Omega-3 emulsion of Rubber (*Hevea brasiliensis*) seed oil

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ABSTRACT

The formulation of omega-3 emulsion using rubber (*Hevea Brasiliensis*) seed oil based on the best performance of the emulsion in terms of higher viscosity, smaller droplet size, lower moisture content and slightly acidic pH value supported by degree of creaming in varying the type and composition of emulsifier used. Rubber seed oil contains significant value of alpha-linolenic acid which plays an important role in maintaining human health. Therefore, formulation of rubber seed oil emulsion is important to become a new source of omega-3 emulsion instead of fish oil. Rubber seed oil was mixed with distilled water and nonionic emulsifier which were lecithin and span 80 by homogenizer. From the analysis conducted, the best formulation was the emulsion with 50% of distilled water, 6% of lecithin and 47% of rubber seed oil.

Keywords: Emulsion; Emulsifier; Lecithin; Span 80

1. INTRODUCTION

Rubber tree or its scientific name *Hevea Brasiliensis* is a perennial plant from South America cultivated as an industrial crop after being introduced to Southeast Asia on 1876. This tree produced rubber seeds that compose 43% of oil [1]. Rubber seed oil (RSO) is a semi-dried substance [2] that is rich in polyunsaturated fatty acids of C18: 2 and C18:3, as it contributes 52% of its total fatty acid composition [3]. RSO has already been shown to have many industrial applications, including possible uses for manufacturing fatty acids [4].

Malaysia has been a major rubber growing country as estimated by Malaysian Rubber Board (2011) where 1,022,780 hectares are used for rubber plantation in 2011. The production of rubber seeds by average in Malaysia is about 1 million metric ton per year. Rubber kernel con-tains 29.6% of fat and 11.4% of proteins per seed [5]. Thus, about 296,000 tones fats and 114,000 tones proteins are wasted each year.

Aigbodion and Bakare [6] reported that rubber seed oil contains oleic acid (22.95%), linoleic acid (37.28%) and linolenic acid; also known as omega-3 (19.22%). Rubber seed oil contains alpha-linoleic acid which is one of the omega-3 fatty acids which is very important in our daily diet. However, it cannot be produced by our body and need to be taken through supplements or food. Normally, omega-3 can be consumed from fish oil and vegetable oil. Still, many people do not realize that rubber seed oil is actually edible; instead they think rubber seed oil has high toxicity. Nonetheless, Babatunde and Pond [7] have once stated if the oil is rich in linolenic acid and linoleic acids, it can be used for food and industrial market as it contains high essential nutrients that is comparable to *buah perah* and soy beans.

As in Jerantut, Pahang, the villagers consume rubber seed as their daily dishes which is known as '*asam rong*'. It is a traditional dish and normally the seeds are added into *sambal* and curries. Therefore, this can be a proof that rubber seed has become people's choices and safe to be eaten.

In this study, the rubber seed oil obtained was used to formulate omega-3 emulsion. Emulsion is a heterogeneous system composed of at least two immiscible liquids, water and oil and considered as liquid-liquid colloid type [8]. Emulsion is commonly classified into oil in water (O/W) or water in oil (W/O) depending on the continuous phase.

Formulations of emulsion require which can influence the properties of emulsion in several ways for example the stability of dairy emulsion [9]. In foods sold in the Europe, emulsifiers are given E numbers beginning with 3 or 4 [10]. Emulsifiers will reduce the dynamic surface tension, thus leading to the formation of small fat droplets during homogenization; displace protein that may otherwise be available for bridging flocculation, from the fat globule surface; interact with interfacial protein,

leading to a thicker and stronger adsorbed layer and increase the viscosity of the aqueous phase through the formation of self bodying mesophase structures.

In this experiment, two types of emulsifiers were used, which were lecithin and span 80. Common food applications of lecithin include use as an emulsifier, a stabilizer, a dispersing aid, and an incidental additive, such as a release agent for baked goods. Regardless of its food application, lecithin is generally used in small amounts, with the result that it is, according to one lecithin manufacturer, present in finished foods at levels rarely exceeding 1% by weight of the final food product. On the other hand, span 80 also known as sorbitan monooleate, a commercial Span 80 does contain monoesters, diesters, as well as triesters and tetraesters. Furthermore, the polar head group of all esters present in Span 80 is not sorbitol, but more likely one of the different forms of anhydrized sorbitol.

Therefore, this study aims to determine the best formulation of omega-3 emulsion using rubber seed oil to obtain the most stable emulsion.

