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OIL-IN-WATER (O/W) EMULSIONABLE CONCENTRATE OF ISHPINK (*Ocotea quixos*) WITH THERMODYNAMIC STABILITY[1]

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ABSTRACT - Ecuador has a vast number of native species with fungicidal, herbicidal, and insecticidal properties, of which several have been studied; however, few plant species have been applied for the development of commercial products. *Ocotea quixos* is an indigenous plant of the Ecuadorian Amazon that has fungicidal properties. In this work, we focus on developing an emulsifiable concentrate that is physically stable for use in the agricultural industry. The study aimed to determine the appropriate formulation to prepare an emulsifiable concentrate with thermodynamic stability. For the formulation, we used *Ocotea quixos* essential oil with cinnamaldehyde as an active ingredient, with solvesso 100 as the solvent, two non-ionic emulsifiers (Span-20 and Tween-20), and calcium phenyl sulfonate as an anionic emulsifier to obtain a stable product. The results showed that the OC5C emulsifiable concentrate has the best stability characteristics with the hydrophilic -lipophilic balance (HLB) within the range of 14 to 16 at room temperature as well as at high and low temperature with a drop size between 3 and 4 µm.

Keywords: Oil-in-Water emulsions. HLB value. Essential oil. Phase diagrams.

CONCENTRADO EMULSIONÁVEL ÓLEO-EM-ÁGUA (O/W) DE ISHPINK (*Ocotea quixos*) COM ESTABILIDADE TÉRMICA

RESUMO - O Equador tem um grande número de espécies nativas com propriedades fungicidas, herbicidas e inseticidas, das quais várias foram estudadas, no entanto, muito poucas espécies de plantas têm sido aplicadas no desenvolvimento de produtos comerciais. O *Ocotea quixos*, é uma planta indígena da Amazônia equatoriana, com propriedades fungicidas. Neste trabalho, nos concentramos na elaboração de um concentrado emulsionável que seja fisicamente estável para uso na indústria agrícola. O estudo teve como objetivo determinar a formulação adequada para preparar um concentrado emulsionável com estabilidade termodinâmica. Para a formulação, foi utilizado óleo essencial de *Ocotea quixos* com de cinamaldeído como ingrediente ativo, com solvesso 100 como solvente, dois emulsionantes não iónicos (Span-20, Tween-20) e fenil sulfonato de cálcio como emulsionante aniônico para obter um produto estável. Os resultados mostraram que o concentrado emulsionável OC5C possui as melhores características de estabilidade com o balanço hidrofílico lipofílico (HLB) dentro da faixa de 14 a 16 em temperatura ambiente assim como alta e baixa temperatura com tamanho de gota entre 3 e 4 µm.

Palavras-chave: Emulsões óleo-em-água. Valor de HLB. Óleo essencial. Diagramas de fase.

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INTRODUCTION

Indiscriminate use of several agricultural products has provoked the development of resistance in pathogens against conventional products (LUCAS et al., 2015). The potential to develop pesticides using essential oils obtained from the plants with *Litsea pungens* has been exploited only recently (ISMAN et al., 2011), and there are only a few products manufactured with essential oils that are being used as agrochemicals.

Other researchers have reported that the vast majority of essential compounds extracted from plants show antibacterial and antifungal activity; therefore, it is of great scientific interest to evaluate the performance of these compounds (BRICEÑO et al., 2011).

The genus *Ocotea* has been studied in the last decade for its high content of alkaloids, lignans, and terpenes, among others, in the essential oil extracts (CHAVERRI et al., 2011), with anti-inflammatory activity (BALLABENI et al., 2010) and antibacterial and antifungal properties (BRUNI et al., 2004; GUERRINI et al., 2006; SCALVENZI et al., 2016). In contrast, Noriega and Dacarro (2008) identified that 12.5 $\mu l.ml^{-1}$ of *Ocotea* essential oil inhibits the growth of *Candida albicans* and *Escherichia coli*, whereas a lower concentration of 6.25 $\mu l.ml^{-1}$ inhibits the growth of *Staphylococcus* species (*S. epidermidis*, *S. aureus*, *S. pyogenes,* and *S. mutans*). However, Noriega et al. (2018) identified that essential oil from *Ocotea quixos* has 30.69% of cinnamaldehyde with high antimicrobial and antifungal activity, along with antioxidant activity. Cinnamaldehyde consists of a benzene ring, an ethane group, and an aldehyde group and presents broad-spectrum antimicrobial activity against bacteria, yeasts, and molds (OTONI et al., 2014; SHEN et al., 2015). However, at present, no essential oil has been used to develop a commercial product that is environment-friendly.

