



## Crocin loaded nano-emulsions: Factors affecting emulsion properties in spontaneous emulsification



Mohammad-Amin Mehrnia<sup>a</sup>, Seid-Mahdi Jafari<sup>a,b,\*</sup>, Behzad S. Makhmal-Zadeh<sup>c</sup>, Yahya Maghsoudlou<sup>a</sup>

<sup>a</sup> Faculty of Food Science, University of Agricultural Sciences and Natural Resources, Gorgan, Iran

<sup>b</sup> Cereals Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran

<sup>c</sup> Nanotechnology Research Center, Ahvaz Jondishapur University of Medical Sciences, Ahvaz, Iran

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### ABSTRACT

Spontaneous emulsification may be used for encapsulating bioactive compounds in food and pharmaceutical industry. It has several advantages over high energy and other low energy methods including, protecting sensitive compounds against severe conditions of high energy method and its ability to minimize surfactant, removal of cosurfactant and thermal stability compared with other low energy methods. In this study, we examined possibility of encapsulating highly soluble crocin in W/O micro-emulsions using spontaneous method which further could be used for making double emulsions. Nonionic surfactants of Span 80 and polyglycerol polyricinoleate (PGPR) were used for making micro-emulsions that showed the high potential of PGPR for spontaneous method. Surfactant to water ratio (SWR%) was evaluated to find the highest amount of aqueous phase which can be dispersed in organic phase. Droplet size decreased by increasing SWR toward the SWR = 100% which had the smallest droplet size and then increased at higher levels of surfactant. By increasing SWR, shear viscosity increased which showed the high effect of PGPR on rheological properties. This study shows in addition to W/O micro-emulsions, spontaneous method could be used for preparing stable O/W micro-emulsions.

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## 1. Introduction

Crocin is one of the three major components of saffron and its principle coloring pigment. Crocin is glycosidic ester of dicarboxylic acid of crocetin which 6 types of it has been detected and among all, trans di gentiobiosyl ester is the most abundant isomer (Fig. 1) [1–3]. Crocin has a high antioxidant activity [4,5] and exhibits beneficial effects on many organs including nervous system, gastrointestinal, cardiovascular, genital, endocrine, immune systems and against cancer [1,6–28].

Crocin is highly soluble in water and is a highly unsaturated carotenoid which makes it susceptible to environmental conditions like low pH, oxygen and light. *Trans-cis* isomerization, oxidation reactions and degradation causes diminishing its color, flavor and nutritive value [29,30]. Due to its properties as an ingredient and medicinal compound, crocin must be protected against environmental conditions and should have a controlled release. There are several ways to encapsulate and protect hydrophilic bioac-

tive components including liposomes, multiple emulsions, solid fat particles, biopolymer complexes, cubosomes (bicontinuous cubic liquid crystalline structure), and biologically derived systems (like yeasts, spores or viruses) [31–33].

Multiple emulsions consist of small droplets of one phase embedded within larger droplets of another phase which are themselves dispersed in a continuous phase [34,35]. One of the most well-known multiple emulsions are double emulsion with two main configurations: water in oil in water (W/O/W) and oil in water in oil (O/W/O) emulsions [36]. The first formulation (W<sub>1</sub>/O/W<sub>2</sub>) consists of an internal water phase (W<sub>1</sub>) trapped as small droplets inside oil droplets (O), which are themselves (W<sub>1</sub>/O) dispersed within an external water phase (W<sub>2</sub>) [37]. Different structural parameters and applications of W<sub>1</sub>/O/W<sub>2</sub> emulsions have been investigated over the last few years such as effect of internal and external phase emulsifier [38–41], volume fraction of different phases [42–44], inclusion of biopolymers [45–49], production procedures, release properties of encapsulants, encapsulation of bioactive compounds [38,41,50], and internal phase particle size. One of the most important parameters determining characteristics of double emulsions and affecting releasing rate of encapsulant is internal phase droplet size.

\* Corresponding author at: Pishro Food Technology Research Group, Gorgan, Iran.  
E-mail address: [smjafari@gau.ac.ir](mailto:smjafari@gau.ac.ir) (S.-M. Jafari).

