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Research Article

Production of Mayonnaise from *Lagenaria siceraria* and *Cucumeropsis mannii* Seeds Oils Supplemented with *Citrus sinensis* and *Cymbopogon citratus* Essential Oils

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Abstract

Mayonnaise, a semi-solid emulsion of oil in water, is much appreciated for its pleasant taste, which can stimulate the appetite. However, many commercial mayonnaises are produced with oils rich in saturated fatty acids, which are responsible for a number of diseases. The aim of this work is to formulate a mayonnaise based on the seed oils of *Lagenaria siceraria* and *Cucumeropsis mannii*, enriched with essential oils (EO) of *Citrus sinensis* and *Cymbopogon citratus*. Five mayonnaise samples were formulated according to their vegetable and essential oil composition. After extraction and analysis of the oils, the prepared mayonnaises were subjected to physico-chemical, microbiological and sensory analyses. The results showed that *L. siceraria* and *C. mannii* oils contained predominantly linoleic (C18:2n-6), oleic (C18:1 n-9), stearic (C18:0) and palmitic (C16:0) acids. C. citratus EOs analyzed by GC/MS yielded four components representing 93.71% of the total composition: geranial (50.32%), neral (33.27%), mircene (8.42%) and geraniol (1.67%). *C. sinensis* epicarp oil yielded three majority compounds representing 96.18%: D-limonene (88.45%), β-pinene (2.94%) and linalool (4.78%). Both oils showed low antioxidant activity. The pH values of the freshly formulated mayonnaises ranged from 4.09 to 4.25. Acid and peroxide values were equal to 4.25 g KOH/g oil and 7.18 meq O2/ kg respectively for all mayonnaises. After 45 days' storage at 4 ℃, these indices increased significantly ($P < 0.05$). The addition of essential oils to mayonnaise samples resulted in absent or lower total bacterial counts. Overall, it was observed that E1 mayonnaise presented the highest sensory evaluation characteristics.

Keywords

Formulation, Mayonnaise, Cucurbit Oil, Essential Oil, Chemical Composition

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1. Introduction

In recent years, there has been a strong demand for natural products due to their many benefits for the human body, and this is evident in our daily lives, as these products have no side effects on health [1]. Many of the most popular products on the market are emulsions. Emulsions containing natural products are the subject of much research [2]. An emulsion is a heterogeneous mixture of two immiscible liquid substances, one dispersed as small droplet in the other [3]. Emulsions are found in many fields: medicine, pharmaceuticals, cosmetics, food, paints, fuels, etc.

The main challenge facing the agri-food industry is to cope with the world's growing population by providing sufficient and available food, but also to offer healthy, varied and innovative food products while ensuring sustainable development, thanks to the formulation of new food products [4]. The new food products formulated are: butter emulsion, cheese, milk, vinegar, mayonnaise.

Mayonnaise emulsion is one of the most popular condiments added to a variety of foods to enhance flavor and taste. Typically, the ingredients used to formulate mayonnaise include egg (whole or yolk), vinegar, water, spices and vegetable oil [5]. In the traditional recipe, the fat content varies from 65% to 80%, so mayonnaise is essentially an oil-in-water emulsion.

In the present study, the mayonnaise emulsion is formulated from cucurbit oils. These oils have numerous health benefits due to their excellent source of antioxidants such as polyphenols, tocopherols, carotenoids and Poly Unsaturated Fatty Acids (PUFAs), contributing to the prevention of diseases such as prostate, intestinal worms, asthma, health promotion and cardiovascular disease [6].

Mayonnaise has become one of the most sought-after food products on the market. According to the research of Mc Clements, mayonnaise, a semi-solid oil-in-water emulsion, is one of the most widely used ancient sauces in the world today [7]. Mayonnaise is used as an accompaniment to salads, sandwiches, French fries and in many family sauces. In the Congo, mayonnaise is served as an accompaniment to most dishes in small local restaurants.

However, most of these mayonnaises are responsible for illnesses such as excess fat or obesity, cardiovascular disease, etc. These illnesses are caused by the vegetable oils rich in saturated fatty acids (palmitic acid) that make up these mayonnaises. To solve this problem, we planned to formulate a mayonnaise based on cucurbit oils rich in polyunsaturated fatty acids and enriched with essential oils with beneficial effects for consumer health. Plants can be used to improve health and preserve food. These plant properties are often attributed to antioxidant components such as plant phenolics, including flavonoids and phenylpropanoids among others. Today, aromatic plants have a considerable advantage thanks to the gradual discovery of applications for their essential oils in healthcare, as well as their use in other areas of economic interest.

The agri-food sector is increasingly interested in essential oils for food seasoning, conservation and preservation of perishable food products. Essential oils or their isolates are used not only for the flavor they impart, but also for their antibacterial and antifungal properties.

Consequently, this study investigated the effects of orange epicarp and lemongrass leaf essential oils on the physicochemical properties, microbial population, shelf life and organoleptic properties of mayonnaise, while revealing their chemical composition, antioxidant potential and antimicrobial activity.

2. Materials and Methods

2.1. Plant Materials

Oilseed species studied: *Lagenaria siceraria* (Nsiya in Lari) and *Cucumeropsis manni* (*Nzaka* in *Nzebi*) seeds were used for vegetable oil extraction.

Lagenaria siceraria seeds (Figure 1a) from Matti village (4°11'12" South and 15°19'38" East) in the Pool department were purchased at the Total market in Arrondissement 2 Bacongo (Brazzaville). Those of *Cucumeropsis manni* (Figure 1b) from the village Mbaya (2°27'54" South and 12°43'50" East) in the Niari department were purchased from a supplier in Mfilou (Brazzaville).

(a) Unhulled seeds of *Lagenaria siceraria*

(b) Unhulled seeds of *Cucumeropsis mannii*

Figure 1. *Plant Squash seed species used in this study.*

Hulled seeds were oven-dried at 70 ℃ for 24 hours. They were then ground using a manual mechanical grinder. The powder of each seed obtained was wrapped in polyethylene film, sealed in bags and placed in a freezer at around -18 ℃ until the oils were extracted.

Aromatic plant species used: *Citrus sinensis* fruit epicarp (Figure 2a) and *Cymbopogon citratus* leaves (Figure 2b) were used for essential oil extraction.

