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Capsaicin emulsions: Formulation and characterization

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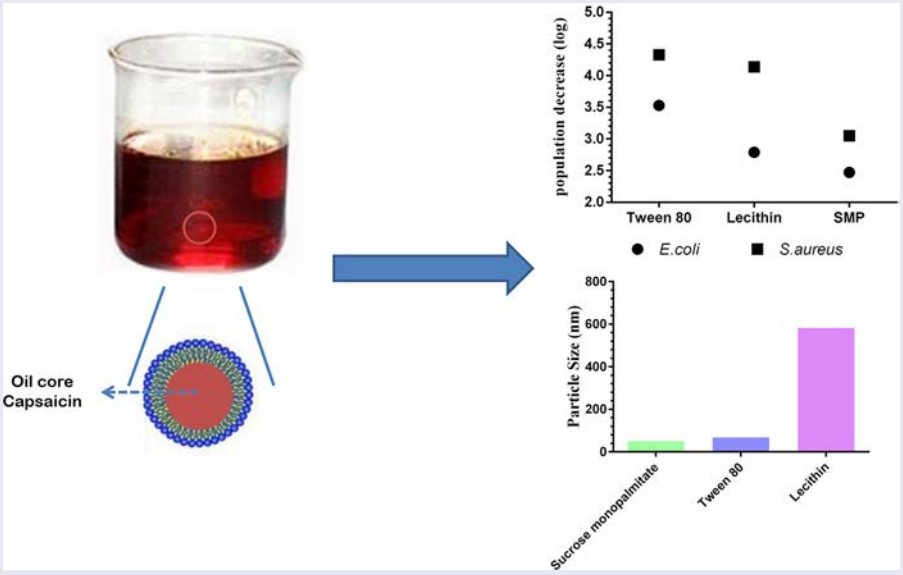
ABSTRACT

Oleoresin capsicum, the oil extract of chili pepper, is mainly composed of capsaicin. Capsaicin is a hydrophobic volatile compound exhibiting antimicrobial activity against various microorganisms. Capsaicin in the form of an emulsion-based carrier system could be a good alternative to enhance bioavailability and simultaneously to increase the shelf-life of food. In this study, capsaicin emulsions were formulated using three different surfactants (Tween 80, commercial soy lecithin, and sucrose monopalmitate/SMP). Effects of aqueous phase composition, pH, and heating the pre-homogenized dispersion were investigated. For characterization, NMR relaxometry, color, turbidity, and antioxidant activity experiments were conducted. Antimicrobial efficacies of the emulsions were also evaluated against *Escherichia coli* and*Staphylococcus aureus*. Mean particle sizes of emulsions with surfactants Tween 80, lecithin, and SMP were found to be 68.30, 582.63, and 50.10 nm, respectively. Lecithin-containing emulsions showed the highest antimicrobial activity against *S. aureus* with 4.60 log reduction, whereas the same effect was observed in Tween 80-containing emulsions against *E. coli* with 3.86 log reduction. Emulsions prepared with SMP showed the highest antioxidant activity with 0.482 mg DPPH/L emulsion. The formulated emulsions have the potential to be used in food industry as antimicrobial food grade solutions.

GRAPHICAL ABSTRACT



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Antimicrobial; antioxidant activity; capsaicin; emulsion; NMR relaxometry

1. Introduction

In the food industry, it is very critical to eliminate microbial hazards away from food products both for public health and for economy. Therefore, new methods for utilizing the synergistic effect of already existing techniques are needed to ensure food safety. Aromatic, oil-based liquids, which are essential oils, could be considered as possible solutions for preservation since these natural antimicrobial agents have proven to exhibit preservative activities for centuries.[1] The key roles of essential oils are penetration and also disruption of cell membranes due to their hydrophobicities that cause leakage of ions or vital components of cells. There are numerous studies in the literature that focused on the determination of antimicrobial activity of essential oils. Effect of 52 essential oils and their extracts on *Acinetobacter baumanii, Aeromonas veronii biogroup sobria, Candida albicans, Enterococcus faecalis,*

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Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica subsp. enterica serotype typhimurium, Serratia marcescens, and *Staphylococcus aureus* were investigated.[2,3] Among these extracts, the ones obtained from lemongrass, oregano, and bay oil showed inhibitory effects against all microorganisms at 2% (v/v).

Capsaicin (8-methyl-N-vanillyl-6-nonenamide), which is the main active agent found in Capsicum species in the Solanaceae family, is an important essential oil and widely used in pharmaceutical applications and food industry.[4] It has been used as a nutritional and medicinal agent for decades. Capsaicin was used in 32 of 437 remedies by Mayan inhabitants of Mesoamerica to treat microbial sourced illnesses.[5] In the literature, capsaicin was proved to be a compound showing great bioactivity against cardiovascular, respiratory, and nervous system diseases and also was found to show antimicrobial activity against *Staphylococcus aureus, Salmonella typhimurium, Bacillus cereus, Listeria monocytogenes*, and *Helicobacter pylori*.[6] It was also found that capsaicin had an inhibitory effect over the widely known pathogen *Bacillus subtilis* within 48-hour incubation period.[7] In another study, where antimicrobial packaging films were designed, capsaicin extracted from four types of chili peppers (Green Malagueta Salvador, Red Malagueta Salvador, Red Thai Capsicum Frutescens, and Red Cayenne) was used. The result of antimicrobial activity assays showed that 50, 100, and 150 **g/L** capsaicin inhibited *E. coli, Streptococcus*, and *B. subtilis*, respectively.[8]

