# *Assessment of citrus flavor nanocapsules efficiency*

Sedigheh Amiri
Department of Food Science and Technology
Yasooj Branch, Islamic Azad university
Yasooj, Iran
sedighehamiri@gmail.com

Soleiman Abbasi
Department of Food Science and Technology
Faculty of Agriculture, Tarbiat Modares University
Tehran, Iran
sabbasifood@modares.ac.ir

Hamid Ezzatpanah
Department of Food Science and Technology
Science and Research Branch, Islamic Azad university
Tehran, Iran
hamidezzatpanah@yahoo.com

***Abstract*—The aim of this study was to encapsulate orange peel oil (OPO) using microemulsion technique. Microemulsification of OPO with T60 and propanol was studied in 1:1, 1:2 and 2:1 surfactant:cosurfactant ratios. Dynamic light scattering results showed that the particle sizes of the OPO capsules in O/W microemulsions with 1% wt. oil were less than 20 nm. O/W microemulsion systems are good OPO vehicles and OPO release from these systems was more efficient than noncapsulated OPO. The effectiveness of microemulsions in encapsulating of OPO was verified by sensory assessment of beverages containing microemulsified OPO in comparison with ones containing free essential oil.**

***Keywords- microemulsion; orange peel oil; encapsulation; nanoparticles***

## I. INTRODUCTION

Orange peel oil (OPO) is a popular flavoring agent in the food and pharmaceutical formulations due to its specific aroma and low cost but its application has been limited in different foods owing to its low aqueous solubility as well as stability during processing and storage which leads to changes in the sensory properties of the OPO and the products containing it [1]. In order to increase the stability of products formulated with OPO and the possibility of OPO usage in aqueous formulations, encapsulation can be used as a method for entrapping the essential oil within a protective layer of coating materials. Microemulsions, potentially can be used for coating volatile compounds such as essential oils in order to increase their stability during storage or processing. Microemulsions are homogeneous, clear, and thermodynamically stable solutions, which consist of different ratios of oil, surfactant, cosurfactant and water [2]. The transparency of the microemulsions is due to their droplet size (5 to 100 nm) which is smaller than the wavelength of the visible light (150 nm) [3]. Surfactants play very important role in the formation of such systems as they have amphiphilic nature and can form a monolayer film between the polar and nonpolar parts of the system, which can be used for encapsulation of different compounds (polar or nonpolar) in a nonpolar or polar environment [4]. Microemulsions have some advantages over macro-emulsions offering sustained drug or flavor release and improved drug bioavailability [5]. Over the past few years, microemulsions have attracted interests for being used in cosmetic, pharmaceutical and food industries to encapsulate and transport compounds or allow solubility of high polar molecules [3]. Therefore, the main objectives of this study were to evaluate as well as to compare the capability of different food grade surfactants on formation and stability of orange peel oil microemulsions as an encapsulation method. Such systems can be used potentially in food as well as pharmaceutical industries for flavoring and therapeutic purposes.

## II. MATERIALS AND METHODS

### *A. Materials*

Orange peel oil (OPO) (100%, w/w) was obtained from Giah Esanse Company (Gorgan, Iran). Propanol and T60 [polyoxyethylene (20) sorbitan monostearate] were purchased from Merck Chemical Co. (Darmstadt, Germany). All other chemicals were reagent grade.

### *B. Preparation of microemulsion*

Microemulsions were prepared using T60 as surfactant in combination with propanol as cosurfactant with the surfactant:cosurfactant ratio of 1:1, 1:2 and 2:1 at ambient temperature. The samples were prepared by diluting water/surfactant:cosurfactant mixtures with OPO, or by diluting OPO/surfactant:cosurfactant mixtures with water. Therefore, mixtures of water (or OPO) with T60:propanol were made with the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1, respectively. Then, the prepared mixtures were diluted by stepwise addition of OPO (or water), until the transparency disappeared and kept for 24 h at room temperature to ensure equilibrium.

Turbidity of mixtures was considered as an indication of non-microemulsion system [6, 7]. The phase diagrams were constructed on the basis of T60:propanol, water and oil titration measurements to find the points that represent transparent, one-phase systems (microemulsions).

C. Dynamic light scattering (DLS)

The particle size distribution of a microemulsion [containing T60:propanol (1:1) 28, water 71 and OPO 1% wt.] was measured using dynamic light scattering technique (Zetasizer, Nano ZS, 4mW He–Ne laser, Malvern Instrument Ltd., UK) at ambient temperature (wavelength of 633 nm, detection angles 70 and 90◦, dynamic viscosity of sample 8.76 mPa.s). The hydrodynamic droplet size, size distribution and polydispersity index were analyzed by DTS software (5.02 version, Malvern Instrument Ltd., UK).

