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Essential Oil Encapsulation Into Nanoemulsion Based Delivery System: Optimization Assay

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Abstract: *This work aimed on the optimization of the encapsulation of clove, cinnamon, ginger and curcuma essential oils into nanoemulsion based delivery system. The optimization protocol was made of three consecutive steps, and nanoemulsion formulations were tested for droplet size diameter and droplet distribution in the medium. The first step consisted on the screening of nanoemulsion components. Three emulsifiers were tested, and the dispersed phase was either made of pure or mixed essential oils with corn oil. Secondly, formulation focused on the homogenization process via sonication and/or ultra-turrax agitation. Finally, the appropriate emulsifier amount was assessed. Results suggested that the use of Tween-40 and 10% of pure essential oil produced the most appropriate nanoemulsions. For clove and cinnamon, only the use of ultra-turrax agitation is required to produce suitable nanoemulsions. However, for ginger and curcuma essential oils, the combination of two physical forces: ultra-turrax and sonication were needed. 1wt% of Tween-40 were able to produce the most appropriate nanoemulsions for clove and cinnamon. However, higher Tween 40 concentrations were needed for curcuma and ginger encapsulation. In conclusion, gathered results present a solid scientific basis to facilitate essential oil valorization in green industries.*

Keywords: *Essential Oils; Nanoemulsion; Formulation; Emulsifier; Physical Forces*

1. INTRODUCTION

Natural molecules like, essential oils (EOs), and herbal extracts are thought to be promising alternative agents to antibiotics, especially with the alarming increase of multidrug resistant bacteria [1]. EOs are among the most economically important plant-derived compounds, as they are known for their numerous interesting biological efficiencies. These substances are derived from a variety of plants found in moderate to warm climates, such as the Mediterranean and tropical regions, and are a major element of traditional pharmacopoeia. EOs are a mixture of low-molecular-weight molecules that include terpenes (monoterpenes and sesquiterpenes), alcohols, aldehydes, and ketones, which are responsible for the aromatic fragrance that these materials frequently show in addition to their bioactive components [2]. Over 3000 EOs have been identified so far, with 10% of them being commercially and economically important. These mixtures have the potential to contain a large number of bioactive chemicals with a variety of health promoting capacities, and they are in the first line with current consumer preferences for natural products [1].

In reality, the extraordinary effectiveness of most EOs against a wide spectrum of pathogenic bacteria responsible for food spoilage and food poisoning has been consistently documented [1; 3; 4]. However, it has been frequently stated that the valorization of EOs in innovative industries is neither economically nor practically feasible [2]. In fact, one of the most important considerations when using EOs as green and efficient bioactive compounds is their impact on the finished product's sensory characteristics. In reality, the high doses of EOs needed to prevent microbial growth may degrade organoleptic qualities (aroma and taste) and have an unfavorable effect [3]. Alternatively, if various techniques based on the cumulative or synergistic effects of antibacterial activity are used, lower doses of EOs may be possible.

Encapsulating EOs appears to be a promising new strategy to facilitate their use in innovative industries. Encapsulation is the process of coating sensitive components with a protective layer known as wall material [1]. EO encapsulation is a multi-step process with many interconnected phases [3]. Several research groups concentrated their works on EO encapsulation, and various techniques were proposed [5]. It has been concluded that the choice of the encapsulation method and encapsulating material to use are a prejudious steps in the formulation process. Accordingly a number of factors, including the target mean particle size, the physicochemical characteristics of the wall and core ingredients, the intended application of the encapsulated material, the capsule content release system, the production capacity, and of course the encapsulation cost have to be considered [1]. In particular, the EO encapsulation into nanoemulsion based delivery systems has persuaded the research community of its usefulness.

Food, cosmetics, pharmaceuticals, and agricultural products are all examples of where emulsions are used [4]. Emulsions are defined as thermodynamically unstable systems mixing at least two immiscible liquids. According to each phase proportion in the medium, there are two forms of emulsions Oil-in-water (O/W) and water-in-oil (W/O) emulsions. Oil-in-water emulsions are widely employed in food products such as milk, drinks, and sauces, and they are also used to provide lipophilic bioactive substances such vitamins and essential oils (EOs) [3]. Coalescence, flocculation, lactation, and Ostwald ripening are some of the events that restrict the stability of emulsions if it is improperly prepared, all of which can significantly diminish its stability and its biological efficiencies [3].