Studies in the literature confirmed the increase in the bioavailability of capsaicin using emulsions as carrier systems. In a pharmacological study, capsaicin nanoemulsions prepared by high-pressure homogenization were used as a potential transdermal delivery system to permeate through rat's skin. It was shown that permeation of the nanoemulsions was 65% higher than 40% ethanol solution of capsaicin, which was used as a control.[9] In addition, it was studied that double-layer and triple-layer capsaicin-loaded nanoemulsions prepared with chitosan/alginate polymers enhanced the bioavailability of capsaicin 131.7 times than the control ones and prolonged the half-life.[10] Oleoresin capsicum was usually used as the capsaicin source in these emulsion systems. Oleoresin is obtained through industrial extraction of the dried ripe fruits of capsicum species and contains a complex mixture of capsaicinoids.[11,12]

The most important challenge about the use of essential oils in food systems is strong odor/taste that could alter the organoleptic properties of food products when they are used directly as antimicrobial additives. Since in emulsion systems, their activities are found to be higher, by using low concentrations it is possible to design emulsion-based carrier systems with minimal effect on organoleptic properties. Recently, emulsion-based systems have been successfully used in food applications as effective lipophilic carrier systems for nutraceuticals, drugs, flavorings, antioxidants, and antimicrobial agents.[13,14] Emulsions also introduce many benefits such as enhanced solubility for lipophilic compounds and bioavailability.[15]

Energy input is necessary to form emulsions due to immiscibility of the lipophilic and aqueous phases. To stabilize the food emulsions, emulsifiers are used. These molecules have an important role as they prevent droplets to coalescence after homogenization. Soy and egg lecithins, sugar esters as well as sorbitan esters (Tweens) are the commonly used surfactants in food emulsion formulations.[16] Surfactants create barriers in the whole colloidal dispersion and keep oil and aqueous phases separate. It is crucial to find the appropriate surfactant type and amount in designing an emulsion system. There are numerous studies about the use of synthetic surfactant Tween 80 and the natural emulsifier soy lecithin in emulsion systems, but studies that use sucrose monopalmitate (SMP) are limited and there is no study in the literature that used SMP to design a capsaicin-loaded emulsion. Also, many factors are able to affect emulsion formation such as pH and heating. It was observed that pH changed the dissolution of surfactant and oil within the dispersion as well as the mean particle size by creating electrical repulsive charge between phases.[17,18] In addition, it was observed that heating had a significant impact on emulsion formation. During heat treatment of an emulsion, the interfacial behavior, oil, water, and surfactant solubility change and these changes could ease the formation of small droplets in an emulsion.[19] When the viscosity difference between oil and water phase in the emulsion increases, the mean particle size of the system increases as well. Therefore, to reduce viscosity difference between dispersed phase and the continuous phase without using a high concentration of the active substance, co-solvents such as glycerol, propylene glycol, or poly ethylene glycol may be used.[20–24] In this study, the effects of all aforementioned factors were evaluated.

Nuclear magnetic resonance (NMR) relaxometry is a convenient and nondestructive analytical method that is successfully used for microstructural analysis. NMR relaxometry provides an advantage to evaluate particle size,[25,26] mobility[27,28] and arrangement of oil molecules[29] within emulsion systems. During NMR measurements, the sample is exposed to a static magnetic field and series of radio-frequency pulses cause movement of hydrogen nuclei. Then, the signal decay is observed during relaxation, which is used to obtain the spin-spin relaxation (T_2) time. Overall, T_2 times are mainly related to the mobility of protons that come from oil and water in emulsion systems.[30]

The objective of this study was to prepare and characterize capsaicin-loaded emulsion systems that were prepared by using a high-speed homogenizer. In the study, effects of surfactant type, pH, continuous phase composition, and heating of the dispersions before homogenization were investigated. For characterization of the emulsions, the nondestructive technique NMR relaxometry (T_2 relaxation), particle size, color, turbidity, and antioxidant experiments were conducted. For determination of antimicrobial activity, emulsions of different formulations were tested against *Escherichia coli* and *Staphylococcus aureus*.

2. Materials and methods

2.1. Materials

Oleoresin capsicum (OC, SHU 1000 000) was supplied from Alfasol (Gaziantep, Turkey). Tween 80, potassium phosphate

monobasic, sodium phosphate dibasic dihydrate, sodium acetate, ethanol, methanol, ethyl acetate, glacial acetic acid, 2, 2-diphenyl-2-pcrylhydrazyl (DPPH), and pure capsaicin (≥95%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glycerol, Violet Red Bile agar (VRBA), nutrient broth, and peptone from meat were purchased from Merck KGaA (Germany). Baired Parker agar (BPA) with egg yolk tellurite was supplied by Nisan Elektronik Ltd., Ankara, Turkey. Soy lecithin was purchased by Smart Kimya (Ankara, Turkey). Sucrose monopalmitate (SMP) was provided by Compass Foods Company (Singapore). Distilled water was used for all preparations.

