Development of cinnamon essential oil blends microemulsion as natural preservatives for topping creams

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Abstract. Food preservatives play an essential role in the production of safe and high-quality food. Natural preservatives are gradually gaining attention because they have less potential health risks compared to chemical preservatives. In this research, the cinnamon essential oil was encapsulated into the microemulsion system. The aim is to assess the effectiveness of cinnamon essential oil blends microemulsion as a preservative by examining its physical characterisation and stability in topping cream. Phase titration method was conducted to formulate microemulsion by mixing cinnamon essential oil, tween 20 and water together according to ratios from 0:10 to 10:0. A pseudo ternary phase diagram was constructed based on the visual appearance of ternary mixtures obtained. Three microemulsions were chosen and further analysed. The results indicated that cinnamon essential oil blends microemulsion exhibits transparent appearance with low acidity (pH 3.5-4.0), moderate conductivity (51-57 µS/cm), low viscosity (<4 mPa second) and shows excellent stability without being influenced by temperature changes and time. Thus, this study has demonstrated the potential of cinnamon essential oil blends microemulsion for its role in topping cream preservation due to the presence of cinnamaldehyde, a major antibacterial compound.

Keywords: Antibacterial, cinnamon essential oil, food preservatives, microemulsion, topping cream.

1. Introduction
One of the major concerns in the food industry is food safety as food spoilage leads to foodborne illnesses among consumers. Therefore, there are many preservation methods applied in the food industry. One of them is preserving food by the addition of preservatives. Generally, preservatives are substances added to food for inhibiting, retarding or arresting the process of decomposition, fermentation, or acidification of such food but exclude herb, spice, vinegar or wood smoke [1]. Even though chemical preservatives such as benzoates and lactates are commonly added in topping cream to
prolong its shelf life, health awareness among consumers are gradually increasing. It has been investigated that some of the chemical preservatives can pose serious health risks. Thus, both consumers and manufacturers keep demanding for more natural and much safer ingredients [2]. Secondary metabolites of plant material like essential oils have been used mainly for food preservation as they are considered Generally Recognised as Safe (GRAS) volatile oils, showing negligible side effects. Cinnamon essential oil is a type of essential oil which is extracted from Cinnamomum verum. It is generally considered to have many desirable and soothing effects and more delicate flavour that is more suitable for desserts as well as promotes several health benefits [3]. Researchers have investigated that cinnamon essential oil possesses bactericidal, antifungal, antiviral, antitermitic, deodorant, larvicidal and nematicidal properties [4].

However, there is a limitation when using essential oils as preservatives in topping cream because it affects organoleptic aspects. Effective antimicrobial doses of essential oils may exceed organoleptically acceptable level, thus altering the food’s flavour [5]. Researchers have acknowledged the advantages of microemulsion technology in food and suggested the application of essential oil as a part of its formulation in order to improve its effectiveness by preventing it from interacting with food constituents [6]. According to Ma, Davidson and Zhong (2016) [7], essential oil-based microemulsion was found to inactivate and significantly decrease bacteria in reduced-fat milk. Microemulsion (ME) is defined as a system of water, oil, and surfactant(s). It is clear, transparent, and a thermodynamically stable liquid mixture that forms spontaneously [8]. A microemulsion is an environmental-friendly and a well-known delivery system that has an excellent component solubilisation and enriched reaction efficacy when combined with other components as well as contributes to longer shelf life. This shows considerable potential in the area of food technology [9,10].

The cinnamon essential oil which makes up the oil phase of the microemulsion system is of interest for its potential as an antimicrobial agent because it has been shown to inhibit the growth of bacteria responsible for food degradation. The oil also displays suppression of fungal growth, including yeast and filamentous moulds [11]. The efficacy of cinnamon essential oil as antimicrobials suggests that the wide range of antibiotic activities are because of its major component, which is cinnamaldehyde, comprising of 85% in the essential oil [12]. This compound can easily penetrate through the cell membranes of bacteria due to their lipophilic nature and de-stabilising the bacteria’s cellular structure [13]. Therefore, the objective of this study was to develop cinnamon essential oil blends microemulsion and evaluate its effectiveness as antimicrobial agents in topping cream.

2. Materials and methods

2.1. Construction of ternary phase diagram

A ternary phase diagram was constructed using a phase titration method. Ten ratios (10:0 to 0:10) of cinnamon essential oil to surfactant (Tweed 20) were titrated with distilled water (1-9 mL) at ambient temperature (22°C), respectively. The blends were mixed and vortexed thoroughly using vortex mixer (VTX- 3000L, labmart, J & H Berge Inc.) for around 1 minute. The blends were centrifuged at 3000 rpm for 15 minutes using a centrifuge (MPW-351R, Med, Poland). After centrifugation, the visual inspection for appearance, phase separation and transparency of the ternary mixtures were observed and recorded for ternary phase diagram construction by using CHEMIX School Software. This diagram identified and distinguished between phase boundaries and distinct regions of ternary mixtures in terms of liquid crystal, oil in water, micro-emulsion, hydrophilic-lipophilic balance and bicontinuous phase.

