challenge for many industries. The stability factor is sometimes affected by the physicochemical characteristics of the gums added during the aqueous phase [3], [4].

The homogeneity of an emulsion is affected by mixing speed and time. For example, Rita [5] using the mixing speed of 6000 rpm, 8000 rpm and 10000 rpm for 1, 3, 4 minutes to produce homogeneous emulsions. Other researchers reported using 10,000 rpm for 15 minutes [6], 10000 rpm for 5 minutes [7], 13,500 rpm for 30 seconds [8] and 11,500 rpm for 5 minutes [9] to achieve homogeneity.

Emulsion stability refers to the ability of an emulsion to resist changes in its properties over time: the more stable the emulsion, the more slowly its properties change [10]. The perceived quality of emulsion based food products are strongly influenced by their stability, rheology and appearance [4]. A main indicator of loss of stability is an increase in droplet mean diameter of the emulsion, and the growth rate of the droplets can reveal the mechanism responsible [11]. The emulsion stability is highly influenced by the specific gravity, droplet size and distribution, and the rheological characteristics. The addition of hydrocolloids to the aqueous phase can yield specific rheological properties to achieve emulsion stability. Some hydrocolloids act as a surface active gums, having the ability to form a film around the oil droplets. As a result of static stabilisation, hydrocolloids aid in delaying this coalescence and prevent emulsion breakdown. Moreover, some hydrocolloids are known to stabilize the emulsions by enhancing the viscosity of the aqueous phase [3].

Forming a stable emulsion for a period of time to increase shelf-life is one of the main challenges of food product formulation. This can be achieved through the addition of emulsifiers and stabilizer. Emulsifiers are surface-active molecules which could lower the surface tension and prevent the droplet flocculation by absorption on the droplet surfaces [10]. Research on emulsions with various emulsifiers have been reported. For example, Permadi [6] investigated the use of Tween 80 and lecithin as emulsifier in the stability of fish oil emulsion, gum arabic [12] lecithin, xanthan gum [13], glycerin fatty ester acid [14], gum Odina [15], roselle extract [16], Tween 80 and sucrose fatty acid ester (P-type S 1570 and 1570) [5].

According to Ansel [17], the surfactant, also called emulsifier plays an important role in emulsion formation by reducing the interfacial tension. They also reported that the surfactants mixture increased the solubility of the bioactive compound in the emulsion. Moreover, the chemical structure of surfactant must be considered because the chain length compatibility of a surfactant and oil is an important factor in the formation of emulsion. The combination of high and low Hydrophilic

ARPN Journal of Engineering and Applied Sciences

Lipophilic Balance (HLB) values of surfactants provides the necessary conditions for the formation of a stable water-oil emulsion [17], [18].

The objective of the present study was to determine the effect of mixed Tween 80 and Span 80 (T80S80) on the physical, chemical properties and stability of VCO emulsion. The effects of VCO-water ratio and the concentration of emulsifier to the stability of VCO emulsion were also studied. It is expected that by understanding those effects more stable VCO emulsions could be produced and the knowledge could be applied in VCO home industries.

METHODOLOGY

Materials and equipment

Virgin coconut oil (VCO) was obtained from a local VCO producer (CV. Avcol, Makassar, Indonesia). Low HLB surfactant, sorbitan monooleate (Span 80, HLB = 4.3, Sigma) and high HLB surfactant, polyoxyethylene sorbitan monooleate (Tween 80, HLB = 15.0, Merck) were purchased from local chemical supplier.

The emulsions were prepared by Ultra Turrax homogenizer (specification: a minimum speed 3600 rpm and maximum 24000 rpm, particle size 1-10 micron). The viscosity were measured using a Brookfield viscometer model DV-I Prime.

Preparation of mixed emulsifiers

Mixtures of emulsifier T80S80 were prepared at the following ratios of T80:S80, namely 100: 0, 80:20, 60:40, 40:60, 20:80, and 0: 100, respectively. The mixture were stirred by using a magnetic stirrer to obtain a homogeneous mixture

Preparation of VCO emulsion

The VCOE was prepared by using VCO-water (ratio 70:30) and 1% mixed emulsifier T80S80 (ratio 100: 0, 80:20, 60:40, 40:60, 20:80, 0: 100) using Ultra Turrax homogenizer operating at 15,000 rpm for 4 minutes. The best combination of the mixtures would be used for the next experimentation.