2.2. Methods

2.2.1. Capsaicin content in oleoresin capsicum

The amount of capsaicin in oleoresin capsicum was analyzed by high-performance liquid chromatography (HPLC) that consisted of a Pursuit C18 column Microsorb MV C18 (4.6 × 250 mm, 5 mm) and UV-Vis (ProStar 330 PDA) detector. The mobile phase was a mixture of methanol:water (70:30 v/v). The flow rate was 0.8 mL/min for 15 minutes at ambient temperature, and detection wavelength was 280 nm.

The amount of capsaicin in oleoresin capsicum was found as 51.0650 ± 0.0919 mg/mL of capsaicin.

2.2.2. Preparation of buffer solutions

Phosphate buffer solution was prepared by dissolving the 4.56 g potassium phosphate monobasic anhydrous and 28.87 g sodium phosphate dibasic dehydrate in distilled water and if necessary adjusting to pH 7.4 using NaOH and/or HCl. Acetate buffer solution was prepared by dissolving 12 mL 0.2 M sodium acetate and 88 mL 0.2 M acetic acid in water and if necessary adjusting to pH 3.8 using NaOH and/or HCl.

2.2.3. Preparation of emulsion

Oil-in-water emulsion was prepared by homogenizing 2 wt% oleoresin capsicum as oil phase in 98 wt% aqueous phase (2 wt% surfactant, 0–50 wt% glycerol, 0.2 M sodium phosphate buffer at pH 7.4, 0.2 M sodium acetate buffer solution at pH 3.8) with UltraTurrax (WiseTis Homogenizer, Witeg Labortechnik GmbH, Germany) at 20,000 rpm for 2 minutes. When lecithin and sucrose monopalmitate were used as surfactants, to investigate the effect of heating in the pre-homogenization period, the dispersion was mixed on a magnetic stirrer and heated to 60°C for 20 minutes. After cooled to ambient temperature, the resulting mixture was homogenized with UltraTurrax at 15,000 rpm for 3 minutes.

2.2.4. Particle size measurements

Particle sizes of capsaicin emulsions were measured with laser diffraction technique by Malvern Mastersizer 3000 system (Malvern Instruments Limited, Worcestershire, U.K.) that used the intensity of light scattered as the laser beam passes through emulsion droplets. The refractive index value of 1.52 was used to calculate mean particle size. Surface area-based mean diameter D[3, 2] was recorded using the instrument's software. These experiments were conducted at 25°C.

2.2.5. Turbidity measurements

The turbidity of all emulsions was measured using a UV-Visible spectrophotometer (T70, PG Instruments Limited, Leicestershire, UK) at 600 nm.

2.2.6. Color

The color of emulsions was measured by a benchtop CM-5 spectrophotometer (Konica Minolta, Inc., Japan) with illuminant D65 and angle of 10° at 740 nm. The parameters of color measurement were L (brightness), a (red/green ratio), and b (yellow/blue ratio). Pure water with values of $L^*\text{ref} = 100.0$, $a^*\text{ref} = 0.0$, $b^*\text{ref} = 0.0$ was used as reference to make white calibration for the instrument standardization. The emulsions were filled in quartz cells, and L^*, a^*, b^* values were recorded.

2.2.7. NMR experiments

NMR experiments were conducted using a 0.5 T (22.35 MHz) benchtop system (SpinCore Technologies, Inc., Gainesville, USA). The T_2 relaxation times of capsaicin emulsions were measured by using Carr, Purcell, Meiboom, and Gill (CPMG) pulse sequence with a 90–180 pulse gap (t) of 1.0 millisecond, the spectral width of 300 kHz, 32 scans, repetition delay of 3 seconds, and number of echoes of 2500. All T_2 measurements were performed at room temperature. Samples were measured in glass tubes with 10 mm sample size.

2.2.8. Antioxidant activity

Antioxidant activities of selected capsaicin emulsions were measured using DPPH method with slight modifications on the methods followed by Wang et al.[31] Each capsaicin emulsion was dissolved in ethanol:acetic acid:water mixture (50:8:42 v/v) and agitated for 1 minute. After filtering the mixture with a microfilter (0.45 µm Chromafil CA-45/25 S, Düren), samples were diluted to mix with 3.9 mL methanol–DPPH solution (1:100). The final mixtures were left in dark for 1 hour at room temperature, and the absorbances of the samples were recorded by UV/Vis spectrophotometer at a wavelength of 517 nm (A2). Methanol that was added to 3.9 mL methanol–DPPH solution was used as the blank sample (A1). Calibration curve was prepared at concentrations of 5, 10, 15, 20, 25 ppm DPPH in methanol. A1 and A2 values were expressed in terms of concentrations C_1 and C_2 (mg DPPH/L). Antioxidant activity was calculated using Equation 1:

$$AA(mg\ DPPH/L) = \frac{C1 - C2}{V_{sample}} \times V_{total} \times d \qquad [1]$$

V_{sample} is the volume of capsaicin emulsion in mL, V_{total} is the total volume of the capsaicin emulsion and ethanol:acetic acid:water mixture in mL, and d is the dilution rate.