2.2. Determination of physical characterisation

2.2.1 pH

The pH values of formulated microemulsions were measured using digital pH meter (pH 700, Eutech Instruments, Thermo Fisher Scientific Inc.) to analyse its acidity and alkalinity. The pH meter was standardised using pH 4, 7 and 10 buffers before use.
2.2.2 Conductivity
The electrical conductivity of formulated microemulsions was measured with a reliable and pocket-sized conductivity meter (DiST 3, HANNA instrument, Romania). The conductivity was made almost instantaneously with a portable meter [8]. The conductivity meter was immersed in the microemulsion sample without exceeding the maximum immersion level. The reading was shown on the LCD digital display. All the conductivity determinations must be made in triplicate.

2.2.3 Viscosity
The viscosity of microemulsion was determined without dilution using a Brook Field Viscometer (model LVF 69726, Brookfield, Ametek) with UL adapter [9]. Each sample with a volume of 10 mL was poured into 20 mL (16 x 150 mm) using LV-4 spindle at different speeds (30 rpm, 60 rpm and 100 rpm). The spindle was fully submerged in the microemulsion sample.

2.3. Stability study
The physical stability of formulated microemulsions was studied regarding the effect of temperature changes in a fixed time. Formulated microemulsions were stored in low (4ºC) and high temperatures (60ºC) for 48 hours and observed for phase separation, flocculation or precipitation.

2.4. Gas chromatography-mass spectrometry analysis
The compounds present in the formulated microemulsions were analysed using gas chromatography-mass spectrometry (GC-MS) (QP2010 Ultra instrument, Shimadze, Kyoto, Japan) equipped with a HP-5MS column (30 m x 0.25 mm x 0.25 µm) film thickness (Agilent Santa Clara, USA). GC-MS spectra were obtained using the following conditions: the carrier gas was helium, the flow rate was 1.2 mL/min with injection temperature of 250ºC, the solvent used was hexane, sample injection volume was 1 µL, the oven temperature program was initially at 60ºC for 2 min and increased at 15ºC/min from 60ºC to 140ºC and was held for 2 min, then increased to 180ºC at 5ºC/min, held at 180ºC for 3 min, then it increased to 250ºC for 3 min at 10ºC/min. The ionisation mode used was operated at 70 eV. Three microemulsion formulation samples were injected automatically into the GC-MS system. The reading was collected in full scan mode (m/z 60 – 600). The relative content of each component was determined by area normalisation.

3. Results and Discussion

3.1 Formulating microemulsion
A microemulsion is a thermodynamically stable system with a transparent appearance [14]. Figure 1 shows an illustration of the ternary phase diagram based on visual observation of ternary mixtures. The ternary phase diagram is a three-dimensional diagram that displays characteristics of ternary mixtures. Thus, this diagram characterised the microemulsion domains. It was observed that cinnamon essential oil blends microemulsion present clear and transparent appearance. The monolayer which makes up the microemulsion is due to the surfactant which is responsible for combining water and oil at the interface. The hydrophobic tails of surfactant molecules tend to dissolve in the oil phase, whereas the hydrophilic heads tend to dissolve in the aqueous phase. There are 20 microemulsions formulated from a different volume of cinnamon essential oil, tween 20 and water. Microemulsion (ME) exists within the grey region in the ternary phase diagram from Figure 1. Other mixtures formed are emulsion (E), two phase (2P) and three phase (3P).

It can be assumed that the ternary phase diagram from Figure 1 corresponds to solubilisation theory which microemulsion consists of oil solubilised due to normal micelle formation and water solubilised by reverse micelle formation [15]. Three stable microemulsion samples were selected based on the preparation, as shown in Table 1 for further analysis.
3.2 Physical characterisation of microemulsion
The effect of pH is associated with bacterial growth. Based on the results shown in Figure 2, it showed that the pH range of micro-emulsion samples is around pH 3.5 to 4.0, indicating that micro-emulsions are acidic. The acidity condition of the microemulsion is expected to prevent the growth of bacteria as most bacteria grow best at neutral or slightly alkaline state [16].

