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Contribution of Free Fatty Acids to Emulsion Building and Stability: Understanding the Mechanism From the Case Study of a Crude Red Palm Oil (*Elaeis guineensis*)—Based Emulsion Consumed in Africa and Known as Achu Soup/Sauce Jaune/Ncha

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ABSTRACT

In edible emulsions, residual free fatty acids from oils are the primary drivers of lipid oxidation which adversely affects appearance, sensory properties and nutritional quality. These free fatty acids can be significantly reduced if converted into emulsifiers during emulsion preparation. This study helps to understand the mechanism of what happens during *Achu Soup/Sauce Jaune/Ncha* preparation, a traditional African emulsion prepared from crude red palm oil (*Elaeis guineensis*) and an alkaline solution without added emulsifiers. The effect of oil type (canola oil, olive oil and crude red palm oil), the nature of solutions used (alkaline and non-alkaline solutions) and the successive refining steps of crude red palm oil (deacidification, degumming and bleaching) were evaluated. Oils and solutions used were characterised (chemical composition and acid value for oils; pH, anions and cations content for constituents used to prepare solutions). Results demonstrate that emulsion formation with crude red palm oil in the presence of an alkaline solution occurs via mild saponification between solution alkalinity and oil free fatty acids. Free fatty acids are a major contributor to both emulsion formation and physical stability. For the same proportion of oil and water, the higher the alkalinity of a solution, the higher the macroscopic homogeneity of the resulting emulsion. For the same alkaline solution, the higher the free fatty acids content of the oil, the higher the macroscopic homogeneity of the resulting emulsion.

1 | Introduction

Emulsions are complex systems comprising at least 2 immiscible phases, one dispersed in the other (Ghelichi et al. 2023). They are widely used in food (chocolate, butter, margarine, mayonnaise, dairy products, seafood, ice cream, whipped cream, etc.), construction (paints), cosmetics and pharmaceuticals (to encapsulate, protect and deliver bioactive compounds; Bao and Pignitter 2023; Ghelichi et al. 2023; Waraho 2011; Watanabe et al. 2017). In food science, the most common emulsions are oil-in-water (o/w) systems prepared from edible oil (palm oil mostly), water and an emulsifier that reduces interfacial tension and stabilises the dispersion. Because the phases are immiscible, emulsions are thermodynamically unstable and prone to

Abbreviations: HLB, Hydrophilic Lipophilic Balance; O/W, Oil-in-Water emulsion; TAS, Traditional Alkaline Salts; W/O, Water-in-Oil emulsion.

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physical instability mechanisms including flocculation, sedimentation/creaming, Ostwald ripening and coalescence (Khan et al. 2011; Marquez et al. 2018). These can be minimised by selecting an appropriate emulsifier (based on Hydrophilic/Lipophilic Balance, HLB), by increasing the viscosity of the continuous phase through the addition of high molecular weight components, using the appropriate oil/water ratio, optimising the emulsifier concentration, stirring intensity/speed and mixing duration used during emulsion preparation (Khan et al. 2011). If these different mechanisms which lead to physical instability of emulsions can be minimised, it is more difficult to control the chemical instability of emulsions. The latter is associated with the presence of fat and is known as lipid oxidation (Bao and Pignitter 2023; Ghelichi et al. 2023; Waraho 2011). In the case of food emulsions, lipid oxidation is more pronounced, faster and complex than physical stability and has negative effects on the appearance, sensory and nutritional characteristics of prepared emulsions (Bao and Pignitter 2023). Its control is more difficult because it is associated with specific characteristics of the oils used. The factors known to affect lipid oxidation are the presence of transition metals, the presence of enzymes (lipases, lipoxigenases) and the fatty acid composition of the oil (Bao and Pignitter 2023; Waraho et al. 2011). The refining process of edible oil generally eliminates enzymes through heating and significantly reduces the levels of heavy metals and free fatty acids (Gharby 2022; Keurentjes et al. 1990) all of which are major pro-oxidants responsible for oxidative deterioration in emulsions. Nevertheless, even refined commercial oils still contain 0.05%–0.70% of free fatty acids (Waraho et al. 2011) and their presence markedly increases the susceptibility of emulsions to lipid oxidation. One effective strategy to improve the oxidative (chemical) stability of emulsions can be done by eliminating or reducing these residual free fatty acids. One possible way to accomplish this can be by allowing these residual free fatty acids to contribute to emulsion building and stability through their transformation into emulsifiers. This can be achieved by converting them into emulsifiers during the emulsification process itself. This seems to occur during the preparation of *Achu Soup*/*Ncha*, an African preparation (emulsion) made from crude red palm oil (*Elaeis guineensis*), water and an alkaline solution.

In Nigeria, this emulsion is known as *Ncha* and it is used in the preparation of a salad known as *Abacha* (Ene-Obong et al. 2013). In Cameroon, the same emulsion often enriched with spices and dried beef skin, meat and/or fishes is known as *Achu Soup* or *Sauce Jaune*, originated from Western highland area of the country and eaten nationwide with pounded taro (*Achu* or *Taro*; Deli et al. 2018; Ngwasiri Ndasi, Ekuh Ikongefuze, et al. 2021; Njintang et al. 2006). For the preparation of this meal in Africa, traditional Alkaline Salts (TAS) are used. This term is used to characterise naturally occurring rocks (lake deposits) and plant-based salts (plant-based ashes, their filtrates and evaporites of these filtrates) which are widely used in food preparation across Africa, Asia, South America and Oceania (Ankrah and Dovlo 1978; Avallone et al. 2008; Bergeson et al. 2016; Doumta and Tchiégang 2012; Ekosse 2010; Mamiro et al. 2011; Navaratne 2014; F. K. Ngoualem et al. 2019; K. F. Ngoualem et al. 2023, 2020; Ngwasiri Ndasi, Adanmengwi Akah, et al. 2021; Ngwasiri Ndasi, Ekuh Ikongefuze, et al. 2021; Okoye et al. 2016; Osano et al. 2013; Saidou et al. 2015; Schemeda-Hirschmann 1994; Senthil et al. 2006; Srinivasan et al. 2000).

The preparation of the emulsion is done using crude red palm oil (unrefined) and an alkaline solution obtained by dissolving TAS in water, without using any other ingredient which may act as an emulsifier. Since crude red palm oil is known to contain free fatty acids, it can be postulated that emulsion formation and stability in *Achu Soup*/*Sauce Jaune* is done through the formation of emulsifiers from free fatty acids during emulsion preparation by saponification reaction involving weak alkaline solution. If true, this principle can be used to increase the physical stability of prepared emulsions (by increasing the concentration of emulsifiers) and decrease their chemical instability (by decreasing residual free fatty acids found in commercial oils). The objective of this study thus is to understand the contribution of free fatty acids to emulsion formation and apparent stability based on the case study of the preparation *Achu Soup*/*Sauce Jaune*/*Ncha*.

