

Physicochemical Properties and Antibacterial Activity of Kaffir Lime Oil Emulsion Applicable to Beverage Product

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Abstract

Essential oil inside beverage emulsion became an interesting subject to get a new value for the fast-moving customer goods (FMCG) market. In this study, kaffir lime oil (KFO) physicochemical stability properties and antibacterial test on formulation of kaffir lime oil in water beverage emulsions was investigated. The main ingredients of beverage emulsions are 800 ml water, 100 mL high fructose corn syrup (HFCS), 5 grams arabic gum as an emulsifier, 0.5 grams vitamin E, and 0.125 mL kaffir lime oil as flavoring and antibacterial. KFO beverage emulsion used instrument with Stability of oil-in-water emulsion was observed by measuring physicochemical properties: pH turbidity, and conductivity. GC-MS characterization on kaffir lime essential oils revealed the top three components, i.e., citronella (46,47%), citronellol (12,22%), and citronellyl acetate (6,48%). FT-IR KFO spectrum had absorbance at 1726 (C=O stretching), 2922, and 2874 cm⁻¹ (C-H stretching from aldehyde). Absorbance was also present on 1454 and 1379 cm⁻¹ (C=C stretching) wavelengths, resembling citronella. Citronella as a main component is an active antibacterial agent against E. Coli. Gum arabic with R-S(=O)₂-OH group addition reduced pH value to 1.1 on each composition addition and increased conductivity by 317.3 μS/cm. Fructose addition reduced the value by 153.4 μS/cm. Turbidity value increased averagely by 46,9 NTU, then reduced by 14,4 NTU. Citronellal in KFO could hinder e-coli bacterial growth and had an alt value >72 CFU/mL. This formulation study produces a beverage product with pH, conductivity, and turbidity values following the standard and has criteria under the microbiological contamination limit of BPOM and SNI standards and key requirement of potable drink product.

Keywords: emulsified beverage, kaffir lime oil, physicochemical properties, stability, perception

INTRODUCTION

Essential oils from natural plants have wide applications in the food industry cosmetic industry and pharmaceutical industry [1]. Essential oils are natural compounds obtained from plants and Herbs are added to foods to enhance their taste and aroma [2]. In particular, essential oils have been reported to be considered good alternatives to anti-microbial additives in the food and beverage field [3]. Consumers are not only looking for products that are healthy and colorful, but also taste and taste in their dietary choices[4]. Therefore, consumer acceptance of food, among other factors, depends on sensory attributes of the final product [5].

Beverages are of great importance in the food industry as they are capable versatile products to satisfy a number of consumer needs due to their appearance and ease of storage and distribution. Beverage substrates are mainly composed of water and thus a combination of Water- and/or oil-insoluble health-related compounds for beverage flavoring pose a challenge to researchers and required an emulsification step.

In addition, these functional compounds can be decompose after they are added to the food as they may undergo some physicochemical stress during food production and storage. In this regard, these functional compounds may be sensitive to to environmental changes such as pH, temperature and the presence of minerals, hence their shelf life depending on processing and storage conditions [6].

If oil is the dispersed phase and water is the dispersing phase, this system is termed oil emulsion in water. Conversely, if water is the dispersed phase and oil is the dispersing phase, the system is termed water emulsion in oil. A suitable emulsifier or surfactant is often added [7]. The stability character in an emulsion should present a constant physicochemical properties value in a time unit during emulsion solution storage. Turbidity can be the physicochemical stability determining parameter. A book concerning emulsion stability discusses emulsion characterization with the sonification technique [8].

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Furthermore, pre-analysis to physicochemical stability properties was determined by the turbidity, pH, conductivity value, where a more stable turbidity value measured in different time intervals means a better stability physicochemical parameters [9].

