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Effects of Microfluidization on Stability, Functional and Antimicrobial Properties of Soy Lecithin - Clove Essential Oil Based Nanoemulsion

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In current study, soy lecithin - clove oil based nanoemulsion were developed using microfluidization approach. The particle size, zeta potential (stability), colour properties, functional groups (FTIR), antioxidant activity (DPPH) and encapsulation efficiency of nanoemulsion were investigated. In addition, the antibacterial and antifungal activity of nanoemulsion against gram positive, gram negative and fungal strains were investigated. The results revealed that, the increasing concentration of clove oils influenced the higher particle size and lower stability in nanoemulsion, whereas, in contrast application of microfluidization showed opposite results. The lowest particle size was found in NE1 (247.5 nm), which is pure soy lecithin but the 75%:25% (v/v) composition (NE2) and 50:50% (v/v) composition of soy lecithin-clove oil showed lower particle size as 254.1 nm and NE3 (273.1 nm) with -33.3 mV and -40.5 mV zeta potential values. The applications of microfluidization improved the flow of behaviour and molecular interaction in the nanoemulsion. The microfludized nanoemulsion showed higher antioxidant and encapsulation efficiency as compared to non-microfludized nanoemulsion. NE5 showed higher antioxidant activity followed by NE4 (93.83%) and NE3 (92.22%) with 97.42 and 95.75% of encapsulation efficiency, respectively. The microfludized soy lecithin-clove oil nanoemulsion exhibited good antimicrobial activities against gram positive, gram negative and fungal strains.

Keywords: Antimicrobial activity; Clove oil; Nanoemulsion; Particle size; Zeta potential.

1. Introduction

Nowadays, the importance of nanotechnology and nanoscience is increasing in agro-food and pharmaceutical sectors for control release drug delivery and active food packaging due to its greatest advantages including nano size, good antimicrobial properties (Prasad et al., 2023). The nanomaterial including nanoparticle, nanocomposites, carbon nanotubes, quantum dots, carbon dots and nano emulsions (NEs) are used for the delivery of active agents and exhibit good mechanical, thermal and antimicrobial properties, and other functional behaviour (Maurya et al., 2021). Each of these nanomaterials possess unique functional properties due to their nanoscale size.

The nano emulsion is considered as one of the novel drug delivery systems, consisting of an oil and water-based system with the droplet size between 100-500 nm in range and favouring excellent drug targeting and absorption (Souto et al., 2022). Nano emulsions are generally categorized in three different types such as oil in water (O/W), water in oil (W/O), and bi-continuous nano emulsions. Furthermore, in the oil in water types of emulsion are three different types of surfactants such as neutral, cationic and anionic used for developing nano emulsion (Mushtaq et al., 2023). The nano emulsion process involved the development and formation of nanoscale droplets of liquid dispersion to improve the properties. The functional properties and stability of the nano emulsions are also dependent on the types of surfactants, oils and their compatibility and molecular interactions. The major precedence of the nano emulsions as carrier of drug delivery agents improved drug solubility and loading, control drug release and improved bioavailability, reduced variability in patients and protect against enzymatic degradation (Prasad et al., 2023). The use of emulsifiers in the NEs may help in the reducing tension (interfacial) between immiscible phases.

The different types of low and high-energy methods such as high-pressure homogenization, ultrasonication, microfluidization, ultrasound generator, phase inversion, and self-emulsification have been used to formation of nano emulsion with nano droplet size with lower poly-dispersity index and higher stability for the various applications (Kumar et al., 2024a). These techniques are potential for the hydrophobic and hydrophilic interaction and good molecular interactions between the water and oil phases. However, the high-energy techniques are generally preferred for the formation of food grade nano emulsion with addition of lower amounts of surfactants than low energy methods. The hydrophobic and nonpolar interactions between the oil/water phased molecules are the major phenomena for indicating the stability of nano emulsion.

In Recent, the consumer gains more attention about the eco-friendly and sustainable food packaging system to maintain good health as well as environment conversion by replacing the use of plastic and synthetic based packaging in food. Eco-friendly based packaging functionalized with the different types of naturally produced active agents such as plant extract, antimicrobial agents and essential oils and essential oil-based nano emulsion as an alternative of fungicides (Kumar et al., 2023). Essential oils possess good antimicrobial, antioxidant and antifungal activity against food borne pathogens due to presence of phenolic and volatile compounds. There are several types of essential oils such as oregano, basil, cinnamon, orange peel, lemon grass, lemon peel, clove essential oils etc. used for the formation of nano emulsion to control release mechanism and their application in edible packaging for food preservations (Kumar et al., 2024a).

Among the numerous types of essential oils, clove essential oil is one of the potential active agents against food borne and fungal pathogens, those are responsible for the food deterioration (Kumar et al., 2024b). The previous researchers have reported the antimicrobial activities of the clove essential oils against gram negative, gram positive and fungal pathogens (de Meneses et al., 2019). Furthermore, the encapsulation of essential oils may significantly improve the properties of nano emulsions due to their bioactivity and phytochemical constitutions, which may make them suitable for the addition in the food processing sector as a preservative and antimicrobial agents in food packaging (Aswathanarayan et al., 2019; Kumar et al., 2023b). Despite the advantages of essential oil-based nano emulsions, there are several challenges such as stability and aroma associated with the NEs for food packaging application. Therefore, it is necessary to develop a nano emulsion with good stability and less impact of its flavour on the natural aroma of food and food products. In current study, the authors attempt to develop a bio based and non-toxic soy-lecithin - clove oil based nanoemulsion for food packaging application using microfluidization approach. The effects of concentrations of clove essential oils and microfluidization process on the particle size, zeta potential (stability), color properties, functional groups (FTIR), antioxidant activity (DPPH) and encapsulation efficiency were investigated. In addition, the antibacterial and antifungal activity of microfludized soy-lecithin - clove oil nanoemulsion against gram positive (Staphylococcus aureus, Bacillus cereus), gram negative (Pseudomonas aeruginosa, Escherichia coli) and fungal strains (Aspergillus niger, Rhizopus oryzae, Candida albicans) pathogens were investigated.