This procedure was repeated with the ratio of VCO to water of 90;10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, respectively, using the the best of mixed emulsifier from previous step. The concentrations of emulsifier were 1%, 0.75% and 0.5%.

Peroxide number test

Five grams of samples were put in a 300 mL Erlenmeyer flask. 10 ml of chloroform and 15 ml of glacial acetic acid was added into the Erlenmeyer and then shaken to mix it. One ml of saturated KI was added and the Erlenmeyer was immediately closed while shaken roughly for 5 minutes in the dark at a temperature of 15-25 ^{0}C. Then, 75 ml of distilled water were added and shaken vigorously. The mixture were titrated with standard solution of 0.2 N sodium thiosulphate and starch solution as an indicator. Peroxide number was expressed in meq / kg sample [19].

Free fatty acid test

30 grams of sample was weighed and put into an Erlenmeyer flask, then 50 ml of hot neutral alcohol and 2 ml of phenolphthalein (pp) were added. Then, it titrated with 0.1 N NaOH which had been standardized until pink color and not disappeared for 30 seconds. Free fatty acid content was expressed as % FFA [20].

Viscosity test

The prepared emulsion was measured using Brookfield viscometer DV-I Prime. Rotation of spindle on the viscometer could be set according to the viscosity of the sample. Viscosity values would be read directly on the device (cP).

Emulsion stability test

Approximately 60 ml of sample was put into a bottle and stored at 5 ^{0}C for 12 hours, then stored at a temperature of 35 ^{0}C for 12 hours. This cycle was repeated for 10 cycles (5 cycles of temperature of 5 ^{0}C and 5 cycles of 35 ^{0}C). The stability of VCOE was determine as follows:
% stability = (height of stable emulsion/height of initial emulsion) x 100 [21].

RESULTS AND DISCUSSION

Characteristics of VCOE with mixed emulsifiers T80S80

Table-1 shows the characteristics of mixed emulsifiers T80S80. The peroxide number ranged from 1.56 to 1.58 meq / kg sample. It shows that the peroxide number in the obtained VCOE met the Asia Pacific Coconut Community (APCC) standard for VCO which stated that the peroxide number of VCO has to be under 3 meq / kg sample.

Table-1. Characteristics of VCOE with mixed emulsifiers.

Tween 80 and Span 80	Peroxide number	Free Fatty Acid	Emulsion Stability	Viscosity
	(meq/kg)	(%)		(cP)
100:0	1.5737	0.0610	Stable	140
80:20	1.5819	0.0526	Stable	220
60:40	1.5705	0.0451	Stable	120
40:60	1.5662	0.0601	Stable	330
20:80	1.5772	0.0528	Stable	190
0:100	1.5778	0.0459	Stable	170

Table-1 also shows the contents of free fatty acids in the emulsions, they ranged between 0.045-0.061 %. The contents of free fatty acid in the emulsions were lower than the APCC of the VCO which has a maximum free fatty acid content of 0.5 percent [22], [23]. The peroxide

VOL. 11, NO. 8, APRIL 2016 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

number and free fatty acid content in the emulsions suggested that the emulsions were not rancid.

It also can be seen from Table-1 that by using T80S80 emulsifiers in six different ratios resulted stable emulsions. However, the viscosity of the emulsions varied. The highest viscosity was achieved at the ratio of T80S80 of 40:60. By calculating the HLB value of the highest viscosity mixed emulsifier, it gave HLB value of 8.58. The combination of high and low (HLB) values of surfactants provides the suitable conditions for the formation of a stable water-oil emulsion [17-18].

Peroxide number and free fatty acid on various VCO: water ratios

Using the best ratio of T80S80 emulsifier (40:60) as a mixed emulsifier, a set of experimentations with various VCO-water ratios at different emulsifier concentrations were performed to study the rancidity (peroxide number and free fatty acid content) of the emulsions. The results were listed at Table-2 and Table-3.

Table-2. Peroxide number of various VCO: water ratios at different emulsifier concentrations (meq / kg sample).