Gum arabic is a hydrocolloid generated by the acacia tree, an effective encapsulating agent with high water solubility and low viscosity compared to other hydrocolloids. Gum arabic is also popular as an emulsifier in the oil in a water emulsion system. Figure 4 illustrates gum arabic consists of simple sugar with many branches, i.e., galactose, arabinose, rhamnose, and glucuronic acid. It also contains protein components covalently bound to the molecule structure [10]

One of the most exciting Indonesian essential oils applied to an emulsion system is the kaffir lime (*Citrus hytrix*, DC). The volatile kaffir lime essential oil is utilized as flavor, perfume ingredient, antioxidant, and good bacteria. Several precedent studies mentioned that kaffir lime crude extract has an active component as cytotoxic [11], anti-cancer [12] and antibacterial [13] [14]. Antibacterial is a particular substance generated by living organisms in a low concentration that hinders a primary process in a microorganism [15].

In this study developed a kaffir lime oil emulsion system in water. It also examined physicochemical of the new formulated KFO-contained beverages is proceeded. Further, the antibacterial activity as the requirement of potable beverages is proceeded

EXPERIMENT

Chemicals and instrumentation

The formulation of emulsion drink utilized Design Expert Ver.10 software. Primary ingredients include RO-UV water H₂O; kaffir lime essential oil obtained from the Essential Oil Institute of Universitas Brawijaya with main component is citronella C₁₀H₁₈O (46.47%), citronellol C₁₀H₁₀O (12.22%), and citronellyl acetate C₁₂H₂₂O (6.48%); Gum arabic powder C₁₅H₂₀NNaO₄ (Chemipan Corporation. ltd), High Fructose Corn Syrup C₆H₁₄O₇ (PT Sorini Agro), and vitamin E alpha-Tocopherol C₂₉H₅₀O₂. Base lactose TTC agar with tergitol 7 media agar. Incubator

KFO emulsion process

Composition variation comprised the comparison of kaffir lime essential oil (KFO) : gum arabic : encapsulated vitamin E : sweetener (HFCS) :water.

The formulation stage was initiated by mixing KFO with other ingredients in a vortex or blender for two minutes under room temperature, followed by a stirring process using a homogenizer to generate emulsion. Then, 500 grams of the emulsion was heated at 60° C (30 minutes), immediately cooled, and stored at room temperature before analyzing its physicochemical properties.

Organoleptic Preception Test

Thirty people used the panel to assay organoleptic. To ensure the quality of the assay, well-trained employees of PT CS2 Pola Sehat from operational QC to the managerial level were chosen as panelist. The researchers employed two stages, i.e., scoring test and hedonic interval test following SNI 2346:2006.

Raw material identification and physicochemical properties and anti micro bacterial activity analysis

The HFCS Brix index analysis used hand-held refractometer Atago PAL 3, pH values of RO water, and gum arabic solution utilized pH meter seven Go Melter toledo. Active components of kaffir lime essential oil were analyzed using Fourier Transform Infrared (FT-IR) and Gas Chromatography Mass Spectroscopy Shimadzu type QP 2010s (GC-MS) analyses. All formulation was analyzed by the Refractive index of sugar dissolved with hand-held refractometer and acidity with pH meter seven Go Melter toledo, turbidity with eutech TB1000, TDS with TDSmeter KL731, conductivity with conductimeter PE02, and ensure all formulation was measured.

RESULT AND DISCUSSION

Composition Formulation Calculate

The primary object of the study was the relationship between composition formulation and physicochemical value, and antibacterial activity as the key requirement of potable drink. Therefore, an appropriate formulation design was required to determine the composition variable of each ingredient. The formulation design is a method aiming to minimize the formulation number to achieve an optimal stable condition. Design Expert Ver.10 software acquired ten formulation variables. An organoleptic test was carried out to obtain product characteristics suitable to the customer's preference following the SNI standard of hedonic and preference test. Samples F1, F2, and F4 were selected due to their high scores.

Water as the primary component of emulsion beverage production was used for approximately 800 to 900 mL on each composition variable. The analysis shows that the water sample contained zero iron and chlorine; hence, it was eligible to use. TDS value was low, i.e., under 100, and therefore, the water was potable. The water pH value was normal by 6.65, indicating neutral water [5].