(a) Epicarp of *C. sinensis* fruits

(b) Leaves of *C. citratus*

Figure 2. *Plant materials used for essential oil extraction.*

Citrus sinensis fruits from Oyo (1 ° 10′ 12″ South, 15 ° 58′ 12″ East) in the Cuvette Centrale department were purchased at the Oyo market. *Cymbopogon citratus* leaves were harvested in central Brazzaville in the kata kata camp (4 °16'15.7 "South 15 °16'03.4 "East) in the poto-poto district.

Citrus sinensis fruit epicarps were cut and shade-dried for 7 days. *Cymbopogon citratus* leaves, after cutting, were dried for three (3) days in the shade.

2.2. Extraction and Analysis of Vegetable Oils

2.2.1. Extraction of Vegetable Oils by the Water Method

250 g of seed powder was mixed with 1000 mL of distilled water in an aluminum pan. The mixture was heated for 4 hours at 150 ℃. After boiling, two phases are obtained. The oil supernatant in the upper phase is transferred to a separating funnel for 24 hours, after which the oil phase is separated from the aqueous phase. The oil obtained is purified by mixing it with 10% salted water, then heating the mixture to boiling point for about 30 minutes. The purified oil is extracted by decantation. The amount of salt water used is 25% by weight of the purified oil. The rate of oil extraction is then determined in relation to the mass of crushed material.

2.2.2. Chemical Characterization of Vegetable Oils

Oil chemical indices such as acid and peroxide values were determined using AOAC methods [8].

2.2.3. Fatty Acid Composition by Gas Chromatography (GC)

Preparation of fatty acid methyl esters (FAME) and GC analysis for fatty acid composition were carried out according to the method described by Piombo *et al.* [9].

2.2.4. Tocopherols Content

Quantification of tocopherols (α, β, δ and γ) was carried out in accordance with ISO 9936 [10].

2.3. Extraction and Analysis of Essential Oils

2.3.1. Extraction of Essential Oils by Hydrodistillation

Essential oils were extracted by hydrodistillation. Several distillations were carried out by boiling 250 g of each plant material impregnated with a sufficient quantity of water for three hours. The oil obtained was then collected by decantation and stored in a bottle until use. The essential oil yield (volume in mL) was determined in relation to 100 g of plant material [11]. The volatile oils were dried over anhydrous sodium sulfate and stored at (-18 ℃) in the dark for analysis and further antibacterial studies [12, 13].

2.3.2. Determination of Essential Oil Composition by Gas Chromatography-mass Spectrometry (GC-MS)

For quantitative analysis of the Essential Oil, a Focus gas chromatograph equipped with a DB5 MS column (20 m x 0.18 mm x 0.18 µm) was used. For qualitative analysis, a ThermoScientific Focus GC gas chromatograph, coupled to a ThermoScientific DSQ II mass spectrophotometer, equipped with a DB5 column (20 m x 0.18 mm x 0.18 µm) was used. Compounds were identified by comparing their mass spectra and retention indices (IR) with those of the databases [14-17].

2.3.3. Assessing the Antioxidant Activity of Essential Oils

The free radical scavenging activity of lemongrass and orange epicarp essential oils was measured by the DPPH assay [18]. The oil sample was dissolved in methanol to give a concentration of 0.2-0.8 µL/mL for lemongrass and citrus oils. Then 1 mL of the essential oil solution was added to 2 mL of 100 µM DPPH methanolic solution. For the control reaction, the essential oil was replaced by 100% methanol. The mixture was incubated for 2 hours in the dark at room temperature. Finally, absorbance was measured at 517 nm using an ultra-violet-visible spectrophotometer (Agilent Technologies Cary 300 UV-Vis). The percentage inhibition of DPPH radicals was plotted against sample concentrations and a regression curve was established to calculate the IC50 value.

2.3.4. Assessing the Antimicrobial Activity of Essential Oils

Three (3) strains of Gram-negative pathogenic bacteria commonly associated with food poisoning were used to evaluate the antimicrobial activity of essential oils. These strains included: *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*. The antibacterial activity of *Citrus sinensis* and *Cymbopogon citratus* essential oils was assessed using the MH agar well diffusion method at concentrations of 20 and 50 µl/well of the compound tested.

Reactivation of pathogenic bacteria was carried out on PCA agar using the streak method and incubated for 24 h at 37 ℃. A portion of the bacterial colony was then transferred to coded sterile Eppendorf tubes containing distilled water, to form a bacterial suspension. This suspension was homogenized for a few seconds using a vortex. The bacterial concentration was adjusted to 105 CFU/ml after measuring the optical density between 0.08 and 0.1 using a spectrophotometer at 625 nm.

2.4. Mayonnaise Production and Characterization

2.4.1. Mayonnaise Formulation

Mayonnaise formulation was carried out as described by Leuschner and Boughtflower [19]. This formulation comprises three successive stages: mixing of all ingredients except oil, addition of oil under controlled conditions during continuous mixing and storage of these mayonnaises [13].

Table 1 shows the various ingredients used in the formulation of the mayonnaises studied.

Table 1. *Mayonnaise ingredients formula.*

Ingredients[a]	% by Mass	Emulsion phase
Vegetable oil	65	Oil
Egg yolk	23	Emulsifier
Vinegar (6% acetic acid)	7	Water
Sugar	1.4	Water
Salt	1.6	Water
Spices	0.3	Water
Water	1.5	Water

[a]Weight % of emulsifying ingredients is inversely related to weight % of oil in formula.

The essential oils were added to the mayonnaise oil. Five mayonnaise samples were formulated according to their vegetable and essential oil composition (Table 2).

The formulated mayonnaise samples were transferred to five (5) glass bottles (100 ml) with stoppers and stored for 45 days at 4 ℃ until analysis.

Table 2. *Composition of each mayonnaise.*

Mayonnaise	Composition
E1	VO*C.mannii* + VO*L.siceraria*+ 100 µL EOcit + 100 µL EOcym
E2	HV*C.mannii* without essential oils
E3	HV*C.mannii* +100 µL EOcym
E4	HV*C.mannii* +100 µL EOcit
E5	HV*C.mannii* + 50 µL EOcit et 50 µL EOcym

*VOC.mannii: Vegetable oil from the seeds of *C. mannii*
VOL.siceraria: Vegetable oil from the seeds of *L. siceraria*
EOcit: Essential oil of *Citrus sinensis* fruit epicarp
EOcym: Essential oil of *Cymbopogon citratus* leaves

2.4.2. Chemical Characterization of Mayonnaises

Formulated mayonnaises were chemically evaluated during production and after 45 days' storage.