Emulsifiable concentrates are typically optically transparent oily liquid formulations that are prepared by dissolving the active ingredient in organic solvents (such as benzene, toluene, xylene and other), which may also contain surfactants and other additives. These systems are then diluted with water prior to utilization, which leads to spontaneous formation of an oil-in-water emulsion that contains the active ingredients inside oil droplets (FENG et al., 2018a). The stability of emulsions may be affected by several factors such as the Hydrophile-Lipophile Balance (HLB) (LOSADA-BARREIRO et al. 2013), concentration of active ingredients (HALLOUARD et al. 2015), and addition of surfactant type (FENG et al. 2016), among others (FENG et al., 2018b).

Due to the importance of promoting the manufacture, distribution, and use of pesticides that meet the basic quality requirements, we used the technical specifications by (Food and Agriculture Organization of the United Nations/World Health Organization) FAO/WHO (2016) and (Collaborative International Pesticides Analytical Council) CIPAC (2016) as a reference. The CIPAC Standards contain pesticide analysis methods and physical chemical methods that are accepted worldwide. This allowed standardization of the methodology to approve a product for agricultural usage.

This study adopted a low-cost method to prepare emulsions loaded with *Ocotea* essential oil (cinnamaldehyde). The main objective was to investigate the optimal conditions for preparing emulsions using the essential oil of *Ocotea* using a mixture of different surfactants. Additionally, we evaluated the thermodynamic stability, droplet size of the emulsifiable concentrates. Finally, the best formulation of the O/W emulsifier with thermodynamic stability was determined. Furthermore, the present work can help develop stable emulsions with different essential oils that have beneficial properties for crops.

MATERIAL AND METHODS

Ishpink Essential Oil (*Ocotea Quixos*)

The essential oil of the Ishpink plant (*Ocotea quixos*) was provided by the CHANKUAP Macas-Ecuador Foundation (2017).

Emulsifiable concentrates preparation from *Ocotea quixos*

The emulsifiable concentrates were prepared following the formulation reported by KNOWLES, (1998), combining 10% *Ocotea* oil, 20% surfactants, and 70% solvent (Solvesso 100). To prepare one liter of the sample, 100 g of the *Ocotea quixos* oil was weighed and added to different concentrations of 3 surfactants (Tween 20 "sorbitan monolaurate PEG20", Span 20 "sorbitan monolaurate", and calcium phenyl sulfonate), the solvent was added to make up the complete volume to one liter. The hydrophilic-lipophilic balance (HLB) was established experimentally (O´LENICK, 2014). The emulsion and re-emulsion stability tests were performed according to CIPAC MT 36.3. (CIPAC, 2016), recording traces of cream at 2 hours and re-emulsification at 24 hours according to the regulations (APVMA, 2005). For quality tests, only the most stable mixtures were chosen, with HLB between 14 and 16. A ternary diagram was prepared from the formulation data using the ProSim Ternary Diagram software (ProSim S.A. Labége, France).

Determination of physical appearance, potential of hydrogen (pH), emulsion stability, and foam

persistence of the emulsifiable concentrates upon aging

To determine the stability of the emulsifiable concentrate over long periods of storage, it is necessary to evaluate its characteristics at room temperature (25°C ± 2) and at high temperature (54° C ± 2) for 14 days (aging test) following the CIPAC MT 46.3. (CIPAC, 2016). For samples at ambient temperature and those that were subjected to aging, we carried out the following tests: 1) Assessment of the physical appearance of the product by documenting the color and phase separation and taking the yellow color with translucent tone and without phase separation as appropriate parameters. 2) Potential of hydrogen (pH) was determined by diluting the concentrate to 1% v/v following the methodology described in CIPAC MT 75.3. (CIPAC, 2016). 3) Emulsion stability was determined according to CIPAC MT 36.3. (CIPAC, 2016) by recording traces of oil and cream < 2 mL at 0, 0.5, 1, 2, 24, and 24.5 h according to regulations (APVMA, 2005). 4). Foam persistence was determined for each of the concentrates at 0, 10 s, and 1, 3, and 12 min according to CIPAC MT 47.2. (CIPAC, 2016).

Qualitative analysis of the emulsifiable concentrates by infrared spectrometry (IR), determination of droplet size, and stability at low temperatures

Qualitative analysis was performed for the sample at room temperature and samples subjected to the aging process, by infrared spectrometry in a spectrophotometer (Perkin Elmer Spectrum Version 10.03.07.) with scanning between 450 and 4000 cm^{-1}. The droplet size was determined using a microscope. We assumed that the most appropriate drop size was ≤ 4 µm. Finally, to evaluate stability at low temperatures, the samples were placed in an environment with temperatures 0°C ± 2 for 7 days, and the presence of crystallization and phase separation was recorded following the CIPAC MT 39.3 standard. (CIPAC, 2016).