2. MATERIALS AND METHODS

2.1. Preparation of Rubber Seed Oil

Rubber seeds were prepared by removed the outer skin. The seeds were dried in oven and ground into fine powder. The rubber seed oil was produced using soxhlet extraction method.

2.2. Formulation of Emulsion

The rubber seed oil was mixed with distilled water and emulsifier at the temperature of 50℃ to 60℃. The mixture was homogenized at 10,000 rpm. The lecithin and span 80 were used as emulsifier in this experiment. Lecithin is a food ingredient that is derived from plant sources, including soy. Meanwhile, span 80 is a molecularly heterogonous non-ionic emulsifier which is cheap and normally is used as food emulsifier and in oral pharmaceuticals. The concentrations of emulsifiers were varied according to **Table 1**.

Table 1. Emulsion composition and codes.

Code	O:W (v/v)	Type of emulsifier	Emulsifier (v/v)% / (w/w)%
E1	50:50	lecithin	3.0
E2	50:50	lecithin	6.0
E3	50:50	lecithin	9.0
E4	50:50	span 80	10.0
E5	50:50	span 80	8.5
E6	50:50	span 80	7.0
E7	50:50	span 80	6.0

v/v. volume/volume; w/w. weight/weight

The further analyses done on the emulsion were viscosity, pH value, droplet size, moisture content and degree of creaming.

2.3. Stability Test

The fresh prepared emulsion was transferred to a graduated cylinder. The amount of free water or oil was separated from the emulsion and observed everyday for a period of 10 days. The volume of water inside the emulsion was calculated using eq.1 to determine the stability of the emulsion with the respect to days.

$$\text{Degree of creaming} = v_1 - v_2 \qquad (1)$$

In **Eq.1**, v_1 represents the total volume of the fresh emulsion while v_2 is the volume of the crude emulsion after a few days.

2.4. Physical Characteristics

The prepared emulsions were observed at room temperature for 24 hours. Observations were done based on the phase separation of the emulsion, and then the emulsion was used for further analysis. The emulsion which stable for 24 hours is usually stable for several days [11].

The viscosity is a measure of the ratio of shearing stress to rate of shear as shown in the **Eq.2**. Viscosity was performed at 25℃ using viscosmeter LVDV-II +P CP Brookfield with the use of Rheocalculator V3.3 Build 49-0 software.

$$\textbf{Viscosity (cP)} = \textbf{100/rpm} \times \textbf{TK} \times \textbf{SMC} \times \textbf{Torque} \qquad (2)$$

where RPM is current viscometer spindle speed, TK is viscometer torque constant, SMC is current viscometer torque % expressed as a number between 0 and 100.

First of all the viscometer was auto zero with no spindle attached for calibration. Then after 10 seconds, the main display flashed at 00.0. The spindle was attached to the viscometer and spindle must not to touch the bottom or sides and should be centered. After that, the spindle was immersed in the sample cup and switched on the button for agitation process. The speed was adjusted from 0 to 10 rpm until the stable reading appeared. Lastly, the dial reading and rpm were recorded after the reading was stabilized.

The moisture content was determined using drying method. Firstly, the sample was weighed and recorded before dried in the oven at 105℃ for 6 hours. After drying process, the weight of sample was measured. The eq.3 showed the calculation of moisture content.

$$\textbf{\% Moisture} = [(\textbf{M}_{wet} - \textbf{M}_{dry}) / \textbf{M}_{wet}] \times 100\% \qquad (3)$$

where M_{wet} is the weight of wet sample while M_{dry} is the weight of dry sample.

The pH value was determined by homogenized the emulsion using vortex to ensure the sample was mixed

well and measured by a digital 3505 pH meter (model Jenway) for 3 times and average pH value was calculated.

The diameter of the droplets was measured by using Olympus BX 5 optical microscope (x40) equipped with a calibrated eyepiece micrometer. The emulsion was put into a glass plate and measurements were taken after 3 to 4 days. The images of size and distribution of particles were taken using digital camera and analyzed by CC12 soft imaging system software.

2.5. Analysis of Variance (ANOVA)

In this study, the parameters were analyzed using Analysis of Variance (ANOVA). The results of ANOVA and degree of creaming were used to determine the best formulation.

3. RESULTS

3.1. Stability of Emulsion

The stability of emulsion was studied through the degree of creaming for a period of 10 days by observing the volume of the oil as a dispersed phase. The results were shown in the **Table 2**. The value 0.0 indicates the emulsion maintain its stability.