The main chemical parameters analyzed in the mayonnaises were: water content, pH, titratable acidity, acid value and peroxide value. All these measurements were carried out in three trials.

The water content of mayonnaise samples was determined according to AOAC method 925.10 [20].

The pH values of the mayonnaise samples were measured directly on a homogenized sample using a pH meter (HANNA Instruments HI 83141 at 25 ℃ [13]. Titratable acidity was determined by titration with a solution of NaOH. Lipids were extracted from mayonnaise samples and the acid and perox-

ide values of the extracted lipids were determined according to AOAC methods [8].

2.4.3. Microbiological Analysis of Mayonnaises

It involved enumerating microbial groups or bacterial species indicative of hygiene and/or food product spoilage. The microbiological parameters concerned were: total aerobic flora (TAF); *Staphylococcus aureus*; coliforms and yeasts and molds.

Four culture media were prepared: Plate Count Agar (PCA) for the study of total flora in mayonnaises; Mannitol Salt Agar (MSA) for the identification of *Staphylococcus aureus*; MacConkey Agar for the identification of Enterobacteriaceae and Sabouraud Dextrose Agar for the isolation of Fungi type micro-organisms (moulds and yeasts). All culture media used were seeded on the surface by spreading. Each dehydrated culture medium was dissolved in distilled water at a weight-to-volume ratio/determined and varied according to the type of medium as recommended by the manufacturer. After heating and homogenization, the medium was autoclaved at 121 ℃ for 15 min.

Sample processing involved preparation of the mother suspension and decimal dilutions. This was carried out in accordance with standard NF EN ISO 6887-2,2017, which defines the general rules for the preparation of stock suspension and decimal dilutions for microbiological analysis.

Ten (10) grams of mayonnaise were aseptically weighed into a flask using a balance. A volume of 90 ml of distilled water was added. The mixture was homogenized for 5 min to obtain the mother suspension corresponding to the 10^{-1} dilution. A quantity of 1 ml of stock suspension was taken and introduced into a test tube containing nine (9) ml of previously prepared distilled water. This gave the 10^{-2} dilution.

The following formula was used to calculate the average count of microorganisms in CFU/g [21]:

$$N=\Sigma c/v.n.d \quad (1)$$

with: N: microbial concentration in CFU/g of mayonnaise; Σc: Sum of the colonies of the boxes retained; v: volume of inoculum inoculated; n: number of boxes retained; d: dilution retained.

2.4.4. Sensory Analysis of Mayonnaises

The parameters for organoleptic analysis of mayonnaise samples were determined according to the following criteria: Taste, color, odor, texture and wholesomeness. In order to assess sensory qualities, a panel of 15 mixed tasters (students) were selected at random. These tasters were non-smokers, and had not consumed any food or drink that might influence their perceptions for a period of one hour prior to analysis. Each mayonnaise attribute was coded (Table 2). A Nine-Point Hedonic Scale was used for the evaluation of sensory attributes. [22].

2.5. Statistical Analysis

The statistical processing of the various data from this study was carried out using the classical statistical method. The calculation of means and standard deviations and the analysis of variance (ANOVA) were carried out using XLSTAT version 2016.02.28451, a Microsoft Excel macro command.

3. Results and Discussion

3.1. Quality and Composition of Vegetable Oils

3.1.1. Chemical Characterization of Vegetable Oils

Chemical analysis of *Cumeropsis mannii* and *Lagenaria siceraria* seed oils yielded the results shown in Table 3. Acid and peroxide values are very important parameters in determining the quality of the extracted oil.

Table 3. *Chemical properties of extracted oils.*

Parameters	*C. mannii*	*L. siceraria*
Extraction yield, (%)	19.03 ±0.78	14.35 ±0.25
Acid value, (mg KOH/g oil)	4.25 ±0.25	2.91 ±0.27
Peroxide value, (meq O_2 /g oil)	7.18 ±0.11	8.76 ±0.39

Table 3 shows that acid number values are low (less than or equal to 4) for all species. The acid value is 4.25 mg KOH/g for *C.mannii* seeds oil and 2.91 mg KOH/g for *L.siceraria* seeds oil. These acid number values comply with the Codex Alimentarus standard, which stipulates that the acid number of virgin oils must not exceed 4 mg KOH/g oil [23]. Examining the peroxide values of these different oils, we note maximum values of around 9 mg O_2 /g oil. These maximum peroxide values are well below the maximum permissible value for virgin edible oils, i.e. 15 mg O_2/g oil [23]. It can therefore be clearly concluded that the oils extracted from *C. mannii* and *L. siceraria* seeds using the water method have not undergone any oxidation affecting the quality of these oils. It should therefore be noted that the seed oils of the two cucurbit species studied are of good chemical quality and can therefore be used in the formulation of lipid-rich mayonnaises.

The percentage yields of vegetable oils show that the seeds of *C. mannii* are more oleaginous than those of *L. siceraria*; these contents vary significantly ($p<0.05$) between the different cucurbit species studied.

3.1.2. Fatty Acid Composition

Table 4 shows the fatty acid composition of *C. mannii* and

L. siceraria seed oils extracted by the water method. The seed oils studied were found to be very rich in unsaturated fatty acids, with linoleic acid predominating at 56.85% and 67.77% for *C. mannii* and *L. siceraria* seed oils respectively. These observations show that *L. siceraria* seeds yield the oils with the highest linoleic acid content ($p<0.05$).