2 | Material and Methods

The mechanism of emulsion formation in the presence of alkaline solutions was investigated in four consecutive steps: (i) determination of the emulsion type in *Achu Soup/Sauce Jaune/Ncha*; (ii) evaluation of the influence of oil type (chemical composition and acid value) using crude red palm oil, commercial canola oil and commercial olive oil; (iii) Assessment of the effect of progressive refining of crude red palm oil on emulsion formation; (iv) Examination of the role of solution alkalinity using both alkaline and neutral salt solutions.

2.1 | Determination of the Type of Emulsion Occurring in *Achu Soup/Sauce Jaune*

A simple mixture made from water, oil and a TAS was used for this purpose. In order to avoid any limitation to a particular mixture and to have a clear idea about all the possible types of emulsions which may form from the mixture of all these primary ingredients (water, oil and TAS), a mixture design of these three components with process variable (stirring speed) was used to prepare emulsions and after colouration with a dye (methylene blue), they were observed through a microscope. Proportions of each component of the mixture used in this study were obtained through a nationwide survey on household's usage of TAS (Figure 1) in the preparation of foods amongst which *Achu Soup*/*Sauce Jaune*. This survey showed that burnt white *Kanwa* (local term used in Cameroon for lake deposits) is the major TAS used in the preparation of these emulsions and 2.90–49.87 g of this TAS was used for the preparation of 1 L of emulsion. The oil/water ratio used varies between 1/9 and 3/7. In order to mimic the burning process done at home for this TAS, white *Kanwa* was crushed, sieved with 1 mm sieve and then burnt at 400°C during 1 hour in a muffle furnace.

As explained by women who use to prepare the emulsion, for the preparation of *Achu Soup*/*Sauce Jaune* at household scale, crude red palm oil is liquefied (by heating a little) and then

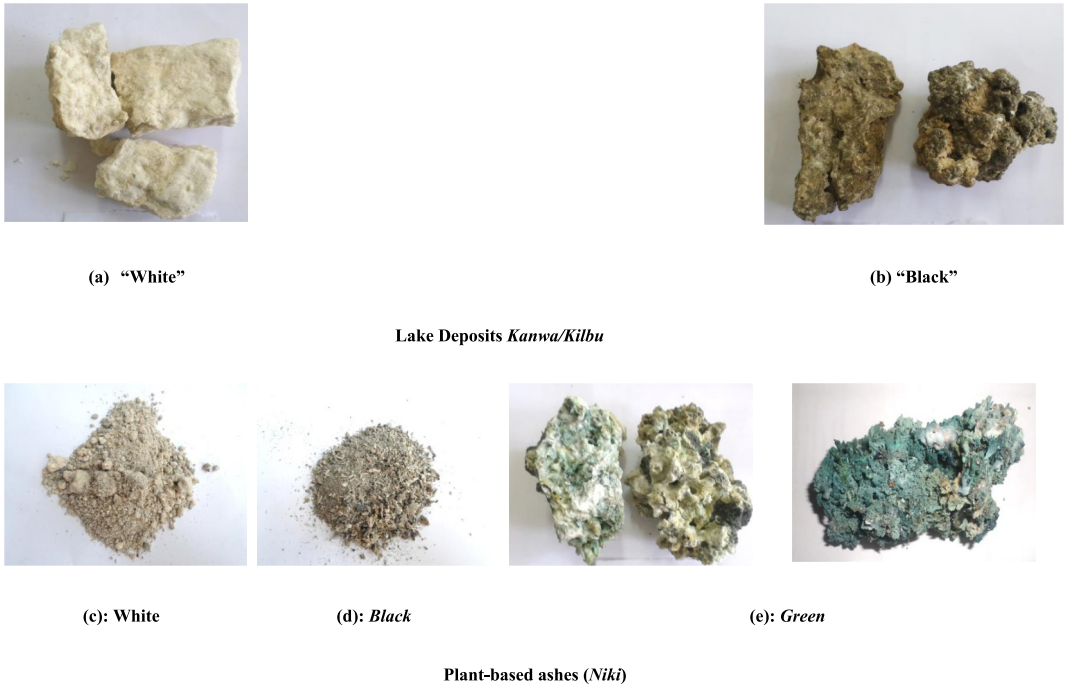


FIGURE 1 | Traditional salts used in the preparation of Achu Soup/Sauce Jaune in Cameroon.

allowed to cool while remaining liquid. TAS (an alkaline) is either added directly (lake deposits) (major process used) or their solution/filtrate is progressively added to red palm oil while shaking the mixture with a hand or a container dipped in the solution.

2.1.1 | Set Up of the Mixture Design and Preparations of Emulsions at Laboratory Level

2.1.1.1 | Set Up of the Mixture Design. A constrained mixture design was developed using household proportions of crude red palm oil, water and burnt white Kanwa (the predominant traditional alkaline salt identified in a nationwide survey; Figure 1).Since mixture design requires the same unit for involved components, all the quantities of components used for 1 L (1000 mL) of the preparation were converted to volume. To this respect, once crushed, sieved and burnt, 2.90 and 49.87 g of white lake deposits have a volume of 2.85 and 50.46 mL, respectively. The remaining volume (949.54 and 997.15 mL) is the sum of the volume of water and oil. The following set of Equations (1–3) was obtained for 100 mL of emulsion.

$$0.285\ \text{mL} \le x_3 \le 5.046\ \text{mL}, \tag{1}$$

$$94.954\ \text{mL} \le x_1 + x_2 \le 99.714\ \text{mL}, \tag{2}$$

$$\frac{1}{9} \le \frac{x_1}{x_2} \le \frac{3}{7}, \tag{3}$$

where x_1: volume of red palm oil in 100 mL of emulsion (mL); x_2: volume of distilled water in 100 mL of emulsion (mL); x_3: volume of white sample of burnt lake deposits in 100 mL of emulsion (mL).

Equation (3) shows that the sum of water and oil proportions is 10 parts and these parts vary as shown in Equation (2). By considering the quantity of each part, the set of Equations (1), (4) and (5) were obtained

$$0.285\ \text{mL} \le x_3 \le 5.046\ \text{mL}, \tag{1}$$

$$9.495\ \text{mL} \le x_1 \le 29.913\ \text{mL}, \tag{4}$$

$$66.465\ \text{mL} \le x_2 \le 89.739\ \text{mL}, \tag{5}$$

where x_1: volume of red palm oil in 100 mL of emulsion (mL); x_2: volume of distilled water in 100 mL of emulsion (mL); x_3: volume of white sample of burnt lake deposits in 100 mL of emulsion (mL).