Based on the results shown in **Table 2**, the E4 and E5 of span 80 did not form creaming at all in 10 days. They were very stable compared to the others. Meanwhile, E6 and E7 started to form creaming at day 2. The E6 emulsion showed the higher rate of creaming formation initially but it had been slowed down and the degree of creaming was the same as E7 in the end

3.2. Physical Characteristics

3.2.1. Effect of Concentration and Type of Emulsifier on Viscosity

The formulation of emulsion with lecithin as emulsifier had the higher viscosity compared to formulation of emulsion with span 80 as shown in **Figure 1**. The maximum value for lecithin was 30 cP while for span 80 was 8 cP.
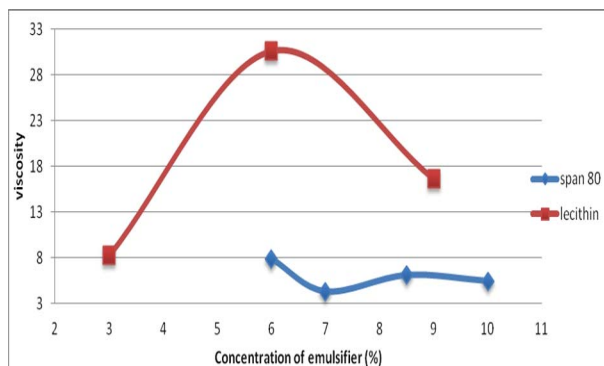

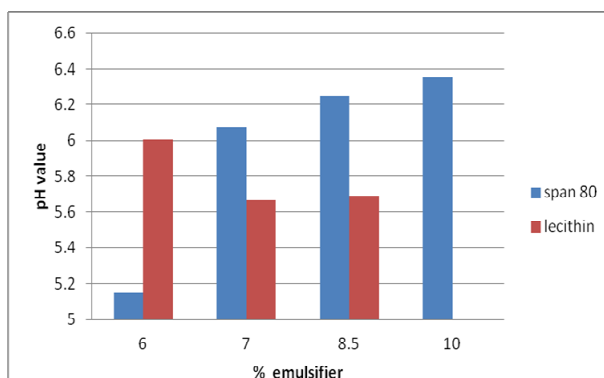**Figure 1.** Viscosity of the formulation of emulsion with the increasing percentage of emulsifiers.

Figure 2. Interaction of pH value with increasing percentage and type of emulsifier.

3.2.2. Effect of Concentration and Type of Emulsifier on pH value

The pH value showed in the **Figure 2** increased as the amount of span 80 increased while the pH values were dereased when the amount of lecithin was increased. The formulation of span 80 mostly approximately neutral while lecthin produced slightly acidic emulsion.

3.2.3. Effect of Concentration and Type of Emulsifier on Moisture Content

Figure 3 showed the effect of the percentage and type of emulsifier on moisture content. The use of span 80, the moisture content increased from 6% to 8.5% and then decreased when the span 80 amounts was further increased. On the other hand, moisture content for lecithin formulation decreased slightly when lecithin increased. All the formulations used lecithin had lower moisture content compared to span 80. The 9% of lecithin emulsifier gave the lowest moisture content which was 23% moisture content.

3.2.4. Effect of Concentration and Type of Emulsifier on Droplet Size

The analysis results of optical microscope were shown in **Figure 4** for span 80 and **Figure 5** for lecithin. The

Table 2. Effect of lecithin and span 80 percentages on degree of creaming.

Code	Day									
	1	2	3	4	5	6	7	8	9	10
E1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4
E2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E3	0.0	0.0	0.0	0.0	0.0	0.5	1.4	1.8	2.0	2.4
E4	0.0	0.6	0.9	1.1	1.2	1.4	1.5	1.6	1.6	1.7
E5	0.0	1.0	1.1	1.1	1.3	1.3	1.4	1.5	1.6	1.7
E6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

microstructure of the emulsions indicated that most of the dispersed phase were present as circular droplets and had many range of sizes. This generally occurred for the emulsions, which were prepared by physical methods like vortex method and mechanical homogenizers [12]. **Figures 4(a)** and **(b)** show the microstructure of the short term stable emulsions after 48 hours which span 80 as an emulsifier. This condition also applied to **Figure 5(c)** which lecithin as an emulsifier.

In addition, **Figures 4(c)** and **(d)** show the microstructure for the most stable emulsion with the percentage of span 80 were 8.5% (E5) and 10%(E4) respectively. **Figures 5(a)** and **(b)** also show the microstructure of the more stable emulsion.