2. Material and methods

2.2 Microbial strains

The microbial strains such as Gram positive (Staphylococcus aureus {NCDC 109}, Bacillus cereus {NCDC 240}), Gram negative (Pseudomonas aeruginosa {NCDC 105}, Escherichia coli {NCIM 2065) and fungal strains (Aspergillus niger {NCDC 244}, Rhizopus oryzae {NCDC 52}, Candida albicans {MTCC 10231}) pathogens were provided by Microbiology lab, NIFTEM, Kundli.

2.3 Preparation of nanoemulsion

The soy-lecithin - clove oil based nanoemulsion was formulated according to the process described by Singh et al. (2023) with some modifications. A total 5 nanoemulsion were formulated with varying the concentrations of soy lecithin and clove essential oils (Table 1). The microfluidization of soy-lecithin - clove oil mixture was 3 passed through microfluidizer (M1110P, Microfluidics, USA) at 15000 psi (Dhaka et al. 2021). The methodology and procedure for the preparation of nanoemulsion and their characterization are graphically shown in Figure 1.

Table 1: Formulization of soy-lecithin - clove oil based nanoemulsion

Nanoemulsion	Soy lecithin (v/v)	Clove essential oil (v/v)	Microfludization
NF1	100	0	-
NF2	75	25	-
NF3	50	50	-
NF4	25	75	-
NF5	0	0	-
NE1	100	0	1500 psi
NE2	75	25	1500 psi
NE3	50	50	1500 psi
NE4	25	75	1500 psi
NE5	0	0	1500 psi

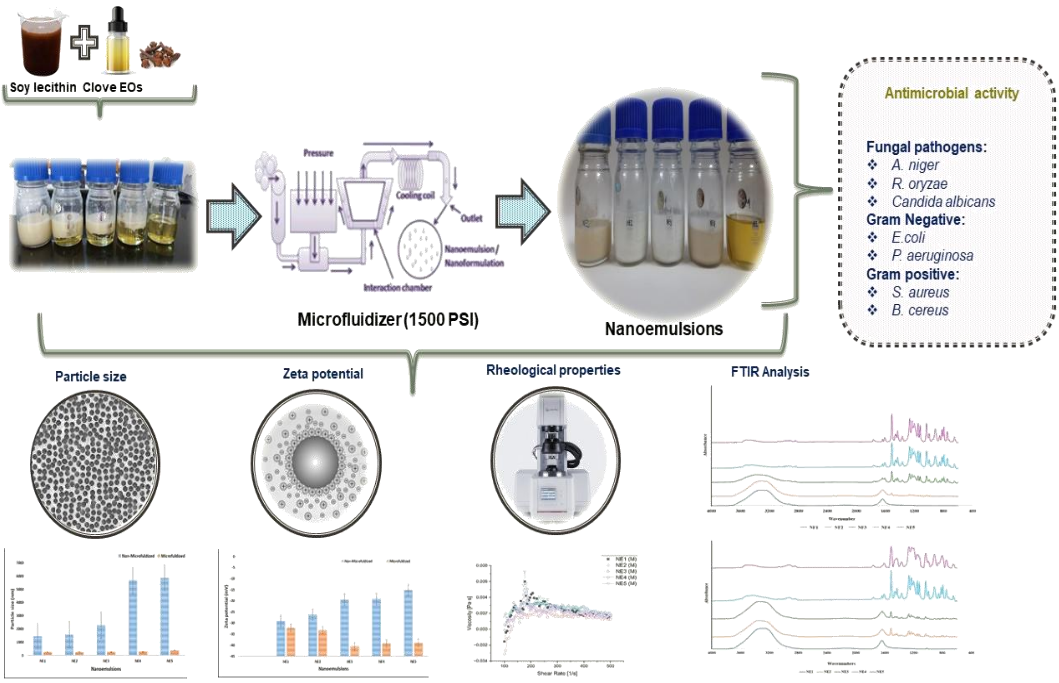


Figure 1: Schematic presentation of preparation of nanoemulsions and characterization

2.4. Characterization of nanoemulsion

2.4.1 Particle size and zeta potential distribution

The electrochemical surface properties of the matrix revealed by measurement of zeta potential and particle size, which indicated the stability and the size distribution of the matrix (Sabbah et al., 2016). The particle size and zeta potential of the soy lecithin and clove oils based nano emulsions were measure using particle size analyser (DLS/ELS, Litesizer 500, Anton Paar, India) with 100 and 1000 numbers of scanning with automatic mode according to process followed by Kumar et al. (2023b). The average value of triplicate reading with standard deviation of particle size (nm) and zeta potential (mV) has been reported as results.

2.5 Rheology characterization

The effects of the shear rate on shear stress and viscosity behaviour of soy-lecithin - clove EOs microfludized and non-microfludized nanoemulsion were determined using rheometer (MCR-52, Anton Paar, Austria) using cylindrical geometry coupled with cooling circulating bath at 20°C by applying 0.01 to 500 per seconds according to the methodology followed by Kumar et al. (2019). The Herschel-Bulkley model was used to calculate the rheology data in terms of consistency (k) and viscosity (n), which also indicated the flow of behaviour of nanoemulsions.

2.6 Color properties

The color properties (LAB) of the microfludized and non-microfludized soy-lecithin - clove EOs based nanoemulsion were determined using digital handheld Chroma meter (CR-400, Konika Minolta Cr-400, Tokya, Japan) (Singh et al., 2023). The L*, a* and b* indicated the Lightness/darkness, greenish/redness and blueness/yellowness of the nanoemulsion. The average value reported as result in terms of L*, a* and b*.

2.7 Fourier Transform Infrared Spectroscopy

FTIR of the microfludized and non-microfludized soy-lecithin and clove EOs based nanoemulsion were performed using ATR-FTIR (Opus, 7.2, Germany) to identify the functional groups in nanoemulsion (Hamid et al., 2021). The spectra of the samples recorded between 600-4000 $^{cm-1}$ of wavelength with 4 $^{cm-1}$ FTIR (Kumar et al., 2024b).