2.2.9. Antimicrobial activity tests

Gram-positive *Staphylococcus aureus* (ATCC 43300) and Gram-negative *Escherichia coli* (ATCC 11229) were used to examine the antimicrobial activity of capsaicin emulsions. All bacteria were provided by Public Health Institution of Turkey, from culture collection, and preserved at the Department of Food Engineering, METU. The antimicrobial activity of capsaicin emulsions was performed according to the method

of Al-Adham et al.,[32] Salvia-Trujillo et al.,[33] and Abbaszadeh et al.[34] with few modifications. Overnight cultures of bacteria were grown to stationary phase in an agitated incubator (New Brunswick Scientific, Edison, N.J., USA). The final concentrations of both bacteria strains were 10^8–10^9 colony forming units/ milliliter (CFU/mL). The working cultures of bacteria were prepared by centrifugation at 3600×*g* for 10 minutes and washing twice with sterile saline (0.85% NaCl) and Tween 80 (0.1%) solution. For testing the antimicrobial activity of emulsions, 1% v/v-aliquot of subcultures of each bacteria strain was mixed with 0.5 mL of the capsaicin emulsion and 4.5 mL of sterile phosphate-buffered solution (PBS, pH 7.4). For *E. coli*, after contacting 15 minutes at 37°C with emulsions, 0.1 mL samples were taken from the mixture and spread on VRBA and left for counting the colony after incubation at 37°C for 24 hours. For *S. aureus*, after contacting 15 minutes at 35°C with emulsions, 0.1 mL samples were taken from the mixture and spread on Baird Parker agar with egg yolk tellurite and left for counting the colony after incubation at 35°C for 48 hours. These experiments were performed in duplicate for *E. coli* and *S. aureus*. All components of emulsions were tested for microbial contamination as controls.

2.2.10. Statistical analysis

All characterization experiments for emulsions were performed in triplicate. The differences between the mean values were assessed with analysis of variance (ANOVA). The significance of mean differences was checked by the Tukey test at 5% significance level using Minitab (ver.16.2.0.0, Minitab Inc., United Kingdom).

3. Results and discussion

3.1. Mean particle size

Mean particle sizes (d_{32}) of emulsions at different formulations are shown in Figure 1. Surfactant level within emulsion was kept low (2% w/w) primarily to avoid Oswald ripening and instability problems. Moreover, it is known that the free surfactants that do not interact with oil molecules promote micelle formation that may end with droplet growing and phase separation.[35]

Surfactant type had a significant effect on mean particle size ($p < 0.05$), **and** the smallest particle size was obtained with SMP ($d_{32} < 71$ nm), whereas the largest particle size was observed in emulsions prepared with lecithin ($d_{32} < 1.4$ µm). Also, Tween 80 produced slightly higher particle size emulsions than SMP ($d_{32} < 84$ nm). It was reported that nonionic surfactants such as Tween 80 and SMP were highly soluble in aqueous phase, whereas a zwitterionic surfactant lecithin was composed of phospholipids that could ease the dissolution mainly in the oil phase. Thus, the absorption behavior of surfactants to oil droplet surfaces could be different resulting in change on the mean particle sizes of emulsions, which were prepared under the same conditions.[17,36] Moreover, Ozturk et al.[17] showed that to obtain smaller particle sizes using lecithin, higher amounts (>2%) were required. Thus, to achieve smaller particle size as with Tween 80 or SMP, higher lecithin concentrations might have been required.

Results in Figure 1 show that pH had a significant effect on mean particle sizes for all surfactant types ($p < 0.05$). Decrease in pH results in changes on repulsive or attractive forces between the particles in colloidal dispersions. In Tween 80 and SMP (without heating) emulsions, **a** decrease in pH caused an increase in mean particle size. Tween 80 and SMP are nonionic surfactants as they do not contain ionizable group. However, in a study conducted by Hsu and Nacu,[37] it was stated that nonionic surfactants might cause oil/water interface to become negatively charged at pH higher than 3. Thus, electrostatic repulsion between particles could be disturbed when pH decreased.

Mean particle size of lecithin- and glycerol-containing emulsions increased at pH 3.8. Lecithin contains ionizable anionic phospholipid groups in acidic pH.[38] As pH decreases, these anionic groups could become protonated, which could have decreased the repulsive force between oil droplets.

Glycerol is added to the emulsions as a water-soluble co-solvent, has an effect on physicochemical properties such as viscosity, refractive index, interfacial tension, and solubility of nonionic or ionic surfactants and thus plays an important role in maintaining the stability of the emulsions.[39] Change in these properties was also reflected in the mean particle size of emulsions (Figure 1). The addition of 50% (w/w) glycerol resulted in a significant decrease in the mean particle size for all emulsions except the ones prepared with Tween 80 at pH 7.4 ($p < 0.05$). The effect was more obvious in lecithin-containing emulsions,