RESULTS AND DISCUSSION

Emulsifiable concentrates from *Ocotea quixos*

The hydrophilic-lipophilic balance (HLB) value of a surfactant plays a large role in determining its functionality. Emulsions with surfactants of HLB < 6 tend to be oil soluble and stabilize water-in-oil, whereas emulsions with surfactants of HLB > 10 tend to be water soluble and stabilize oil-in-water (FENG et al., 2018a). The samples OC4C and OC4D had an HLB of 15.6, whereas the samples OC5C and OC5D had a HLB of 14.6 (Table 1). From the formulation of concentrates, a ternary diagram was obtained in which the HLB position of the 4 emulsifiable concentrates was shown (Figure 1). The emulsions show HLB values higher than 10, suggesting that the emulsions dissolve in water, thus decreasing the cost of application.

Table 1. Emulsifiable concentrates selected.

Raw Material	OC4C ($g.L^{-1}$)	OC4D ($g.L^{-1}$)	OC5C ($g.L^{-1}$)	OC5D ($g.L^{-1}$)
Ocotea quixos oil	100	100	100	100
Tween 20	120.4	86	103.6	74
Span 20	19.6	14	36.4	26
Phenyl sulfonate calcium	60	100	60	100
Solvesso 100	Up 1L	Up 1L	Up 1L	Up 1L
Referential HLB	15.6	15.6	14.6	14.6
Up = Up to 1 L				

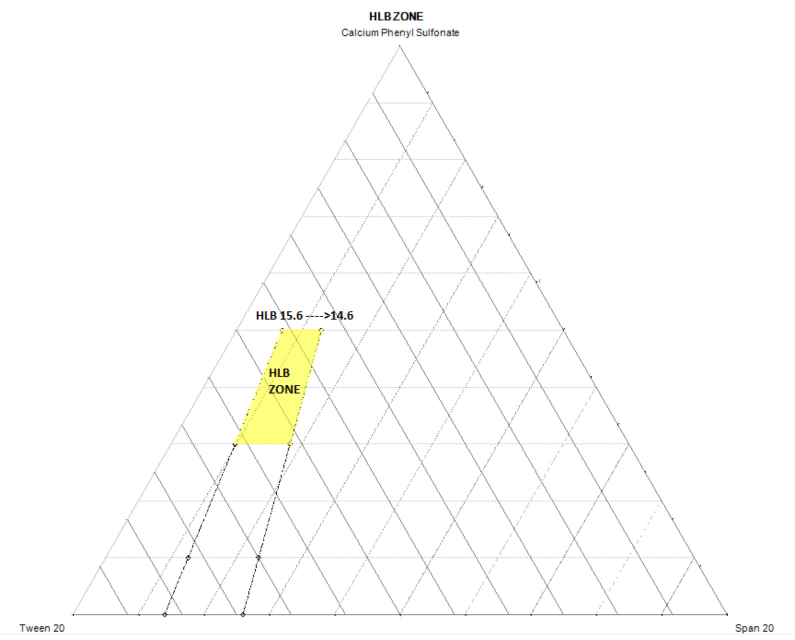

Figure 1. A ternary phases diagram displaying the proportion of HLB variables. HLB Zone is indicated in yellow.

Determination of physical appearance, potential of hydrogen (pH), emulsion stability and foam persistence of the emulsifiable concentrates upon aging

Emulsifiable concentrates are typically optically transparent oily liquid formulations that are prepared by dissolving a certain amount of active ingredient (essential oil or pesticide) in organic solvents, which may also contain surfactants and other additives (FENG et al., 2018a). The 4 emulsifiable concentrates were subjected to a temperature of 54 ± 1°C for 14 days (aging). The physical appearance of the EC showed no differences in color, hue, and phase separation in the concentrates at room temperature and with aging (Table 2). All emulsions exhibited excellent stability after being subjected to the aging treatment, maintaining the yellow coloration and translucent shade; likewise, no phase separation was observed at both room temperature and after the aging treatment. These results were similar to those reported by Shao et al. (2018) when evaluating the stability of a microemulsion with the active ingredient norcantharidin for controlling *P. xylostella*.

Table 2. Physical appearance of the formulation after the period of aging.