Conductivity measurement provides useful information on the behaviour of microemulsion by identifying the nature of the continuous phase. It is performed on the microemulsion to determine its ability to transfer electric current. The minimum conductivity and maximum conductivity for conductivity meter are 0 µS/cm and 99.9 µS/cm, respectively. Figure 3 revealed that the conductivity of samples is in the range between 51 to 57 µS/cm. As conductivity is the reciprocal of resistivity [17], it can be indicated that the microemulsion gives moderate conductivity, showing low resistivity. Additionally, conductivity tends to increase with an increase in water content. Since sample C was formulated with a high amount of water, it shows the highest conductivity compared to the rest with 56.88 µS/cm while sample A consisting of a low amount of water shows the lowest conductivity of 51.66 µS/cm. This proves that the samples contain water as the continuous phase as oil-in-water (O/W) microemulsion has relatively high conductivity. Increase in conductivity is due to the presence of micelles, where oil droplets in a continuous aqueous phase had increasing interaction with each other.
Conversely, water-in-oil (W/O) should give relatively low conductivity as determined by the continuous oil phase. Electrical conductivity has shown to be related to antibacterial activity as it is used to measure the cell membrane permeability. An increase in conductivity corresponds to complete loss of viability of bacteria [19]. Based on the results, it shows that the high conductivity of microemulsion plays a role in antibacterial activity in topping cream. Viscosity is the measure of fluid’s resistance under an applied force. According to Bloor and Wyn-Jones (2012) [20], microemulsion has low viscosity generally. The graph from Figure 4 shows that sample C has the highest viscosity while sample A has the lowest viscosity at speed 30 rpm, 60 rpm and 100 rpm. The viscosity of microemulsion systems depends on oils and surfactant used. The changes in viscosity are due to changes in molecular interaction [21]. More internal force is applied to sample C, causing higher viscosity due to more molecules movement in sample C compared to other samples. This means that sample C is thicker compared to other samples. Therefore, the particles move slower than the others. The microemulsion becomes more viscous with increasing speed. It can be assumed that the samples are shear-thickening fluids as their viscosity increases with increasing shear rate. The results indicate that the average viscosity of all the samples is not more than 4 mPa second, showing low viscosity. It can be assumed that the fluid of the samples is thin due to the presence of small molecules.

3.3 Stability study
After storing the microemulsion samples at a low temperature of 4˚C and high temperature of 60˚C for 48 hours, it is observed that all the samples do not display any cracking, creaming and phase separation. It is clear, transparent and consists of only the phase. This shows that the microemulsion system is in stable condition to be incorporated into food. This proves that the microemulsion is not susceptible to any changes in terms of physical appearance and has the ability to withstand heating and cooling temperatures [22]. During long-term storage, it is also observed that there were no single changes in the condition for microemulsion.

3.4 Gas-chromatography-mass spectrometry analysis
Gas chromatography-mass spectrometry analysis has verified the presence of cinnamaldehyde, cinnamic acid, cinnamyl acetate and eugenol in cinnamon essential oil blends microemulsion. Table 2 showed that cinnamaldehyde is the major bioactive component found in cinnamon comprising more than half of the components in oil [23].
Table 2. Percentage of antibacterial components in cinnamon essential oil

| Components of cinnamon essential oil | Percentage of components (%) |
|-------------------------------------|-----------------------------|
| Cinnamaldehyde                      | 56.3                        |
| Cinnamic acid                       | 30.3                        |
| Cinnamyl acetate                    | 7.1                         |
| Eugenol                             | 2.0                         |
| Others                              | 4.3                         |

A number of experimental studies have shown increasing evidence that cinnamaldehyde is effective against Escherichia coli and Staphylococcus aureus. These bacteria, as well as moulds and yeasts, are commonly known to cause spoilage in bakery products and induce food poisoning. Cinnamaldehyde inhibits their growth by damaging their membrane permeability and integrity. It is reported that cinnamaldehyde attributes antifungal activity against Candida albicans by inhibiting mycelial growth, thus affecting its structure and causes membrane damage. The low-molecular-weight and highly lipophilic components of cinnamon essential oil easily gain access through cell membranes and cause damages to the yeast cell [24]. The bactericidal effect by eugenol could be attributed to cell membrane since this component alter fatty acids composition [25]. Cinnamic acid is associated with Adenosine triphosphate (ATP) production and intake of glucose by acting on sulfhydryl groups of enzymes [26]. The acid showed higher inhibition against fungal species compared to bacteria.

Additionally, the antibacterial efficacy depends on the components’ chemical structure, specifical the presence of hydroxyl groups. Hydrocarbon monoterpenes that is usually inactive exhibits the lowest antibacterial activity [27]. Meanwhile, aldehydes are characterised by having the highest and powerful antimicrobial activity, followed by alcohols. It can be assumed that cinnamyl acetate and cinnamic acid, which are alcohols and carboxyl respectively will show lower antibacterial effect whereas aldehydes such as cinnamaldehyde and eugenol will attribute greater antibacterial activity.

4. Conclusion
It can be concluded that cinnamon essential oil blends microemulsion is suitable as a preservative in topping cream because it is safe and has numerous useful properties. The advantage of microemulsion technology proves that cinnamon essential oil is effective by stabilising and preventing it from interacting with other important constituents in food. The results obtained concluded that cinnamon essential oil blend microemulsions possess clear and transparent appearance, low acidity (pH 3.5 - 4.0), moderate conductivity (51 - 57 μS/cm), low viscosity (<4 mPa second) and showed great stability without being influenced by changes in temperature and time. Verification of the presence of cinnamaldehyde, cinnamic acid, cinnamyl acetate and eugenol by GC-MS analysis shows that these components exist in the cinnamon essential oil blends microemulsion and have an efficient antibacterial effect. Thus, this research has demonstrated the strong potential of cinnamon essential oil blends microemulsion to be used as a natural preservative in food.

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