VCO : water	Peroxide number (meq / kg sample)		
	Emulsifiers Concentrations		
	1%	0.75%	0.5%
90 : 10	1.52	1.55	1.55
80 : 20	1.51	1.52	1.53
70 : 30	1.51	1.52	1.52
60 : 40	1.50	1.51	1.52
50 : 50	1.49	1.56	1.50
40 : 60	1.47	1.49	1.48
30 : 70	1.47	1.49	1.48
20 : 80	1.44	1.45	1.44
10 : 90	1.42	1.44	1.43

Based on Table-2, peroxide numbers resulting from various ratio of VCO-water were from 1.42 to 1.56 meq / kg sample. Analysis of peroxide number aimed to determine total peroxide as a temporary product of the oxidation of fats which can cause rancidity in oil. Based on the Codex-Stan 210-1999 the maximum level of peroxide number in virgin fat and oils is 15 meq/kg sample. The maximum peroxide in VCO based on the APCC standard is 3 meq / kg sample. Peroxide number in VCO is low because VCO contains approximately 90% of unsaturated fatty acids which are more resistant to rancidity due to oxidation process in comparison with unsaturated fatty acids [22].

It can be seen from Table-3 that the contents of free fatty acids in the emulsions ranged from 0.065% to 0.011% which is quite low compared to the maximum free fatty acid content in VCO of 0.5% based on APCC standard. In addition, as a comparative content of free fatty acids, coconut oil has a maximum of 5% free fatty acids [23]. According to Ketaren [24], the content of free fatty acids of 0.2 wt. % in food products will result in undesirable flavor.

Table-3. Free fatty acid (% FFA) of various VCO: water ratios at different emulsifier concentrations.

VCO: water	Free Fatty Acids (%)		
	Emulsifier concentrations		
	1%	0,75%	0,5%
90 : 10	0.101	0.110	0.107
80 : 20	0.092	0.102	0.103
70 : 30	0.093	0.097	0.101
60 : 40	0.087	0.081	0.092
50 : 50	0.079	0.082	0.090
40 : 60	0.071	0.077	0.079
30 : 70	0.069	0.070	0.075
20 : 80	0.065	0.067	0.070
10 : 90	0.067	0.066	0.066

The stability and viscosity of VCO emulsions

Table-4 shows the stability of emulsions in different mixtures of VCO and water. The stable emulsions were achieved in the ratios of VCO: water of 90:10 and 80:20, respectively. The most stable emulsion which was indicated by inseparable emulsion was the emulsion with VCO: water ratio of 80:20.

With an increase in the water content in the emulsion, the stability decreased. It reached the lowest stability of 13 % at VCO: water ratio of 10:90 and emulsifier concentration of 0.5% (Table-4). In the high water content at each emulsifier concentration, a separation (coalescence) occurred at the bottom of the emulsion. The separation process also took place on the top of emulsion (creaming) at the VCO: water ratios of 70:30 to 30:70. It caused by inability of emulsifiers to bind all of the available water. In this case, the hydrophilic and lipophilic groups will determine the capacity of emulsifier in producing emulsions.

Table-4. Emulsion stability (%) of various VCO: water ratios at different emulsifier concentrations.

VCO: water	Stability (%)		
	Emulsifier concentrations		
	1%	0.75%	0.5%
90 : 10	90	90	87
80 : 20	100	100	100
70 : 30	74	81	71
60 : 40	65	65	61
50 : 50	55	58	58
40 : 60	42	52	48
30 : 70	32	42	39
20 : 80	20	26	35
10 : 90	20	22	13

VOL. 11, NO. 8, APRIL 2016 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

From a physical appearance, VCOE with ratio of 80:20 did not form creaming. Creaming could not directly break the emulsion but it could trigger a coalescence which then creates unstable emulsion [25]. In the VCO-water ratio of 70:30, creaming has occurred and it was found that approximately 75% of the emulsion could not be separated.

It was reported that VCOE which contained 75% of oil was successfully formulated [18]. It required surfactant: water ratio of 4.5:1 or higher. When the microemulsions were formulated to contain 77.78% oils, the surfactant: water ratio was at least 5.5 part or higher. These microemulsions remained stable during storage, even after centrifugation, but they were not stable when subjected to heating at 70 ºC or higher. A stable water-oil microemulsions could also be obtained by utilizing combinations of nonionic surfactants with the following proportion: 16.6% of Tween 20, 15.0% of Span 20, and 68.4% of Span 80. Transparent water-oil microemulsion can only be formed when the ratio of water and surfactants was at least 1:4.5 and the ratio of water/surfactants and VCO was not more than 1:3.5 [18].