2.8 Antioxidant activity and encapsulation efficiency of nanoemulsion

The antioxidant activity of the microfludized soy-lecithin and clove EOs based nanoemulsion were investigated according to the standard DPPH (2, 2, Diphenyl, 1, Picrylhydrazyl) assay. The samples absorbance was recorded at 517 nm of wavelength using UV-spectrophotometer and results reported as % inhibition activity (Zhao et al., 2020). The encapsulation efficiency was determined by the difference between the antioxidant activity of microfludized and non-microfludized nanoemulsion and pure clove essential oils (NE5) (Wang et al., 2023). The DPPH activity of the nanoemulsion calculated using below equation:

% inhibition = $^{Abs\ 517}$Control- $^{Abs\ 517}$Sample/ $^{Abs\ 517}$ Control*100

2.9 Antibacterial and antifungal activity

The antibacterial and antifungal activity of the soy lecithin-clove oil-based nano emulsions against gram positive (Staphylococcus aureus, Bacillus cereus), gram negative (Pseudomonas aeruginosa, Escherichia coli) and fungal strains (Aspergillus niger, Rhizopus oryzae, Candida albicans) microbial strains were investigated using by agar well diffusion method according to Kumar et al. (2023b) with minor changes. The inoculated microbial culture was applied in prepared Muller-Hinton agar and Potato dextrose media plates. Thereafter the 6 mm of well were prepared in the media using sterile cork borer and nano-emulsions were placed in it. The petri- plates allowed incubating at 35℃-37℃ for bacterial and 25℃ for fungal strains up to 48 h and 120 h respectively. The results measured and reported in terms of zone of inhibition in mm

2.10 Statistical analysis

The average value of the triplicate data reported as results with standard deviation and standard errors. ANOVA was use to analyse the data with appointed Posthoc Duncan multiple range test with 0.05 of significance level using SPSS software. Microsoft excel (2016) used to prepared graph.

3. Results and discussion

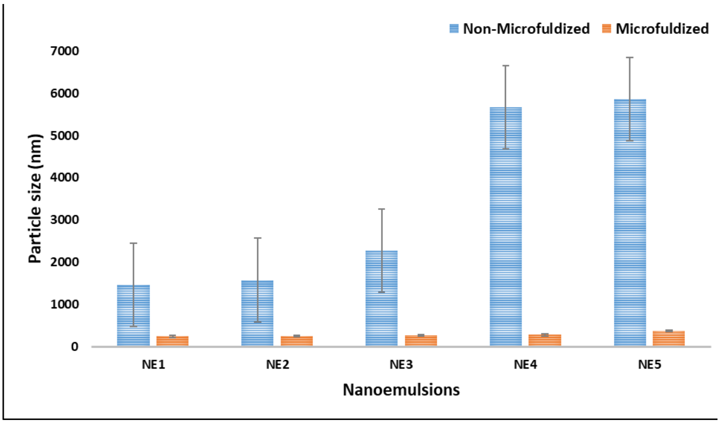
3.1 Particle size and zeta potential

The effects of increasing concentration of clove essential oils and microfludized treatment on the particle size and stability of nano emulsion shown in Table 2 and Figure 2. The results revealed that the increasing concentration of clove essential oils in soy lecithin significantly ($P<0.05$) increased the particle size of nano emulsion due to hydrophobicity (Shenbagam et al., 2023). The higher particle size in non-microfludized nano emulsion were found in NE5 (5855 nm) followed by NE4 (5672 nm) nano emulsion with lower zeta potential value as -15.1 mV and -19.2 mV respectively. The nano emulsion without clove oil showed 1468.5 nm of particle size with -29 mV of stability. In addition, the application of microfludization as high-pressure homogenization process significantly ($p<0.05$) reduced the particle size of nano emulsion with enhancement of stability (zeta potential) value. The particle size in the nano emulsion were reduced from 1468.6 nm to 247.5 nm in NE1, 1581.3 nm to 254.1 nm in NE2, 2280 nm to 273.1 nm in NE3, 5672 nm to 285.1 nm in NE4 and 5855 nm to -15.1 nm in NE5 nano emulsions respectively. The stability of the, nano emulsion was significantly improved with the application of microfludization application. Therefore, the significantly higher zeta potential vale (stability) was found in NE3 (-40.5 mV) followed by NE4 (-39.2 mV) respectively with the particle size in nano range as 273.1 nm and 285.1 nm respectively. Overall results indicated that the application of microfludization is potential to reducing the particle size of the nano emulsions with improving their stability. The emulsion prepared with equal amounts of soy lecithin and clove essential oils (50:50) showed higher stability due to microphase separation and intermolecular interaction between the hydrophobic and hydrophilic molecules of the soy lecithin and clove essential oil nano emulsions. The microfludization process is potential to produce highly stable and homogenous nano emulsions due to its combined forces of ultra-high pressure, high velocity, intense shear rate and cavitation and which is potential to improved molecular interaction between the oil and water phase in nano emulsions (Ozturk and Turasan, 2021). The results of present investigation are in line with the previous findings of Banasaz et al. (2022), they have developed vitamin A oil and water emulsion using microfludization and reported that the microfludization process significantly reduced the particle size of emulsion and improved stability due to exhibited good molecular interaction between hydrophobic and hydrophilic molecules of oil and water in nano emulsion. In addition, Kumar et al. (2023b) results are also in line with current findings. They have developed nano coating for food packaging using cornstarch with orange peel essential oil by ultrasonication process as high-pressure homogenization and reported reduction in particle size distribution, which leads to higher stability of formulations.

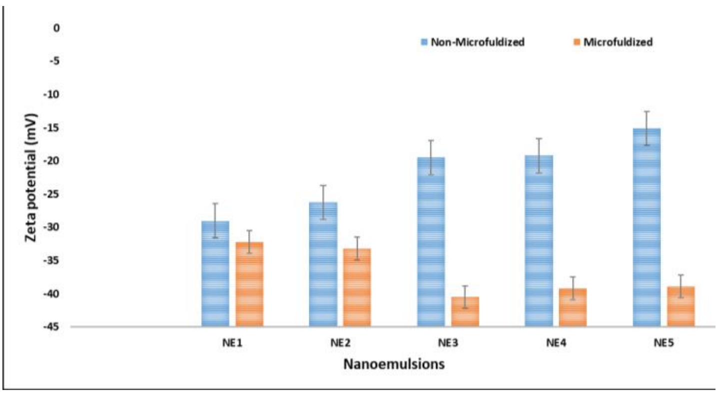
Table 2: Particle size (nm) and zeta potential (mV) distribution in soy lecithin-clove oil-based nano emulsions