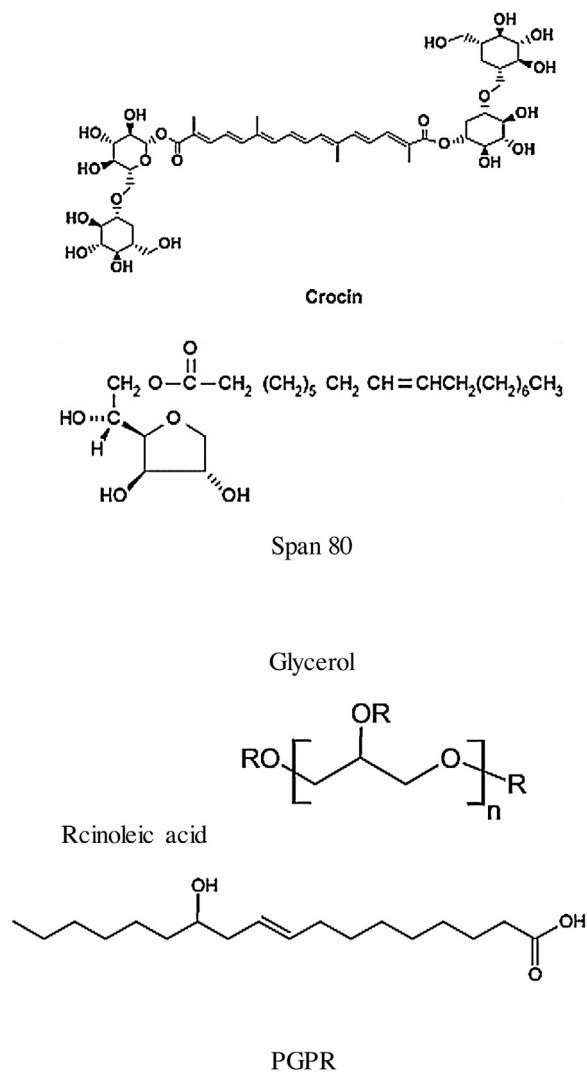


Fig 1. Chemical structure of crocin, Span 80 and PGPR.

Nanoemulsions (also called mini-emulsions) compared with conventional emulsions (also called emulsions or macro-emulsions) have very small particles, i.e., mean radii between 100 and 1000 nm. They have some advantages over conventional emulsions including high stability against gravitational separation and droplet aggregation, optical clarity, increasing bioavailability of encapsulated substances which makes them suitable for food, beverage and pharmaceutical industries [51–54].

All emulsions could be produced in two methods: high energy and low energy emulsification. High energy methods like high speed homogenizer, high pressure homogenizer and ultrasonic homogenizer are traditionally used in industrial operations because of the flexible control of emulsion droplet size distribution and ability to produce fine emulsions from a wide variety of materials [55]. Nanoemulsions produced using low energy methods are called micro-emulsions. Unlike nano-emulsions, micro-emulsions are thermodynamically stable isotropic liquids which are produced using different techniques like phase inversion composition (PIC), phase inversion temperature (PIT), and spontaneous emulsification [53].

Spontaneous emulsification consists of mixing dispersed phase with a surfactant having high affinity toward continuous phase (affinity roughly could be determined using HLB value), then adding the homogeneous mixture to continuous phase. High affinity of surfactant causes turbulence at dispersed phase/continuous phase

interface and surfactant displacing toward the continuous phase makes very small droplets of dispersed phase covered with surfactant in continuous phase (Fig. 2) [56–59]. To increase turbulence at two phases interface, co-surfactants like ethanol, acetone, propylene glycol, ethyl acetate and methyl acetate could be used for producing O/W micro-emulsions. In such cases, organic phase consists of oil, surfactant, co-surfactant; aqueous phase consists of water. Homogenous mixture of organic phase will be added to aqueous phase drop wise over a period of time, while mixing using a magnetic stirrer [56–58]. Droplet size of dispersed phase depends on level and type of surfactant and co-surfactant, surfactant structure, surfactant to dispersed phase ratio, level and type of two phases, level and type of encapsulant, additive or nutritive constituents in dispersed phase, and viscosity of dispersed and continuous phases, which the influence of all of them will be on amount of turbulence and spontaneity [56–58,60–62].

High levels of surfactant and co-surfactant limits application of low energy methods for food and pharmaceutical industries. Recently, some efforts have been made for limiting or reducing co-surfactant and decreasing surfactant to dispersed phase ratio [56,58,61,62].

