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Effect of vitamin C load on preparing water in oil microemulsions using spontaneous method

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Abstract

Water in oil emulsions could be used for preparing low fat food products or encapsulating water soluble sensitive constituents. In this research, vitamin C loaded water in oil microemulsions prepared using spontaneous method without any co-surfactant. In spontaneous method, microemulsions are formed based on surfactant affinity toward continues phase and are thermodynamically stable. Results showed that by increasing vitamin C concentration, droplets size of emulsions increased from 66.7 nm for pure water to 214.3 nm for 3% loaded microemulsions and poly dispersity index increased from 0.15 to 0.501. Visual appearance of microemulsions changed from transparent for pure water containing microemulsions to opaque for 3% vitamin C loaded emulsions. By increasing vitamin C concentration, viscosity decreased from 115.4 to 87.9 mPa.s. This research showed that up to 3% vitamin C containing microemulsions could be prepared without co-surfactants.

Keywords: microemulsion, spontaneous method, vitamin C load, dynamic viscosity

Introduction

Emulsions are a suspension of water in oil or oil in water, in which the disperse phase droplets are stabilized by covering with emulsifier (Hoffmann & Reger 2014). There are two ways for construction of nanoemulsions: high energy methods and low energy methods. High energy methods include an energy source with the ability to exert extensive forces like high pressure, high shear or ultrasonic energy to emulsion. Compared to high energy methods, low-energy methods are not currently widely used in the food industry. However, studies have shown that low-energy methods are more efficient in producing small droplets than high energy methods and also have some advantages like lower preparation cost, high energy efficiency and the simplicity of implementation. Low energy methods for forming nanoemulsions include spontaneous (self-assemble) method, phase inversion temperature (PIT), and phase composition (PIC) (Yan *et al*. 2014). Structurally, nanoemolsions and microemulsions are relatively similar, and include surfactant, disperse phase and continuous phase. Nanoemulsions and microemulsions also have similar physicochemical properties such as high optical clarity, good stability against gravity separation and ability to entrap water soluble or oil soluble compounds in disperse phase. However, nanoemolions are thermo-dynamically unstable systems, while microemulsions are thermodynamic stable systems. Hence, the final structure and properties of nanoemulsions depend on the pathway used for their production, while the microemulsions do not (Peng *et al*. 2010).

Microemulsions are currently the subject of many researches due to their wide range of potential and actual uses. High capacity of microemulsions to encapsulate drugs and nutritional compounds make them attractive formulations for medications. These systems also have several benefits for oral intake, including increased absorption, increased clinical potency and reduced toxicity (Singh, 2011). Many foods such as yogurt, ice cream, low fat products and mayonnaise are examples of emulsion-based foods (Niu *et al.*, 2017).

The quality of products based on emulsions such as foods, petrochemicals, medications,

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health care products and cosmetics, is determined by a combination of different physicochemical properties such as stability, rheology and appearance (Park *et al*., 2013).

Encapsulation is a process in which active and sensitive compounds (central nuclei), such as oils, flavor compounds, and bioactive compounds are surrounded by a layer or outer wall. Based on the size of the particle, the process output is subdivided as follows: Macroencapsulation (greater than 5000 µm), microencapsulation (1- 5000 µm) and nanoencapsulation (less than 1 µm) (Jafari *et al.*, 2008).

Nanoencapsulation, in comparison to Microencapsulation, have enhanced bio-availability, improved controlled release, and more precise targeting of the compounds in the core. (Mozafari *et al*., 2006, Assadpour and Jafari, 2018). Central core compositions, protective walls, and microcoating methods are effective on the properties of capsules or spheres created. One of the most important methods for producing nanocapsules is the production of nanoemulsions (Ezhilarasi *et al.*, 2012).

Vitamin C (ascorbic acid) is a water-soluble vitamin. This vitamin is found in fruits and vegetables such as guava, orange, strawberries, kiwi fruit and cauliflower. It is essential for the production of collagen protein, wound healing, immune and nervous system as well as antioxidant properties to prevent diseases. Environmental factors such as temperature, pH, oxygen, metal ion, UV, and X-rays affect sustainability of vitamin C (Wilson *et al.*, 1995, Parhizkar *et al.*, 2018).