Nano emulsions	Non-Microfludized		Microfludized	
	Particle size (nm)	Zeta Potential (mV)	Particle size (nm)	Zeta Potential (mV)
NE1	1468.5±2.31	-29±1.9	247.5±2.34	-32.2±1.6
NE2	1581.3±2.04	-26.2±2.2	254.1±1.90	-33.2±1.5
NE3	2280±3.06	-19.5±2.3	273.1±1.56	-40.5±1.67
NE4	5672±4.91	-19.2±1.06	285.1±2.47	-39.2±1.2
NE5	5855±4.98	-15.1±2.39	376.9±1.89	-38.9±2.3

Where, NE1 (Soy lecithin 100: 0 clove essential oils), NE2 (Soy lecithin 75: 25 clove essential oils), NE3 (Soy lecithin 50: 50 clove essential oils), NE4 (Soy lecithin 25: 75 clove essential oils), NE5 (Soy lecithin 0: 100 clove essential oils)



2a



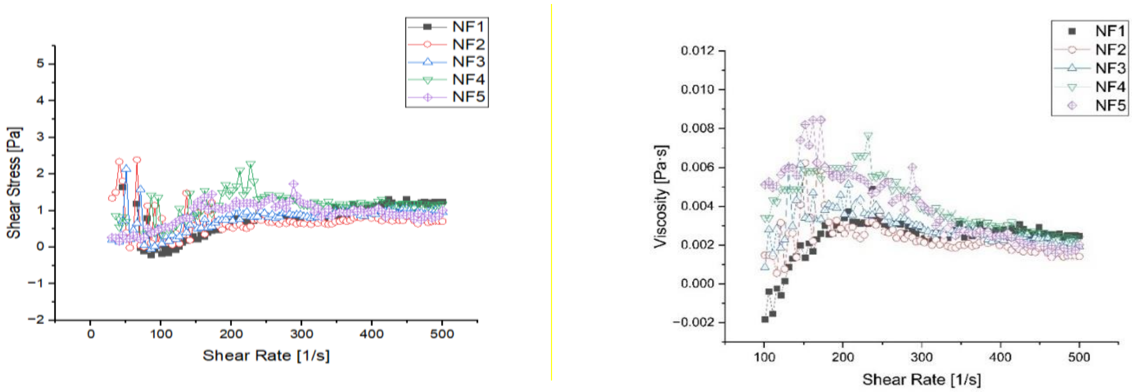
2b

Figure 2: Particle size (nm) (2a) and zeta potential (mV) (2b) distribution in soy lecithin-clove oil-based nano emulsions

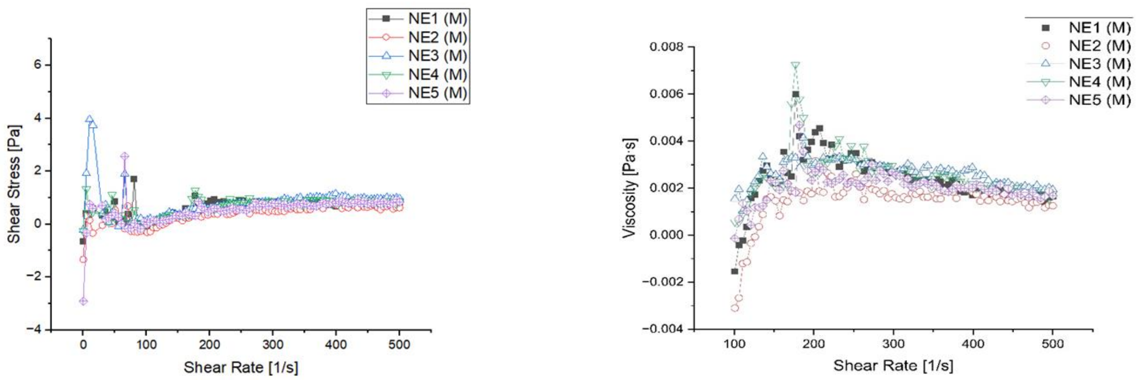
Where, NE1 (Soy lecithin 100: 0 clove essential oils), NE2 (Soy lecithin 75: 25 clove essential oils), NE3 (Soy lecithin 50: 50 clove essential oils), NE4 (Soy lecithin 25: 75 clove essential oils), NE5 (Soy lecithin 0: 100 clove essential oils)

3.2 Rheology characterization

The effects of shear rate on the shear stress and viscosity of microfludized and no-microfludized soy lecithin - clove oil nanoemulsion are shown in Figure 3. The results showed that, all the nanoemulsions had difference thickness and consistency based on the soy lecithin and clove oil compositions. Both (microfludized and non-microfludized nanoemulsion showed shear thinning and non-Newtonian deportment and the viscosity increased with increasing concentration of clove essential oil due to reduction of polarity and interaction between hydrophilic and lyophobic phases (Singh et al., 2023; Primozic et al., 2017). The viscosity in the microfludized nanoemulsions was less as compared to non-microfludized nanoemulsion due to increase collision between the droplets and density difference between water/oil phases (Haro-González et al., 2023). Overall, results indicated that the concentration of clove oils and microfludization have significant effects on the consistency and flow of behaviour of nanoemulsions.



Non-microfludized (3a)



Microfludized (3b)

Figure 3: Effects of shear rate on shear stress and viscosity of non-microfludized (3a) and

microfludized (3b) soy lecithin - clove oil nanoemulsions

Where, NF1/NE1 (Soy lecithin 100: 0 clove essential oils), NF2/NE2 (Soy lecithin 75: 25 clove essential oils), NF3/NE3 (Soy lecithin 50: 50 clove essential oils), NF4/NE4 (Soy lecithin 25: 75 clove essential oils), NF5/NE5 (Soy lecithin 0: 100 clove essential oils), NE= Microfludized nanoemulsions, NF= Non-microfludized nanoemulsion