The purpose of this research was investigating possibility of making micro-emulsions of water containing crocin in olive oil to finally make a double emulsion for protecting and controlling release of crocin. In this research, first of all, an appropriate surfactant was chosen and then amount of crocin, surfactant to water ratio, and preparation conditions were investigated.

## 2. Materials and methods

Crocin (MW: 976.96 g/mole, Purity  $\geq 95\%$ ) was purchased from Sigma–Aldrich Co. (St. Louis, MO), polyglycerol polyricinoleate (PGPR) 4175 kindly donated by Palsgaard. Span 80 was purchased from Samchun Chemicals Co. (South Korea). Extra virgin olive oil was purchased from a local market. Double distilled water was used for preparing W/O micro-emulsions.

### 2.1. Micro-emulsion production

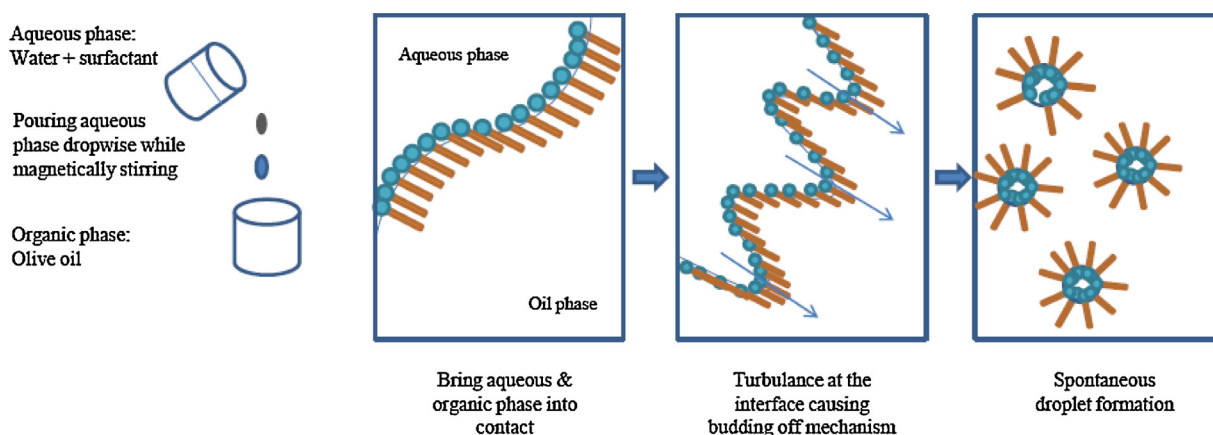
W/O micro-emulsions were prepared by spontaneous emulsification according to previously mentioned procedures for making O/W emulsions [56,58] with some modifications. Aqueous phase was prepared by mixing crocin solution and surfactant using a magnetic stirrer (RCT Basic, IKA, Germany) at 1000 rpm and then added drop wise to oil phase while magnetically stirring. First of all, the procedure was standardized: (i) water content of 10 wt.%, surfactant content of 10 wt.% (SWR = 100%), and oil content of 80 wt.%, (ii) magnetic stirrer speed of 700 rpm, (iii) all procedures were performed at room temperature and aqueous phase added to oil phase in 1.5 h and stirring continued for another 0.5 h to get isotropic conditions.

### 2.2. Surfactant to water ratio (SWR%)

Capacity of surfactant to emulsify the highest level of aqueous phase and its effect on droplet size was evaluated using SWR. In all samples, amount of olive oil was maintained constant (80 wt.%) and levels of surfactant and aqueous phase changed from SWR 25 to 175%.

### 2.3. Effect of stirring speed

To investigate effect of stirring speed of mixing during addition of aqueous phase into oil phase, different speeds i.e., 400, 700 and 1000 rpm were used.



**Fig. 2.** Schematic representation of spontaneous method for preparing water in oil micro-emulsions of crocin. Aqueous phase (water + surfactant) was added into organic phase (olive oil) drop wise, while magnetically stirring at ambient temperature (Idea adapted from Anton and Vandamme [56]).

#### 2.4. Effect of mixing time

After 2 h mixing as a standard procedure, two samples were stirred for additional 0.5 and 1 h, i.e., 2 h (standard), 2.5 h and 3 h.

#### 2.5. Effect of crocin level in aqueous phase

In addition to standard samples containing 0.1% crocin, a sample with 0.2% crocin in aqueous phase was prepared.