In this study we tried to encapsulate vitamin C using water in oil microemulsions and evaluate the effect of vitamin C concentration on microemulsions droplet size. At next stage, rheological and optical properties of emulsions were studied. Results could be used to enrich water in oil emulsions.

Material and Methods

Polyglycerol polyrecioleate (PGPR) 4175 kindly donated by Palsgaard**.** Vitamin C was purchased from Merck Germany**.** Sesame oil was purchased from a local market. Distilled water was used for preparing W/ O micro-emulsions.

Microemulsion production

Water in oil microemulsions were prepared by spontaneous method (Mehrnia *et al.*, 2016). Aqueous phase was prepared by mixing water and surfactant using a magnetic stirrer (IKA-ULTRA-TURRAX T25BASIC, Germany) at 13500 rpm, and then added drop wise into oil phase while magnetically stirring.

All emulsions prepared at same conditions: (i) 18 wt.% water, surfactant 10 wt% (wor= 25%) and oil content 72 wt.%; (ii) magnetic stirrer speed of 700 rpm and (iii) stirring time of 2 hours. All emulsions were prepared at room temperature and kept overnight for further analysis.

Effect of vitamin C levels in the aqueous phase

Different concentrations of vitamin C were added to internal aqueous phase to monitor their formation properties and physical characteristics. Vitamin C was dissolved in pure water at 1, 2 and 3% concentration and aqueous phase containing vitamin C was then added dropwise into sesame oil. Up to 3% vitamin C was selected based on solubility of vitamin C in aqueous phase and monitoring its sedimentation visually. Higher concentrations of vitamin C were disregarded due to instability and sedimentation of vitamin C.

Droplet size measurement

Droplet size of microemulsions was measured using a dynamic light scattering method by Zetasizer *Nano*-ZS instrument (ZEN3600, Malvern Instruments). All samples diluted using sesame oil to 1:1000 concentration to prevent multi scattering. To measure droplet size, the refractive index of emulsifier (PGPR= 1.464) and viscosity of sesame oil (viscosity= 62 cp) at 25°C were set in software. All measurements were done in triplicate after overnight storage of samples at ambient temperature (Leong *et al*., 2017).

Color

To evaluate the composition and preparation conditions on the micromelution optical properties, the values of L*, a*, b* were measured using a chromatometer (Konica Minolta CR-400). L* indicates the brightness of the sample while a* and b* represent color coordinates. +a* is red, and -a* is green. +b* is in the direction of yellow and -b * is blue. The color coordinates were measured from the top of the container (Mehrnia *et al*., 2016).

Rheological properties and flow behavior

Viscosity and flow behavior of the emulsions were measured by a Brookfield viscometer (DV2T-LV) equipped with UL-adapter with spindle No. 18 at ambient temperature. Measurements were performed in triplicate and data were calculated on average.

Flow behavior of emulsion samples were described by fitting the experimentally measured shear stress– shear rate data to five different common models namely Power law (Eq. 1), Herschel– Bulkley (Eq. 2), Bingham (Eq. 3), Casson (Eq. 4), and NCA4 /CMA Casson (Eq. 5):

$$\tau = k\gamma^{n} \quad (1)$$

$$\tau = \tau 0 + k\gamma n \quad (2)$$

$$t = t_0 + k\gamma \quad (3)$$

$$t = t_0^{0.5} + ky^{0.5} \quad (4)$$

$$t = \sqrt{t_{CA}} + \sqrt{n_{ca}} * \sqrt{y} \quad (5)$$

Where y is shear rate (1/s), t is the shear stress (Pa), K is the consistency coefficient ($Pa.s^n$), n is the flow behavior index (dimensionless), t_0 is the yield stress (Pa), t_{CA} is the Casson yieid value (Pa), and n_{CA} is the Casson plastic viscosity(Pa s).

Statistical analysis

All experiments were performed on at least two freshly prepared samples. The results are reported as averages and standard deviations calculated from these measurements using a statistical software package (SPSS, version 20). Means were subjected to Duncan's test and a P-value of <0.05 was considered statistically significant.

Results and discussion

The average droplet size can be regarded as the most important characteristics for evaluating emulsification process and stability of emulsion systems (McClements, 2007).

In vitamin C loaded emulsions, by increasing vitamin C concentration in aqueous phase, the average droplet size increased from 66.7 to 214.3 nm. (Table 1).