Table 4. *Fatty acid composition of C. mannii and L. siceraria seed oils.*

Type of fatty acid	*L. siceraria*	*C. mannii*
C14: 0	0.080 ±0.001	0.060 ±0.000
C16: 0	12.110 ±0.008	15.879 ±0.000
C16: 1	0.062 ±0.000	0.077 ±0.000
C17: 0	ND	0.084 ±0.001
C17: 1	ND	ND
C18: 0	6.860 ±0.006	11.754 ±0.022
C18: 1 (n-9c)	12.040 ±0.007	14.416 ±0.004
C18: 2 (n-6)	67.770 ±0.017	56.846 ±0.045
C20: 0	0.480 ±0.005	0.381 ±0.003
C18: 3 (n-6)	0.350 ±0.000	0.298 ±0.001
C18: 3 (n-3)	0.120 ±0.001	0.151 ±0.000
C21:0	0.120 ±0.009	0.079 ±0.001
%SFA	12.80	16.48
%UFA	87.21	83.54
%MUFA	18.96	26.25
%PUFA	68.25	57.29

ND: Not detected; SFA: Saturated Fatty Acid; UFA: Unsaturated Fatty Acid; MUFA: Mono Unsaturated Fatty Acid (MUFA); PUFA: Poly Unsaturated Fatty Acid

Regardless of the seed species considered, the following four major fatty acids were found: linoleic acid (C18:2 (n-6)), oleic acid (C18:1 (n-9)), stearic acid (C18:0) and palmitic acid (C16:0). These four fatty acids have cumulative contents of 98.78% and 98.91% respectively for *Lagenaria siceraria* and *Cucumeropsis mannii* seed oils.

Lagenaria siceraria and *Cucumeropsis mannii* seed oils have qualitatively identical major fatty acid profiles (C18:2 (n-6) > C16:0 > C18:1 (n-9) >C18:0).

3.1.3. Tocopherols Composition

Tocopherols form a family of compounds comprising four (4) substances: α-tocopherol, which is vitamin E proper, β-tocopherol, γ-tocopherol and δ-tocopherol [23]. Vitamin E is recognized as an antioxidant, thanks to its ability to inhibit lipid peroxidation [24]. In this respect, it participates, along with many other substances, in the fight against reactive oxygen species (ROS), i.e. the fight against free radicals and non-radical elements produced during the formation of free radicals [23].

Table 5 shows the results of tocopherol analysis by High-Performance Liquid Chromatography of extracted oils.

Table 5. *Tocopherol content of extracted oils.*

Tocopherol (mg/kg)	*L. siceraria*	*C. mannii*	Colza oil	Colza oil*	Sunflower oil*	Olive oil*	Soya bean oil*	Corn oil*
Alpha	12.92	ND	92.46	170.00	490.00	200.00	100.00	110.00
Beta	ND	412.73	ND	50.00	ND	10.00	ND	50.00
Gamma	338.21	4.17	154.65	600.00	50.00	10.00	590.00	600.00
Delta	Nd	Nd	Nd	20.00	10.00	ND	260.00	20.00

* [25]; Nd: Not determined. ND: Not detected

The oxidative stability of oils depends in particular on their unsaturated fatty acid (UFA) content and composition. The more unsaturated oils are the less stable to oxidation, the higher the number of double bonds on the fatty acids. Thus, according to the results in Table 4, *Lagenaria siceraria* oil (over 87% UFAs, including 68% PUFAs) will be more oxidizable than *Cucumeropsis mannii* oil (around 83% UFAs, including 57% PUFAs). This stability will also depend on the oil's tocopherol content (including vitamin E), which can exert a protective antioxidant action [26-28]. The most active form is α-tocopherol, which is found most frequently in nature. The β and γ tocopherols have reduced vitamin activity (respectively around 40% and 15% of the activity of the α form, while the δ is practically inactive. Table 5, which presents the results of the tocopherol composition of the oils studied, shows that *Lagenaria siceraria* oil is composed of alpha-tocopherol and gam-

ma-tocopherol with respective contents of 12.92 and 338.21 mg/kg of oil. *Cucumeropsis mannii* oil, on the other hand, contains 412.73 mg/kg beta-tocopherol and 4.17 mg/kg gamma-tocopherol. Compared with other oils found in the literature (Table 5), these values are very low. Water-extracted Lagenaria siceraria oil contains 37 times less alpha-tocopherol than sunflower oil and 15 times less than olive oil. For analyses carried out under the same conditions, *Lagenaria siceraria* oil contains 7 times less alpha-tocopherol than rapeseed oil. In contrast, Table 5 shows the absence of alpha-tocopherol in *Cucumeropsis mannii* oil. Given their high polyunsaturated fatty acid content, *Lagenaria siceraria* and *Cucuperopsis mannii* oils appear to be more susceptible to oxidative stress.

3.2. Properties and Composition of Essential Oils

3.2.1. Essential Oil Content of Studied Plants

Analysis of the results of essential oil extraction from *Citrus sinensis* zest or epicarp in Table 6 shows that the yield obtained after 7 days drying in the shade gives a value of 0.86±0.49% obtained by hydrodistillation. This value is almost similar to the results of Jeannot *et al*. and Fusseli *et al*., who reported essential oil yields of *Citrus sinensis* sweet orange peels of 0.6 and 0.8% respectively [29, 30]. Extraction of essential oils from *Cymbopogon citratus* leaves gives a yield value of 0.47±0.16% after 3 days drying. This yield is low compared to those reported by Mohamed (2.12%) on the extraction of essential oils from *Cymbopogon citratus* leaves dried in the shade for 2 days and El-Kholany on the extraction of essential oils from *Cymbopogon nardus* which is 1.29% [13, 31]. The harvesting period, essential in terms of yield and essential oil quality, climate, geographical area, plant genetics, leaf drying method, etc. could explain this difference.

3.2.2. Chemical Composition of Essential Oils

Table 6 shows the chemical composition of the *Citrus sinensis* and *Cymbopogn citratus* essential oils studied. This table shows that the essential oils obtained do not have the same composition.