	RO water	KFO	HFCS	GA	Vit E
F1	800 ml	0.125 ml	100 ml	15 gram	0,5 gram
F2	900 ml	0.2 ml	200 ml	10 gram	0,5 gram
F3	900 ml	0.125 ml	200 ml	10 gram	1 gram
F4	800 ml	0.125 ml	100 ml	5 gram	0,5 gram
F5	800 ml	0.5 ml	200 ml	10 gram	0,5 gram
F6	800 ml	0.125 ml	300 ml	10 gram	0,5 gram
F7	800 ml	0.125 ml	200 ml	10 gram	0,5 gram
F8	800 ml	0.2 ml	200 ml	10 gram	1 gram
F9	800 ml	0.125 ml	300 ml	10 gram	1 gram
F10	800 ml	0.125 ml	300 ml	15 gram	0,5 gram

Table 1. Design Expert 10 formulation calculation.

Kaffir lime essential oil increase an aroma and taste additive in the emulsion beverage was added for approximately 0.125 to 0.5 mL. Kaffir lime essential oil addition was much lower than water since excessive addition of kaffir lime essential oil will lead to a bitter taste. Kaffir lime oil also has volatile components, e.g., limonene and citronellal [16], where even a slight addition will deliver kaffir lime aroma and fresh taste received by human sensors [17].

In this study, kaffir lime essential oil (KFO) used as the sample was acquired by distillate a mixture of twigs and kaffir lime leaves at the Essential Oil Institute Field Laboratory of Universitas Brawijaya, Kesamben, Blitar Regency, East Java. citrus hystrix leaves are distilled using the water and steam method up to 4 hours, 2L of kaffir lime oil obtained from the fractional distillation of kaffir lime branch was put into round flask then distilled at low pressure until 5 mbar and ratio reflux 20/10 [15]. The result was a clear yellow solution with a fresh, distinctive lime aroma. GC-MS characterization of kaffir lime essential oil from the Essential Oil Institute of Universitas Brawijaya (warsito) shows three top components being citronella (46.47%), citronellol (12.22%), and citronellyl acetate (6.48%) with a chemical structure as depicted in figures 2 [18].

KFO was characterized using an FT-IR spectrophotometer to discover the constituting

functional groups. The obtained KFO spectrum was compared with the FT-IR spectrum from standard citronellal (Sigma Aldrich). The KFO FT-IR spectrum had absorbance on 1726 (C=O stretching), 2922, and 2874 cm⁻¹ (C-H stretching from aldehyde). Absorbance was also present on 1454 and 1379 cm⁻¹ (C=C stretching) wavelengths illustrated in Figure 1. Comparing the KFO spectrum and standard citronellal spectrum, both spectrums were alike. Thus, it was concluded that the primary constituent component of KFO is citronellal. In determining other components constructing KFO, this study applied an approach by comparing the fragmentation pattern of each component with the substance fragmentation pattern in library WILEY.7 MS with a similarity index $\geq 95\%$. KFO characterization results using GC-MS obtained chromatogram and mass spectra illustrated in Figure 3.

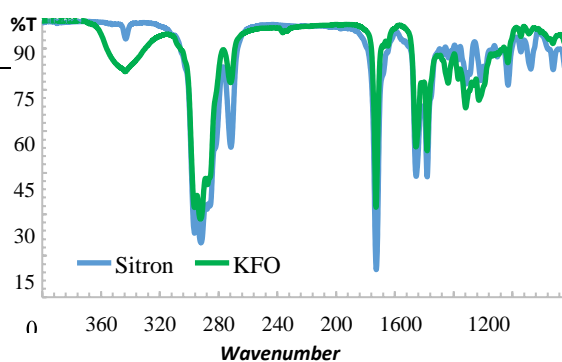


Figure 1 KFO spectrum

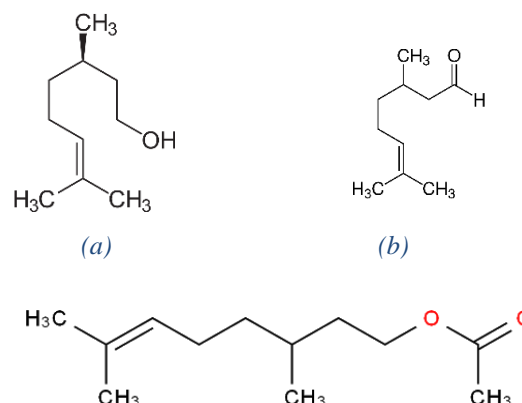


Figure 2 Three top component (a) citronella (b) citronellol (c) Citronellyl acetate