Table 1- Effect of vitamin C concentration on emulsions droplet size

sample	Droplet size (nm)	PDI
vitamin C 0%	66.730	0.15
vitamin C 1%	107.65	0.2241
vitamin C 2%	170.65	0.423
vitamin C 3%	214.30	0.501

Results showed that except for microemulsions containing 3% vitamin C, nanoemulsion was formed (r<100 nm). Same results were observed in water in oil emulsions containing salts (Sapei *et al*., 2012, Zhu *et al*., 2016) and organic encapsulants (Mehrnia *et al*., 2016). It seems that droplet size is highly affected by surfactant and encapsulant concentration (Lutz *et al*., 2009, Nabi-Meibodi, *et al*., 2013, Rocha-Selmi *et al*., 2013).

Increasing droplet size by increasing encapsulant may be affected by hydrophilicity of encapsulant. Highly hydrophilic compounds entrap more water and prevent disperse phase break to small droplets.

By increasing vitamin C concentration, polydispersity index (PDI) increased from 0.15 for pure water to 0.501 for 3% containing emulsions. Increasing polydispersity index may be due to non-uniform separation of disperse phase droplets in process of microemulsion formation. Visual appearance (Figure 1) of microemulsions showed that by increasing encapsulant emulsion, transparency decreased that is due to increasing droplet size at higher concentrations of vitamin C.

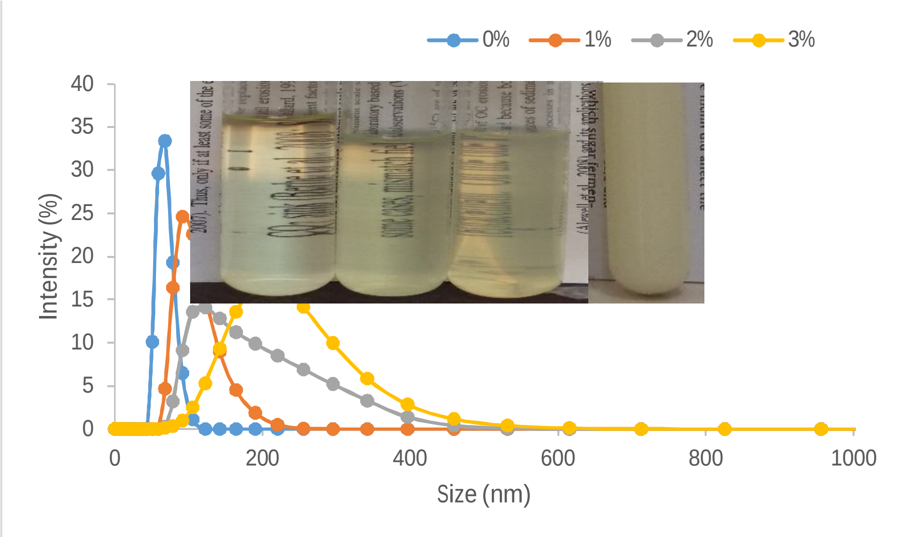

Fig. 1. Effect of vitamin C concentration on visual appearance of water in oil microemulsions

Shear viscosity

Flow characteristic of emulsions is an important and fundamental issue affecting their usage in emulsion based products (Tadros, 2004). Viscosity of emulsions are highly influenced by droplet size and disperse phase concentration (Tadros, 1994, Fanun, 2008).

Comparing viscosity of vitamin C loaded microemulsion (Fig. 2) showed that by increasing vitamin C concentration, viscosity decreased from 115.4 to 87.9 mPa.s. At same concentrations of dispersed phase, smaller droplets separation from disperse phase consequently produce more droplets comparing to separation of large droplets. Higher viscosity of lower vitamin C loaded microemulsions may be due to their lower droplet size, higher number of droplets and consequently higher collision of droplets. Same results were seen in other researches reports that by decreasing droplet size viscosity of microemulsion increased (Mehrnia *et al*., 2016).