		Temperature (25 ± 1°C)			
Test Code		OC4C	OC4D	OC5C	OC5D
Appearance of the formulation	Color	Yellow	Yellow	Yellow	Yellow
	Tonality	Translucent	Translucent	Translucent	Translucent
	Phase Separation	no	no	no	no
Hydrogen Potential	pH	6.29	6.26	6.28	6.39
		Accelerated Storage (54 ± 1ºC, 14 days)			
Test Code		OC4C	OC4D	OC5C	OC5D
Appearance of the formulation	Color	Yellow	Yellow	Yellow	Yellow
	Tonality	Translucent	Translucent	Translucent	Translucent
	Phase Separation	no	no	no	no
Hydrogen Potential	pH	6.48	6.51	6.56	6.51

Potential of hydrogen (pH) can indicate the stability of the EC in long periods of storage. The change in pH over long storage periods or in the aging treatment can represent degradation of the active component, bacterial proliferation, instability, or incompatibility of some components; however, the samples upon aging showed an increase in pH, wherein the sample OC4C showed pH 6.48 as the lowest and the sample OC5C showed pH 6.56 as the highest (Table 2) remaining within the acceptable range of changes. The emulsifiable concentrates showed pH values close to 7.

The stability of emulsifiability with different levels of water hardness is an important factor for a product of agricultural use due to the type of water hardness present in different agricultural regions. Some agricultural regions use groundwater that often has significant levels of hardness, but some surface water supplies also have the same issue. Calcium concentrations up to and exceeding 100 mg. L^{-1} are common with groundwater. In contrast, magnesium usually occurs at lower concentrations compared to calcium in groundwater (about 50 mg. L^{-1} and rarely about 100 mg. L^{-1}), and calcium-based hardness usually predominates (WHO, 2011). Considering its importance, water of different hardness was used, called waters CIPAC A and D, and the characteristics of emulsions were examined as indicated in the CIPAC MT 36.3 standard (Table 3).

Addition of water may cause redistribution of surfactant throughout the system, wherein part of the surfactant diffuses from the interface to the aqueous phase, leading to a decline in the association of the essential oil, surfactant, and solvent, resulting in an increase in particles (WANG et al., 2015). For this reason, is important to identify the appropriated bloom (rapid and complete emulsification in water with minimal agitation (HILL, 2009)) and cream formation.

All the emulsions presented appropriated bloom, formed milky bluish emulsions, and did not show cream formation until the time of 1 h. At 2 h, they showed traces of cream. After 24.5 h, re-emulsions of emulsions formed with the OC4C and OC5C samples at room temperature were shown to be stable. Emulsions formed with the OC4D and OC5D samples subjected to aging were unstable with cream formation, which was similar to those observed at with emulsions of samples that were at room temperature (Table 3). Emulsifiable concentrates when exposed to high temperatures may exhibit accelerated degradation and physical and chemical changes, affecting the emulsifiability capacity (KREILGAARD, 2002; LAWRENCE; REES 2012).

Table 3. Emulsion characteristics of emulsifiable concentrates (CIPAC MT 36.3).

	Temperature (25 ± 1°C)								Accelerated Storage (54 ± 1ºC, 14 days)							
Test Code	OC4C		OC4D		OC5C		OC5D		OC4C		OC4D		OC5C		OC5D	
Type of water hardness	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D
Bloom	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Emulsionability 0 h	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA
Emulsionability 0.5 h	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA
Emulsionability 1 h	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	SC LA	TC LA	TC LA	SC LA	SC LA
Emulsionability 2 h	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA	TC LA
Emulsionability 24 h	C LA	C LA	C LA	C LA	C LA	C LA	C LA	C LA	C LA	C LA	C LA	C LA	C LA	C LA	C LA	C LA
Emulsionability 24.5 h	SC LA	SC LA	C LA	C LA	SC LA	SC LA	C LA	C LA	SC LA	SC LA	C LA	C LA	SC LA	SC LA	C LA	C LA

B: Bloom, SC: emulsion without cream, LA: Bluish Milky Emulsion, TC: Emulsion with Traces of Cream, C: Emulsion with Cream < 2 mL.

In the analysis of foam persistence, 1) for samples maintained at room temperature, the OC4C concentrate presented 20 mL foam which was decreased until 12 minutes of evaluation, where 2.5 mL foam was observed. However, OC4D, OC5C, and OC5D presented 4 mL foam which was reduced to 0 mL of foam, where the OC5D concentrate did not show any foam at 12 min. In contrast, the concentrate OC4D did not present foam after 3 min. Finally, the OC5C concentrate ceased to present foam after 1 min (Table 4). 2) For samples maintained at high temperature (aging 54 ± 1°C): the concentrates presented 10 mL of foam which was decreased. The OC4D and OC5C concentrates presented similar behavior by decreasing the foam up to the time of 12 min with 3.5 mL foam. In contrast, the OC5D formulation decreased the foam to the time of 12 min with 2.1 mL foam. Finally, the OC4C formulation decreased the foam to the time of 12 min with 0.5 mL of foam (Table 4).