To further study the stability of the emulsions, a viscosity test was performed and the results are presented at Figure-1.

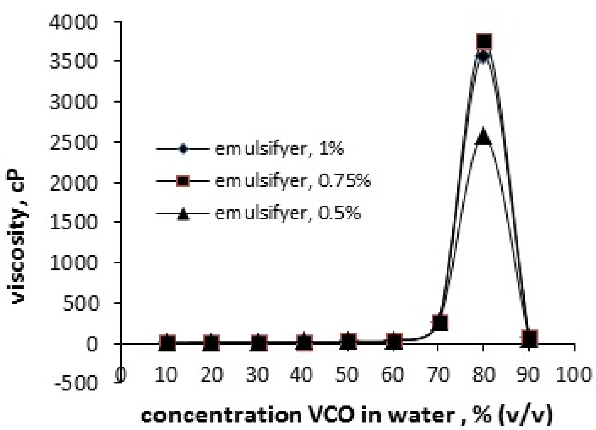

Figure-1. Viscosity of VCOE at various VCO-water ratios.

It can be seen from Figure-1 that a low oil concentration in VCOE resulted in a low viscosity. The lowest viscosity was 6cP which was achieved by VCOE emulsion with oil concentration of 10%. The viscosity slowly increased until the VCO-water ratio of 60: 40 (34 cP) with 1 percent concentration of emulsifier. A significant increase in the VCOE viscosity occurred from ratio 70:20, peaked at 80:20 and then gradually decreased at a ratio of 90:10. The highest viscosity achieved in this research was 3573 cP at the ratio of oil-water of 80:20 and the emulsifier concentration of 0.75%. The viscosity patterns of emulsion is quite similar regardless the added concentrations of emulsifiers. Low viscosity emulsion was more prone to be creaming and syneresis. This is due to differential creaming speeds of individual small and large droplets, making them to stick together. VCO emulsion showed lower phase separation due to higher viscosity of the emulsions [26].

CONCLUSIONS

The best mixed emulsifier T80S80 was achieved at the ratio of T80:S80 of 40:60. While the most stable VCO emulsion was produced by adding mixed emulsifiers T80S80 at the VCO-water ratio of 80:20, with the emulsifier concentrations of 0.5%, 0.75% and 1%. The emulsions had peroxide numbers between 1.51 and 1.53 meq peroxide / kg sample. The content of free fatty acids in the solutions ranged from 0.09 to 0.103%. The highest viscosity was 3759 cP which was achieved in the emulsion with VCO-water ratio of 80:20 and emulsifier concentration of emulsifier of 0.75%.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the funding of this research from the Ministry of Research Technology and Higher Education, the Republic of Indonesia.

REFERENCES

[1] Villarino, B. J., Dy B. J. and Lizada M. C. C. 2007. Descriptive sensory evaluation of virgin coconut oil and refined, bleached and deodorized coconut oil. LWT. Vol 40, p. 193-199.

[2] Wiyani, L., Aladin, A. and Abdullah. 2013. Coconut Processing into Virgin Coconut Oil (VCO) Export quality, based Environmentally Friendly. (in Bahasa Indonesia). Competitive Grants. Hibah Bersaing 2nd Year Report. Indonesian Muslim University. Makassar, Indonesia.

[3] Khor, Y. P., Koh, S. P., Long and K., Long, S. 2014. A Comparative Study of the Physicochemical Properties of a Virgin Coconut Oil Emulsion and Commercial Food Supplement Emulsions. mdpi.com

[4] Mirhosseni, H., Tan, C., Aghlara, A., Hamid, N., Yusof, S. and Chern, B. 2008. Optimization of the contents of arabic gum, Xanthan gum and orange oil affecting turbidity, average particle size, polydispersity index and density in orange beverage emulsion. Food Hidrocolloid. 22: pp 1212-1223.

[5] Rita I. 2011. Emulsification process and cost production analysis of beverage emulsion of Red Palm Oil. (In Bahasa Indonesia). Thesis. . Graduate Program, Bogor Agricultural Institute, Bogor.