#### 2.6. Droplet size measurement

Droplet size of micro-emulsions was measured using a dynamic light scattering method (Zetasizer Nano Zs, Malvern Instrument, Malvern, UK). To avoid multiple scattering, all samples diluted using olive oil. All measurements were conducted after overnight storage of samples at ambient temperature [58].

#### 2.7. Shear viscosity

Effect of composition and preparation conditions on viscosity was measured using a Brookfield viscometer (LVDV Pro II, Brookfield Engineering Laboratories, USA) by a spindle s34.

#### 2.8. Color

For evaluating the effect of composition and preparation conditions on color of micro-emulsions, tristimulus values ( $L^*$ ,  $a^*$ ,  $b^*$ ) of color coordinates were measured using a Chroma meter (Konica Minolta CR-400).  $L^*$  represents the lightness of the sample while  $a^*$  and  $b^*$  provide color coordinates.  $+a^*$  is the red direction and  $-a^*$  is the green direction.  $+b^*$  is the yellow direction and  $-b^*$  is the blue direction [63]. About 1 cm<sup>3</sup> of samples poured in a container with height of 7 cm and diameter of 4 cm and the color coordinates were measured from the top of the container.

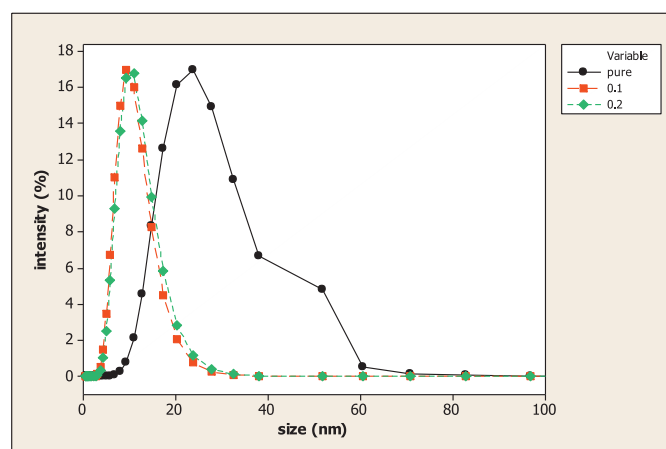
#### 2.9. Statistical analysis

Experiments (Table 1) repeated two or three times and all measurements were done on fresh samples and results reported as the mean and standard deviation. Statistical analysis was performed using SPSS (version 16). Mean values were compared using Duncan's test at 5% significant level.

**Table 1**

Experimental parameters and their levels.

No	Parameter	Level
1	Crocin level (%)	0.1, 0.2
2	PGPR, Span 80	SWR 100
3	SWR	25–175
4	Stirring time (h)	2, 2.5, 3
5	Stirring speed (rpm)	400, 700, 1000



**Fig. 3.** Effect of crocin load on particle size of micro-emulsions at standard conditions: oil phase 80%, stirring speed 700 rpm at ambient temperature.

### 3. Results and discussion

#### 3.1. Effect of crocin load and surfactant type

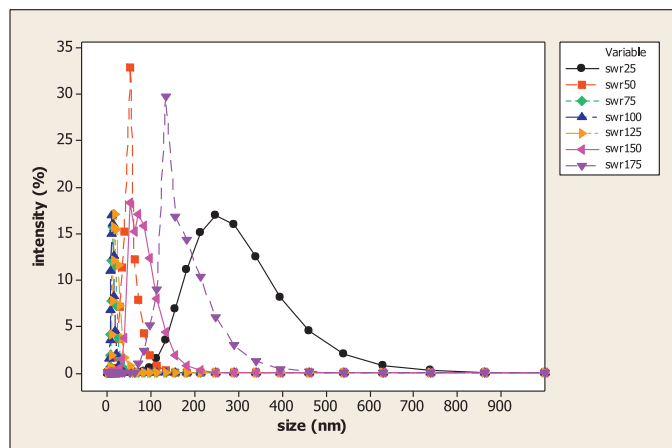
To investigate the influence of surfactant type and structure on micro-emulsion properties, two surfactants of Span 80 and PGPR were used in standard formulations, i.e., water content: 10 wt.% crocin solution 0.1%, surfactant content: 10 wt.% (SWR = 100%), oil content: 80 wt.% and stirring speed of 700 rpm at ambient temperature.