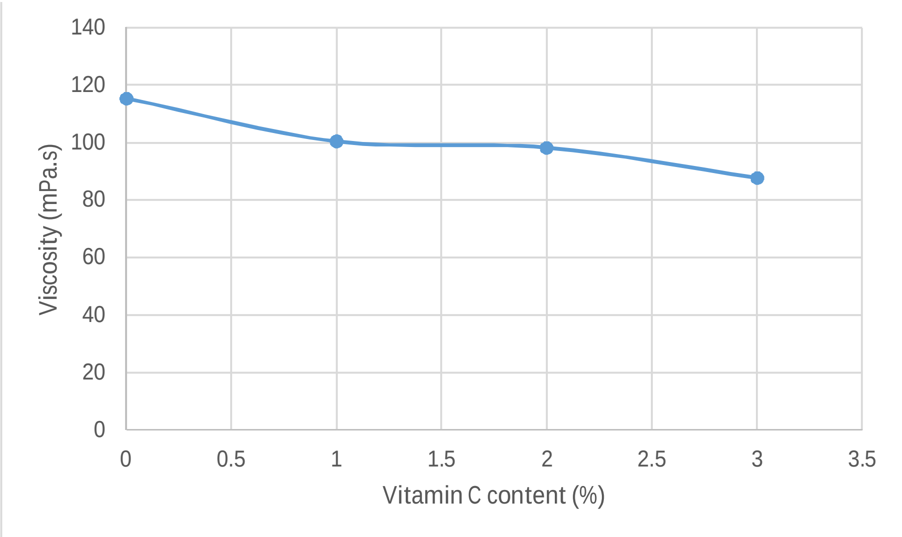

Fig. 2. Viscosity of vitamin C loaded emulsions at 20 rpm

Flow behavior

Evaluating flow behavior of vitamin C loaded emulsions (Table 2) showed that emulsions without vitamin C are mostly fitted on casson models. By adding vitamin C in disperse phase mostly power law or bingham models can predict flow behavior of samples.

Table 2- Flow behavior of vitamin C loaded microemulsions fitted to rheological models

Vitamin C	Model	K (mPa s^n)	n	T_0 (Pa)	Confidence of fit (%)
0%	**Herschel–Bulkley**	121.017±8.890	0.49997±.707	801.127±1103.26	0.49997±.707
	Power law	145.232±34.669	0.919±0.093	-	0.99465±0.007
	Bingham	106.816±10.790	-	89.655±98.848	0.9929±0.009
	Casson	10.151±0.617	-	5.162±6.532	0.99657±0.004
	NCA/CMACasson	103.238±12.525	-	5.162±6.532	0.99657±0.004
1%	**Herschel–Bulkley**	89.754±0.078	1.030±0.0002	56.039±2.946	0.99999±0
	Power law	105.235±0.768	0.988±0.001	-	0.99991±0.0008
	Bingham	100.495±0.004	-	22.071±2.679	0.99995±0.0003
	Casson	9.863±0.012	-	0.937±0.172	0.99983±0.0007
	NCA/CMACasson	53.491±61.686	-	0.937±0.172	0.99983±0.0007
2%	**Herschel–Bulkley**	89.802±0	1.031±0	58.425±2.828	0.99999±0
	Power law	105.937±0.821	0.988±0.002	-	0.99990±0.0008
	Bingham	100.936±0	-	23.185±2.828	0.99995±0
	Casson	9.878±0.012	-	1.022±0.185	0.99983±0.0007
	NCA/CMACasson	97.582±0.254	-	1.022±0.185	0.99983±0.0077
3%	**Herschel–Bulkley**	90.126±2.368	1.026±0.007	45.500±5.800	0.99999±0.0002
	Power law	102.686±0.886	0.991±0.002	-	0.99993±0.0007
	Bingham	99.320±0.005	-	16.503±1.853	0.99996±0.0001
	Casson	9.838±0.002	-	0.564±0.020	0.99989±0.0002
	NCA/CMACasson	96.798±0.056	-	0.564±0.020	0.99989±0.0028

Optical properties of microemulsions

One of the most important advantages of nanoemulsion and microemulsion in comparison to conventional emulsifiers is there transparency or semi transparency that makes them suitable for use in foods and beverages (Mehrnia *et al.*, 2016). Evaluating optical properties of emulsions showed that (Table 3) by increasing vitamin C concentration, lightness decreased from 18.92 for pure water microemolsions to 12.28 for vitamin C 3% emulsion. a value for all samples were negative or slighlty green but vitamin c concentration didn't affected a value significantly. b value for all samples were positive or in yellow area. Yellowness of smaples decreased by increasing vitamin C concentration comparing to pure water. During two weeks storage, lightness decreased, a value and b value increased. Increasing lighness may be due to decreasing droplet size of microemulsions during storage (Barzegar *et al.*, 2018).