3.3 Color properties

The effects of the increasing concentrations of clove essential oils and microfludization process on the color (LAB) properties of nano-emulsions are shown in Table 3. The results demonstrated that the combination (water/oil droplets) of the different soy lecithin and clove essential oils showed higher L* values in NF3 (87.77), NF2 (86.29) and NF4 (81.36), respectively as compared to the NF1 (66.67) and NF5 (31.33) in non-microfluidizer nano-emulsions. The a* value was higher found in pure soy lecithin (NF1) with -3.67 as compared to others, which indicated the higher green color appearance in the sample. In addition, a* value was gradually found lower in NF4 (-1.09), NF2 (-0.98) and NF3 (-0.80), respectively. Therefore, b* value was significantly ($p<0.05$) decreased with decreasing concentration of clove essential oils in nanoemulsion, which might be possible due to presence of phytochemical compounds in the clove essential oils. Moreover, the microfluidizer nanoemulsion indicated the same trends of results like non-microfluidizer nanoemulsion but the values of L* were higher in NE3 (90.5) and NE2 (88.63). There is no significant ($P=0.05$) difference observed in L* value of NE1, NE4 and NE5 nanoemulsion. The b* values in microfluidizer nanoemulsion was influenced in NE1 (9.71) and NE5 (7.79), respectively due to cavitation of particle size and homogenous mixing of the pure soy lecithin and clove essential oils. The b* value in NE2 (8.73), NE3 (5.14) and NE4 (4.24) nanoemulsion were gradually declined as compared to non-microfluidizer (NF2, NF3 and NF4) nanoemulsion. Overall results demonstrated that the addition of different concentrations of clove essential oils and microfludization process have significant effects on the color attributes of nanoemulsions. This might be possible due to presence of phytochemical or bioactive compounds in clove essential oil and homogeneous distribution of particles in nano emulsion by microfludization process (Dhaka et al., 2021).

Table 3: Color properties of non-microfludized and microfludized nano-emulsions

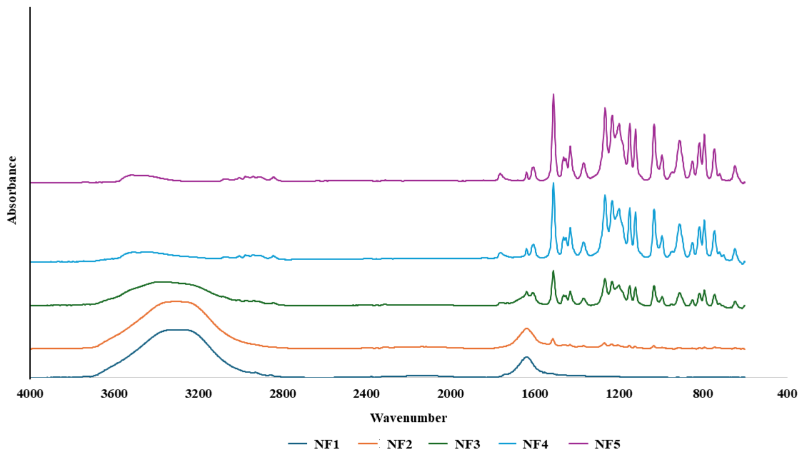
NE	Non-Microfludized			NE	Microfludized		
	L*	a*	b*		L*	a*	b*
NE1	66.67±0.30	-3.67±0.05	7.38±0.53	NF1	66.08±0.3	-3.99±0.01	9.71±0.02
NE2	86.29±0.45	-0.98±0.03	8.92±0.26	NF2	88.63±0.04	-2.42±0.03	8.73±0.07
NE3	87.77±1.25	-0.80±0.12	6.5±0.06	NF3	90.5±0.18	-0.76±0.30	5.14±0.01
NE4	81.36±2.13	-1.09±0.32	7.1±2.07	NF4	81.42±2.18	-1.01±0.09	4.24±0.22
NE5	31.93±0.75	-2.1±0.01	2.25±0.16	NF5	29.22±0.30	-1.39±0.15	7.79±0.83

Where, NF/NE1 (Soy lecithin 100: 0 clove essential oils), NF2/NE2 (Soy lecithin 75: 25 clove essential oils), NF3/NE3 (Soy lecithin 50: 50 clove essential oils), NF4/NE4 (Soy lecithin 25: 75 clove essential oils), NF5/NE5 (Soy lecithin 0: 100 clove essential oils), NE= Microfludized nanoemulsions, NF= Non-microfludized nanoemulsions

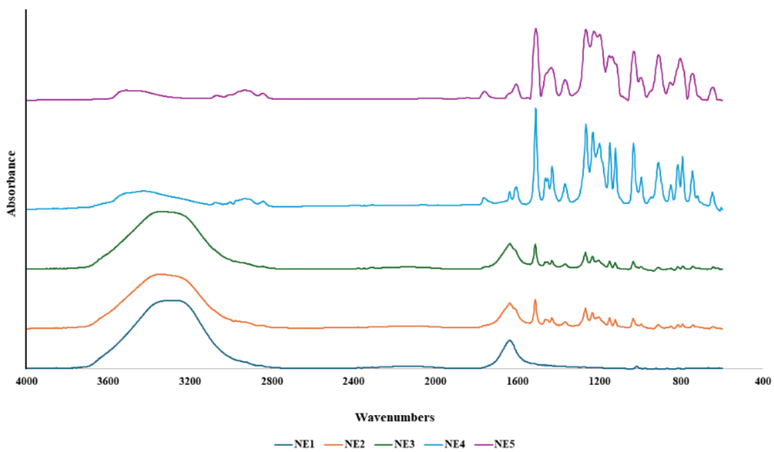
3.4 Fourier Transform Infrared Spectroscopy (FTIR) of Nanoemulsion