Since mixture designs are based on proportions and that the sum of proportions should always be equal to 100% in this case (or 1 L of preparation when all the components are concerned), quantities of components of Equations (1), (4) and (5) were divided by 100, leading to a set of Equations (6–8) below.

$$0.09495 \le x_1 \le 0.29913, \tag{6}$$

$$0.66465 \le x_2 \le 0.89739, \tag{7}$$

$$0.00285 \le x_3 \le 0.05046, \tag{8}$$

where x_1: proportion of red palm oil in the emulsion; x_2: proportion of distilled water in the emulsion; x_3: proportion of white sample of burnt lake deposits in the emulsion. The sum of proportions is always 1% or 100%.

In order to mimic the agitation done with hands in the preparation of the emulsion as it is done at household scale, stirring speeds have been added to these initial constraints of the

mixture design. In this respect two stirring speeds have been selected: 400 and 800 rpm. So, the resulting design is a mixture design with constraints on components proportions and a process variable (stirring speed). The set of Equations (9–12) are those of the mixture design used.

$$0.09495 \leq x_1 \leq 0.29913, \quad (9)$$

$$0.66465 \leq x_2 \leq 0.89739, \quad (10)$$

$$0.00285 \leq x_3 \leq 0.05046, \quad (11)$$

$$Z = 400 \text{ rpm}, 800 \text{ rpm}, \quad (12)$$

where x_1: Proportion of oil in the mixture; x_2: Proportion of water in the mixture; x_3: Proportion of salt in the mixture; Z: Stirring speed used during the emulsification.

2.1.1.2 | Preparations of Emulsions. Using constraints on the mixture components and process variables, 38 experiments have been generated with the software Minitab 18. Each experiment has been done in duplicate and altogether, 76 experiments have been done (Table 1).

For each experiment, the total volume of the mixture was fixed to 100 mL, the required volume of each component of the mixture was calculated accordingly and their masses determined (by using their density since oil, water and white burnt lake deposits have different densities). Since TAS always have insoluble matter (Gopalakrishnan 2015; K. F. Ngoualem et al. 2023; Saidou et al. 2015), for each experiment, aqueous solutions of burnt white lake deposits were prepared in a more important volume but at the required concentration for the experiment. In this respect, the solution obtained by adding burnt lake deposits to water was sonicated (Elma E 30H Elmasonic) during 10 min at 30°C and filtered with Whatmann filter paper number 42. The necessary volume for the experiment was then measured and poured in a burette. The corresponding volume of red palm oil (previously maintained at 40°C in a water bath) for the experiment was weighed in a 500 mL beaker for the preparation of the emulsion. An agitator (Scilogex OS20-S with a helix probe of 6 cm of diameter) previously set at the stirring speed of the experience was dipped into the beaker containing red palm oil in such a way that the helix was in contact with oil. The agitator was then switched on before beginning the dropwise addition of the aqueous solution contained in the burette. During this addition of the solution, the agitator was continuously working up to the total addition of the solution contained in the burette. A Video 1 showing the preparation of the emulsion as done in the laboratory is found in this article.

Red palm oil (*E. guineensis*) used in this work was the crude one (not refined) and was bought in Africa by a woman who used to prepare *Achu Soup*/*Sauce Jaune*. This choice was made because during the survey, women insisted on the fact that it is always crude red palm oil which is used to prepare a good *Achu Soup*/*Sauce Jaune*. Once bought, it was kept in a closed container far away from light (by covering the closed container with a black polyethylene paper and keeping it in a cupboard).

2.1.2 | Determination of the Nature of Prepared Emulsions

In order to determine the nature of prepared emulsions, methylene blue, an aqueous soluble dye, was used to prepare solutions at 100 ppm. Once each emulsion was prepared (Table 1), around 15 mL was added in a wire previously containing 1 mL of methylene blue solution. The mixture was closed with an airtight lid and hand-shaken for a while (around 2 min) to allow homogenisation with the dye. A drop of the mixture was then put between two slides and observed with a microscope (Euromex L Scope series) at objective 40 (40 times extension). Methylene blue is an aqueous soluble dye which can only be able to colour water, regardless of the fact that it is the dispersed or the continuous phase. Possibilities of emulsions were oil in water (O/W), water in oil (W/O), oil in water in oil (O/W/O) or water in oil in water (W/O/W). During microscopic observations of prepared emulsions, the colour of the droplets of the dispersed phases was also noted. The photographs of microscopic observations of a droplet of some of the prepared emulsions placed between two slides was done with a fluorescent microscope (Nikon Eclipse Ni) operating up the right bright field extended 200 times in order to effectively show the nature of prepared emulsions. Once prepared, a volume of emulsion was centrifuged (Eppendorf Centrifuge 5810 R) at 12,000 g during 1 h at 30°C. This was done in order to see the different phases which will be obtained after centrifugation.

2.2 | Study of the Effect of the Type of Oil on Emulsion Formation

In order to study the effect of the type of oil, two other commercial oils were bought from a supermarket and used: olive (*Olea europaea*) and canola (*Brassica napus* L.) oils. With all these oils (olive, canola and crude red palm oil), the same sample of alkaline was used (burnt white lake deposits). In order to be given an opportunity to observe the formation of emulsion for all these oils (especially for olive and canola oils), the maximum proportions of oil (28.49%) and burnt white lake deposits (5.046%) as well as the minimum proportion of water (66.47%) as defined for the mixture design (Table 1) were used. These three oils were kept in a water bath at 40°C. Solutions of burnt lake deposits were prepared as previously described but at the concentration indicated for this study and added in a wire previously containing the quantity of oil of the study. The wire was airtightly closed and vigorously hand-shaked for 5 minutes and then allowed to stand up for around 1 hour before taking photographs. All these oils were also tested with solutions of other TAS (Figure 1) prepared at the concentration of the experiment.

