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Formulation and Characteristic of Ascorbic Acid Microemulsion in the Scale-up Production

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Abstract

Ascorbic acid microemulsion has been successfully formulated using ternary food grade nonionic surfactants. The preparation of that microemulsion was a laboratory-scale technique which had to be scaled up for technical use. Controlled technical-scale production of ascorbic acid microemulsion was a very important aspect to be studied. The objectives of this study were to determine the optimal conditions of ascorbic acid microemulsion production in the technical-scale and to observe its characteristic. Ascorbic acid microemulsion was prepared by dissolving ascorbic acid into deionized water and then mixed with surfactants mixture and virgin coconut oil. The production was scaled up to forty-fold. To define their characteristic, these microemulsions were subjected to stability test during storage at room temperature by measuring turbidity index and photooxidative stability. The results indicated that in a technique which was done in previous study, the formation of ascorbic acid microemulsion can only be scaled up to tenfold. Ascorbic acid microemulsion production was strongly influenced by stirring speed, mixing time, and container: magnetic stirrer size ratio. There was no significant difference stability of ascorbic acid microemulsion during storage. This study confirmed that ascorbic acid microemulsion which was formulated using ternary food grade nonionic surfactants could be produced in technical scale.

Keywords ascorbic acid, microemulsion, nonionic surfactant, virgin coconut oil, scale-up production.

1. Introduction

Microemulsion is a liquid dispersion of immiscible solutions which is made homogenous by adding relatively large amounts of surfactant and co-surfactant and having diameter of the droplets in the range of 100-1000 Å or 10-100 nm [1]. In contrast to the ordinary emulsion (macroemulsion), microemulsion is a thermodynamically stable, transparent isotropic solution, low viscosity, and spontaneous formation from the hydrophobic or hydrophilic parts of surfactant molecules [2].

Microemulsion is composed of three main components i.e. water, oil, and surfactant and/or co-surfactant. Surfactant formed a monolayer in the interfacial area. Co-surfactant is often required to lower the interfacial tension of oil/water interface which is needed to form microemulsion [3].

Microemulsion can be classified to three types, i.e. oil-in-water (o/w), water-in-oil (w/o), and bicontinuous. Changes in conditions or the concentration of the components used in the microemulsion formulation can change the type of microemulsion formation. At low oil concentration (< 30%) microemulsions are in the o/w form. Conversely, at low aqueous phase concentration, w/o microemulsions are formed. Inside the specific o/w and w/o microemulsion areas, many different defined systems, such as micellar, inverse/reverse micellar, lamellar, and bicontinuous phases may exist at distinct oil-water-surfactant concentration [2, 4].

Generally, w/o microemulsion can be formed by using surfactants that have low Hydrophilic-Lipophilic Balance (HLB), range of 3-8, whereas o/w microemulsion can be formed with surfactant having a HLB range of 8-18 [2].

In contrast to the other colloid systems, microemulsions have specific characteristics, i.e. relatively wide surface area, low interfacial tension, and high solubilizing capabilities. According to Patel *et al.* [1], the important factors to be considered during microemulsion preparation are: (1) Surfactants must be carefully chosen so that an ultra low interfacial tension (< 10^{-3} mN/m) can be attained at the oil/water interface which is a prime requirement to produce microemulsions, (2) Concentration of surfactants must be high enough to provide the number of surfactant molecules needed to stabilize the microdroplets to be produced by an ultra low interfacial tension, and (3) The interface must be flexible or fluid enough to promote the formation of microemulsions.Food-grade microemulsions have been increasing attention of researchers and exhibited great potential in industrial applications [2].

However, food-grade microemulsions are difficult to be formulated due to the complexity of food systems and the need for all of the components to be approved as being of food grade limited the choice of components (especially the type of surfactants), leading to difficulties in formulations [5]. We had successfully formulate food-grade w/o microemulsion using deionized water as aqueous phase, dehydrated virgin coconut oil as oil phase, and a mixture of Tween 20 (polyoxyethylene sorbitan monolaurate), Span 20 (sorbitan monolaurate), and Span 80 (sorbitan monooleat) as surfactants [6].

The microemulsion characteristics can be determined by characterization method. The first step in the characterization of microemulsion is the determination of where a clear isotropic region lies within the different concentrations of water, oil, and surfactant. According to Flanagan and Singh [2], characterization of microemulsion can be divided into two main areas, i.e. characterization at the macroscopic level and microscopic level. Viscosity, conductivity, and dielectric measurements provide useful information at the macroscopic level. While the size, shape, and particle dynamics used to provide information on the microscopic level [1].