The maximum droplet size in **Figure 6** of lecithin slightly increased as the percentage of emulsifier increased. The span 80 with 8.5% shows the smallest value of maximum droplet size between all the emulsifier concentrations followed by the 10% of span 80 and 3% of lecithin.
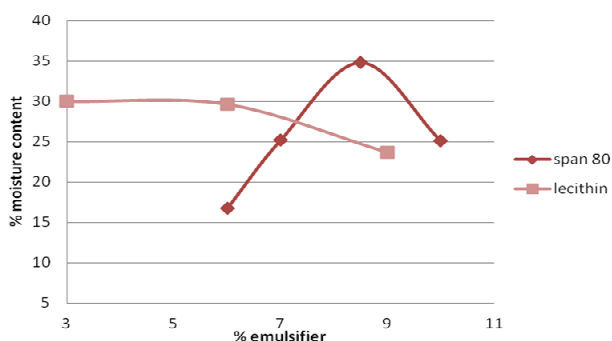

Figure 3. The effect of percentage and type of emulsifier on moisture content.
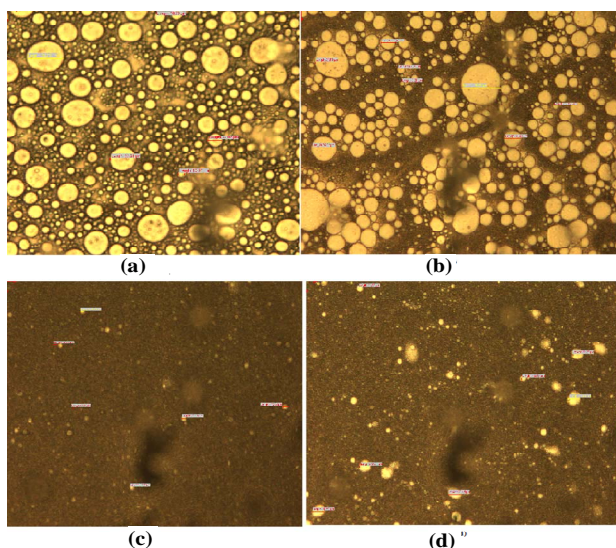

Figure 4. The microstructure of emulsion with different concentration of span 80 (a) E7-6%; (b) E6-7%; (c) E5-8.5%; (d) E4-10%.

3.2.5. Effect of Concentration and Type of Emulsifier on Moisture Content

The one-way ANOVA test was carried out to determine the best formulation of omega-3 emulsion in terms of moisture content, pH value, viscosity and droplet size for various formulations and it was expressed as mean ± SD. Abbreviation (a, b, c) were arranged according to small value to the big value. The data tabulated in **Table 3** and the results of stability analyses were used to determine the best formulation of omega-3 emulsion.
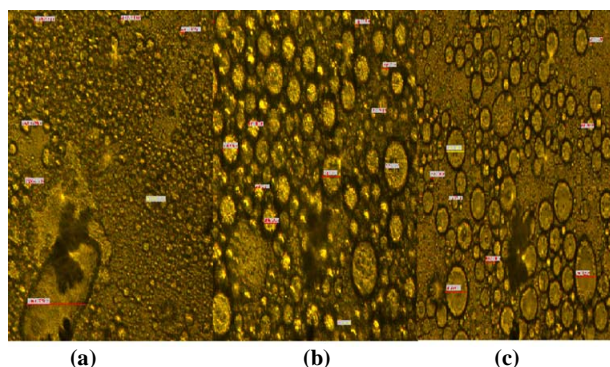

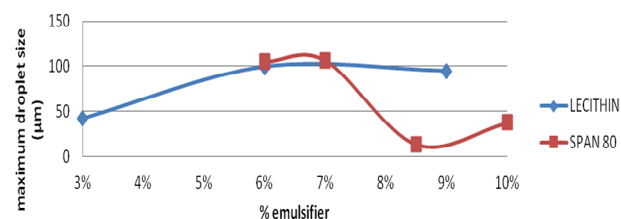**Figure 5.** The microstructure of emulsion with different concentration of lecithin (a) E1-1%, (b) E2-6%, (c) E3-9%.

Figure 6. Maximum droplet size based on the concentration of emulsifiers.

Table 3. The analysis of omega-3 emulsion samples for various formulations.