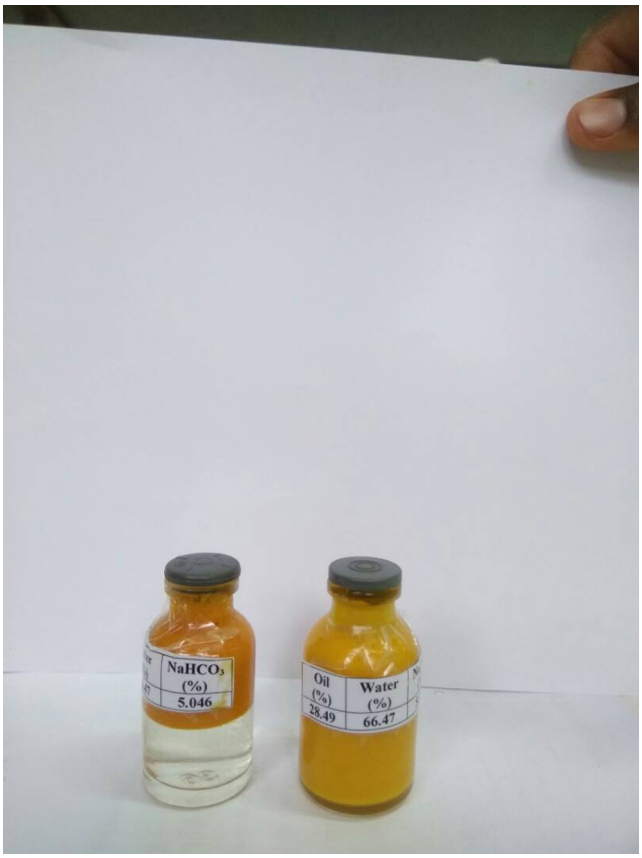
The chemical analysis (fatty acid profile, acid value and free fatty acids content) of used oils was done in order to understand the basis of emulsion formation, if any. In this respect, the fatty acids profile was done by using gas chromatography (Shimadzu GC-2010, Shimadzu Corporation, Japan) with flame ionisation detector (FID-2010 plus) after the preparation of samples with the method described by Sahena et al. (2010). The acid value of these 3 oils was done by using the method defined by the Food Safety and Standards Authority of India (FSSAI 2012). The free fatty acid content (%) was obtained by dividing the acid value of the oil by 2 (Chinedu et al. 2017).

TABLE 1 | Parameters of the mixture design used (single experiments obtained).

Experiment	Oil (%)	Water (%)	Salt proportion (%)	Stirring speed (rpm)
1	28.489	66.465	5.046	800
2	9.976	89.739	0.285	400
3	9.495	87.599	2.906	800
4	9.495	85.459	5.046	400
5	29.913	66.465	3.622	400
6	9.736	89.739	0.526	400
7	9.495	89.739	0.766	400
8	29.913	68.134	1.954	800
9	19.547	77.945	2.508	400
10	24.018	72.205	3.777	400
11	19.945	79.771	0.285	800
12	9.495	87.599	2.906	400
13	29.201	66.465	4.334	400
14	24.018	72.205	3.777	800
15	14.521	81.702	3.777	400
16	24.730	72.205	3.065	400
17	18.992	75.962	5.046	400
18	19.9445	79.7705	0.285	400
19	29.201	66.465	4.334	800
20	14.521	83.842	1.637	400
21	9.495	85.459	5.046	800
22	14.761	83.842	1.397	400
23	9.976	89.739	0.285	800
24	24.730	72.205	3.065	800
25	19.547	77.945	2.508	800
26	14.521	83.842	1.637	800
27	28.489	66.465	5.046	400
28	29.913	69.802	0.285	400
29	14.521	81.702	3.777	800
30	29.913	68.134	1.954	400
31	24.730	73.873	1.397	400
32	9.495	89.739	0.766	800
33	29.913	69.802	0.285	800
34	24.730	73.873	1.397	800
35	14.761	83.842	1.397	800
36	9.736	89.739	0.526	800
37	29.913	66.465	3.622	800
38	18.992	75.962	5.046	800

2.3 | Study of the Effect of the Refining Process of Crude Red Palm Oil on Emulsion Formation

Crude palm oil, as it is the case with any crude oil, is known to contain many compounds which, because of their structure, can work as emulsifiers and thus contribute to emulsion formation and/or stability. Those of importance are free fatty acids, phospholipids, mono and diacylglycerols. In order to understand their contribution to emulsion formation in *Achu Soup*/*Sauce Jaune*, they were progressively eliminated through the



VIDEO 1 | Red palm oil emulsion making with laboratory equipments. To view this video in the full-text HTML version of the article, please visit https://onlinelibrary.wiley.com/doi/10.1002/FCI2.70052.

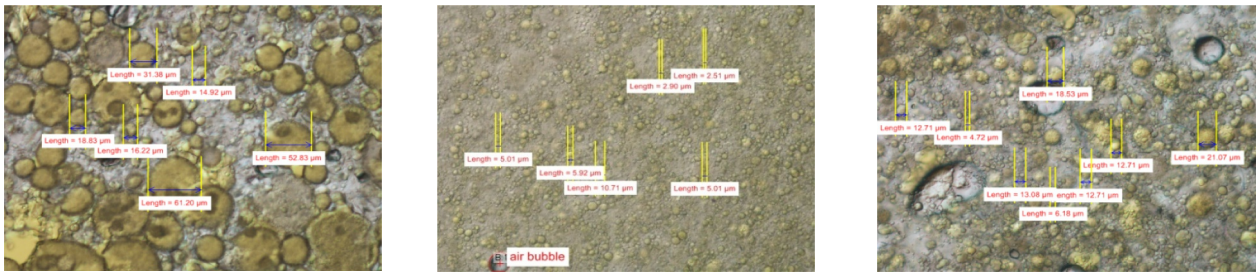
refining process of crude red palm oil and each resulting fraction was used to see their ability to form apparently physically stable emulsions. The proportion of ingredients used was the same as those used to understand the effect of the type of oil (Section 2.2). Briefly, in a wire containing the refined fraction of red palm oil (28.49%), the prepared solution of TAS, at a volume corresponding to the proportion of water (66.47%) and containing the required proportion TAS was added. The wire was air-tightly closed and vigorously hand-shaken during around 5 minutes and allowed to stand up for around 1 h, the observations were done and their photographs taken.