The effects of clove essential oil (EOs) concentrations and microfludization on the functional groups of the nanoemulsion are shown in Figure 4. The results showed that, all the nanoemulsion (microfludized and non-microfludized) showed a broad-spectrum peak between 3514 $^{cm-1}$-3274 $^{cm-1}$, which correspondence to the presence of -OH group and strength of the phenol peak. In addition, these peaks shifted higher with increasing the concentration of clove essential oils in nanoemulsion. NE5 and NF5 showed higher peak range at 3514 $^{cm-1}$ and 3509 $^{cm-1}$, respectively due to presence of pure clove essential oils. C-H stretching peak of clove EO was shifted at higher spectra with increasing concentration of clove EOs (Elsherif and Shrief, 2021). The presence of soy lecithin was observed at broad spectrum peak at 2378 $^{cm-1}$ -2229 $^{cm-1}$ in the nanoemulsion, which was shifted lower with increasing the concentration of clove EOs and NE5 nanoemulsion indicated the absence of soy lecithin. The incorporation of higher concentration of clove EOs in nanoemulsion and microfludized application significantly influenced the C-H stretching of the nanoemulsion. The presence of aromatic ring of clove EOs were observed in all the microfludized and non-microfludized nanoemulsion between 1638 $^{cm-1}$ -1607 $^{cm-1}$ peak spectrum (Elsherif and Shrief, 2021). This indicated the interactions between water molecules, soy lecithin and clove EOs, which also leading for enhancing the OC=H vibrations. The interaction between the matrixes also confirmed by the peak at 912 $^{cm-1}$ – 910 $^{cm-1}$. The bending vibration of the -OH and –CH groups peak were observed between 1438 $^{cm-1}$ - 1431 $^{cm-1}$ spectra peak, which is attributed to the presence of love essential oils in the nanoemulsion (Hameed et al., 2021). The aromatic alkyl C=C-H stretching band at 2970^{cm-1} - 2931 $^{cm-1}$ was observed in the nanoemulsion with higher concentrations of clove EOs. A band between 1531^{cm-1} - 1511 $^{cm-1}$ was observed in all the microfludized and non-microfludized nanoemulsion except NF1 and NE1, which correspondence to –C-C stretching band due to presence of clove EOs (Nagaraju et al., 2021; Gul et al., 2022).

Overall, result suggested that the application of microfludization process for the preparation of nanoemulsion significantly improved the molecular interaction and homogenous structure between the water-oil molecules. These findings are good corroborate by the previous studies of Kumar et al. (2024b) and Kumar et al. (2023b), those indicated that the application of high-pressure homogenization and shearing technology influenced the molecular interaction between the matrixes due to micro phase separation. The results are in line with the previous findings of Hameed et al. (2021) and Gul et al. (2022), those have developed and characterized the clove essential oil based nanoemulsion.



Non-Microfludized Nano-emulsions (4a)



Microfludized Nanoemulsions (4b)

Figure 4: FTIR analysis of non-microfludized (3a) and microfludized (3b) nanoemulsions

Where, NF1/NE1 (Soy lecithin 100: 0 clove essential oils), NF2/NE2 (Soy lecithin 75: 25 clove essential oils), NF3/NE3 (Soy lecithin 50: 50 clove essential oils), NF4/NE4 (Soy lecithin 25: 75 clove essential oils), NF5/NE5 (Soy lecithin 0: 100 clove essential oils), NE= Microfludized nanoemulsion, NF= Non-microfludized nanoemulsion

3.5 Antioxidant activity and encapsulation efficiency of nanoemulsion

The results of DPPH antioxidant activity and encapsulation efficiency of nanoemulsions are shown in Table 4. The results indicated that the application of microfludization treatment significantly ($p<0.05$) in increased the antioxidant activity in all the samples. Furthermore, the increasing concentration of clove essential oils in the soy lecithin also increased the DPPH antioxidant activity of the nanoemulsion. The higher antioxidant activity was showed by microfludized NE5 (96.31%), which is significantly higher as compared to the other samples and non-microfludized NE5 (94.04%) respectively. the 100% soy lecithin based nanoemulsion showed significantly lower antioxidant activity before microfludization (15.81%) and after microfludization (17.65 %). The higher antioxidant activity in the nanoemulsion increasing with increased clove oil concentration due to presence of higher phenolic constituents such as eugenol, which is responsible for the higher antioxidant activity of clove oils (Shahavi et al., 2016). The previous studies, Yin et al. (2014) and Fan et al. (2024) were reported that the presence of phenolic compound groups in the clove oil (eugenol) potential as antimicrobial, antioxidant, antifungal, and anticancer properties. In addition, the presence of phenolic groups in the clove essential oils has connecting ability with the emulsifier and improved affinity for aqueous (Hashem et al., 2024). In addition, the application of microfludization techniques as high-pressure homogenization may also significantly potential to improve the biological activity of nanoemulsion due to reduce the particle size of the matrix and improving their molecular interaction and micro phase separation, which resulted development of nanoemulsion in more uniform droplet size with higher stability and antioxidant activity (Dhaka et al., 2021).

These results are in good agreement with the previous findings of Shahavi et al. (2019) and Kumar et al. (2023b), they developed nanoemulsion and edible coating (ultrasonic) with addition of clove and orange peel essential oils and improved their antimicrobial and antioxidant activity. Banasaz et al. (2021) was applied microfludization technique for developing nanoemulsion (corn oil, water, whey protein and vitamin A) and maintained the higher content of α- tocopherol in the emulsion with reducing particle size and improved stability as compared to non-microfludized samples.

Table 4: Antioxidant (DPPH) and encapsulation efficiency of soy lecithin-clove oil based nanoemulsions

Nanoemulsions	Non-microfludized	Microfludized	Encapsulation efficiency (%)
	Antioxidant activity (%)		
NE1	15.81±1.84	17.65±0.95	0
NE2	68.21±2.10	73.96±1.06	76.79±0.21
NE3	83.65±0.87	92.22±0.67	95.75±0.23
NE4	89.26±1.25	93.83±0.84	97.42±0.12
NE5	94.04±0.94	96.31±0.71	100±0.01

Where, NE1 (Soy lecithin 100: 0 clove essential oils), NE2 (Soy lecithin 75: 25 clove essential oils), NE3 (Soy lecithin 50: 50 clove essential oils), NE4 (Soy lecithin 25: 75 clove essential oils), NE5 (Soy lecithin 0: 100 clove essential oils).