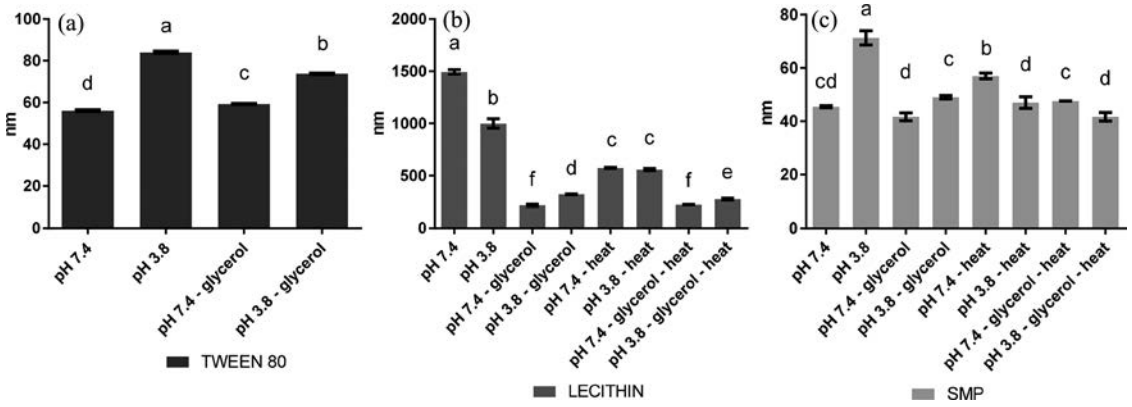

Figure 1. Mean particle sizes of capsaicin emulsions (a) Tween 80, (b) Lecithin, and (c) SMP.

and almost 7 times smaller particle sizes were obtained at pH 7.4. Shchipunov and Schmiedel[40] stated that lecithin was able to maintain stability by creating an interfacial film between oil and water interface. Also, glycerol has a higher viscosity than water and its addition increases the aqueous phase viscosity.[21] According to Dima et al.,[6] an increase in viscosity of aqueous phase could result in a reorientation of surfactant molecules at the interface. Thus, it was hypothesized that change in viscosity was likely to cause a decrease in particle size by increasing adsorption of lecithin around the oil particles due to reorientation at the interface.

Heating of lecithin-formulated emulsions during preparation helped to dissolve the lecithin and formed smaller particles in the colloidal dispersion during continuous stirring in the pre-homogenization process. This was explained due to the changes in the molecular structure of the surfactants with temperature.[22,41] After mild heat treatment (60°C), lecithin became more soluble and helped to form smaller droplets regardless of the pH. On the other hand, heating could also break the interfacial repulsive force between droplets and caused the particle to grow. This might be the case observed in SMP emulsions prepared at pH 7.4 w/wo glycerol. On the other hand, results showed that effect of pH and heating had a synergistic effect and decreased the particle size in the absence of glycerol ($p < 0.05$), whereas no effect was observed with glycerol addition.

3.2. Color

Visual quality is one of the most important attributes for a food product. In this study, the color of the formulated emulsions was also evaluated. It is known that emulsion color is effected from oil concentration, refractive index of both aqueous and oil phase, and mean particle sizes of droplets.[42] The oleoresin capsicum used in this study had very dark red color (almost black) in which L^*, a^*, b^* values were recorded as 0.18, +0.25, −0.11, respectively. The results for the color analyses are given in Table 1. The strong color of oleoresin capsicum was diminished within emulsions. SMP-containing emulsions were the brightest and red colored followed by Tween 80 emulsions. Lecithin emulsions were yellowish in color and had lower L^*, a^*, b^* values compared to other two surfactants. While oil concentration of all emulsions was fixed (2% w/w), it was considered that droplet size and refractive index difference were responsible for the difference. As particle sizes of SMP-containing emulsions were less than Tween 80 and lecithin, the highest L^*, a^*, b^* values were obtained with SMP and the lowest values with lecithin. On the other hand, glycerol had **a** higher refractive index (1.47) than water (1.33). Its addition increased the refractive index of aqueous phase that was reflected in the color results.[43] Glycerol-containing emulsions had higher L^*, a^*, b^* values. When Pearson correlation analysis was conducted between color values and mean particle sizes, a positive correlation of > 0.67 was obtained ($p < 0.05$) with surfactant Tween 80 and lecithin. The change in color of the emulsions in the presence of glycerol or heat treatment before homogenization or pH changes was associated with the differences in the mean particle size of emulsions.

3.3. Turbidity

Transparency is related to the light passed through, whereas opacity comes from an object that scatters or absorbs the light. Most of the emulsions remain between these properties and thus called as translucent.[44] As with the color values, turbidity is an important parameter to maintain natural and appealing looking of a food product and is affected by particle size and refractive index difference of the emulsion. In this study, the turbidities of the emulsions were evaluated and the results are given as in Figure 2. Emulsions were not completely transparent but slightly opaque. Opacity decreased by the addition of glycerol to the aqueous phase. Similar results were observed in nanoemulsions containing glycerol by Qian and McClements.[21] They observed that opaque emulsion turned to slightly turbid with increasing glycerol to 50% in the aqueous

Table 1. Effect of the different surfactants on the color of capsaicin emulsions.