Table 3- Effect of vitamin C concentration on optical properties of microemulsion

Vitamin C	Fresh emulsion			Fresh emulsion			1st week			2nd week		
	L	a	b	L	a	b	L	a	b	L	a	b
0%	10.78^{bC}	-1.68^{bA}	18.92^{cD}	18.92^{cD}	-1.68^{bA}	10.78^{bC}	13.88^{aC}	-2.09^{aB}	9.60^{aC}	15.19^{bB}	-2.18^{aC}	10.91^{bB}
1%	10.45^{bB}	-1.59^{bA}	17.24^{cC}	17.24^{cC}	-1.59^{bA}	10.45^{bB}	14.93^{aD}	-2.37^{aAB}	9.73^{aC}	15.68^{bC}	-2.45^{aAB}	11.58^{cC}
2%	9.57^{bA}	-1.69^{bA}	15.93^{bB}	15.93^{bB}	-1.69^{bA}	9.57^{bA}	13.57^{aB}	-2.49^{aA}	8.36^{aB}	16.49^{cD}	-2.38^{aBC}	11.46^{cC}
3%	9.68^{cA}	-1.50^{cA}	12.28^{cA}	12.28^{cA}	-1.50^{cA}	9.68^{cA}	10.52^{aA}	-2.46^{bA}	7.39^{aA}	11.23^{bA}	-2.67^{aA}	8.07^{bA}

Conclusion

In this study effect of vitamin C loading on formation, droplet size, rheology and optical properties of water in oil microemlsions were evaluated. Results showed that using spontaneous method, microemulsions could be formed without any co-surfactant. By increasing vitamin C concentration, droplet size of microemulsions increased and visual appearance changed from transparent to turbid. Increasing droplet size resulted in decreasing viscosity of microemulsions and the lowest viscosity was observed in microemulsions containing 3% vitamin C. Optical properties were in agreement with visual appearance and by increasing vitamin C concentration, lightness was decreased. In two weeks storage, lightness decreased which may be due to decreasing droplet size of microemulsions.

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اثر غلظت ویتامین سی بر ساخت میکروامولسیون‌های آب در روغن به روش خودبه‌خودی

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چکیده

امولسیون‌های آب در روغن می‌توانند برای تهیه محصولات غذایی کم‌چرب یا پوشش‌دهی ترکیبات محلول در آب حساس به شرایط محیطی مورد استفاده قرار گیرند. در این تحقیق میکروامولسیون‌های آب در روغن حاوی ویتامین سی در فاز آبی داخلی به روش خودبه‌خودی و بدون استفاده از ترکیبات فعال سطحی کمکی تهیه شدند. در روش خودبه‌خودی، میکروامولسیون‌ها در روش خودبه‌خودی بر اساس تمایل ترکیب فعال سطحی به فاز پیوسته تهیه شده و از نظر ترمودینامیکی پایدار می‌باشند. نتایج نشان داد با افزایش غلظت ویتامین سی اندازه ذرات از 66/7 nm برای آب خالص تا و 214/3 nm برای میکروامولسیون‌های حاوی 3% ویتامین و شاخص پراکندگی اندازه ذرات از 0/15 تا 0/501 افزایش یافت. ظاهر میکرامولسیون‌ها از حالت شفاف برای آب خالص تا کدر برای میکروامولسیون‌های حاوی 3% ویتامین سی تغییر کرد. با افزایش غلظت ویتامین سی، ویسکوزیته از 115/4 تا 87/9 mPa.s کاهش یافت. نتایج نشان داد که با افزایش غلظت ویتامین سی تا 3%، همچنان می‌توان بدون استفاده از ترکیبات فعال سطحی میکروامولسیون‌ها را به روش خودبه‌خودی تهیه نمود.

واژه‌های کلیدی: میکرومولسیون، روش خود به‌خودی، بارگذاری ویتامین C، ویسکوزیته دینامیکی

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