Our results (Table 2 and Fig. 3) showed that it is possible to make nano-emulsions with both Span 80 and PGPR but, the smallest droplet size belongs to the micro-emulsions prepared by PGPR. Experiments with Span 80 and 0.2% crocin showed some sedimentation (crystallization) of encapsulant. Stability of dispersed phase in an emulsion depends on amount of surfactant to cover all droplets of dispersed phase and its speed for adsorbing at two

**Table 2**  
Effect of surfactant type and crocin concentration on mean particle diameter and span of micro-emulsions.

Surfactant/Chemical structure	HLB	Crocin level%	Mean particle diameter (nm)	Span
Span 80/Sorbitan monooleate	4.3	0	17 ± 2	0.947 ± 0.03
		0.1	26 ± 5	0.973 ± 0.02
		0.2	40 ± 5	0.970 ± 0.06
PGPR/Polyglycerol polyricinoleate	1.5 ± 0.5	0	21 ± 4	0.963 ± 0.04
		0.1	9.7 ± 3	0.964 ± 0.03
		0.2	10 ± 4	0.975 ± 0.05

10 wt.% aqueous phase, 10 wt.% surfactant and 80 wt.% oil phase, stirring speed of 700 rpm.



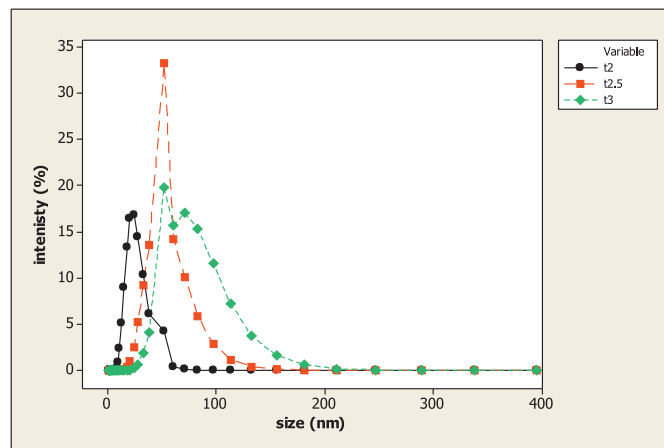
**Fig. 4.** Effect of SWR on particle size of micro-emulsions at standard conditions: oil phase 80%, stirring speed 700 rpm at ambient temperature.

phase interfaces [64]. It seems small molecules of span compared with PGPR were unable to cover all droplets which causes sedimentation of crocin solution [41,65]. Previous studies have shown good emulsifying properties of PGPR with vegetable oils which is due to excellent anchoring properties of both hydrophilic and hydrophobic moieties of PGPR. Moreover, by increasing encapsulant concentration, droplet size decreased in micro-emulsion prepared with PGPR and increased in their counterparts with Span 80. Same results have been observed in W/O emulsions containing electrolytes in the aqueous phase [41–67]. Reduction of droplet size may be due to increasing adsorption density of surfactant and high hydrophobicity of crocin which causes some kind of shrinkage in water droplets.

All spans (emulsion size distributions) were in the same range i.e., about 95% of droplets were in the same range which could be due to increased time of mixing.

### 3.2. Effect of surfactant concentration (SWR)

One of the most important parameters in preparing emulsions using low energy methods is minimizing surfactant level. We obtained different SWRs from 25 to 175% by changing surfactant and water content, while oil phase was kept constant based on standard conditions; surfactant: PGPR, crocin concentration: 0.1%, stirring speed: 700 rpm at ambient temperature. Our results showed that except SWR 25% ( $d \approx 232$  nm), all other surfactant levels resulted in nano-emulsions (Fig. 4). Like other studies in preparation of W/O micro-emulsions by spontaneous methods, droplet size decreased by increasing surfactant level (from SWR 25 to 100%) and then increased (from 100 to 175%) [56,58,68]. Previous studies considering the influence of higher surfactant concentrations on decreasing droplet size to a certain value have suggested two theories for decreasing droplet size. The first one is decreasing interfacial tension due to adsorbing surfactant to the interface and the second is diffusing more surfactant from the outer phase to



**Fig. 5.** Effect of stirring time (standard, 2.5 h and 3 h) on particle size of micro-emulsions.

inner phase which both of them could affect W/O micro-emulsions stabilized with PGPR according to its anchoring property and high affinity toward dispersed phase [58]. Increasing droplet size by higher SWRs in W/O micro-emulsions prepared using spontaneous method have reported to be due to composing a liquid crystalline phase with high viscosity which retards self-emulsifying capabilities and increases droplet size [68].