The specific objective of the present work was to optimize the encapsulation of clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*), curcuma (*Curcuma longa*) and ginger (*Zingiber officinale*) EOs into nanoemulsion based delivery system, with the smallest possible droplet size and the most homogenous distribution of droplet sizes. Accordingly, different nanoemulsion formulations were tested for each EO, such as emulsifiers (type and concentration), lipid phase (compositions), and the homogenization method and apparatus.

2. MATERIAL AND METHODS

2.1. Plant Treatment and EOs Extraction

The dried cinnamon barks, curcuma and ginger rhizomes and clove flower buds were purchased from a local market and cutted into small pieces before extraction. The samples were individually subjected to hydrodistillation for 5-6 hours, using a Clevenger type apparatus. Recovered EO were treated with anhydrous sodium sulphate (Na_2SO_4) before being stored at -20°Cfor further analyses.

2.2. Nanoemulsion Formulation Protocol

For the realization of the NE, many formulations were tested. Indeed, components were screened such as emulsifiers (type and concentration), lipid phase (compositions) and the homogenization process and apparatus had been examined. With this respect, three chemical substances were tested as emulsifier comprising two anionique (Arabic Gum and Alginate) and one non-ionique (Tween 40) emulsifiers with two high energy methods (sonication and ultra-turrax) which were also assessed alone or combined for their effectiveness in stabilizing cinnamon, curcuma, ginger and clove EOs.

Emulsifier type and dispersed phase composition effect.

Firstly, to evaluate the emulsifier type and dispersed phase composition effect, emulsions were made by the same physical forces (30 min of sonication) and emulsion phases (dispersed and continuous) were varied (Table 1). In fact, the continuous aqueous phase was prepared by dissolving different emulsifiers (Alginate, Arabic gum, Tween 40) in deionized water at 1%. The dispersed phase was set at 10% of the total emulsion volume and was either made of pure tested EO or composed of 1:1 mixture one of tested EOs and fixed oil (corn oil).

The most suitable formulations, in terms of emulsion visual stability, its droplet size average and the minimization of the emulsifier amount, were considered for further formulation optimization.

Physical forces effect.

The evaluation of the physical forces effect in the formulation of EO nanoemulsions was made secondly as detailed in Table 2. Thus, the dispersed phase was made of pure tested EOs, and the continuous phase composition was fixed at 1% of Tween 40 solution. Tested physical forces were Ultra-turrax agitation (0 and 1 min) and sonication (0 and 30 min).

The most suitable formulations, in terms of emulsion visual stability, its droplet size average and the minimization of the emulsifier amount, were considered for further formulation optimization.

Emulsifier concentration effect.

The last step of EO encapsulation into nanoemulsion based delivery system was devoted to the assessment of the emulsifier concentration effect. Thus, for the previous tested parameters, only best levels were fixed for each tested EO and different emulsifier concentrations were tested as detailed in Table 3.

For every tested formulation, oil-in-water emulsions had been prepared by means of a two-step procedure. At the first, oil-in-water NEs were prepared by homogenizing the dispersed phase with the surfactant suspension at ambient temperature. Thereafter, the NEs were made by passing the coarse emulsion through physical forces.

For every tested formulation, the mean diameter of dispersed oil droplets, polydispersibility index (PDI) were measured using the Zetasizer Software (version 7.03) to collect and analyze data.

2.3. Statistical Analysis

At least three replicates were used for all tested parameters. Means were compared using the Duncan test at the $p < 0.05$ level, when significant differences were found using the Statistical package SAS 9.1 (2002, 525).

3. RESULTS

In order to encapsulate *Z. officinale*, *C. zeylanicum*, *C. longa* and *S. aromaticum* EOs into stable nanoemulsions that preserve EOs biological activities and facilitate their valorization in numerous fields, formulation optimization was made to fix emulsifiers type and concentration, lipid phase composition and the used physical forces.

3.1. Emulsifier Type and Dispersed Phase Composition Effect

In this work, the evaluation of the emulsifier type and the dispersed phase composition effect was assessed. Gathered results (Table 4) demonstrated that the emulsifier type and the dispersed phase composition influence significantly ($p<0.05$) the droplet size average of produced emulsions, as well as their PDI.

Data shown in Table 4 suggested that, the uses of anionic emulsifiers (Arabic gum and alginate) lead to a creaming phenomenon while using Tween 40, nonionique emulsifier, resulted a good homogeneity (PDIs <0.5, except for pure curcuma). In the same range, the inclusion of fixed oil in water formulas (50% of the dispersed phase) produced inappropriate emulsions with either droplet size diameters (over 3872 and 1271 nm for clove and ginger, respectively), or with creaming phenomena (for cinnamon and curcuma EOs). For the four-tested EOs, a formula with Tween 40 as emulsifier and 10% of pure EO produced the most appropriate nanoemulsions for this step of formulation.