Table 4. Determination of foaming (CIPAC MT 47.2).

	Temperature (25 ± 1°C) (mL)				Accelerated Storage (54 ± 1ºC, 14 days)			
Test Code	OC4C	OC4D	OC5C	OC5D	OC4C	OC4D	OC5C	OC5D
0 s	20	4	4	4	10	10	10	10
10 ± 1 s	16	2.5	1.5	1.5	6	6	6	6
1 ± 0.1 min	13	1.5	0	1.5	5	5	5	4
3 ± 0.1 min	10	0	0	1.5	4	4	4	2.5
12 ± 0.1 min	2.5	0	0	0	0.5	3.5	3.5	21

The emulsion stability, physical appearance, potential of hydrogen (pH), and the foam persistence are determining factors for the selection of an emulsifiable concentrate as these factors can affect the drop size, durability, activity of the active ingredient, and production costs, among others. The four emulsifiable concentrates showed good characteristics of stability at room temperature and during the aging treatment; however, the concentrate OC5C showed better characteristics of emulsifiability and foam persistence, followed by the concentrate OC4C.

Qualitative analysis by infrared (IR) spectrometry, stability of the concentrate at low temperatures, and determination of droplet size of the emulsion concentrates.

During storage, the emulsifiable concentrates are exposed to different changes in temperature, ranging from high (in summer) to low (in winter) and can be stored for long times. This can generate changes in the composition of the formulation. For this reason, the concentrates at room temperature and upon aging treatment were evaluated by infrared spectrometry (IR) to determine whether the composition of emulsifiable concentrates is altered in these conditions. The four emulsifiable concentrates do not show changes in their composition during long periods of storage (aging treatment) with correlations of 0.99 in each of the concentrates when compared between room temperature and storage (aging treatment) (Figure 2).

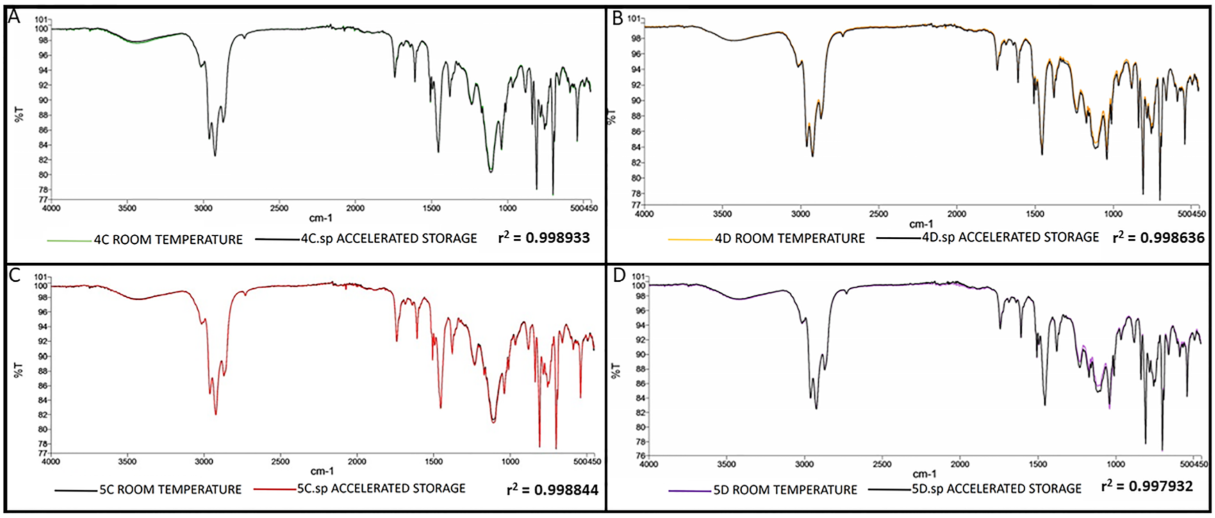

Figure 2. Comparison of emulsifiable concentrates A) OC4C, B) OC4D, C) OC5C, and D) OC5D by infrared spectrometry (Perkin Elmer Spectrum Infrared Spectrum Version 10.03.07) between EC at room temperature (25 ± 1°C) and EC accelerated storage (54 ± 1°C, 14 days).