The refining process started with crude red palm oil. On that oil, free fatty acids were eliminated by neutralization with a 0.1 N sodium hydroxide solution, followed by the elimination through centrifugation (Eppendorf Centrifuge 5810 R at 8000 g during 30 min) of the mixture of oil with soap. A fraction (Fraction 1) of the deacidified red palm oil fraction was kept and the remaining quantity used for degumming. Although crude red palm oil is known to be poor in gums, degumming was done using both the water degumming process and the dry degumming process as explained by Gharby (2022). For water degumming, 20 mL of warm water heated at 85°C was added to 200 mL of the deacidified red palm oil. The mixture was slowly agitated for 20 min and allowed to stand for 2 h. A separating funnel was then used and the collected oily fraction used for dry degumming. To carry out the dry degumming, citric acid monohydrate 99% (Sigma-Aldrich) was dispersed into the heated fraction of red palm oil (80°C) at a concentration of 1 g/100 g of oil as well as activated charcoal at a concentration of 3 g/100 g of oil. The mixture was shaken for 30 min and then filtered using activated charcoal. The mixture was then centrifuged (Eppendorf Centrifuge 5810 R at 10,000 g during 30 min) and the upper oily fraction collected and dried at 50°C under vacuum in order to eliminate the residual water. A part of the degummed and deacidified red palm oil was kept (Fraction 2) and the remaining quantity kept for bleaching. Bleaching was done as explained by Ferfuia et al. (2023). In this respect, oil was heated by maintaining it in the water bath at the temperature of 95°C for 30 min (time considered when the temperature of oil reached 95°C). When the temperature of oil was 95°C, activated charcoal was progressively added and the mixture was stirred for 30 min while heating. After the required time, the mixture was filtered with activated charcoal and the upper phase was collected (Fraction 3). The crude red palm oil and the commercially refined red palm oil were used along the different fractions (Fraction 1, Fraction 2 and Fraction 3) for comparison purposes.

2.4 | Study of the Effect of the Nature of Solutions on Emulsion Formation

In order to understand the contribution of the alkalinity of solutions to emulsion formation, different salts with the ability to form alkaline solutions or not were used with crude red palm oil. In this respect, salts of analytical grade which can't form alkaline solutions (Sodium chloride [NaCl, JHD, 99.5% of purity], potassium chloride [KCl, Sigma-Aldrich Laborchemikalien GmbH, 99%–105% of purity] and calcium chloride [$CaCl_2$, Riedel-De Haen Ag Seelze-Hannover, 84%–86% of purity]) as well as those which form alkaline solutions (Sodium bicarbonate [$NaHCO_3$, Sigma-Aldrich, 99%–105% of purity], sodium carbonate [Na_2CO_3, Sigma-Aldrich, 99% of purity minimum], potassium hydroxide [KOH, Wilkinson Vickers LTD, 80% of purity minimum] and burnt white sample of lake deposits) were used. Crude red palm oil (kept in a water bath at 40°C) was the only oil used during the experiments. The proportion of ingredients used was the same as those used to understand the effect of the type of oil (Section 2.2). A mixture of distilled water (without any salt on it) and red palm oil was used as control. Solution of TAS was prepared as previously described (Section 2.1). For analytical grade salts and alkaline, since they were all totally soluble at concentration used, they were not filtered. In a wire containing the defined proportion of oil (28.49%), the prepared solution (alkaline or not), at a volume corresponding to the proportion of water (66.47%) and containing the required proportion of salt was added. The wire was air-tightly closed and vigorously hand-shaken during around 5 minutes and allowed to stand up for around 1 h. Observations were done and their photographs taken.

Since the objective here was to understand the contribution of the alkalinity of solutions in emulsion formation and stability, the pHs of solutions of all the salts and alkaline used were measured (pHmeter Adwa AD1020). For TAS, since their alkalinity was attributed to their carbonate and bicarbonate



Emulsion prepared with 19.95% of red palm oil, 79.77% of water, 0.285% of burnt lake deposits and 400 rpm of agitation speed

Emulsion prepared with 24.02% of red palm oil, 72.2% of water, 3.776% of burnt lake deposits and 400 rpm of agitation speed

Emulsion prepared with 19.95% of red palm oil, 79.77% of water, 0.285% of burnt lake deposits and 800 rpm of agitation speed

(a): Microscopic observations (Nikon Eclipse Ni fluorescent microscope, x200) of some prepared emulsions



Emulsion prepared with 29.92% of red palm oil, 68.13% of water, 1.942% of burnt lake deposits and 400 rpm of agitation speed

Emulsion prepared with 24.02% of red palm oil, 72.2% of water, 3.776% of burnt lake deposits and 400 rpm of agitation speed

Emulsion prepared with 9.74% of red palm oil, 89.74% of water, 0.52% of burnt lake deposits and 800 rpm of agitation speed

(b): Macroscopic observations of some emulsions after centrifugation (12000g, 1 hour)

FIGURE 2 | Microscopic observations of some prepared emulsions (a) and macroscopic observations of centrifuged emulsions (b).

content (K. F. Ngoualem et al. 2023; Osano et al. 2013; Saidou et al. 2015), the quantity of these compounds was determined by titration using the method described by K. F. Ngoualem et al. (2023).

2.5 | Statistical Analysis

Data treatment, which has mainly consisted at the determination of means and standard deviations, was done with the software Microsoft Excel 2013 whereas the Analysis of Variance (ANOVA) was done with the software STATGRAPHICS Centurion XVI.I.

3 | Results and Discussion

3.1 | Determination of the Nature of *Achu Soup/Sauce Jaune* Emulsion

Once prepared, it was observed that many mixtures were foamy, some being uniformly foamy and relatively viscous whereas others were either not viscous or just foamy in the upper part of the prepared emulsion. This foamy aspect of many mixtures shows that they contain emulsifiers since the formation of foam at air-water interface requires the presence of emulsifiers (Holmberg et al. 2003; Pashley and Karaman 2004; Tadros 2005). The colour of the prepared mixtures was varying from yellow to orange, the latter colour being observed when important proportion of oil and very few proportion of burnt lake deposits (mixture prepared with 19.95% of red palm oil, 79.77% of water, 0.285% of TAS and 400 rpm of agitation speed; mixture prepared with 29.92% of red palm oil, 69.80% of water, 0.285% of TAS and 800 rpm of agitation speed for instance) were used. When observed in a microscope, the continuous phase was always coloured in blue (Figure 2a), meaning that water is always the continuous phase of *Achu Soup*/*Sauce Jaune*. Concerning the dispersed phase, it was generally made of homogeneous yellow, red and translucent separated droplets. This means that the dispersed phase was oil and owing to the homogeneity of these colours, it can unambiguously be said that *Achu Soup*/*Sauce Jaune* is oil-in-water (O/W) emulsion. Yellow and translucent droplets were always visible in all the prepared emulsions, the translucent one being generally of greater size (diameter) and of lessened quantities when compared to yellow ones. For emulsions in which red dispersed droplets were found (emulsion prepared with 14.76% of red palm oil, 83.84% of water, 1.4% of TAS and 400 rpm of agitation speed; emulsion prepared with 14.52% of red palm oil, 81.70% of water, 3.77% of TAS and 400 rpm of agitation speed for instance), it was generally observed an



(a): Heated canola oil (on the left) and mixture prepared by using canola oil, water and burnt white lake deposits

(b): Heated olive oil (on the left) and mixture prepared by using canola oil, water and burnt white lake deposits

(c): Heated red palm oil (on the left) and mixture prepared by using canola oil, water and burnt white lake deposits

FIGURE 3 | Prepared mixtures of oil (28.49%), water (66.47%) and traditional alkaline salts (5.046%).