Samples		L^* value[a]	a^* value[a]	b^* value[a]
Oleoresin Capsicum		0.18 ± 0.00	0.25 ± 0.11	−0.11 ± 1.00
Tween 80	pH 7.4	23.3 ± 0.06 c	37.49 ± 0.03 c	40.17 ± 0.1 c
	pH 3.8	14.95 ± 0.01 d	31.98 ± 0.03 d	25.72 ± 0.03 d
	pH 7.4- glycerol	31.54 ± 0.14 a	43.93 ± 0.08 a	54.38 ± 0.25 a
	pH 3.8- glycerol	23.90 ± 0.06 b	40.00 ± 0.03 b	41.17 ± 0.08 b
Lecithin	pH 7.4	0.34 ± 0.01 c	1.35 ± 0.02 c	0.33 ± 0.02 c
	pH 7.4- glycerol	3.44 ± 0.01 ab	15.03 ± 0.0 ab	5.66 ± 0.02 ab
	pH 7.4 - heat	0.45 ± 0.00 c	1.98 ± 0.01 c	0.62 ± 0.02 c
	pH 7.4- glycerol- heat	3.59 ± 0.02 a	15.97 ± 0.00 a	6.10 ± 0.02 a
	pH 3.8	0.29 ± 0.01 c	0.95 ± 0.06 c	0.22 ± 0.03 c
	pH 3.8- glycerol	2.66 ± 0.00 b	12.17 ± 0.04 b	4.39 ± 0.00 b
	pH 3.8- heat	0.26 ± 0.01 c	1.03 ± 0.02 c	0.30 ± 0.02 c
	pH 3.8- glycerol- heat	2.73 ± 0.01 b	12.56 ± 0.00 b	4.56 ± 0.01 b
SMP	pH 7.4	27.28 ± 0.57 d	41.10 ± 0.29 e	47.04 ± 0.98 d
	pH 7.4- glycerol	41.96 ± 0.06 a	52.15 ± 0.02 a	72.35 ± 0.11 a
	pH 7.4 - heat	18.50 ± 0.05 e	34.84 ± 0.04 f	31.89 ± 0.09 e
	pH 7.4- glycerol- heat	37.02 ± 0.04 c	48.86 ± 0.01 c	63.83 ± 0.08 c
	pH 3.8	39.50 ± 0.53 b	49.84 ± 0.32 b	68.11 ± 0.91 b
	pH 3.8- glycerol	41.31 ± 0.13 a	51.98 ± 0.03 a	71.22 ± 0.23 a
	pH 3.8- heat	36.10 ± 0.48 c	47.54 ± 0.28 d	62.24 ± 0.83 c

[a]Data are the mean ± standard error results. Means in the same column indicated by different letters are significantly different ($p < 0.05$). ANOVA was conducted for each surfactant type.

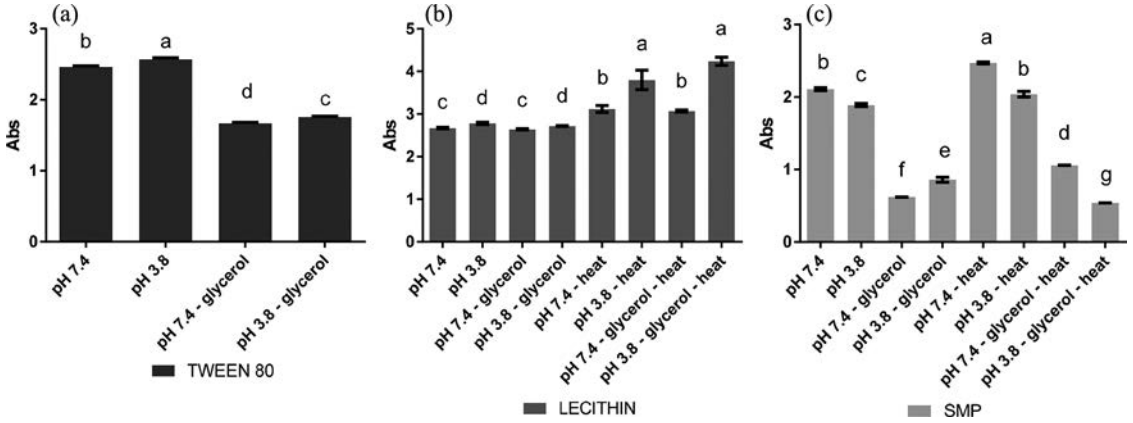

Figure 2. Turbidity values of capsaicin emulsions (a) Tween 80, (b) Lecithin, and (c) SMP.

phase. This was an expected result for two reasons. First, glycerol affects the refractive index contrast between the two phase in the emulsion and consequently turbidity changes. Second, particle sizes could be different when glycerol is added and that results in different scattering property of emulsions.[45] As the smaller particles less scattered the light than larger particles, emulsions containing larger particles had higher turbidity values. Pearson correlation analysis showed that particle size and turbidity of surfactant SMP-containing emulsions were well correlated ($r = 0.396$, $p < 0.10$).