The emulsifiable concentrates can present problems at low temperature due to non-ionic surfactants composed of a number of oxygen containing groups, whose solubility in water decreases with decreasing temperature due to dehydration of their polar head groups. A surfactant solution may become turbid when a certain temperature, called the cloud point is reached (LINDMAN et al., 2016; BATIGÖÇ; AKBAŞ, 2017; FENG et al., 2018a). This phenomenon is reversible; the solution again becomes transparent and homogeneous after heating. In our study, the emulsifiable concentrates were subjected to 0ºC without observing crystallization or phase separation (Table 5).

Table 5. Stability of emulsifiable concentrates (EC) at 0ºC (CIPAC MT 39.3).

		Temperature (0 ± 1°C, 7 days)			
Test Code		OC 4C	OC 4D	OC 5C	OC 5D
Appearance of the formulated	Phase Separation	No	No	No	No
	Crystallization	No	No	No	No

The droplet size of the emulsifier helps determine the type of the emulsifier; the emulsifiable concentrates (ECs) have droplet sizes close to molecular dimensions whereas the oil-in-water (O/W) emulsions have dimensions close to 2-5 µm. In contrast, microemulsions (MEs) have dimensions ranging from 10-100 nm, and nanoemulsions have dimensions ranging from 20-500 nm droplet size (FENG et al., 2018a). In our study, OC4C and OC5C concentrates at room temperature showed a drop size of 3 µm whereas OC4D and OC5D concentrates have a drop size > 4.5 µm (Table 6). This may be a result of essential oils tending to produce emulsions with small droplet sizes because of their low viscosity and interfacial tension (QIAN; McCLEMENTS, 2011). On the contrary, addition of an emulsifier with higher viscosity prior to homogenization could decrease the size of the oil droplets produced by increasing the disruptive shear stresses generated within the homogenizer (QIAN; McCLEMENTS, 2011). However, Lu et al. (2018) determined that nanoemulsions with citral essential oil, and an HLB of 2 showed a droplet size of 410 nm whereas with the HLB value increased to 12, the droplet was the smallest at 28 nm. On the other hand, FENG et al., (2018b) determined that the mean droplet size increased from around 0.44 to 4.27 µm together with the HLB value of the surfactants increasing from 10.5 to 15.5 when evaluating the oil-in-water emulsions with lambda-cyhalothrin and different types of surfactants. Finally, the size of the droplets produced depends on system composition, mixing order, agitation rate, stored time, dropping rate, temperature, and other environmental factors (PERAZZO et al., 2015).

High temperature and long storage time can result in 5%-10% reduction in the concentration of the active component due to degradation (FENG et al., 2018a). For this reason, we determined the droplet size in the concentrate under aging treatment. The concentrates OC4C, OC4D, and OC5D showed a droplet size > 4.5 µm, whereas the OC5C concentrate had a droplet size < 4.5 µm (Table 6). These results suggest that the OC5C concentrate did not present a major change in droplet size and was maintained in the range of oil-in-water emulsifiers.

Table 6. Determination of drop size.

	Temperature (25 ± 1°C)				Accelerated Storage (54 ± 1ºC, 14 days)			
Test Code	OC4C	OC4D	OC5C	OC5D	OC4C	OC4D	OC5C	OC5D
Drop size (µm)	3.04	5.3	3	4.8	4.7	5.9	4	5.5

The present work used the essential oil of *Ocotea* for formulating emulsifiable concentrates. Noriega et al. (2018) determined that the essential oil of *Ocotea* with 30.69% cinnamaldehyde has high antimicrobial and antifungal activity. On the other hand, JARDIM et al., (2017) determined that emulsions of the essential oil of *Cinnamomum cassia* with 83.3% cinnamaldehyde show nematicidal activity without phytotoxicity in soybean plants. This suggests that the emulsifiable concentrates of *Ocotea* possess antimicrobial and antifungal activity, without presenting phytotoxicity. FENG et al., (2018a) described the typical characteristics of emulsifiable concentrates as follows: transparent appearance, a droplet size of molecular dimensions, thermodynamic stability, no water content, high emulsifier dosage, and high production costs. Considering these characteristics in our study, we determined that the four emulsifiable concentrates satisfy the characteristics of thermodynamic stability, but showed translucent appearance, a droplet size in the range 3–6 µm, medium water content, and low production costs. This suggests that the concentrates have many characteristics of oil-in-water (O/W) emulsions as described by FENG et al., (2018a). However, O/W emulsions have the problem the thermodynamic stability according to FENG et al., (2018a); this problem was not observed in our concentrate. This indicated that the concentrates have the best characteristics of emulsifiable concentrates and O/W emulsions.