3.2. Physical Forces Effect.

Based on the obtained results from the previous step of nanoemulsion formulation, only formulations where pure EOs were encapsulated with Tween 40 will be subjected to further optimization. Concerning the physical forces effect on the nanoemulsion formulation, two encapsulating physical forces were tested: Ultra-turrax agitation (0 and 1 min) and sonication homogenization (0 and 30 min). Table 5 results showed that both tested physical forces influence significantly ($p<0.05$) obtained emulsion droplet size and PDI.

Obtained results suggested that for clove and cinnamon EOs, an increase in droplet size following the combination of two physical forces was observed as $d_{3,2}$ raised (from 754.2 nm to 830.9 nm, for clove EO). In turn, the high-energy method (ultra-turrax) produced emulsions with smaller droplet size diameters (in the range of 344.9 and 88.703 nm for clove and cinnamon, respectively). A formula with Tween 40 and the use of ultra-turrax agitation for 1 minute will be chosen for the optimization of clove and cinnamon nanoemulsions.

For ginger and curcuma nanoemulsions, a decrease in droplet size following the combination of two physical forces: ultra-turrax and sonication was observed. According to Table 5, this decrease ranges from 1181 nm to 328.9 nm for ginger. A formula with Tween 40 and ultra-turrax combined with sonication forces will be chosen for the optimization of ginger and curcuma nanoemulsions.

3.3. Emulsifier Concentration Effect.

Based on the obtained results from the previous steps of nanoemulsion formulation, only formulations where pure EOs were encapsulated with Tween 40 will be subjected to further optimization. For ginger and curcuma nanoemulsions, the combination of 1 minute agitation using UltraTurrax and 30 minutes sonication will be used. However, for clove and cinnamon nanoemulsions, only one minute of UltraTurrax homogenization will be used. Concerning the emulsifier concentration effect on the nanoemulsion formulation, for each tested EO, 3 different concentrations will be assessed according to previous gathered results. Table 6 results showed that Tween 40 concentration influence significantly ($p<0.05$) obtained emulsion droplet size and PDI.

Considering clove and cinnamon nanoemulsions, the increase of emulsifier concentration up to 10 wt% induced a significant increase ($p<0.05$) of produced droplet size. For instance, at 1; 7.5 and 10 wt% emulsifier, nanoencapsulated clove EO presented a wide range of droplet diameter averages varying from 483.8 to 344.9 nm (10 and 1 wt%, respectively).

The focus on ginger nanoemulsion showed that increasing the emulsifier concentration up to 7.5 wt% induced a significant increase ($p<0.05$) of produced droplet size. Indeed, the encapsulating capacity of Tween 40 at 1wt% produced a 329 nm droplet size diameter. However, the use of 7.5 wt% of the same emulsifier has managed to decrease this droplet size up to 151.06 nm.

Interestingly, curcuma nanoemulsion presented the most appropriate droplet size diameter when using 5 wt% of Tween 40.

Table1. *Formulation of clove, ginger, cinnamon and curcuma nanoemulsions: Emulsifier type and dispersed phase composition effects. EO: Essential oil, FO: Fixed Oil, Al : Alginate, AG: Arabic gum; T40: Tween 40*

Dispersed phase			Continues phase
%	EO	FO	Emulsifier
10	50	50	Al
10	50	50	AG
10	50	50	T40
10	50	50	Al
10	50	50	AG
10	50	50	T40
10	50	50	Al
10	50	50	AG
10	50	50	T40
10	100	0	Al
10	100	0	AG
10	100	0	T40
10	100	0	Al
10	100	0	AG
10	100	0	T40
10	100	0	Al
10	100	0	AG
10	100	0	T40

Table2. *Formulation of clove, ginger, cinnamon and curcuma nanoemulsions: Physical forces effect. EO: Essential oil, T40: Tween 40.*

Dispersed phase			**Aqueous phase**		**Treatment**	
%	**EO**	**FO**	**Emulsifier**	**%**	**Ultra-turrax**	**Sonication**
10	100%	0%	T 40	1	0	30 min
10	100%	0%	T 40	1	1 min	30 min
10	100%	0%	T 40	1	1 min	0

Table3. *Formulation of clove, ginger, cinnamon and curcuma nanoemulsions: emulsifier concentration effect. EO: Essential oil, T40: Tween 40.*