*D. Release rate of OPO in microemulsion*

The OPO release profile was determined by UV-Vis spectroscopy [7], where 10 g sample from prepared microemulsion [containing T60:propanol (1:1) 30, water 64 and OPO 6% wt.] were placed in a dialysis bag (molecular weight cut off 14 kDa) which was kept in a beake**r** containing 20 g of propanol. The whole system was kept in a thermostatic bath at 30 °C, under constant stirring. At certain time intervals, 2.0 mL of surrounding medium (propanol) were withdrawn and analyzed by UV-Vis. The higher absorbance of solvent shows higher transmission of oil from inside the bag to outside.

*E. Sensory analysis*

Three kinds of beverages (water, a citrus-like beverage and tea (at temperature of 85°C)) were considered as experimental samples for assessing the efficiency of OPO encapsulation. A citrus-like beverage containing 10% sucrose and 1.5 ppm citric acid was prepared. To each sample, OPO (free and in the microemulsion form) was added to give a final concentration of 10 ppm and their sensory properties were evaluated. As it was intended to compare the aroma of capsulated OPO in compared to free one, the panelists were asked to compare the sensory properties of two samples from each kind of beverages (one containing free essential oil and the other containing capsulated essential oil). 30 ml samples were presented in 100 ml opaque plastic cups. Assessors were using water between samples. The samples were coded with 3-digit random numbers. Panelists were then asked to score each sample and also pointed their preferences when answered each question. 12 assessors evaluated the intensity of odor and taste in each sample and rated their liking score (0 to 10) for odor and taste intensity and also overall acceptability of each sample.

*F. Statistical analysis*

The experiments were performed in triplicates. Analysis of variance (ANOVA) was performed using the Duncan's multiple-range test to compare treatment means. Significance was defined at $p<0.05$.

## III. RESULTS AND DISCUSSION

*A. Preparation of microemulsion*

The phase diagrams of OPO/propanol/water with T60 were constructed (Fig. 1). The transparent, one phase regions were considered as the microemulsion regions where the OPO, surfactant, propanol and water were soluble. The microemulsion regions which was produced by T60:propanol with the ratio of 1:1, 1:2 and 2:1 (w/w) were 38.61, 31.51 and 35.29%, respectively. Any changes in surfactant:cosurfactant content led to a significant decrease on microemulsion regions where the 1:1 surfactant:cosurfactant ratio produced the broadest region of one phase microemulsion ($p<0.05$). These results were quite comparable with ones reported on mint [6], as well as eucalyptus oil microemulsion systems [4].

One microemulsion can exist in three forms: W/O, bicontinuous and O/W phase, which are not distinguishable by observing the microemulsion [6, 9]. As it was intended to encapsulate OPO, finding O/W region was very important. For this purpose, the electrical conductivity of the microemulsion [6, 8, 9] was investigated which was then followed by measuring the viscosity [5] (data not shown). Based on electrical conductivity and viscosity data, the boundaries between three regions of O/W, bicontinuous and W/O for the OPO, water and T60:propanol system were determined (Fig. 1). As it can be seen, the O/W region in T60 microemulsion system was almost evident (6.35% of total phase diagram). As T60 is considered as a hydrophilic surfactant and is truly water soluble with high HLB (14.9), it can form O/W microemulsions [10]. O/W region is an important region as this region is more applicable in the food industry. Such region was also limited to a small area in the AOT and CrEL or AOT and Brij35 microemulsions with ethanol and mint oil [6].

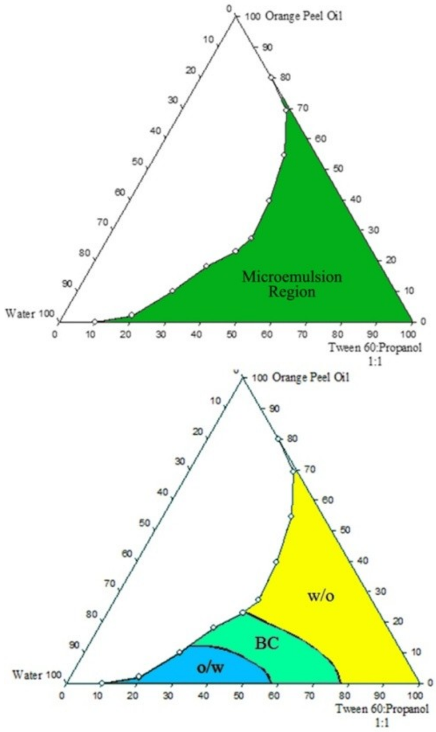


Figure 1. Phase diagrams of OPO/T60 :propanol (1 :1)/water microemulsions at ambient temperature. The yellow, light green and blue colors show the W/O, bicontinuous and O/W microemulsion areas. All ratios were by weight.