Table 6. *Components of Citrus sinensis and Cymbopogon citratus essential oils.*

Essential oil content (%)	***Citrus sinensis*** **0,86 ±0,49**		***Cymbopogon citratus*** **0,47 ±0,16**	
No.	**Compounds**	**Content (%)**	**Compounds**	**Content (%)**
1	α-pinene	0.196	α-pinene	0.2498
2	Sabinene	0.157	Mircene	8.4281
3	β-pinene	2.947	β-cis-ocimene	0.6984
4	D-limonene	88.457	5,8,10-undecatrien-3-ol	0.1439
5	NI	0.057	β-pinene	0.2582
6	NI	0.860	Linalol	0.6984
7	Linalol	4.783	trans-chrysanthemal	0.1296
8	NI	0.068	Verbenol	0.6793
9	NI	0.061	cis-verbenol	0.9711
10	NI	0.048	β-citronellol	0.1099
11	Terpinen-4-ol	0.121	Neral	33.278
12	α-terpineol	0.391	Geraniol	1.677
13	Citral	0.461	Geranial	50.327
14	NI	0.049	geranyl acetate	0.1427
15	α-copaene	0.161	caryophyllene	0.2302
16	Caryophyllene	-	α-bergamotene	0.1162
17	Germacrene D	0.337	2-tridecanone	0.0871

Essential oil content (%)	*Citrus sinensis* 0,86 ±0,49		*Cymbopogon citratus* 0,47 ±0,16	
No.	**Compounds**	**Content (%)**	**Compounds**	**Content (%)**
18	NI	0.081	caryophyllene oxide	0.0858
19	Cadinene	0.517	eudesm-7-(11)-en-4-ol	0.0807
20	NI	0.053	selinen-11-en-4-α-ol	0.0382
21	NI	0.073	-	-
22	NI	0.070	-	-
23	7-selinen-4-ol	-	-	-

NI: Not identified

Cymbopogon citratus oil contains four major compounds accounting for 93.71% of the total composition: geranial (50.32%), neral (33.27%), mircene (8.42%) and geraniol (1.67%). Ihaddadene and Merrouche obtained two major compounds in *C. citratus* essential oils from Brazil and Cuba, namely geranial (53.2 and 51.4%) and neral (36.37 and 35.21%) [32].

With regard to the composition of *Citrus sinensis* fruits epicarp essential oils, there are three main compounds accounting for 96.18% of the total composition: D-limonene (88.45%), β-pinene (2.94%) and linalool (4.78%). These results are almost in line with those obtained by Boumenikhra, who, after identification, of these three compounds in the essential oils studied found in *Citrus volkameriana, Citrus aurantium and citrumelo 4475* values of 96.45%, 96.05% and 96.019% respectively [33]. On the one hand, limonene is the majority compound in the essential oils of *Citrus volkameriana* (72.027%), *Citrus aurantium* (83.82%) and *Citrumelo 4475* (68.077%) [33].

3.2.3. Evaluation of DPPH Free-Radical Scavenging Activity of Essential Oils

Figure 3 shows the results of the effect of concentration on the scavenging power of DPPH free radicals.

Absorbance was measured spectrophotometrically at 517 nm, and percentage inhibitions were calculated from the results. The values obtained were used to draw curves representing the variation in percentage inhibition as a function of essential oil concentrations for *Citrus sinensis* epicarps and *Cymbopogon citratus* leaves (Figure 3).

According to the results (Figure 3), at all concentrations tested, the two essential oils showed significantly different activities. The essential oil of *Citrus sinensis* epicarp showed lower activity than that of *Cymbopogon citratus* leaves. Indeed, the essential oil of *Cymbopogon citratus* leaves reached almost its maximum activity at 0.01 mg/ml, with a percentage inhibition (PI) of 13 %. For *C. sinensis* epicarp essential oil, the highest percentage of inhibition (PI) at the same concentration was of 8%.

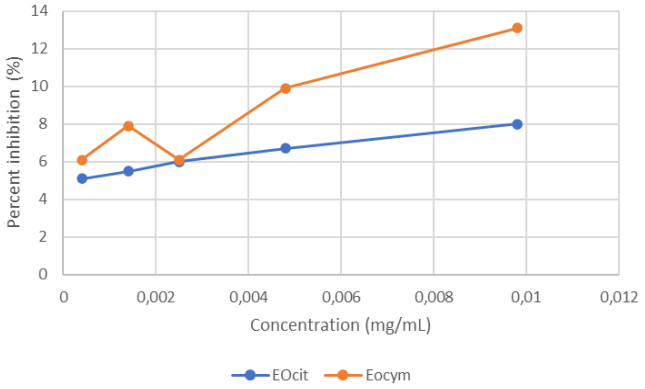

Figure 3. *DPPH free radical scavenging Citrus sinensis and Cymbopogon citratus oils.*

The DPPH free radical reduction kinetics obtained are shown for each concentration of essential oils (Figure 4).

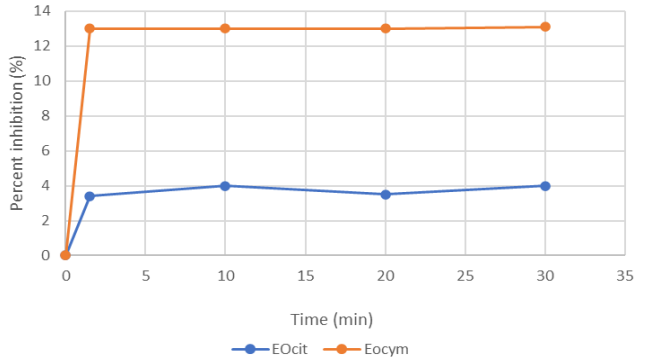

Figure 4. *Percentage inhibition versus time of Cymbopogon citratus and Citrus sinensis oils.*

For both essential oils examined, the reaction is biphasic, with a rapid increase in the percentage of inhibition in the first few minutes, followed by a slower stage, corresponding to the attainment of equilibrium, so two zones can be distinguished: a Zone of low radical trapping kinetics observed at the end of the first two minutes for both oils. A zone of high DPPH radical trapping kinetics, or a zone of tendency towards equilibrium, observed after 2 minutes.

The results show that the reaction between DPPH and essential oils reaches equilibrium within a very short time.

To better compare the activities of the two plant oils tested, the parameters for calculating antioxidant activity, i.e. IC50, TEC50 and ARE, were determined and summarized in Table 7 below:

Table 7. *Parameters for calculating antioxidant activity.*

Substances	IC50 (µg /mg)	TEC50 (min)	ARE (ml/ µg.min)
EOcit	295094.828	110.551	3.07E-08
EOcym	143.000	226.684	3.09E-06
Gallic Acid	154.000	4.224	1.54E-03
Vitamin C	58.000	0.759	2.27E-02

ARE: Anti-free radical effectiveness

IC50 is inversely related to a compound's antioxidant capacity, as it expresses the amount of antioxidant required to decrease the free radical concentration by 50%. The lower the IC50 value, the greater the antioxidant activity of a compound. IC50 values have been determined. *Citrus sinensis* fruit epicarp oil has a much higher IC50 than *Cymbopogon citratus* leaves oil, with values of 295094.828 µg/ml and 143 µg/ml respectively. Ascorbic acid showed an IC50 equal to 58 µg/ ml, as shown in Table 7.