In our previous study, we used the w/o microemulsion as ascorbic acid delivery system and named as ascorbic acid microemulsion. That microemulsion effectively inhibits photooxidation of virgin coconut oil during storage under fluorescent light [7]. The preparation that microemulsion was a laboratory-scale technique which had to be scaled up for technical use. The objectives of this study were to determine the optimal conditions of ascorbic acid microemulsion production in the technical-scale and to observe its characteristic.

2. Materials and Methods

2.1. Formulation of ascorbic acid microemulsion

Referring to our previous study [6], ascorbic acid microemulsion was prepared by adding the following components to the final stated percentages (w/w): 4.55% of aqueous phase, 20.45% of surfactants mixture, and 75.00% of dehydrated virgin coconut oil as oil phase. The aqueous phase consists of ascorbic acid (1% microemulsion formula) which was dissolved into the deoinized water whereas the surfactants mixture composed of 16.60% of Tween 20, 15.00% of Span 20, and 68.40% of Span 80. Aqueous phase dissolved ascorbic acid was added with the surfactants mixture and mixed on a hot plate stirrer at 40°C and then the oil phase was added dropwise while stirring. Mixing is conducted in a glass beaker equipped with a magnetic stirrer. The formation of ascorbic acid microemulsion was characterized by the presence of pale yellow, clear, and transparent liquid solution without phase separation. For application in technical scale, this formulation then to be scaled up to twice, four, ten, twenty, and forty-fold. Ascorbic acid microemulsion samples that can be obtained were then allowed to equilibrate at room temperature (30 ± 1°C) for at least 24 h before characteristic determination.

2.2. Characterization of ascorbic acid microemulsion

Ascorbic acid microemulsions were then characterized on physical properties and photooxidative stability test.

2.2.1. Physical properties of ascorbic acid microemulsion

The physical properties of ascorbic acid microemulsion were evaluated by measuring the viscosity, conductivity, and interfacial tension. Viscosity determinations were performed in a model LVT Brookfield viscosimeter. Electrical conductivity was measured by a conductivity probe (Vernier Con-BTA), and the drop method was used to perform the interfacial tension measurements.

To evaluate the physical stability of ascorbic acid microemulsion during storage, formulations stored at room temperature (30 ± 1°C) were characterized by visual inspection and monitored by turbidity measurements using a UV/Vis spectrophotometer (UV-1650 PC, Shimadzu, Japan) every two weeks for up to two months. The turbidity was calculated as described by Fletcher and Morris [8, 9].

2.2.2. Photooxidative stability test

Photooxidative stability of ascorbic acid microemulsions were performed under accelerated condition using fluorescent lights with intensity of approximately 4000 lux. A portion of 20 mL ascorbic acid microemulsions each formulation were placed in a 30 mL transparent serum bottles with rubber caps and exposed to the light for up to 5 hours at room temperature (30 ± 1ºC). Another set of microemulsion without ascorbic acid was also prepared and used for comparison. Peroxide values (PV) of the samples were measured according to the method proposed by the AOCS Official Method [10].

2.3. Statistical analysis

The data were represented as the mean value of three replications. Significant differences ($P < 0.05$) between means were determined using Duncan's multiple range tests.

3. Results and Discussion

3.1. Scale-up production of ascorbic acid microemulsion

In our previous study, we have found a technique to formulate ascorbic acid microemulsion. This technique was a laboratory-scale production, which was as much as 25 g. Ascorbic acid microemulsion production was carried out using 150 ml glass beaker (diameter 6 cm) by 3 cm of magnetic stirrer size and fast stirring speed. With this technique, ascorbic acid microemulsion obtained after mixing all components for 10 minutes. When the production was increased to twofold, newly formed ascorbic acid microemulsion can be obtained after 15 minutes of mixing process, however, when it was increased to fourfold, for up to 30 minutes of mixing processes have not been able to obtain an ascorbic acid microemulsion. This suggests that even if often no distinction is made between invention and innovation, the difference is definitely not a subtle one. As a matter of fact, it is not always possible to turn a good idea into an innovation and put it into practice: an invention can sometimes lay unused for ages without paying back in terms of industrial realization and of profitable business [11].