TABLE 2 | Fatty acids profile[A], acid value and free fatty acids content of canola, olive and red palm oils.

	Olive oil	Canola oil	Red palm oil
Margaric acid (%)	ND	0.022	0.04
Myristic acid (14:0) (%)	ND	ND	0.98
Pelargonic acid (15:0) (%)	ND	ND	0.04
Palmitic acid (16:0) (%)	9.87	6.35	46.59
Arachidic acid (20:0) (%)	0.52	1.32	0.44
Lignoceric acid (24:0) (%)	1.06	ND	ND
Oleic acid (18:1 (9)) (%)	82.68	53.56	37.86
Linoleic acid (18:2 (9, 12)) (%)	5.03	28.23	9.54
Linolenic acid (18:3 (9, 12, 15)) (%)	0.55	8.12	0.22
Total identified fatty acids (%)	99.77	98.08	95.71
Acid value[B] (mg of KOH/g of oil)	2.41 ± 0.08^{a}	2.03 ± 0.44^{a}	371 ± 18^{b}
Free fatty acid (%)	0.123 ± 0.002^{a}	0.101 ± 0.022^{a}	18.6 ± 0.9^{b}

Abbreviation: ND, Not Detected during the analysis.
[A]Identification percentages of fatty acids are 99.71%, 98.08% and 95.71% for olive oil, canola oil and red palm oil, respectively.
[B]Number with the same letter in the index are not significantly different at 95% of the confidence intervals.

upper red palm oil layer after their centrifugation (Figure 2b), meaning that these dispersed red droplets were non-emulsified red palm oil droplets. After the centrifugation of all the prepared emulsions, it was always observed a yellow layer (Figure 2b), meaning that observed yellow droplets were emulsified red palm oil droplets.

From these observations, it is clear that *Achu Soup*/*Sauce Jaune* is oil-in-water emulsion which always contains emulsified droplets of red palm oil (yellow) and sometimes non emulsified droplets of red palm oil (red) and/or translucent droplets.

3.2 | Study of the Effect of the Type of Oil on Emulsion Formation

The preparation of mixture made of oil (canola oil or olive oil or red palm oil), water and burnt white lake deposits allowed obtaining mixtures of different nature (Figure 3).

Red palm oil forms a homogenous emulsion whereas the remaining oils (canola and olive) just form a small emulsified layer (Figure 3). The same observations were done with other TAS. This result shows 2 things: (i) commercial refined oils contain compounds which can react with alkaline solutions to form emulsifiers, although the emulsified layer is small and (ii) crude red palm oil contains more compounds which can react with alkaline solution to form more emulsifiers and thus to form a more stable emulsion. The difference in emulsion potential of these different oils can be due to their chemical composition and/or their properties. In order to understand these differences, the fatty acids profile, the acid value and the free fatty acid content of these oils were examined (Table 2).

These three oils have some common fatty acids (Table 2), although at different proportions: oleic acid, linoleic acid, linolenic acid and palmitic acid. Since only red palm oil was forming the emulsion, the comparison of its fatty acids profile with other non-forming emulsion oils is of interest. In this respect, with respect to the 2 major fatty acids of crude red palm oil and olive

oil (oleic and palmitic fatty acids), it comes out that they are identical although in different proportions, meaning that if the nature of oil was the main factor of emulsion formation, we should have observed the formation of emulsion with olive oil. But this is not the case. When comparing red palm oil with canola oil in terms of their fatty acids profile, it is observed that both oils contain important quantity of oleic acid as their main fatty acid and again, if the nature of fatty acids was responsible of emulsification, canola oil should have shown some important emulsified layer. From these comparisons of these three oils in terms of their fatty acids profile, it can be concluded that it is neither the nature of fatty acids of oils nor their proportion which determine their emulsifying ability.

When comparing the acid value of these oils, it comes out that red palm oil has an acid value which is 155 times more important than that of canola oil and olive oil. Commercial canola and olive oil have free fatty acids content (0.10% and 0.12%, respectively) within the range for commercial oils (0.05%–0.7%; Waraho et al. 2011). The free fatty acids content for crude red palm oil (18.6%), although very high, was reported in some factories (Edyson et al. 2022) and is due to post-harvest operations and the fact that crude red palm oil used for *Achu soup/Sauce Jaune* was not refined. It is well-known that the refining process allows removing free fatty acids (deacidification) and many others compounds (phospholipids, some odour and taste imparting substances, waxes, pigments, sulphur-containing compounds, phenolic compounds, trace metal ions, contaminants) which can lead to the oxidation of oil and after this process, no more than 0.05% of free fatty acids should remain in the refined oil (Belitz et al. 2009). From these observations, it can be suggested that free fatty acids significantly contribute to emulsion formation and stability. Since crude red palm oil also contains, beyond free fatty acids, other compounds (carotenoids, tocopherols, tocotrienols, phytosterols, phospholipids, squalene, ubiquinons, aliphatics alcohols, triterpene alcohols, methyl sterols and aliphatics hydrocarbons; Mba et al. 2015) which can contribute to emulsion building and stability, they were eliminated during a progressive refining process and the different remaining fractions were used with a solution of TAS in order to understand their contribution to emulsion formation and apparent physical stability.

3.3 | Study of the Effects of the Refining Procedure of Red Palm Oil on Emulsion Formation

Figure 4 clearly demonstrates the critical role of free fatty acids (FFAs) in emulsion formation and apparent physical stability. When crude red palm oil is mixed with water in the absence of an alkaline solution, no emulsion forms at all. This also shows that free fatty acids are not emulsifiers and in order to be effective, the medium (emulsion) must have an alkaline pH. The addition of TAS (*Kanwa* in the picture refers to the burnt lake deposits) allows to obtain mixtures with different degrees of apparent physical stability and the most stable being the emulsion prepared with crude red palm oil (Figure 4). The removal of free fatty acid through deacidification is associated with emulsion formation but with a reduced stability as well as the modification of its colour from yellow (for the stable emulsion) to orange (Figure 4). Since the same proportion of components was used for the preparation of these samples (Figure 4), it implies that the orange colour obtained when removing free fatty acids is due to the reduction of the quantity of pro-emulsifiers (free fatty acids). This is consistent with the observation reported in Section 3.1 where orange colour emulsions were obtained with a high proportion of crude red palm oil and low proportion of TAS. Since crude red palm oil and deacidified red palm oil differ primarily in the elimination of free fatty acids, it can be concluded that free fatty acids in alkaline medium generate a substantial amount of emulsifiers (probably carboxylates) which significantly contribute to emulsion formation and apparent physical stability. However, it is worth noting that they may not be alone to produce emulsifiers during emulsion formation because their elimination is still associated with emulsion formation, although the apparent physical stability of resulting emulsions are low when compared to the ones produced using crude red palm oil.