Steady state was chosen as the measurement period when the reaction is shown to progress slowly. The time to equilibrium depends on the reactivity of the antioxidants and the concentrations used. Ascorbic acid reacts most rapidly with DPPH at TEC50 = 0.759 min. The equilibrium time for the EOs studied was 226.684 min for *Cymbopogon citratus* leaves oil and 110.551 min for *Citrus sinensis* oil. To characterize the efficacy of these antioxidants, the free radical scavenging efficacy parameter is calculated.

Anti-free radical effectiveness combines the two parameters (IC50 and TEC50). This parameter was defined to easily characterize the antioxidant behavior of a substance.

According to these results, *Cymbopogon citratus* essential oil has the highest antioxidant activity, with an anti-free radical efficacy of 3.09E-06 ml/µg.min, compared with *Citrus sinensis* epicarp essential oil, which has an anti-free radical efficacy value of 3.07E-08 ml/µg.min. On the other hand, compared with ascorbic acid, *Cymbopogon citratus* EO has a 7346-fold lower anti-free radical efficacy.

With ARE values< 1.10^{-3} ml/µg.min, *Cymbopogon citratus* and *Citrus sinensis* oils show low antioxidant activity.

3.2.4. Screening of the Antimicrobial Activity of Essential Oils

Evaluation of the potency of *Citrus sinensis* fruit epicarp essential oil on bacterial strains including *Enterobacter spp, Klebsiella pneumoniae* and *Escherichia coli* showed that these bacterial strains are resistant to the antimicrobial activity of this oil.

Figure 5 shows the evolution of the inhibition diameter on bacterial strains tested for the antimicrobial activity of *Citrus sinensis* essential oil.

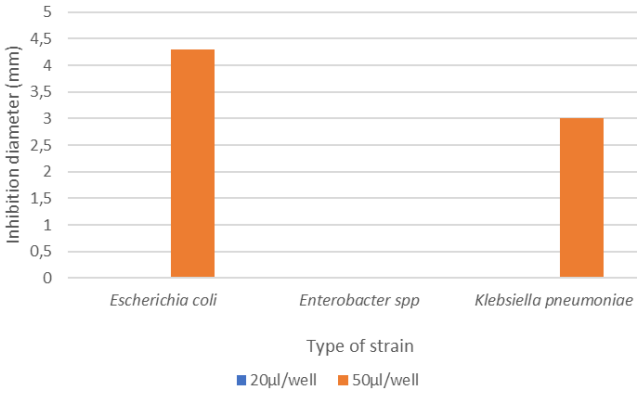

Figure 5. *Antimicrobial activity of Citrus sinensis essential oils.*

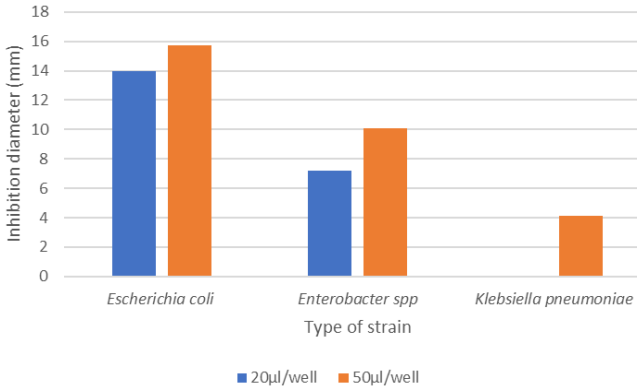

Figure 6. *Antimicrobial activity of Cymbopogon citratus essential oils.*

Assessment of the antimicrobial activity of *Cymbopogon citratus* essential oil on *Escherichia coli*, *Enterobacter spp* and *Klebsiella pneumoniae* strains showed that these bacterial strains are sensitive to the *Cymbopogon citratus* oil studied. This oil therefore has antimicrobial activity. Figure 6 shows the evolution of the inhibition diameter on the bacterial strains tested.

The results in figures 5 and 6 show that the activity of *Citrus sinensis* and *Cymbopogon citratus* oils varies according to the target strains. No zone of inhibition around the wells was observed for *Enterobacter spp*. This bacterium has a very high resistance potential to the antibacterial action of

Citrus sinensis oils. *Escherichia coli* and *Klebsiella pneumoniae* strains showed relative resistance to this oil, despite the weak zones of inhibition observed. These bacteria have a very high resistance potential to the antibacterial action of *Citrus sinensis* oil. *Cymbopogon citratus* oil was found to be effective against most of the strains observed, with the exception of *Klebsiella pneumoniae*. This bacterium is potentially resistant to the activity of *Cymbopogon citratus* oil, compared with strains showing promising resistance to the activity of *Cymbopogon citratus* oil.

3.3. Quality of Formulated Mayonnaises

3.3.1. Chemical Quality of Formulated Mayonnaises

Five mayonnaises were formulated. Mayonnaise samples were stored for 45 days. The results of physicochemical analysis of the 5 mayonnaise samples are summarized in Table 8.

The pH values of the freshly prepared mayonnaise samples and on the 45th day after storage at 4 ℃ are shown in Table 8. The pH values of the freshly prepared mayonnaise samples ranged from 4.09 to 4.25, reflecting the acidic nature of the formulated mayonnaises. This acidic nature of the mayonnaises confirms the results of Pons *et al.* who obtained pH values ranging from 3.6 to 3.9 [34]. Muhammad *et al.*, found pH values ranging from 4.30 to 4.47 for mayonnaises enriched with sesame germ powder [22]. On the other hand, Rasmy *et al.* found pH values equal to 4.43 for mayonnaise samples treated with beta hydroxy acid (BHA) and sage essential oil at different concentrations [35].

During storage, pH values decreased continuously in all mayonnaise samples. This result corroborates those found by El-bostany *et al.*, Kishk and Elsheshetawy, who had also found that pH values decreased continuously in mayonnaise samples during the storage period [36, 37]. Examination of the results of pH measurements shows values that increase with the addition of *C. citratus* essential oils. Mayonnaises E1 and E3 showed the highest pH values at initial time. This may be due to the fact that adding or increasing the concentration of *C. citratus* essential oil leads to a reduction in antibacterial activity and delays the decrease in pH values as a result of its antibacterial effect [13].