3.4 T_2 (spin-spin) relaxation time

The ability of water molecules to move is related to interactions between other molecules such as oils and surfactants. These interactions could restrict the water molecules completely as well as some free water could remain in the colloidal dispersion.[46] These molecular motions reflect the properties of dispersions by the changes on the T_2 relaxation times. Likewise, oil has short relaxation times and surfactants decrease the molecular mobility by solubilizing in two dispersions.[29]

The changes in T_2 times of the capsaicin emulsions are shown in Figure 3. pH decrease, glycerol addition, and heat treatment before homogenization significantly affected the T_2 values ($p < 0.05$). In all emulsions, the addition of glycerol lowered the T_2 times. One of the main reasons for this is that glycerol drops the mobility of water molecules due to high viscosity. Thus, glycerol-added samples showed fast relaxations in **a** magnetic field. The other reason for the decrease in T_2 times with glycerol addition might be the increase in capsaicin solubility due to co-solvent glycerol and the reduced the mobility of water. This result showed that T_2 may be used to define and quantify the solubility of oil molecules in an emulsion system.

T_2 times were longer at lower pH values. When acetate buffer was added to the colloidal dispersion, the free H^+ protons were increased. Thus, this could result in an increase in T_2 when the pH was 3.8 for Tween 80 and SMP-containing emulsions. On the contrary, the same relation was not observed on lecithin-stabilized emulsions. The polar head group of lecithin molecule interacted with water molecules predominantly, and T_2 times were found lower than other surfactants.

3.5. Antioxidant activity

An organic radical, DPPH is widely used to test the antioxidant activity of food samples.[47] It is known that antioxidant activity comes either from the inactivation of free radicals ($^{-}$OH, $O2^{-}$, and ROO-) or from the inhibition of their formation. The radical scavenging activity is related to reactivity and the amount of antioxidant agent in the medium. Especially, in a multiphase medium such as emulsions, the antioxidant behavior of oil is expected to be different than in its bulk form due to interactions with aqueous phase or surfactants. Therefore, localization of oil phase and mobility is important to show the effect on antioxidant activity.[48,49] In this study,

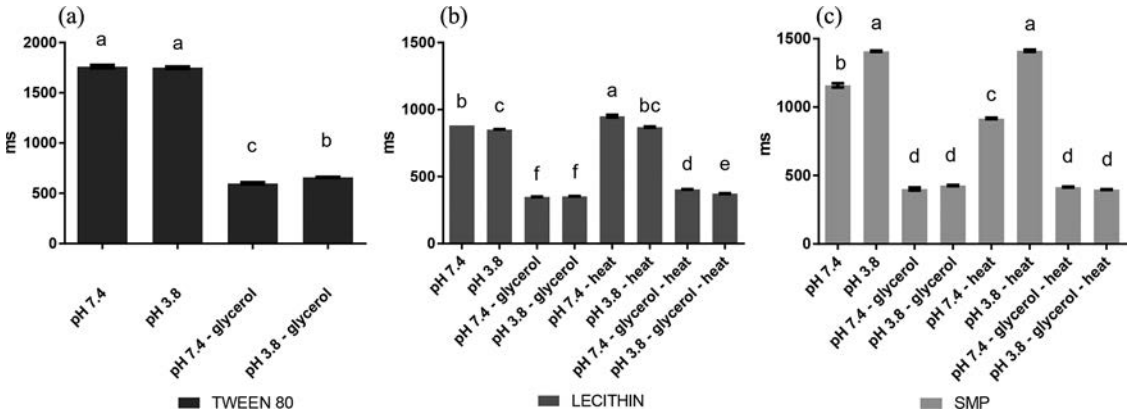

Figure 3. T2 relaxation times of capsaicin emulsions (a) Tween 80, (b) Lecithin, and (c) SMP.

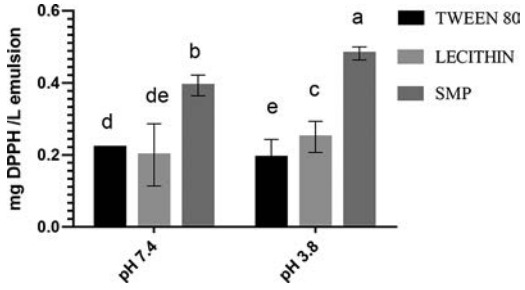

Figure 4. Antioxidant activity of selected capsaicin emulsions for surfactants Tween 80, lecithin, and SMP.

antioxidant activities of selected emulsions were evaluated to observe the effect of surfactant type.

In a study, capsaicin is known to exhibit high antioxidant activity due to its phenolic OH group.[50] The antioxidant activity of oleoresin capsicum was measured as 1.95 mg DPPH/L (data not shown). As surfactants affected the absorption kinetics on oil–water interface and reorganized the interface molecules, they directly affected the antioxidant activities of emulsions.[51] In Figure 4, results show that the highest antioxidant activity was found on the emulsions prepared with SMP at pH 3.8 by using only 2% (w/w) oleoresin capsicum. The lecithin-containing emulsions at pH 7.4 were not found to be significantly different from Tween 80 emulsions ($p > 0.05$). The study confirmed that emulsions prepared with same concentration but different surfactants could differ in their antioxidant activity.