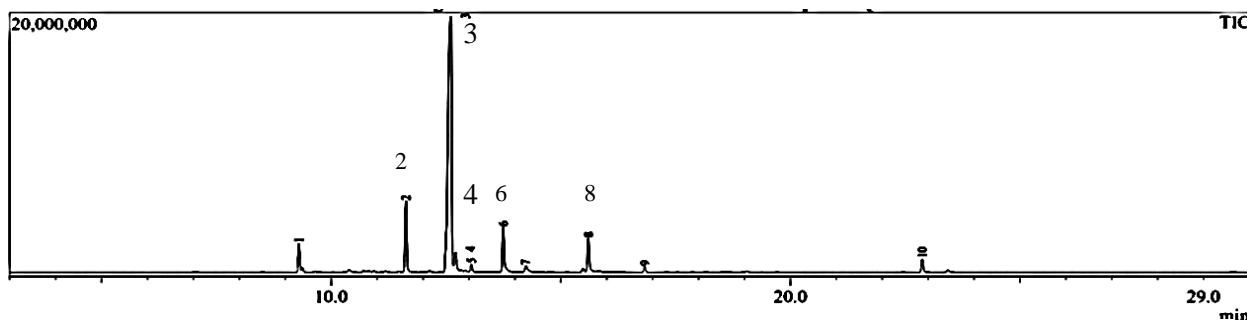


Figure 3 KFO GC-MS chromatogram

Gum arabic acts as the emulsifier and stabilizer of the emulsified beverage to keep its stability during storage. Gum arabic polysaccharide acts as an emulsifier adsorbing the oil phase that creates a thin layer with a high viscosity difference [19]. Gum arabic ability to create the thin layer is affected by the number of protein components covalently bound with carbohydrate molecules. Emulsion droplets containing gum arabic are stable upon flocculation and creaming due to the steric barrier effect generated from polysaccharides protruding towards the continuous emulsion phase, preventing one droplet from meeting other droplets [20].

Gum arabic physicochemical properties and characteristics can be observed in parameters of pH value, conductivity, and different mixture turbidity after adding fructose and gum arabic. Water and HFCS parameters before adding gum arabic and fructose can be observed in the table no 2,3,4. Then, these values changed after adding gum arabic and HFCS components

	GA	HFCS	add Gum Arab*	add HFCS*
F1	15 gram	100 ml	488*	269*
F2	10 gram	200 ml	360*	178,3*
F4	5 gram	100 ml	221*	161,4*

*Conductivity value $\mu\text{S}/\text{cm}$

Table 2. Conductivity value change

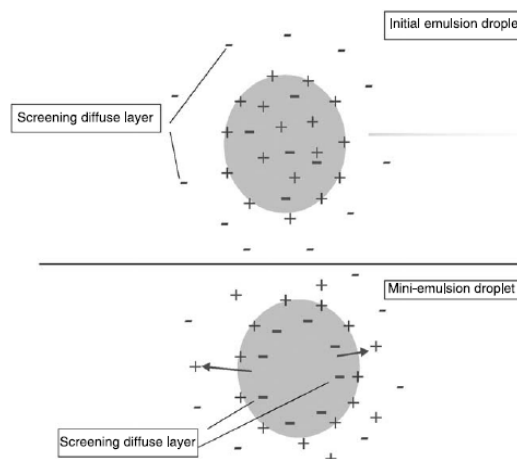


Figure 5. Ion exchange between the inner part and the outer part of emulsion droplets (dickinson,2009)

Physicochemical properties: Conductivity value change on each composition, $\mu\text{S}/\text{cm}$

Beverage manufacture looking a new design product with do not change in visual apperance, and conductivity value may measure potential energy value which can be starting point to determine physicochemical properties of emulsion drink.