CONCLUSION

This study showed that the four emulsifiable concentrates satisfy the APVMA (2005) standards. The most stable emulsion, OC5C, was obtained using two non-ionic surfactants, Span-20 (36.4 g. L^{-1}) and Tween-20 (103.6 g. L^{-1}), and an anionic surfactant calcium phenyl sulfonate (60 g. L^{-1}) with 14.6 HLB. The characteristics of the concentrate OC5C indicate that the concentrate is an O/W emulsifier with a droplet size of 3 µm and pH 6.28 at room temperature. On the other hand, the concentrate OC5C showed thermodynamic stability without presenting changes in composition, pH, and droplet size. The findings of these studies should facilitate the formulation of more stable and effective emulsions using different essential oils that have beneficial properties for crops.

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REFERENCES

AUSTRALIAN PESTICIDES AND VETERINARY MEDICINES AUTHORITY - APVMA. **Guidelines for generation of storage stability data for agricultural chemical products**. Disponível em: <https://apvma.gov.au/node/1042> Access in: 25 jun. 2017.

BALLABENI, V. et al. Ocotea quixos Lam. essential oil: in vitro and in vivo investigation on its anti-inflammatory properties. **Fitoterapia**, v. 81 n. 4, p. 289-295, 2010.

BATIGÖÇ, Ç.; AKBAŞ, H. Thermodynamic parameters of clouding phenomenon in nonionic surfactants: the effect of the electrolytes. **Journal of Molecular Liquids**, v. 231, s/n., p. 509-513, 2017.

BRICEÑO, G. et al. Efecto de extractos etanólicos de ruda y neem sobre el control de bacterias fitopatógenas del género “Erwinia 1”. **Agronomía Tropical**, v. 61, n. 2, p. 141-148, 2011.

BRUNI, R. et al. Chemical composition and biological activities of ishpingo essential oil, and traditional Ecuadorian spice from Ocotea quixos (Lam.) Kosterm. (Lauraceae) flower calices. **Food Chemistry**, v. 85, n. 3, p. 415-421, 2004.

CHANKUAP, F. **La Fundación Chankua**. Disponível em: <http://chankuap.org/wp-content/uploads/2014/02/7890.FICHA-TECNICA-AE-ISHPINK.pdf>. Access in: 25 jun. 2017.

CHAVERRI, C. et al. Chemical analysis of essential oils from Ocotea gomezzi W. C. Burger and Ocotea morae Gómez-Laur. (Lauraceae) collected at “Reserva Biológica Alberto M. Brenes” in Costa Rica and their Cytotoxic Activity on tumor cell lines. **Journal of the Brazilian Chemical Society**, v. 22, n. 4, p. 741-745, 2011.

CIPAC, C. I. **Collaborative International Pesticides Analytical Council**. Disponível em: <http://www.cipac.org/>. Acesso em: 1 jun. 2016.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS/WORLD HEALTH ORGANIZATION - FAO/WHO. **Manual on development and use of FAO and WHO specifications for pesticides 2016**. 1. ed. 3 re. WHO and FAO, Roma, 2016. cap. 7, p. 137-152.

FENG, J. et al. Application of nanoemulsions in formulation of pesticides. In: JAFARI, S. M.; McCLEMENTS, D. J. (Eds.). **Nanoemulsions (Formulation, applications, and characterization). Part 4. Application of nanoemulsions**. Academic Press. Elsevier Inc. India, 2018a. cap. 12, p. 379-413.

FENG, J. et al. Formulation of oil-in-water emulsions for pesticide applications: impact of surfactant type and concentration on physical stability. **Environmental Science and Pollution Research**, v. 25, n. 22, p. 21742-21751, 2018b.

FENG, J. et al. Effect of emulsifying process on stability of pesticide nanoemulsions. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 497, s/n., p. 286–292, 2016.

GUERRINI, A. et al. Composition of the volatile fraction of Ocotea bofo Kunth (Lauraceae) calyces by GC MS and NMR fingerprinting and its antimicrobial and antioxidant activity. **Journal of Agricultural and Food Chemistry**, v. 54, n. 20, p. 7778-7788, 2006.

HALLOUARD, F. et al. Preparation and characterization of spironolactone-loaded nano-emulsions for extemporaneous applications. **International Journal of Pharmaceutics**, v. 478, n. 1, p. 193–201, 2015.