After 45 days' storage of mayonnaises at a temperature of 4 ℃, we obtained the lowest titratable acidity value with mayonnaise E2, which is composed of *Cucumeropsis mannii* oil and no essential oil.

Mayonnaise is susceptible to deterioration through auto-oxidation of the unsaturated and polyunsaturated fatty acids in the oil. Lipid peroxidation in food emulsions leads to the production of bad tastes and odors, shortening the shelf life of these products [38]. Variations in the peroxide value of mayonnaises prepared at initial time and after 45 days' storage are shown in Table 8.

The peroxide value in the freshly prepared mayonnaise samples was 8.53 meq O_2/ kg (for E2, E3, E4 and E5) and 8.33 meq O2/ kg (for E1). This value was not affected by the addition of citronella and citrus essential oils at time zero. This same finding was made by El-Kholany when adding different concentrations of lemongrass and geranium essential oils at time zero to fresh mayonnaises [13].

Table 8. *Chemical characteristics of formulated mayonnaises.*

samples	Mayonnaise E1		Mayonnaise E2		Mayonnaise E3		Mayonnaise E4		Mayonnaise E	
Days	0	45	0	45	0	45	0	45	0	45
pH	4.22 (0.01)	3.88 (±0.01)	4.19 (±0.00)	3.64 (±0.01)	4.25 (±0.00)	3.78 (±0.00)	4.09 (±0.01)	3.71 (±0.00)	4.17 (±0.00)	3.78 (±0.00)
Titratable acidity	0.38 (±0.02)	0.66 (±0.03)	0.40 (±0.01)	0.47 (±0.03)	0.37 (±0.03)	0.55 (±0.08)	0.44 (±0.04)	0.60 (±0.03)	0.41 (±0.03)	0.64 (±0.00)
Water content	Nd	20.27 (±1.14)	17.86 (±0.37)	18.20 (±0.51)	21.01 (±0.81)	21.05 (±0.90)	Nd	18.99 (±0.86)	Nd	18.76 (±1.03)
Peroxide value	8,33 (±0.44)	16.67 (±0.00)	8.53 (±0.44)	29.09 (±1.58)	8.53 (±0.44)	17.68 (±0.87)	8.53 (±0.44)	19.39 (±1.05)	8.53 (±0.44)	28.18 (±1.58)
Acid value	4.67 (±0.33)	5.98 (±0.32)	4.25 (±0.25)	14.77 (±0.86)	4.25 (±0.25)	5.61 (±0.56)	4.25 (±0.25)	4.48 (±0.00)	4.25 (±0.25)	15.34 (±0.32)

Nd: Not determined

After storage, the data revealed that the peroxide index values for all mayonnaise samples increased significantly ($P \leq 0.05$).

The peroxide values of mayonnaises E1 and E3 were signifi-

cantly lower (16.67 and 17.68 meq O2/ kg respectively) than those of the other mayonnaises. However, E2 and E5 mayonnaises gave the highest values (29.09 and 28.18 meq O2/ kg respectively). This clearly shows that treatment of the E5 mayonnaise sample with *Cymbopogon citratus* (50 µl) and *Citrus sinensis* (50 µl) oils failed to inhibit oil oxidation in the mayonnaise. The antioxidant activity of Cymbopogon and Citrus essential oils being low, during storage, the mayonnaises formulated were affected by a number of deterioration factors (chemical oxidation...), hence the high peroxide index values obtained and close to those of mayonnaise without essential oil (E2).

The acid value of mayonnaise has been used as a measure of the hydrolysis of triacylglycerols, leading to the formation of free fatty acids. According to Codex this acid value should not exceed 4 g KOH/g oil [39]. According to Kishk, free fatty acids can be produced by oxidation of the double bonds of unsaturated fatty acids [40]. Thus, according to the data in Table 8, E2 and E5 mayonnaises had the highest acid index values after 45 days' storage at 4 ℃, at 14.77 and 15.34 g KOH/g respectively. We note an increase in the acid value of mayonnaise with a significant difference ($P < 0.05$) during storage. Pourkomailian. and Karas *et al.* explained this increase in acid number in mayonnaise by the activity of lactic acid bacteria present in the aqueous phase [41, 42]. In addition, Stefanow had reported that these increases were probably due to the hydrolytic activity and oxidizing enzymes present in eggs [43]. El-Kholany asserted that in the advanced stages of oxidation, low-molecular-weight free fatty acids could be formed through the accumulation of acidic cleavage products, resulting in an increase in the acid number [13].

3.3.2. Microbiological Quality of Mayonnaises

The quality of mayonnaises prepared with different concentrations of *C. citratus* and *C. sinensis* essential oils was evaluated microbiologically. Table 9 shows the results of microbiological analysis of control mayonnaise (E2: no essential oil) and mayonnaises treated with essential oils (E1, E3, E4 and E5) after 45 days' storage at 4 ℃.

Table 9. *Effect of storage on the microbiological characteristics of mayonnaise.*

Researched germs	E1	E2	E3	E4	E5	Norms
			(in CFU⁄mL)			
TAMF	6.0×10^1	9.0×10^1	Abs	3.0×10^1	5.0×10^1	10^5
Staphylococcus aureus	5.5×10^1	10.5×10^1	3.0×10^1	1.5×10^1	6.0×10^1	10^3
Enterobacteriaceae	Abs	Abs	Abs	Abs	2.0×10^1	10^2
Moulds, Yeasts	Abs	Abs	Abs	Abs	3.0×10^1	10^3
Interpretation	Satisfactory quality	Satisfactory quality	Satisfactory quality	Satisfactory quality	Satisfactory quality	

*TAMF: Total aerobic mesophilic flora; CFU: Colony Forming Units; Abs: Absence

According to the results, mayonnaise E3 (100 µL of *C. citratus* oil) showed an absence of TAMF germs, while the lowest total bacterial count (3.10^1 CFU⁄mL) was found in mayonnaise E4 (100 µL of *C. sinensis* oil) followed by mayonnaise E5 and the highest total bacterial count (9.10^1 CFU⁄ml) was found with mayonnaise E2 (control).