Conductivity value change upon adding gum arabic was higher and went lower when fructose was added. When water meets gum arabic, they create charged molecules that transfer electrons from one atom to another atom [9], increasing conductivity by $317.3 \mu\text{S}/\text{cm}$ on average. This may ensue to the presence of a surfactant whose

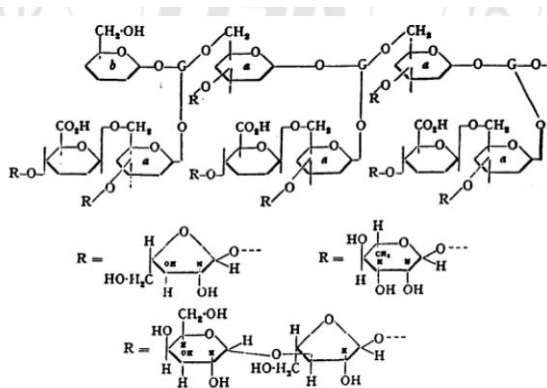


Figure 4 Gum Arab Molecule Structure (william,2009)

specific conductivity is many orders of magnitude below that of the dispersed phase and also the dispersion medium. The surfactant forms a focused layer on the surface of the droplets, which might produce an extra barrier for interphase ion transport [21]

However, adding fructose decreased the conductivity value averagely by 153.4 $\mu\text{S}/\text{cm}$, and it was stable for five storage days in room temperature. Big fructose molecules are hydrolyzed into monomers, reducing the charge from surfactant molecules, which partly bind with fructose monomer [22]

Each emulsion droplet has a surface tension and different pole groups because the emulsion surface is covered by an organic substance layer [20]. A previous study asserted that ions from the diffused layer are partly distributed beyond the organic substance layer and partly into the layer [7]. The ion exchange phenomenon also occurs in the adsorption layer, where electrostatic interaction happens, resulting in information regarding ion-exchange out of the adsorption layer measured in conductivity value [7]. Figure 5 shows ion exchange between the inner part and the outer part of emulsion droplets.

Electrical conductivity is a parameter used to starting point to study the kind of emulsion, determinant section inversion temperature also as identification of potential instability phenomena like flocculation and coalescence [9].

Physicochemical properties: pH value change on each composition

pH value changed from its initial neutral value of 6.95 to -1.0 when gum arabic was added. This pH value reduction may be caused by gum arabic with its anion surfactant properties, reducing pH value [23] Anion property from gum arabic has a negatively charged carboxylic acid [8]. During mixing, the visual observation demonstrated foam generation, identic with the ionic surfactant physical property that creates foam during mixing

	GA	HFCS	add Gum Arab*	add HFCS*
F1	15 gram	100 ml	54,6	20,1
F2	10 gram	200 ml	46,4	41,1
F4	5 gram	100 ml	40,4	38,87

*pH value

Table 3. pH value change

pH analysis for five days also experienced changes, possibly due to the sunlight intensity penetrating the clear cup. The light intensity can

increase the surface temperature of emulsified beverages [24] The second possibility is the changes in room temperature during storage. A study concerning water acidity condition (pH) stated that a relatively low water temperature might increase CO₂ solubility and decrease water pH. Low temperature will also increase DIC (dissolved inorganic carbon) concentration, decreasing water pH [25].

This formulation study acquired an average pH value of 6.3, following the potable water quality parameter that ranges between 6.2 to 8.5 [26] It also falls under the standard pH range of 6 – 8.5 stated in SNI 3553-2006

Physicochemical properties: Turbidity value change on each composition (NTU)

During the mixing process, the physical appearance changed, and the mixture turned turbid. This change is depicted from the turbidity value [27]. Gum arabic addition into water averagely increased turbidity value by 46.9 NTU. However, fructose addition decreased turbidity value by 14.4 NTU because surfactant molecules react with fructose monomers in water, reducing the turbidity value [28] However, visual observation did not reveal this difference. The turbidity value was stable in five days of storage in room temperature with the final value after fructose addition [8]