*B. Dynamic light scattering (DLS)*

Particle size distribution of a sample from the O/W region of T60:propanol microemulsion was measured using DLS technique. Narrow distributed dispersions with low particle size polydispersity were observed for the O/W region of microemulsion. The mean particle size of over 90% of particles were 2.495 nm and only some 10% had greater sizes (18.44 nm) for T60:propanol (1:1). The polydispersity index was 0.394, indicating the homogenicity of the microemulsion. The polydispersity index is the indicator of particle homogenicity and varies from 0 to 1. When the value is closer to zero, the system is more homogenous [11]. The small sizes of the micelles obtained in this study can be related to high ratio of surfactant to OPO, as demonstrated by Polizelli et al. (2009) [6]. They showed that the micelle size depended on surfactant:oil ratio and it decreased when the surfactant:soybean oil ratio increased.

*C. Release rate of OPO in microemulsion*

According to GC-MS results, limonene is the most component of OPO (>90%). Pharmaceutical and pesticide effects of this compound have been proved [11]. Studies have verified the red palm weevil *Rhynchophorus ferrugineus* larval and adult mortality effect of limonene [11]. These effects as well as anti-spasmodic and anti-septic effects [11] make the release profile of the essential oil more important as a drug or insecticide. The UV-Vis spectra of OPO microemulsion system with T60:propanol (1:1) was measured between 280 to 400 nm to assess the release profile of OPO. As maximum absorbance occurred at 291 nm, the absorbance of microemulsion in comparison with control was evaluated at 291 nm as a function of time (Fig. 2). The higher absorbance is the indicator of higher release of OPO from the systems. As it is shown in Fig. 2, the release rate of microemulsion sample was such higher than that of pure OPO/propanol solution during experiment. Although, the difference of OPO release amount between microemulsion and control decreased with time, but was higher for microemulsion at all stages of measurement. The greater amount of OPO released from microemulsion, especially at the first hours of measurement is so valuable, which makes the system favorable for being used for different compounds especially drugs that need rapid release and absorption. Microemulsion systems have demonstrated potential to improve the bioavailability of the poorly permeable drugs. There are drugs with poor permeability that their oral bioavailability is limited. Consequently such drugs are administered at significantly higher doses than required. Microemulsification can be considered as a solution to increase the membrane permeability of the therapeutic agents. The result of this study was in good agreement with one reported on vitamin E microemulsion systems [2].

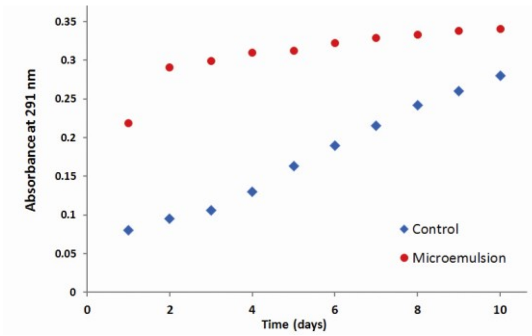


Figure 2. The absorbance of OPO released from OPO microemulsion and propanol solution versus time, at 291 nm.

*D. Sensory analysis*

In sensory evaluation, microemulsified OPO were dissolved in the solutions very well. It was completely in contrast with what happened for the free essential oil as it was spreading on the surface of the solutions. Statistical analysis revealed significant differences ($p<0.05$) between the samples from each category containing free and nanocapsulated OPO. The panelists notified that in all cases, the flavor intensity in samples containing microemulsified OPO was more than free OPO containing samples as the microemulsified essential oil was dissolved in the solutions completely which results in good distribution of flavor and therefore higher citrus sense in the products containing nanocapsulated OPO. In addition, the odor intensity of microemulsified samples was higher than samples containing free oil. After 3 and 6 days, the odor intensity of all samples were decreased, but in all cases the intensity was much higher in nanocapsulated OPO containing samples, whereas the odor intensity were completely disappeared in free essential oil containing samples after 6 days. Encapsulation of OPO caused controlled release of aroma during sample storage. In all cases, the beverage samples containing OPO microemulsions were recognized as preferred samples in compared to samples containing free OPO ($p<0.05$), especially for hot tea. The high temperature of tea at the time of consumption caused the higher release rate of OPO aroma, resulted in undesired taste and odor in tea containing free OPO, while tea containing OPO microemulsion was preferred due to its milder aroma and flavor and its natural citrus flavor sense. The overall acceptability of all samples containing OPO microemulsion was significantly higher than the samples with free oil ($p<0.05$).

## IV. CONCLUSIONS

T60:propanol (1:1) microemulsion system was able to form OPO nanocapsules with particles smaller than 20nm. The UV patterns of these capsules were less affected after 30 minutes exposure to UV light when compared to pure OPO, indicating the protective effect of the OPO enveloping wall. In addition, the microemulsified OPO showed higher sensory attributes in some popular drinks in contrast to free OPO containing samples. Such results indicated the applicability of formulated microemulsion for being used in different aqueous food systems, which is very valuable from the view point of industry.

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