### 3.3. Effect of stirring time and speed

For evaluating the influence of stirring speed on droplet size, standard conditions were used with different speeds, i.e., 400, 700 and 1000 rpm. At 400 rpm, no nano-emulsion was formed ( $d \approx 1260$  nm). The smallest droplet size was related to speed of 700 rpm. By increasing stirring speed from 700 to 1000 rpm, droplet size increased from 9.79 to 24.4 nm which may be due to re-coalescence of emulsion droplets. In O/W emulsions produced using spontaneous method, previous studies have shown that increasing speed from 200 to 800 rpm decreased droplet size [58]. Increasing droplet size in W/O micro-emulsions may be due to high mobility of water droplets due to increasing external energy which may cause some coalescence.

Same results were observed in investigating the effect of stirring time using standard conditions, except changing time of preparation (Fig. 5). By increasing stirring time from 2 to 3 h, droplet size increased from 20.7 to 64.5 nm which could demonstrate re-coalescence and then, increase in droplet size. Our results show that preparing micro-emulsions need a balance between time and speed. By decreasing stirring speed, the required time for making micro-emulsions will be increased.

### 3.4. Effect of SWR, stirring time and speed on viscosity

All emulsion showed Newtonian behavior rheologically. Stirring time and speed showed no significant effect on viscosity of

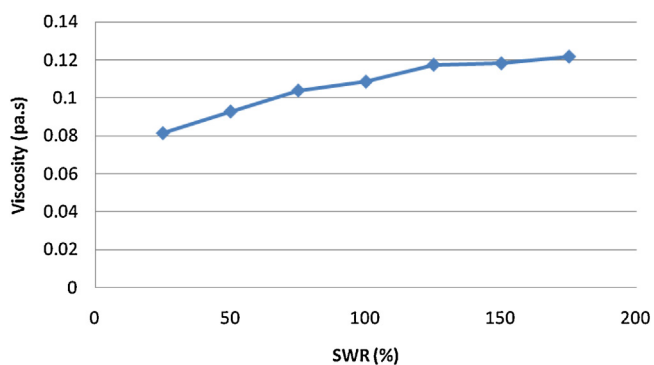


Fig. 6. Effect of SWR on viscosity of micro-emulsions containing crocin.

micro-emulsions but, by increasing SWR, i.e., increasing surfactant content and decreasing water content at constant amount of oil, viscosity increased from 0.081 to 0.144 Pa.s (Fig. 6). In contrast to O/W micro-emulsions [69], in crocin W/O micro-emulsions by decreasing dispersed phase volume fraction and increasing surfactant (increasing SWR), viscosity is increased. It sounds that the effective parameter influencing W/O micro-emulsions is the liquid crystalline and viscous layer of surfactant around the water droplets. Effect of surfactant content on increasing the viscosity have been confirmed in other studies [70]. In W/O emulsions produced using the rotor-stator method, the same results as O/W micro-emulsions have been observed, i.e., increasing viscosity by increasing dispersed phase volume fraction [71].

### 3.5. Optical properties of micro-emulsions

One of the most important advantages of nano-emulsions and micro-emulsions over usual emulsions is their transparent or translucent appearance making them suitable for using in foods and beverages. To achieve such conditions, it is important to prepare micro-emulsions with droplet sizes smaller enough than wavelength of light to prevent light scattering.

Our findings showed that all tristimulus color coordinates ( $L^*$ ,  $a^*$ ,  $b^*$ ) changed significantly ( $P < 0.01$ ) by increasing SWR. It seems the best way for interpreting color coordinates in spontaneous method is based on SWR or dispersed phase volume fraction rather than particle size, as it changes irregularly.

In freshly made micro-emulsions, lightness increased by increasing SWR which means effective role of surfactant in scattering of light in spontaneous method. It may be due to crystalline nature of PGPR at water/oil interface which causes more scattering and higher lightness. SWR 175 and 125 showed the highest and lowest redness/greenness ( $a^*$ ), respectively but for blueness/yellowness ( $b^*$ ), SWR 25 showed the highest value. Same patterns were observed after one and two weeks.

Monitoring color parameters during storage could be used as a good parameter for evaluating emulsions stability. Although, the smallest particles were related to SWR 100, but the highest stability indices according to optical parameters were belonged to SWR 125–175 since all color parameters did not changed significantly ( $P > 0.05$ ) during two week storage (Table 3) which shows importance of surfactant concentration on physical properties of emulsions produced using spontaneous method.