3.4 | Study of the Effect of the Type of Salts on Emulsion Formation

The role of alkalinity was confirmed by preparing mixtures of crude red palm oil and water with a range of salts of different chemical nature (Figure 5) and comparing them with a control containing no salt.

Water and neutral salts ($CaCl_2$, KCl, NaCl) don't form emulsions whereas alkaline solutions ($NaHCO_3$, Na_2CO_3, KOH and traditional salts) do (Figure 5). Moreover, when comparing emulsion prepared with sodium bicarbonate (Figure 5h) with those prepared by using either sodium carbonate (Na_2CO_3, Figure 5c) or potassium hydroxide (KOH, Figure 5e) or Traditional Alkaline Salts (Figure 5f), it is evident that sodium bicarbonate forms a less stable emulsion. In order to understand this behaviour, the pHs of used solutions were measured (Table 3).

When comparing the pH of sodium bicarbonate ($NaHCO_3$) with that of other salts and base forming alkaline solutions (sodium carbonate [Na_2CO_3], potassium hydroxide [KOH] and burnt white lake deposits), it can be observed in (Table 3) that sodium bicarbonate had the lowest basic pH. Salts forming neutral solutions (NaCl, KCl, $CaCl_2$) as well as water didn't form emulsion at all. This conclusively proves that free fatty acids are not emulsifiers at all, otherwise their important quantity would have been sufficient to produce an emulsion. These salts are said to form neutral solutions owing to the fact that they are considered as formed by reaction between a strong acid and a strong base (reaction between sodium hydroxide [NaOH] and chlorhydric acid [HCl] in the case of NaCl for instance; Zumdahl and Zumdahl 2007). When considering their pH (of salts forming neutral solutions), with the exception of calcium chloride ($CaCl_2$), they are around the pH of distilled water ($pH \approx 7$). Even in the case of calcium chloride ($CaCl_2$), the fact that their pH is different of the pH of distilled water (they were



FIGURE 4 | Mixtures of crude and commercial red palm oil and refined fraction of crude red palm oil with water and traditional alkaline salts.

supposed to have the same pH as that of distilled water used for their preparation) is due to impurities present in these used analytical grade salts. In fact, the analytical grade calcium chloride used in the study contains 0.05% sulphate, whereas the analytical grade sodium chloride used contains 0.001% of phosphate, anions which considered alone have effect on pH (sulphates yield to more basic solutions whereas phosphates, depending on the form present, which can produce either acidic or basic solutions). Since the pH is a measure of the activity of hydrogen or hydroxide ions (Christian et al. 2014; Zumdahl and Zumdahl 2007), the activity of hydroxide anions is very low in solutions of neutral salts (NaCl, KCl, $CaCl_2$) and more important in basic solutions (KOH, Na_2CO_3, $NaHCO_3$ and burnt lake deposits). This activity increases with increasing pH. Thus, the activity of hydroxide anions in KOH (pH ≈ 13.91) solutions are more important than in Na_2CO_3 (pH ≈ 11.24) solutions, which

in turn are more important than in solution of burnt lake deposits (pH ≈ 10.67), which are also more important than in solution of $NaHCO_3$ (pH ≈ 8.83). This clearly shows that the alkalinity of solutions (as measured by the pH) is an important determinant of emulsion formation and stability. The higher the alkalinity of solution, the more stable the emulsion prepared from it. Solvation equations which result from the addition in aqueous solution of these salts and bases are reactions (1–8) below.

The alkalinity of solutions of salts (Na_2CO_3, $NaHCO_3$ and lakes' deposits) and base (KOH) as shown by their pH (Table 3) results from the excess of hydroxide anions (OH^-) formed in solution when solubilised. For water and neutral salts (NaCl, $CaCl_2$ and KCl), there are not any excess protons or hydroxide ions. Thus, it is this anion (OH^-) which determines the formation of this emulsion and the higher is their activity in solution (measured by the pH), the more stable the emulsion is.

Sodium chloride	$NaCl + H_2O \longrightarrow Na^+ + Cl^- + H^+ + OH^-$	(1)
Potassium chloride	$KCl + H_2O \longrightarrow K^+ + Cl^- + H^+ + OH^-$	(2)
Calcium chloride	$CaCl_2 + H_2O \longrightarrow Ca^+ + 2\,Cl^- + H^+ + OH^-$	(3)
Potassium hydroxide	$KOH + H_2O \longrightarrow K^+ + 2\,OH^- + H^+$	(4)
Sodium bicarbonate	$NaHCO_3 + H_2O \longrightarrow Na^+ + HCO_3^- + H^+ + OH^- \longrightarrow H_2CO_3 + OH^- + Na^+$	(5)
Sodium carbonate	$Na_2CO_3 + 2H_2O \longrightarrow 2\,Na^+ + CO_3^{2-} + 2H^+ + 2\,OH^- \longrightarrow H_2CO_3 + 2\,OH^- + 2\,Na^+$	(6)
Traditional salts	$NaHCO_3 + Na_2CO_3 + 3H_2O \longrightarrow 3\,Na^+ + HCO_3^- + CO_3^{2-} + 3H^+ + 3\,OH^- \longrightarrow 2H_2CO_3 + 3\,OH^- + 3Na^+$	(7)
Water	$H_2O + H_2O \longrightarrow H_3O^+ + OH^-$	(8)



(a) $CaCl_2$ **(b) NaCl** **(c) Na_2CO_3** **(d) KCl** **(e) KOH** **(f) TAS** **(g)Any salt** **(h) $NaHCO_3$**



(a) $CaCl_2$ **(b) NaCl** **(c) Na_2CO_3** **(d) KCl** **(e) KOH** **(f) TAS** **(g)Any salt** **(h) $NaHCO_3$**

(a): Calcium chloride; (b): Sodium chloride;(c): Sodium bicarbonate; (d): Potassium chloride; (e): Potassium hydroxide; (f): burnt Traditional Alkaline Salts (TAS); (g); Control prepared without any salt; (h): Sodium carbonate

FIGURE 5 | Prepared mixtures of red palm oil, water and miscellaneous salts and base.