3.6 Antibacterial and antifungal activity of nano-emulsions

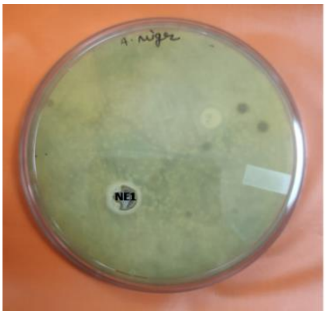
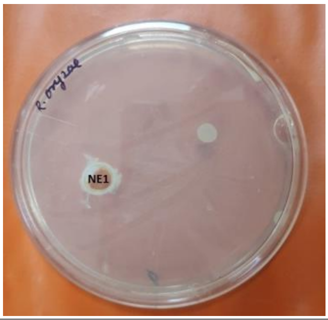
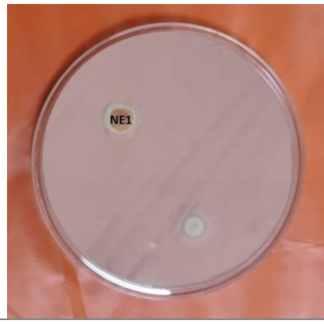
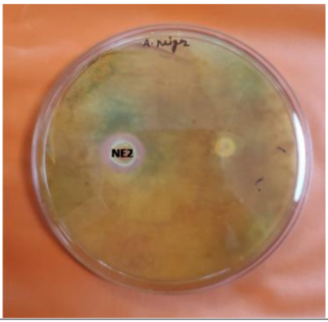
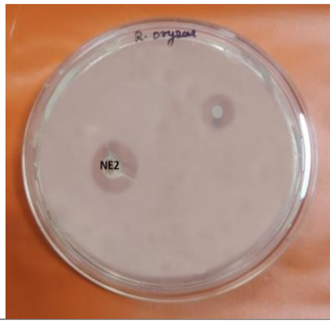
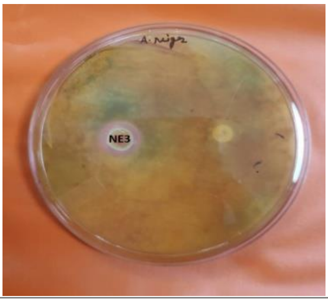
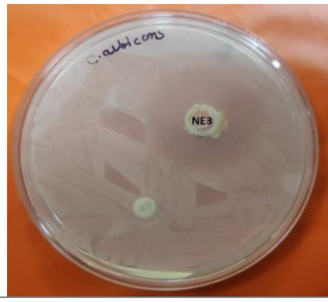
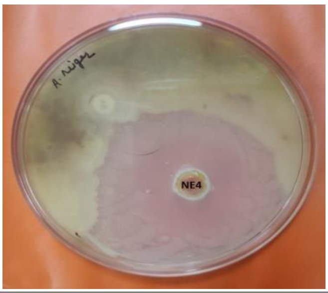
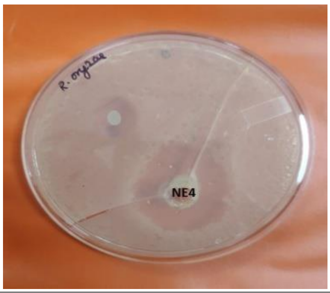
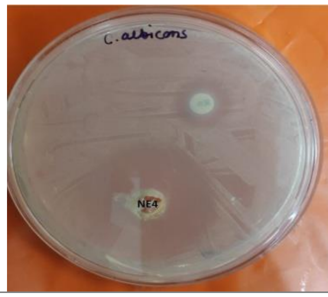
The results of the antimicrobial activity of soy lecithin-clove oil based nano-emulsion prepared using microfludization are shown in Figure 5 (a/b) against fungal (A. niger, C.albicans and R. oryzae), gram positive (B. Cereus and S. aureus), gram negative (E.coli and P. aeruginosa) microbial strains, respectively. As Table 5 showed, that the antimicrobial activity of nanoemulsions was significantly ($p<0.05$) increased with increasing concentration of clove essential oils. The NE1 (control) nanoemulsion did not show antimicrobial activity against R. oryzae, B. Cereus, S. aureus, and P. aeruginosa microorganisms due to absence of clove essential oils. The higher zone of inhibition against fungal and bacterial strains such as A. niger (32 mm), C.albicans (17 mm), R. oryzae (20mm), B.Cereus (30 mm), S. aureus (12 mm), E.coli (18 mm) and P. aeruginosa (20 mm), respectively shown by pure clove essential oils (NE5). This might be possible due to presence of higher phenolic and bioactive compounds such as eugenol in the clove essential oil, which possess good antimicrobial and free radical scavenging activity (Kumar et al., 2024b). The application of pure clove essential oil on fruits and vegetables preservation may affect the natural flavour and aroma of fresh produce, which reduces the consumer acceptability due to presence of higher volatile compounds (Kumar et al., 2024a).

Table 5: Zone of inhibition of nanoemulsions in mm

Nano-emulsions	Fungal strains			Gram positive		Gram Negative	
	A. niger	C.albicans	R. oryzae	B. Cereus	S. aureus	E.coli	P. aeruginosa
	Zone of Inhibition (mm)						
NE1	1.00	1.00	ND	ND	ND	1.00	ND
NE2	5.00	10.0	5.0	20.0	5.0	10.0	5.00
NE3	20.0	12.0	9.0	20.0	7.0	15.0	10.0
NE4	25.0	14.0	12.0	25.0	10.0	15.0	16.0
NE5	32.0	17.0	20.0	30.0	12.0	18.0	20.0

Where, NE1 (Soy lecithin 100: 0 clove essential oils), NE2 (Soy lecithin 75: 25 clove essential oils), NE3 (Soy lecithin 50: 50 clove essential oils), NE4 (Soy lecithin 25: 75 clove essential oils), NE5 (Soy lecithin 0: 100 clove essential oils)

NE4 (75% of clove essential oils and 25% of soy lecithin) nanoemulsion was also demonstrated good antimicrobial activity against fungal and bacterial strains such as A. niger (25 mm), C.albicans (14 mm), R. oryzae (12mm), B. Cereus (25 mm), S. aureus (10 mm), E.coli (10 mm) and P. aeruginosa (16 mm), followed by NE3 and NE2 nanoemulsion. The NE3 nanoemulsion would be a great choice for the incorporation in edible coating and films for food applications due to good antimicrobial activity with fewer aromas. These findings are well corroborated with the previous studies of Haro-González et al. (2023) and Sun et al. (2022), those have reported the antimicrobial behaviour and mechanism of clove essential oil based nanoemulsion against E.coli, S. aureus, L. monocytogenes, S. thyhimurium, Lbp. Plantarum, Lb. acidophilus and Lcb. rhamnosus, respectively.