## 4. Conclusion

In this study, we examined possibility of making W/O micro-emulsions using spontaneous method with the aim of fixing two most important defects of low energy methods for food and pharmaceutical applications i.e., high level of surfactant and use of

Table 3

Effect of SWR on optical properties at standard conditions: oil phase: 80%, stirring speed: 700 rpm and ambient temperature.

SWR %	Fresh emulsions			1st week			2nd week		
	L	a	b	L	a	b	L	a	b
25	11.13	-0.2	4.75	11.95	-0.35	5.62	13.11	-0.91	6.2
50	13.48	-0.3	6.69	13.54	-0.6	6.99	13.75	-0.91	6.83
75	13.83	-0.42	6.7	13.83	-0.68	6.84	14.09	-0.63	6.63
100	13.88	-0.74	6.81	14.08	-0.74	6.98	14.2	-0.84	6.90
125	14.04	-0.94	6.71	14.09	-0.83	6.84	14.17	-0.86	6.79
150	14.1	-0.81	7.05	14.15	-1.04	6.93	14.29	-1.01	6.98
175	14.11	-1.08	6.87	14.14	-1.19	7.01	14.1	-1.05	6.67

cosurfactant. At first, two non-ionic surfactants (Span 80 and PGPR) were evaluated for dispersing various amounts of crocin solutions. Finally, effect of emulsification parameters on droplet size, viscosity and optical properties were evaluated. Our results showed high ability of PGPR for preparing nanoscale emulsions and their stability for a long time. The optimum concentrations were measured based on surfactant to water ratio (SWR) that was around SWR = 100% for standard conditions, i.e, crocin level: 0.1% and stirring speed: 700 rpm at ambient temperature. Results of this study may be used in: (i) encapsulating sensitive compounds through spontaneous method in food, pharmaceutical and cosmetic industries, (ii) ability of producing nanoscale emulsions without using high energy methods, lots of surfactant and without cosurfactants.

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## References

- [1] S.H. Alavizadeh, H. Hosseinzadeh, Bioactivity assessment and toxicity of crocin: a comprehensive review, *Food Chem. Toxicol.* 64 (2014) 65–80.
- [2] J.P. Melnyk, S. Wang, M.F. Marcone, Chemical and biological properties of the world's most expensive spice: saffron, *Food Res. Int.* 43 (2010) 1981–1989.
- [3] M. Sarfarazi, S. Jafari, G. Rajabzadeh, Extraction optimization of saffron nutraceuticals through response surface methodology, *Food Anal. Methods* 3 (2015) 1–13, <http://dx.doi.org/10.1007/s12161-014-9995>.
- [4] T.Q. Pham, F. Cormier, E. Farnworth, V.H. Tong, M.-R. Van Calsteren, Antioxidant properties of crocin from *Gardenia jasminoides Ellis* and study of the reactions of crocin with linoleic acid and crocin with oxygen, *J. Agric. Food Chem.* 48 (2000) 1455–1461.
- [5] K. Zeka, K.C. Ruparelia, M.A. Continenza, D. Stagos, F. Vegliò, R.R.J. Arroyo, Petals of *Crocus sativus* L. as a potential source of the antioxidants crocin and kaempferol, *Fitoterapia* (2015) <http://dx.doi.org/10.1016/j.fitote.2015.05.014>.
- [6] M. Alicezah, R. Ahmad, T. Rahman, G.R.A. Froemming, H. Nawawi, The effect of saffron & its bioactive compound; crocin against monocyte-Endothelial cell interaction in human coronary arterial endothelial cells, *Atherosclerosis* 241 (2015) e74.
- [7] E. Altinoz, Z. Oner, H. Elbe, Y. Cigremis, Y. Turkoz, Protective effects of saffron (its active constituent, crocin) on nephropathy in streptozotocin-induced diabetic rats, *Hum. Exp. Toxicol.* (2014), <http://dx.doi.org/10.1177/0960327114538989>.
- [8] K. Mahdavee Khazaei, S.M. Jafari, M. Ghorbani, A. Hemmati Kakhki, Optimization of anthocyanins extraction from saffron petals with response surface methodology, *Food Anal. Methods* (2015), <http://dx.doi.org/10.1007/s12161-015-0375-4>.
- [9] A.R. Bandegi, A. Vafaei Abbas, B. Ghaderdoost, A. Rashidy-Pour, The effects of *Crocus sativus* L. extract and Crocin on oxidative damage of brain, liver and kidneys that induced by chronic stress in rat, *Clin. Biochem.* 44 (2011) S112.
- [10] M.T. Boroushaki, B. Naghizadeh, H. Mofidpoor, Protective effects of crocin against cisplatin-induced acute renal failure and oxidative stress in rats, *Toxicol. Lett.* 180 (Supplement) (2008) S230.
- [11] R. Bortolomeazzi, N. Sebastianutto, R. Toniolo, A. Pizzariello, Comparative evaluation of the antioxidant capacity of smoke flavouring phenols by crocin bleaching inhibition, DPPH radical scavenging and oxidation potential, *Food Chem.* 100 (2007) 1481–1489.
- [12] Q. Ding, H. Zhong, Y. Qi, Y. Cheng, W. Li, S. Yan, X. Wang, Anti-arthritis effects of crocin in interleukin-1 $\beta$ -treated articular chondrocytes and cartilage in a rabbit osteoarthritic model, *Inflamm. Res.* 62 (2013) 17–25.
- [13] H.A. El-Beshbishy, M.H. Hassan, H.A.A. Aly, A.S. Doghish, A.A.A. Alghaithy, Crocin saffron protects against beryllium chloride toxicity in rats through