Therefore we try to vary the conditions and the results were showed in Table 1.

Table 1 shows that in order to obtain larger quantities of ascorbic acid microemulsion takes longer and faster speed. This could be due to produce higher amounts of ascorbic acid microemulsion require larger glass beaker, so it also takes a longer magnetic stirrer. Stirring is intended to provide energy to the surfactant molecules form a layer immediately surrounds interface and stabilize the microdroplet of microemulsion. That is the leading to successfully of a microemulsion formation. Micoremulsion although theoretically be formed by the arrangement of surfactant molecules in the interfacial layer spontaneously, but in some cases it require energy to accelerate the rearrangement of molecules surfactant or to overcome the small barrier of kinetic energy required for the formation of a microemulsion [1, 2, 12]. Therefore, production of ascorbic acid microemulsion in a large number require stronger and longer of mixing process as shown in Table 1.

Table 1 also shows that the formation of a microemulsion is strongly influenced by the ratio between the size of magnetic stirrer and the diameter of glass beaker. Mixing becomes less perfect if ratio smaller and it can not produce a microemulsion which is characterized by the formation of a turbid or slightly cloudy solution.

Table 1. Evaluation of ascorbic acid microemulsion production

Production scale	Quantity of ascorbic acid microemulsion	Magnetic speed	Mixing time (min)	Glass beaker diameter (cm)/ volume (ml)	Magnetic stirrer size (cm)	Appearance
one fold	25 g	fast	10	6 / 150	5	transparent
twofold	50 g	fast	15	6 / 150	5	transparent
fourfold	100 g	fast	30	7 / 250	5	slightly cloudy
		very fast	25	7 / 250	6	transparent
tenfold	250 g	very fast	40	9 / 600	6	slight cloudy
		very fast	30	9 / 600	8	transparent
twenty-fold	500 g	very fast	45	10 / 1000	8	turbid
forty-fold	1000 g	very fast	60	13 / 2000	8	turbid

On a production scale of fourfold and tenfold, ascorbic acid microemulsion can be obtained by replacing the magnetic stirrer with the longer one. However, it can not be applied on a production scale of twenty-fold and forty-fold. In a production of higher than 250 g, the mixing process is required not only horizontally, so ascorbic acid microemulsion can not be obtained if the mixing process used a magnetic stirrer. Ascorbic acid microemulsions which were obtained from the production scale up to tenfold then were used for further experiment.

3.2. Physical properties of ascorbic acid microemulsion

The physical properties of ascorbic acid microemulsion appear in Table 2. Viscosity, conductivity, and interfacial tension measurements provide useful information at the macroscopic level of ascorbic acid microemulsion in this study. The transition between microemulsion structures can be implied by changes in viscosity [2, 4, 13]. Viscosity is expected to increase in swelling microemulsion systems [14]. Conductivity measurements can be used to determine whether a microemulsion is oil-continuous or water-continuous, and may also be used to monitor percolation or phase inversion phenomena [15]. The conductivity depends not only on droplet size but also on mean droplet-to-droplet distance or droplet concentration [16], and it's closely related to the rate of mass transfer between droplets [17]. Interfacial tension is the surface tension caused by intermolecular interactions at the surface separating two immisible fluids [18].

Table 2. Physical properties of ascorbic acid microemulsion

Production scale	Characteristic *)		
	Viscosity (cp)	Conductivity (µs/cm)	Interfacial tension (mN/m)
one fold	107.5 ± 4.6 [a]	0.9 ± 0.0 [a]	22.55 ± 0.39 [a]
twofold	108.5 ± 3.5 [a]	0.9 ± 0.0 [a]	22.82 ± 0.26 [a]
fourfold	107.5 ± 2.5 [a]	0.9 ± 0.0 [a]	22.67 ± 0.24 [a]
tenfold	108.0 ± 5.0 [a]	0.9 ± 0.0 [a]	22.78 ± 0.22 [a]

*): an average of three replications
Values followed by the same letters in the same column indicate no significant difference (P > 0.05)

Table 2 shows that there was no significant difference (P > 0.05) in viscosity, conductivity, and interfacial tension. This indicates that ascorbic acid microemulsion could be obtained in the scale-up production without physical changes. Low values of conductivity for all of ascorbic acid microemulsions indicated that these are w/o microemulsions and that ascorbic acid incorporation did not change the structure of the system. These values are equal to conductivity of virgin coconut oil which acts as oil phase.