3.6. Antimicrobial activity

The antimicrobial activity results of capsaicin emulsions are shown in Figure 5. All emulsions under the same contact time showed antimicrobial activity against *E. coli* and *S. aureus*. The highest inactivation of *E. coli* for Tween 80, lecithin, and SMP obtained was 3.86, 3.56 and 2.86, respectively. Glycerol addition alone significantly increased the *E. coli* inactivation on the emulsions prepared with Tween 80 and SMP ($p < 0.05$). Also, decreasing pH to 3.8 significantly increased the Tween 80-formulated emulsion's antimicrobial activity, whereas it decreased lecithin-containing emulsion's antimicrobial activity against *E. coli* ($p < 0.05$). *E. coli* is a tough microorganism, which can survive harsh environmental conditions such as pH fluctuations and high temperature.[52] Therefore, decreasing the pH may not be an effective strategy to kill the bacteria along with the capsaicin addition as expected. Freidman et al.[53] examined several essential oils against *E. coli* O157:H7 and *Salmonella* in apple juice at a pH range of 2.8–3.0 and showed that acidity did not contribute to the inhibition of *E. coli*. It was considered that inhibitory activities of capsaicin emulsions against *E. coli* were related to the partition of capsaicin within cell membrane of bacteria. As stated before, capsaicin showed good dispersion in the presence of glycerol within emulsion and that might have eased the permeation to the bacterial membrane. Penetration of active molecules, which in our case is oil phase, through the bacterial membrane is known as an important parameter in antimicrobial activity.[54] Thus, this study revealed that to maximize the antimicrobial activity of capsaicin, glycerol addition was significant to distribute capsaicin in both phases and ease penetration into the cell.

On the contrary, *S. aureus* inhibition was not affected by the surfactant type ($p > 0.05$). Reduction in microbial population for *S. aureus* was in the range of 2.83 to 4.60 log, whereas it was 1.85–3.85 log for *E. coli*.

Emulsions with lecithin exhibited the highest inactivation which confirmed that phospholipid parts of the membranes eased the entrance of lecithin-covered capsaicin droplets. The mode of actions of essential oils to the Gram-positive and Gram-negative bacteria is not known exactly, but it was reported that the main reason behind antimicrobial activity against these two groups was the hydrophobicity differences between cell membranes.[54,55] Gram-negative bacteria contain a hydrophilic cell membrane outside the cell wall that resists antimicrobial actions of hydrophobic essential oils.[56,57] This could explain the lower inactivation toward *E. coli*.

4. Conclusions

In this study, physical, chemical, and antimicrobial properties of capsaicin emulsions prepared with Tween 80, a commercial soy lecithin, and sucrose monopalmitate (SMP) were evaluated. Effect of pH, glycerol addition to the aqueous phase, and heating the dispersion before homogenization were the factors that were investigated. The smallest mean particle size of 41.70 nm was obtained using SMP as the surfactant at a pH of 3.8 in the presence of glycerol with heating. Bright, red colored and slightly turbid emulsions were obtained with Tween 80 and SMP. NMR relaxometry was used successfully

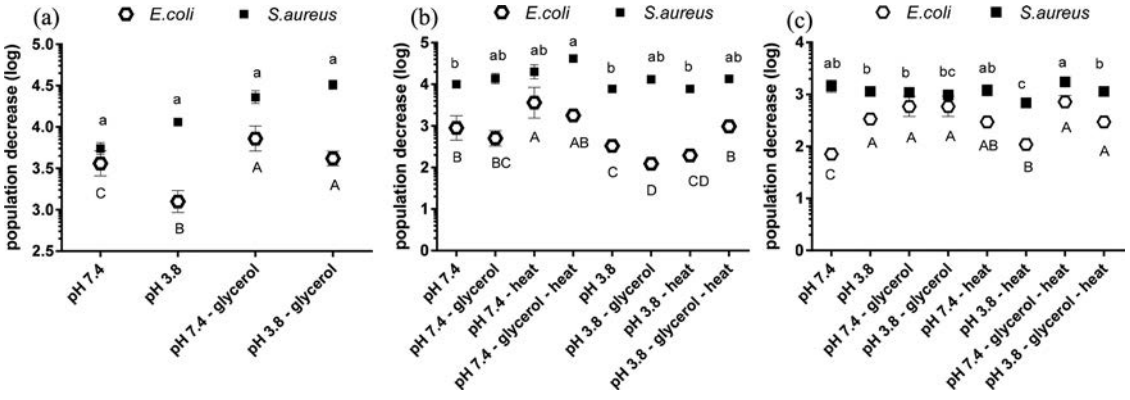

Figure 5. Antioxidant activity of selected capsaicin emulsions for surfactants Tween 80, lecithin, and SMP.

to explain the structural changes between the different formulations. Also, antioxidant properties of capsaicin were protected within emulsions, and the highest antioxidant activity was achieved using SMP at pH 3.8 in the presence of glycerol with heating (0.482 mg DPPH/L emulsion). The highest reduction in *E. coli* population of 3.86 was obtained with emulsions formulated using Tween 80 at pH 7.4 in the presence of glycerol, whereas the highest reduction of 4.60 on *S. aureus* population was achieved using heat-treated lecithin emulsion at pH 7.4 with glycerol. It was shown that glycerol addition to the aqueous phase enhanced the physical properties as well as the solubility of oil within the emulsion. Overall, by using a natural preservative, promising antimicrobial systems were formulated and physical properties were well assessed. The findings of the study could act a guide to design new food product formulations containing capsaicin.

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