	Dispersed phase		**Aqueous phase**		**Treatment**	
	%	**EO**	**Emulsifier**	**%**	**Ultra-turrax**	**Sonication**
Ginger	10	100%	T 40	1	1 min	30 min
	10	100%	T 40	5	1 min	30 min
	10	100%	T 40	7.5	1 min	30 min
Curcuma	10	100	T 40	2.5	1 min	30 min
	10	100	T 40	5	1 min	30 min
	10	100	T 40	7.5	1 min	30 min

Cinnamon	10	100	T 40	1	1 min	0 min
	10	100	T 40	5	1 min	0 min
	10	100	T 40	10	1 min	0 min
Clove	10	100	T 40	1	1 min	0 min
	10	100	T 40	7.5	1 min	0 min
	10	100	T 40	10	1 min	0 min

Table4. *Droplet size average and PDI measurement of clove, ginger, cinnamon and curcuma nanoemulsions: Emulsifier type and dispersed phase composition effects. EO: Essential oil, FO: Fixed Oil, Al : Alginate, AG: Arabic gum; T40: Tween 40 Equal letters indicate no significant difference (p > 0.05) between samples within a column.*

		Clove		**Ginger**		**Cinnamon**		**Curcuma**	
EO	**Emulsifier**	**d3,2**	**PDI**	**d2,3**	**PDI**	**d2,3**	**PDI**	**d2,3**	**PDI**
50	Al	Creaming		Creaming		Creaming		Creaming	
50	AG	Creaming		Creaming		Creaming		Creaming	
50	T40	3872^{b}	0.347^{a}	1271^{b}	0.378^{b}	Creaming		Creaming	
100	Al	Creaming		Creaming		Creaming		Creaming	
100	AG	Creaming		Creaming		1.9$^{e4\ b}$	0.395^{a}	Creaming	
100	T40	754.2^{a}	0.463^{b}	1181^{a}	0.300^{a}	1466.33^{a}	0.492^{b}	2890.53	0.841

Table5. *Droplet size average and PDI measurement of clove, ginger, cinnamon and curcuma nanoemulsions: Physical forces effect. Formulations were made of pure EO and Tween 40 (1%). 1: 0 min agitation, 30 min sonication; 2: 1 min agitation, 30 min sonication; 3: 1 min agitation, 0 min sonication.* **$d_{3,2}$** *expressed in nm. Equal letters indicate no significant difference (p > 0.05) between samples within a column.*

	1		**2**		**3**	
Essential Oils	**$d_{3,2}$**	**PDI**	**$d_{3,2}$**	**PDI**	**$d_{3,2}$**	**PDI**
Clove	754.2^{a}	0.463^{b}	830.9^{c}	0.45^{c}	344.9^{b}	0.533^{c}
Ginger	1181^{b}	0.300^{a}	328.9^{a}	0.211^{a}	339.5^{b}	0.265^{a}
Cinnamon	1466.33^{c}	0.492^{b}	322.567^{a}	0.587^{d}	88.703^{a}	0.316^{b}
Curcuma	2890.53^{d}	0.841^{c}	386.467^{b}	0.277^{b}	477.1^{c}	0.291^{a}

Table6. *Droplet size average and PDI measurement of clove, ginger, cinnamon and curcuma nanoemulsions: Emulsifier concentration effect. Formulations were made of pure EO and Tween 40.* **$d_{3,2}$** *expressed in nm. Equal letters indicate no significant difference (p > 0.05) between samples within a column.*

Essential Oils	**Emulsifier**	**NE**	
	%	**$d_{3,2}$**	**PDI**
Ginger	1	328.9^{c}	0.211^{a}
	5	188.5^{b}	0.285^{b}
	7.5	**151.06^{a}**	**0.224^{a}**
Curcuma	2.5	386.467^{b}	0.277^{b}
	5	**215.23^{a}**	**0.247^{a}**
	7.5	466.9^{c}	0.298^{c}
Cinnamon	1	**88.703^{a}**	**0.316^{a}**
	5	547.73^{b}	0.405^{c}
	10	842.466^{c}	0.381^{b}
Clove	1	**344.9^{a}**	**0.533^{b}**
	7.5	408.43^{b}	0.664^{c}
	10	483.8^{c}	0.484^{a}

4. DISCUSSION

EO formulation aimed to produce stable oil in water emulsion preferably at the nano-scale. An important point in the production of a nanoencapsulated product is the choice of the wall material, generally it is a polymer selected depending on the physicochemical properties of the active agent and the intended application [6].