Parameter	Formula						
	E7	E6	E5	E4	E1	E2	E3
%(w/w) Moisture	16.748 [a]	25.187 [bc]	34.800 [d]	25.134 [bc]	30.006 [cd]	29.647 [cd]	23.662 [b]
pH	5.15 [a]	6.07 [c]	6.25 [d]	6.35 [d]	6.01 [c]	5.67 [b]	5.69 [b]
Viscosity (cP)	7.887 [d]	4.2767 [a]	6.0833 [c]	5.4300 [b]	8.2167 [d]	30.580 [f]	16.613 [e]
Drop Size (μm)	105.13 [f]	106.42 [g]	13.54 [a]	37.41 [b]	42.57 [c]	99.27 [e]	94.81 [d]

The average of all the analysis conducted were expressed as mean of three replication ± standard deviation. Abbraviation (a, b, c) represent statistical comparison made for each measurement for each formualtion. The mean with different letters for each formulation and samples were significantly different ($p < 0.005$).

4. DISCUSSION

Based on the statistical analysis and the results of stability test, the best formulation was determined. The formulation with lower moisture content was needed because it could prevent microbiological present in the sample. E4 showed a good stability among the emulsions and E4 is the best formulation in term of moisture content while E1 showed the lowest moisture content but this emulsion is not stable.

The emulsion must be slightly acidic to prevent the growth of microbial which will cause the emulsion being spoiled easily. This is because most bacteria grow at pH range 6 to 8 and pathogens do not grow well below pH 4.5. The pH of the Scott Emulsion was taken to be compared with the experimental rubber seed oil emulsion. The pH value was 3.14 which were very acidic. E2 that gave the best stability showed the pH ranges between 5.67 - 5.69 for rubber seed oil omega-3 emulsion. Flavoring that is high acidic such as lemon oil can be included to improve the pH of emulsion.

Viscosity can be defined as an important aspect of product quality, the ability to detect differences in the viscosity of beverages allows more satisfying and delicious food product to be created [13]. For the purpose of commercialization, the properties of high viscosity with low oil content and fine droplet size are required. As E5 had the best viscosity value in this experiment when added with span 80 as emulsifier while E2 showed the best viscosity with added lecithin, so their viscosity value were used to compare with Scott's Emulsion. E5 and E2 had lower viscosity compared to the Scott's Emulsion which has 37 cP, but E2 showed the nearest viscosity with the commercial product and it can be concluded that lecithin is the best type of emulsifier for omega-3 emulsion of rubber seed oil in term of viscosity. Therefore, in order to improve the viscosity of emulsion, additives such as thickeners, co-surfactants and so forth for specific application can be added into the emulsion during formulation.

Furthermore, E5 was the best formulation in term of droplet size. In this case, E2 which had the moderate droplet size but it gave the most stable emulsion. Theoretically, when the droplet diameter is large, bacteria reproduce more easily than smaller droplet diameter, as the bacterial growth is reduced due to the lack of nutrients inside the droplets. The droplet sizes are expected to not substantially exceeding 1000 μm for microbiologically stable emulsion [14]. Hence, the less stable emulsion which were E7 and E6 added with the emulsifier of span 80 will not affected with the growth of microorganism as the maximum droplet size was only about 110 μm but the stability will be affected due to the creaming, flocculation of small droplet particles.

5. CONCLUSIONS

From the analysis conducted, there were two best formulations which using 6% (v/v) lecithin and 8.5% (v/v) span 80 as the emulsifier. For span 80, the best formulation was E5 with 50% (v/v) distilled water, 8.5% (v/v) span 80 of the total volume of emulsion and 41.5% (v/v) rubber seed oil (RSO). The other formulation which considered was 50% (w/w) distilled water, 6% (w/w) lecithin of the total weight of oil only and 47% (w/) of RSO. These formulations had nice texture and the emulsion can be kept longer.

However, from this two formulation of emulsion, formulation with lecithin as an emulsifier was the best as the viscosity, stability, pH value and its moisture content were achieved the requirement for the commercial purposed. It can be used as a benchmark for formulation.

From this study, the concentration of span 80 in the formulation will be affecting the omega-3 emulsion. The range between 8.5% to 10% of the span 80 is suggested to produce the most stable emulsion that can be maintained up to a week with a fine droplet size. So, further study should be conducted to use combination of emulsifiers according to hydrophilic-lipophilic balance (HLB) value to improve the physical stability. Besides, the further studies also may include what type and how much of preservatives, antioxidants and other food additives are necessary to increase the shell life of this product.

As a conclusion, the omega-3 emulsion of rubber (*Hevea Brasiliensis*) seed oil has the potential to be commercialized as omega-3, alpha-linolenic acid supplement which is extracted from local source and sustainable. Therefore, more research should be carried out to study the potential of rubber seed oil so that can be applied into different industries such as food, cosmetics and pharmaceutical.

6. ACKNOWLEDGEMENTS

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