For *Staphylococcus aureus* germs, the lowest total bacterial count ($1.5,10^1$ CFU⁄mL) was found in E4 mayonnaise and the highest TBC ($10.5,10^1$ CFU⁄mL) was found with E2 mayonnaise (control). However, we note the absence of Enterobacteriaceae, Moulds and Yeasts in all formulated mayonnaises except for the presence of a few colonies in mayonnaise (E5).

Finally, we note that increasing the concentration of essential oils in mayonnaise samples results in absent or lower total bacterial counts. The presence of germs was not detected on the microbiological quality of the products, as they were all below the tolerance threshold of the standards. Even without the addition of essential oils, the E2 mayonnaise still complies with standards; the addition of essential oils to mayonnaises nevertheless results in lower total bacterial count. And this could be better justified for even longer storage periods.

3.3.3. Sensory Analysis of Formulated Mayonnaises

The sensory analysis was carried out on five (5) prepared mayonnaises. The success of these mayonnaises depends heavily on consumer decisions. The effect of cucurbit oils and essential oils of *Cymbopogon citratus* and Citrus sinensis on mayonnaise quality was studied. The various parameters analyzed are taste, odor, color, texture and wholesomeness.

The 5 samples were prepared, containing 65% oil and the same aqueous phase composed of water, vinegar, sugar, sodium

chloride and minor ingredients (antioxidants and preservatives).

The highest taste score was for sample E1, followed by E2, while samples E3, E4 and E5 showed relatively low scores. The highest odor score was for sample E1, then E4, followed by samples E2, E3, E5 with the lowest scores. The color score of the mayonnaise samples was highest for sample E5, followed by E3 with a similar score. Compared with the other mayonnaise samples studied, samples E1, E2 and E4 had the lowest scores. The highest texture score was for sample E5 and E2 followed by E1, E3, E4. For texture, the highest score was for samples E2, E5 which are similar and the worst score for samples E1, E3, E4 which are also similar. The largest and highest food safety score was for sample E5, followed by E3, E4 and then similar E1, E2, which had a poor score.

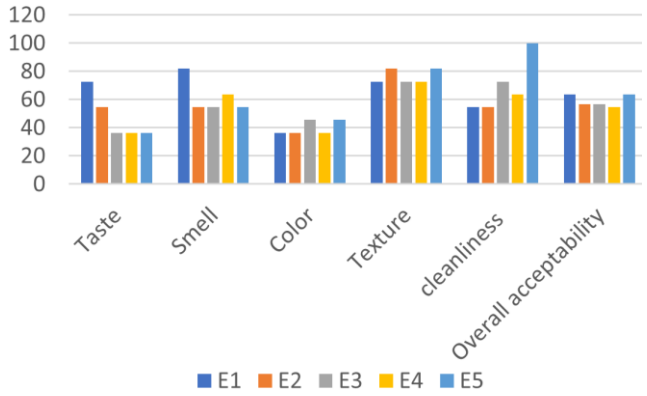

Figure 7. *Histograms of organoleptic parameters of formulated mayonnaises.*

Overall, the blend of *Cucumeropsis manni* and *Lagenaria siceraria* oils and the addition of *Citrus sinensis* and *Cymbopogon citratus* essential oils to mayonnaise were found to have the highest sensory evaluation characteristics. Thus, in terms of overall acceptance of mayonnaises, the E1 mayonnaise (blend of two vegetable oils and blend of two essential oils) ranked first, followed by the E5 mayonnaise (blend of two essential oils).

4. Conclusions

Mayonnaise is an oil-in-water emulsion much appreciated by a large number of consumers for its pleasant taste, which can stimulate the appetite. The formulation used consists of 65% fat and various ingredients. Five mayonnaises were formulated according to essential oil concentrations. Several analyses were carried out to determine the physico-chemical, microbiological and sensory characteristics of the formulated mayonnaises. Two cucurbit species, *Lagenaria siceraria* and *Cucumeropsis mannii*, were selected for this study. The oils from these seeds were extracted by the water method, and extraction yields were equal to 19.03 and 14.35% for *Lagenaria siceraria* and *Cucumeropsis mannii* seeds respectively. Their acid and peroxide values met Codex Alimentarius standards. Analysis of fatty acid composition showed that four major fatty acids were present in all seed species: linoleic acid, oleic acid, stearic acid and palmitic acid.

The hydrodistillation process resulted in the extraction of *Citrus sinensis* and *Cymbopogon citratus* essential oils with contents of 0.86% and 0.47% respectively. Both oils showed low antioxidant activity. *Escherichia coli* and *Klebsiella pneumoniae* strains showed relative resistance to *Citrus sinensis* oil. *Cymbopogon citratus* oil was found to be effective against most strains observed, except *Klebsiella pneumoniae*. The mayonnaises formulated had a good texture and were well appreciated by the panelists. After 45 days' storage at 4 ℃, an increase in mayonnaise acid index with a significant difference ($P < 0.05$) was observed. This increase was all the more remarkable for E2 and E5 mayonnaise. No germs were detected on the microbiological quality of the products, as they were all below the tolerance threshold of the standards.

Generally speaking, the blend of *Cucumeropsis manni* and *Lagenaria siceraria* oils and the addition of *Citrus sinensis* and *Cymbopogon citratus* essential oils to the mayonnaise presented the highest sensory evaluation characteristics.

Abbreviations

TAMF	Total Aerobic Mesophilic Flora
CFU	Colony Forming Units
TBC	Total Bacterial Counts
E1	Mayonnaise Sample No. 1
E2	Mayonnaise Sample No. 2
E3	Mayonnaise Sample No. 3
E4	Mayonnaise Sample No. 4
E5	Mayonnaise Sample No. 5
BHA	Beta Hydroxy Acid
DPPH	2,2-Diph énvl 1-Picrylhydrazyle

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Author Contributions

Bob Wilfrid Loumouamou: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing

C ésestine Kiminou Ngounga: Data curation, Methodology

Eliane Therese Biassala: Formal Analysis

Chanty Ulysse Guen-Koud Auckana Ngala: Formal Analysis, Investigation

Alain Brice Vouidibio Mbozo: Validation

Anicet Fr éd éric Binaki: Methodology

Jean-Mathurin Nzikou: Validation

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Data Availability Statement

No data was used.

Conflicts of Interest

The authors declare no conflicts of interest.

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