Nanoemulsions	Aspergillus niger	Rhizopus oryzae	Candida albicans
NE1			
NE2			
NE3			
NE4			

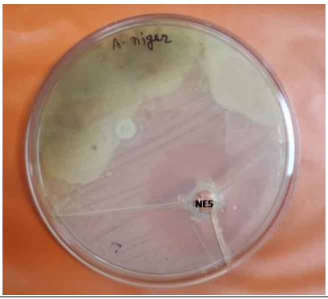
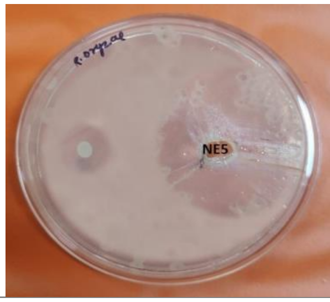
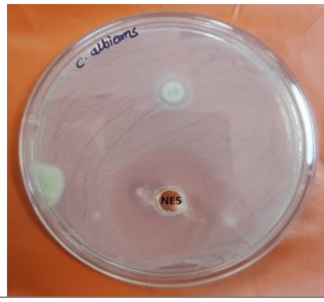
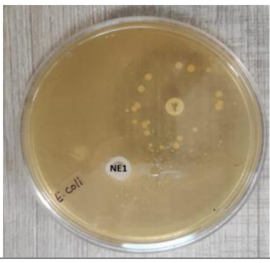
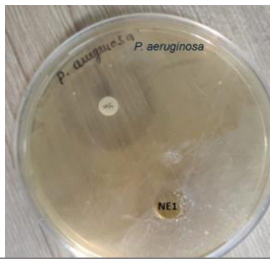
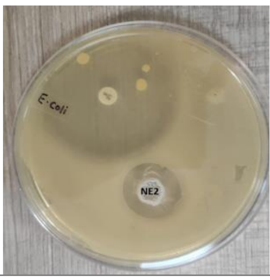
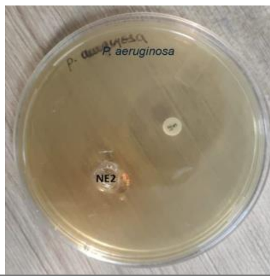
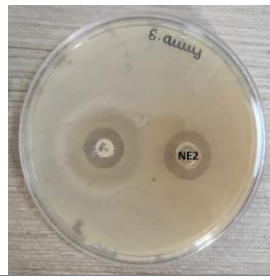
NE5			

Figure 5a: Antifungal activity of soy lecithin- clove oil based nanoemulsion against Aspergillus niger, Rhizopus oryzae and Candida albicans

Where, NE1 (Soy lecithin 100: 0 clove essential oils), NE2 (Soy lecithin 75: 25 clove essential oils), NE3 (Soy lecithin 50: 50 clove essential oils), NE4 (Soy lecithin 25: 75 clove essential oils), NE5 (Soy lecithin 0: 100 clove essential oils)

Nano-emulsions	E.coli	P. aeruginosa	B. cereus	S. aureus
NE1				
NE2				
NE3				

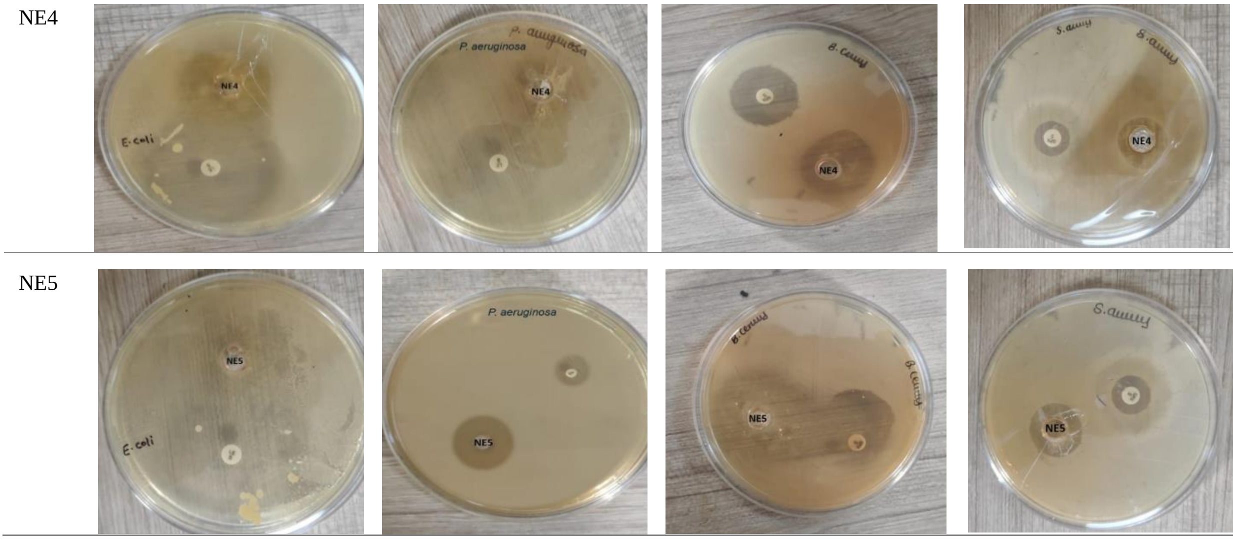


Figure 5b: Antimicrobial activity of soy lecithin- clove oil based nanoemulsion against gram negative (E.coli, P. aeruginosa) and gram positive (B. cereus, S. aureus)

Where, NE1 (Soy lecithin 100: 0 clove essential oils), NE2 (Soy lecithin 75: 25 clove essential oils), NE3 (Soy lecithin 50: 50 clove essential oils), NE4 (Soy lecithin 25: 75 clove essential oils), NE5 (Soy lecithin 0: 100 clove essential oils)

4. Conclusions

In current study, the authors successfully prepared biobased and anti-microbial rich soy-lecithin - clove oil based nanoemulsion for food packaging application. This study indicated that the addition of clove essential oils and microfludization process have significant impacts on the particle size, stability, viscosity, and color attributes of the nanoemulsions. The soy lecithin - clove oil (NE2, NE3 and NE4) nanoemulsions exhibited good properties due to molecular, hydrophobic, and hydrophilic interactions, which make it suitable for incorporation in food packaging as compared to pure soy-lecithin (NE1) and pure clove essential oils (NE5), respectively. The nanoemulsion NE3 showed good physical appearance, lowest particle size and masking of clove essential oil with good antimicrobial activity as compared to others. Therefore, further study should be required to investigating the effects of soy-lecithin - clove oil on food packaging (coating and films) and their application for food preservation.

Conflict of Interest

Authors declared no conflict of interest.

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