HILL, R. L. Detergents in Agrochemical and Pesticide Applications. In: ZOLLER, U. (Ed.). **Handbook of Detergents. Part E: applications. CRC Press**. Taylor & Francis Group, Boca Raton, London, New York, 2009. v. 141, cap. 12, p. 313.

ISMAN, M. B. et al. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. **Phytochemistry Reviews**, v. 10, n. 2, p. 197-204, 2011.

JARDIM, I.N. et al. (E)-cinnamaldehyde from the essential oil of Cinnamomum cassia controls Meloidegyne incognita in soybean plants. **Journal of Pest Science**, v. 91, n. 1, p. 479-487, 2017.

KNOWLES, D. A. Formulation of agrochemicals. In: KNOWLES, D. A. (Ed.). **Chemistry and Technology of Agrochemical Formulations**, Springer Science+Business Media, B.V., Tombridge, Kent, UK, 1998. cap. 3, p. 42-48.

KREILGAARD M. Influence of microemulsions on cutaneous drug delivery. **Advanced Drug Delivery Reviews**, v. 54, n. 1, p. 77-98, 2002.

LAWRENCE, M. J.; REES, G. D. Microemulsion-based media as novel drug delivery systems. **Advanced Drug Delivery Reviews**, v. 64, n. 1-6, p. 175-193, 2012.

LINDMAN, B. et al. Clouding of nonionic surfactants. **Current Opinion in Colloid and Interface Science**, v.22, s/n., p. 23-29, 2016.

LOSADA-BARREIRO, S. et al. Effects of emulsifier hydrophile–lipophile balance and emulsifier concentration on the distributions of gallic acid, propyl gallate, and α-tocopherol in corn oil emulsions. **Journal of Colloid and Interface Science**, v. 389, n. 1, p. 1–9, 2013.

LU, W. C. et al. Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. **Journal of Food and Drug Analysis**, v. 26, n. 1, p. 82-89, 2018.

LUCAS, J. A. et al. Chapter Two - The evolution of fungicide resistance. In: SARIASLANI, S.; GADD, G. M. (Eds.). **Advances in Applied Microbiology**. Academic Press. Elsevier Inc. India, 2015. v. 90, p. 29-92.

NORIEGA, P.; DACARRO, C. Aceite Foliar de Ocotea quixos (Lam.) Kosterm.: Actividad antimicrobiana y antifúngica. **La Granja**, v. 7, n. 1, p. 3-8, 2008.

NORIEGA, P. et al. Antimicrobial and Antioxidant bioautography activity of bark essential oil from Ocotea quixos (Lam.) Kosterm. **Journal of Planar Chromatography-Modern TLC**, v. 31, n. 2, p. 163-168, 2018.

O´LENICK, A. J. Chapter 3 - Anionic Surfactants; Chapter 5 – Nonionic Surfactants In; O´LENICK, A. J. (Eds.). **Surfactants: Strategic Personal Care Ingredients**. Allured Publishing Corporation. Illinois, USA, 2014. cap. 3-5, p. 43-178.

OTONI, C. G. et al. Antimicrobial and physical-mechanical properties of pectin/papaya/puree/ cinnamaldehyde nanoemulsion edible composite films. **Food Hydrocolloids**, v. 41, s/n., p. 188-194, 2014.

PERAZZO, A. et al. Phase inversion emulsification: current understanding and applications. **Advances in Colloid and Interface Science**, v. 222, s/n., p. 581-599. 2015.

QIAN, C.; McCLEMENTS, D. J. Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. **Food Hydrocolloids**, v. 25, n. 5, p. 1000-1008, 2011.

SCALVENZI, L. et al. Actividad Antifúngica in vitro de aceites esenciales de Ocotea quixos. **Bioagro**, v. 28, n. 1, p. 39-46, 2016.

SHAO, H. et al. Microemulsion formulation of a new biopesticide to control the diamondback moth (Lepidoptera: Plutellidae). **Scientific Reports**, v. 8, n. 10565, p. 1-9,2018.

SHEN, S. et al. Effects of cinnamaldehyde on Escherichia coli and Staphylococcus aureus membrane. **Food Control**, v. 47, s/n., p. 196-202, 2015.

WANG, W. et al. Formation and characterization of fully dilutable microemulsion with fatty acid methyl esters as oil phase. **ACS Sustainable Chemistry & Engineering**, v. 3, n. 3, p. 443-450, 2015.

WORLD HEALTH ORGANIZATION - WHO. **Hardness in Drinking-water**. Background document for development of WHO Guidelines for Drinking-water Quality, Washington, DC, USA, 2011.