The evaluation of the dispersed phase composition effect on nanoemulsion formation and homogeneity depicted that the best nanoemulsions (droplet size and distribution) were created with pure EOs in the dispersed phase. Since EO viscosities are lower than vegetable oils [3], any decrease in EO concentrations, the dispersed phase viscosity rises, making it more difficult to break up viscous oil droplets. As a result, bigger droplets will form in nanoemulsions [3].

Obtained results depicted that the uses of Arabic Gum and Alginate produces unstable emulsions (creaming) and that only Tween 40 showed a high miscibility with the four-tested EOs. Moreover, nanoencapsulating pure EO (without corn oil as carrier) produces stable emulsions. In this context, [7] Zhang et al., declared that among the hydrophilic surfactants, Tween has a very good solubility for essential oils. Considering the chemical intervention, emulsion formation can only occur if the interfacial tension between two immiscible phases decreased sufficiently. Such change in interfacial tension is basically achieved using an appropriate emulsifier [6]. Actually, since Tween 40 has both hydrophilic (water-soluble) and lipophilic (oil-soluble) groups, it can then partially dissolve in both phases, bringing them together into a homogeneous emulsion. Also, Tween 40 is known to rapidly coat the surface of the created oil-water interface during emulsification and reduce interfacial tension to prevent droplet coalescence [8]. Consequently, the use of Tween 40 can lower the amount of work necessary to make a homogenous mixture of two ordinarily immiscible layers (in this study case EO and water), and boost the ease of production and the stability of emulsions [9]. Accordingly, to minimize the used emulsifier amount, different concentrations of Tween 40 in the aqueous phase were tested in order to determine the critical micellar concentration (CMC) of Tween 40 with each tested EOs. Indeed, as the emulsifier concentration is increased, the adsorption amount at the oil–water interface gradually increases, and when the adsorption amount is saturated, it reaches critical micellar concentration (CMC); at this point, the interfacial tension reaches a steady-state [10].

A part for the surfactant effect, the ultimate size of a homogenized emulsion deeply depends on the used physical forces [3]. In this case study, the effect of Ultra-turrax (intensive and instant grinding of liquid droplets) and sonication (ultrasonic waves at a high frequency) were assessed as physical forces to obtain stable nanoemulsions, as they are usually recommended to obtain the smallest possible particle sizes [11]. Interestingly, for ginger NE the combined physical forces (ultra-turrax 1 min and sonication 30 min) showed a stable nanoemulsion even if zingibrene have very low solubility in water due to their partial hydrophobic nature equal to 0.01498mg/L [12].

In this study, two approaches were detected. Actually, the finest clove and cinnamon nanoemulsions (droplet size= 344.9 and 88.703 nm, respectively) were formulated using only ultra-turrax agitation for 1 min, while the finest ginger and curcuma nanoemulsions (droplet size= 151.06 and 215.23 nm, respectively) were formulated with ultra-turrax agitation for 1 min combined to sonication homogenization during 30 min. These results are in good accordance with those of Sharma et al., and Majeed et al., [13; 14] who studied Clove nanoemulsion (droplet size 181.9 and 200 nm, respectively). In the same trend, ginger nanoemulsion was better in the actual study than that found by Noori et al., [15] that droplet size is in the rage of 167.3 and in good agreement with the findings of Mostafa [16] formulated nanoemulsion droplet size equal to 151.4 nm.

5. CONCLUSION

The encapsulation of *Z. officinale*, *C. zeylanicum*, *C. longa* and *S. aromaticum* EOs into nanoemulsion based delivery systems was optimized based on three steps approach in order to fix emulsifier type and concentration, lipid phase composition and the used physical forces. Gathered results suggested that for the four-tested EOs, a formula with Tween 40 as emulsifier and 10% of pure EO produced the most appropriate nanoemulsions for this step of formulation. Concerning the second formulation step, obtained findings demonstrated that for clove and cinnamon encapsulation, only the use of ultra-turrax agitation for 1 minute is required to produce appropriate nanoemulsions. However, for ginger and curcuma EOs, the combination of two physical forces: ultra-turrax and sonication were needed. Concerning the emulsifier concentration effect on the nanoemulsion formulation, obtained results highlighted that 1wt% of Tween 40 were able to produce the most appropriate nanoemulsions for clove and cinnamon EOs. However, higher Tween 40 concentration (5 and 7.5 wt%) were needed to successfully encapsulate curcuma and ginger EOs, respectively.

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