The stability of ascorbic acid microemulsions were evaluated by measuring their turbidity changes during storage at room temperature (30 ± 1°C). Turbidity is the most common way to measure microemulsions stability because it's proportional to the average particle diameter [9]. Thus, the turbidity changes can be used to derive changes in the particle volume resulting from either clustering or growth of microemulsion droplets, and to obtain information on stability changes. Visual inspection indicated that all of ascorbic acid microemulsions did not suffer from significant changes in their appearance over the storage period. And also no significant differences (P > 0.05) on turbidity index (Fig. 1). According to Cho *et al.* [9], microemulsion with transparent appearance and a turbidity value less than 1% was defined as a stable microemulsion. This confirms the evidence that ascorbic acid microemulsion formed remained stable despite produced on a larger scale.

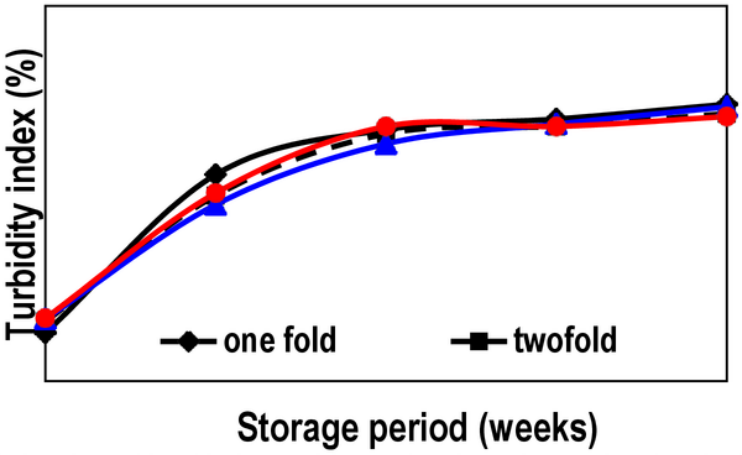

Figure 1. Turbidity index of ascorbic acid microemulsion produced at various scales of production during storage at room temperature (30 ± 1°C)

3.3. Photooxidative stability of ascorbic acid microemulsion

Beside the physical stability of the scale-up production study, to prevent or decrease the oxidative damage, the ascorbic acid microemulsions have to maintain their photooxidative stability. Photooxidation is a reaction that initiates quality deterioration which is indicated by the formation of peroxide compounds. Intense light exposure in photooxidation induces an increased rate of peroxide formation [19]. The peroxide value of ascorbic acid microemulsions which were exposed to fluorescent light were showed in Table 3.

Table 3. Peroxide value of light exposed ascorbic acid microemulsions

Production scale	Peroxide value (meq/kg)	
	Before light exposure	After light exposure
one fold	0 [a]	0 [a]
twofold	0 [a]	0 [a]
fourfold	0 [a]	0 [a]
tenfold	0 [a]	0 [a]

*) an average of three replications
Values followed by the same letters indicate no significant difference (P > 0.05)

Table 3 shows that the peroxide values of all ascorbic acid microemulsions both before and after light exposure is zero. Microemulsion without containing ascorbic acid before and after exposure to light has a peroxide value 0.098 ± 0.009 and 1.298 ± 0.024, respectively. This result indicated that ascorbic acid which was incorporated in w/o microemulsion system could play as oxygen quencher and inhibited photooxidation reaction. Even after they were exposed to fluorescent light, ascorbic acid microemulsions had a peroxide value of zero.

Table 3 also shows that scale-up production of ascorbic acid microemulsions did not affect their photooxidative stability. Thus, the production of ascorbic acid microemulsions appropriate to scale-up for tenfold in a technique as it has done.

4. Conclusion

Production of ascorbic acid microemulsion in greater amounts can not be done with the exact same conditions with production in much smaller amounts. Ascorbic acid microemulsion formation is strongly influenced by the stirring speed, mixing time, and the ratio between the diameter of the container and the size of the magnetic stirrer. In this study, ascorbic acid microemulsion can be scaled-up to tenfold without significant differences in the physical properties. In addition, there was no significant difference physical and photooxidative stability of that ascorbic acid microemulsion during storage. This study confirmed that ascorbic acid microemulsion which was formulated using ternary food grade nonionic surfactants could be produced in technical scale. It would be very interesting for future studies to develop a technique to obtain ascorbic acid microemulsion in larger quantities.

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