[6] Permadi, A. 1999. The stability of Fish Oil Lemuru (Sardinella lemuru) and Its Effect on Encapsulation Efficiency. (in Bahasa Indonesia). Thesis. Graduate Program, Bogor Agricultural Institute, Bogor.

[7] Fatimah F dan Rindengan B. 2011. Effect of Virgin Coconut Oil (VCO) Emulsion Diet on Lipid Profile of White Rats (Rattus norvegicus). J. Littri 17 (1) pp. 18-24.

[8] Moreau D. L. and M. Rosenberg. 1996. Oxidative Stability of Anhydrous Milkfat Microencapsulated in Whey Protein. Journal of Food Sciece. Vol 61 No. 1.

[9] Dian N. L. H. M., Sudin N. and Yusoff, M. S. A. 1996. Characteristic of Microencapsulated Palm-Based Oil as Affected by Type of Wall Material. J.Science Food Agric. No.70: 213-217.

[10] McClements, D. J. 2004. Food Emulsions: Principles, Practice and Techniques. CRC Press, Washington, USA.

[11] Silva, K. A., Rocha-Leao M. H. and Coelho M. A. Z. 2010. Evaluation of aging mechanisms of olive oil-lemon juice emulsion through digital image analysis. Journal of Food Engineering. 97: pp.335-340.

[12] Tensiska, Setiasih, I. S. dan Irawati, D. 2007. Description VCO (Virgin Coconut Oil) Emulsion Drink at Various Sum of Water Addition. (in Bahasa Indonesia). Proceedings of the Indonesian Association of Food Technologist Seminar, 17/18 July, Bandung, Indonesia.

[13] Traynor M. P., Burke R., Frias, J.M., Gaston, E. And Barry-Ryan, C. 2013. Formation and Stability of an Oil in Water Emulsion Containing Lecithin, Xanthan Gum and Sunflower Oil. International Food Research Journal 20(5): 2173-2181.

[14] Bao Chengwei, An Shulin, Wang Wenli., Wang Fei, Y. Shuangchun and Pan Yi. 2013. Research Progress in Glycerin Fatty Acid Ester Emulsifier. International Journal of Scientific & Engineering Research. Volume 4: 924-925.

[15] Samanta, A., Ojha D., Mukherjee B., 2010. Stability Analysis of Primary Emulsion Using a New Emulsifying Agent Gun Odina. Journal Natural Science. Vol 2, No. 5: 495-505.

[16] Ibrahim N. H., Lee T. S., Rozaini M. Z. H. 2013. Potential Application of Rosella Extract in Functional Food Emusions. J. Tech and Food Industry. Vol 24, No. 1: 22-26.

[17] Ansel H. C. 1985. Introduction to Pharmaceutical Dosage Forms. Lea and Febiger Publ.

[18] Rukmini A., Raharjo S., Hastuti P, and Supriyadi S. 2012. Formulation and stability of water-in-virgin coconut oil microemulsion using ternary food grade nonionic surfactants. International Food Research J. 19: 59-65.

[19] Indonesian National Standard. 1994. How to Test Oils and Lipid. (in Bahasa Indonesia). (SNI 01-3555-1994). Jakarta, Indonesia.

[20] Sudarmadji, S., Haryono, B. dan Suhardi. 1999. Procedure of Food and Agriculture Analysis. (in Bahasa Indonesia). Liberty. Yogyakarta, Indonesia.

[21] Tabibi dan Rhodes. 1996. Disperse Systems. In: Modern Pharmeceutics, Revised and Expanded. Banker G. S. and Rhodes C. R. (Eds.). CRC Press, USA.

[22] Syah A. N. 2005. Virgin Coconut Oil, The conqueror Oil of Various Diseases. (in Bahasa Indonesia). Agro media Pustaka, Jakarta, Indonesia.

[23] APCC. 2009. APCC Standards For Virgin Coconut Oil Asian and Pacific Coconut Community [cited: 07 july 2015. http://www.apccsec.org/document/VCO standard.pdf.

[24] Ketaren S., 1986. Introduction to The Technology of Oils and Fats Food (in Bahasa Indonesia). UI Press, Jakarta, Indonesia.

[25] Technical brief. 2011. Particle science Drug development service. Volume 2. http://www. particlesciences.com/.

[26] Dickinson E. and Leser M. E. 2007. Food Colloids: Self-Assembly and Material Science. RSC Publishing, New York.