TABLE 3 | pH at 25°C of solutions (5.046%) of salts used to determine the effects of salts.

Salt	pH	Carbonate content (mg of Na_2CO_3 equivalent[a]/g)	Bicarbonate content (mg of $NaHCO_3$ equivalent[b]/g)
Potassium chloride (KCl)	6.36 ± 0.05[a]	ND	ND
Sodium chloride (NaCl)	6.69 ± 0.09[b]	ND	ND
Calcium chloride ($CaCl_2$)	8.10 ± 0.02[c]	ND	ND
Potassium hydroxide (KOH)	13.91 ± 0.03[d]	ND	ND
Sodium carbonate (Na_2CO_3)	11.24 ± 0.03[e]	1036 ± 49[a]	0
Sodium bicarbonate ($NaHCO_3$)	8.83 ± 0.01[f]	0[b]	1066 ± 64[a]
Burnt lake deposits	10.67 ± 0.02[g]	937 ± 2[c]	52 ± 8[b]
Distilled water	6.97 ± 0.16[h]	ND	ND

Note: Values with the same letter in index in the same column are not significantly different at 95% of confidence intervals.
Abbreviation: ND, Not Determined.
[a]Equivalent Na_2CO_3 is the mass of Na_2CO_3 containing the same quantity of carbonate as 1 g of the compound.
[b]Equivalent $NaHCO_3$ is the mass of $NaHCO_3$ containing the same quantity of bicarbonate as 1 g of compound.

3.5 | Mechanism of the Contribution of Free Fatty Acids to Emulsion Formation and Homogeneity

The results of this study clearly demonstrate that free fatty acids alone are not capable of acting as emulsifiers. An alkaline environment is essential for their conversion into surface-active compounds. Taking all these observations together, the reaction occurring during emulsion preparation is the following mild saponification (Reaction 9):

$$RCOOH + OH^- + H_2O \longrightarrow RCOO^- + 2H_2O. \quad (9)$$

With R which stands for the alkyl radical of the fatty acid; RCOOH is the free fatty acid and $RCOO^-$, the carboxylate anion resulting from the reaction between free fatty acids and hydroxide anion.

In fact, carboxylates are well-known to be used as emulsifiers in the preparation of many types of emulsions in different areas (Belitz et al. 2009; Goodwin 2004; Holmberg et al. 2003; Pashley and Karaman 2004; Tadros 2005). Their ability to work as emulsifiers is due to the simultaneous presence of a hydrophilic head (the carboxylic anion COO^- for carboxylates and phosphate end for phospholipids) and a lipophilic tail (the aliphatic chain R associated to carboxylates or those branched on glycerol in the case of phospholipids). Carboxylates, which belong to anionic class of surfactants (surfactants are molecules which can adsorb at the interfaces of two phases of different nature whereas emulsifiers are a specific subclass of surfactants in which one phase is water and another is oil), are the oldest surfactants known and actually represent 60% of total produced surfactants (Goodwin 2004; Holmberg et al. 2003; Tadros 2005).

For emulsifiers, Griffin (1949, 1954) developed a hydrophilic lipophilic balance (HLB) concept which can be used to classify emulsifiers according to their ability to form either water-in-oil emulsion (W/O) or oil in water emulsion (O/W). Although initially defined for non-ionic emulsifiers, this concept has later on been extended to ionic emulsifiers by Davies (1957). According to the latter author, the HLB of anionic emulsifiers can be computed according to Formula (1).

$$HLB = 7 + m \times Hh - nHl. \quad (1)$$

With HLB: hydrophilic Lipophilic Balance; m: number of hydrophilic group of the molecule; Hh: Contribution value of the hydrophilic group, which is 21.1 for potassium carboxylate anion (Belitz et al. 2009; Davies 1957; Holmberg et al. 2003; Tadros 2005); n: number of hydrophobic group of the molecule; Hl: Contribution value of the hydrophobic group, which is 0.475 for –CH–, $–CH_2–$ and CH_3 according to the same authors.

Since palmitic fatty acid has been identified as being the main fatty acids of red palm oil (Table 2), if their carboxylate of potassium are considered as the main emulsifiers formed (potassium palmitate [CH_3-$(CH_2)_{14}$-COO^-K^+]), the use of the formula for HLB value of ionic emulsifier (Formula 1), allows to obtain an HLB value of 20.175. According to Davies (1957), an emulsifier with HLB between 8 and 18 allows preparing oil-in-water emulsion because they are soluble in water. Moreover, according to Bancroft's rule which states that the phase in which the surfactant is most soluble will form the continuous phase (Goodwin 2004; Tadros 2005), the continuous phase of *Achu soup*/*Sauce Jaune* will be water (because palmitate are more soluble in water) and the dispersed phase is oil as observed in all the prepared emulsions on microscope. The nature of emulsifiers (carboxylate of fatty acid) then justifies why *Achu Soup*/*Sauce Jaune* is always oil-in-water (O/W) emulsion.

4 | Conclusion

Achu Soup/*Sauce Jaune* is an oil-in-water emulsion. The fatty acids composition of the oil is not the determinant of emulsion formation, but mostly it is free fatty acid content. With the elimination of free fatty acids, red palm oil is still able to form emulsion, but this resulting emulsion is physically less stable than the one which is obtained in presence of free fatty acids.

Having an alkaline medium is a requirement for emulsion formation involving water, crude red palm oil and salt. This is due to the fact that the alkalinity of solution allows forming emulsifiers, both with free fatty acids and esterified fatty acids. Increasing the alkalinity of solutions used increases the physical stability of the resulting prepared emulsion.

Author Contributions

Ngoualem Kégah Franklin: investigation, writing – original draft, methodology, validation, writing – review and editing, software, formal analysis, data curation, conceptualization, visualization. **Nguimbou Richard Marcel:** writing – review and editing, methodology, software, formal analysis, supervision, validation, data curation, conceptualization. **Ndjouenkeu Robert:** conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, software, formal analysis, data curation, supervision. **Asad Muhammad Khan:** funding acquisition, writing – review and editing, formal analysis, resources, supervision.

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Ethics Statement

I ensure that all research conducted adheres to ethical guidelines and contributes to the academic community with honesty and integrity. Respondents gave written consent for review and signature before starting interviews.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Figshare at https://figshare.com/articles/journal_contribution/Mechanism_of_emulsion_building_without_emulsifiers/28589876, reference number 28589876.

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