The results indicate that the turbidity has increased by increasing of the particle sizes, due to rise of emulsifier concentration, which was completely expected . Increase in the turbidity can be attributed to the increase in the particle sizes and the existence of either the light scattering free atocopherol droplets in the aqueous environment, or the acetone in the aqueous phase which increases the system refractive index [29]

Turbidity can be the pre analysis to determine physicochemical stability parameter. Turbidity value is also utilized to determine droplet size distributing in an emulsion system [30]. Turbidity also functions as a reference to discover relative stability. The higher the number of irregular droplet sizes, the higher the turbidity value[24]. A modern analysis to measure turbidity is stated in the Nephelometric Turbidity Unit (NTU). The USEPA drinking water regulation recommends this unit. The analysis can be performed quickly and easily using the

nephelometric method.[5]

	GA	HFCS	add Gum Arab*	add HFCS*
F1	15 gram	100 ml	5,68*	5,64*
F2	10 gram	200 ml	5,93*	5,86*
F4	5 gram	100 ml	6,47*	6,31*

*Turbidity value NTU

Table 4. Turbidity value change

Antibacterial activity

The antibacterial test was conducted on pathogenic bacteria of *E. coli* (Gram-negative). Antibacterial activity was determined by measuring the barrier zone generated surrounding the disc paper. Samples F1, F2, and F4 utilized for the antibacterial activity test had a 100% concentration (b/v) [31]. The negative control employed was chloramphenicol. The barrier zone diameter generated from the antibacterial activity test is presented in the table. All samples had F1, F2, and F4 activities after five-day storage on the *Escherichia coli* bacteria observed from the visual presentation in the figure 6.

The barrier zone diameter was positive on all samples. The barrier zone diameter is observed from the absence of a bacterial colony on each sample surrounding the sample point. The more extensive the clear zone, the stronger the substance to hinder bacterial growth. ALT analysis also shows values of 46 cfu/ml, 72cfu/ml, and 12cfu/ml on each sample. ALT value under 100 cfu/ml has passed the BPOM test following the SNI ISO 4833-1 guideline [32].

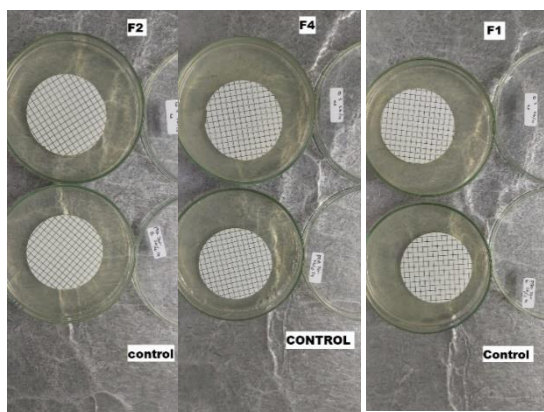


Figure 6. *Escherichia coli* bacteria analysis test results from samples F1, F2, and F4

CONCLUSION

This formulation study obtained a pH value of 6.3 and conductivity of 202.6 $\mu\text{S}/\text{cm}$. This pH value follows the potable water parameter index, ranging from 6 to 8.5 under SNI 3553-2006. The average turbidity of 47.1 NTU and conductivity of 202.6 $\mu\text{S}/\text{cm}$ had a stable eyes visual appearance. Hence, it can be proposed as a decent fruit-scented water product.

Gum arabic plays a pivotal role in the psychochemical characters of emulsified beverages since it changes the turbidity, pH, and conductivity value after addition to the sample. They are determining pre-analysis of physicochemical stable properties.

Turbidity change is caused by the reaction between surfactant molecules and fructose monomers. Gum arabic as the anion surfactant has a negatively charged carboxylic acid RCOO^- (or RCO_2^-) affecting pH value in the composition. Gum arabic and water crates charged molecules that transfer their electrons from one atom to another, increasing and change the conductivity value.

Citronella in kaffir lime essential oil is an active, natural antibacterial substance that hinders bacterial growth and has a barrier power with an alt value under 100 cfu/ml on each formulation.

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