- diminution of oxidative stress and enhancing gene expression of antioxidant enzymes, *Ecotoxicol. Environ. Saf.* 83 (2012) 47–54.
- [14] J. Escribano, G.-L. Alonso, M. Coca-Prados, J.-A. Fernández, Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro, *Cancer Lett.* 100 (1996) 23–30.
- [15] B. Ghadrdoost, A.A. Vafaei, A. Rashidy-Pour, R. Hajisoltani, A.R. Bandegi, F. Motamedi, S. Haghghi, H.R. Sameni, S. Pahlvan, Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats, *Eur. J. Pharmacol.* 667 (2011) 222–229.
- [16] H. Hosseinzadeh, J. Ghenaati, Evaluation of the antitussive effect of stigma and petals of saffron (*Crocus sativus*) and its components, safranal and crocin in guinea pigs, *Fitoterapia* 77 (2006) 446–448.
- [17] H. Hosseinzadeh, F. Talebzadeh, Anticonvulsant evaluation of safranal and crocin from *Crocus sativus* in mice, *Fitoterapia* 76 (2005) 722–724.
- [18] M. Karami, S.Z. Bathaie, T. Tirahi, M. Habibi-Rezaei, J. Arabkheradmand, S. Faghhihazadeh, Crocin improved locomotor function and mechanical behavior in the rat model of contused spinal cord injury through decreasing calcitonin gene related peptide (CGRP), *Phytomedicine* 21 (2013) 62–67.
- [19] F. Khosrojerdi, S. Kazemi Noureini, Study of telomerase activity in cell line MCF7 treated with crocin, *Clin. Biochem.* 44 (2011) S113.
- [20] P. Lari, K. Abnous, M. Imenshahidi, M. Rashedinia, M. Razavi, H. Hosseinzadeh, Evaluation of diazinon-induced hepatotoxicity and protective effects of crocin, *Toxicol. Ind. Health* (2013), <http://dx.doi.org/10.1177/0748233713475519>.
- [21] X. Li, T. Huang, G. Jiang, W. Gong, H. Qian, C. Zou, Synergistic apoptotic effect of crocin and cisplatin on osteosarcoma cells via caspase induced apoptosis, *Toxicol. Lett.* 221 (2013) 197–204.
- [22] M. Mashmoul, A. Azlan, B.N.M. Yusof, H. Khaza'ai, N. Mohtarrudin, M.T. Boroushaki, Effects of saffron extract and crocin on anthropometrical, nutritional and lipid profile parameters of rats fed a high fat diet, *J. Funct. Foods* 8 (2014) 180–187.
- [23] B. Naghizadeh, M.T. Mansouri, B. Ghorbanzadeh, Y. Farbood, A. Sarkaki, Protective effects of oral crocin against intracerebroventricular streptozotocin-induced spatial memory deficit and oxidative stress in rats, *Phytomedicine* 20 (2013) 537–542.
- [24] B. Naghizadeh, S.M.T. Mansouri, N.V. Mashhadian, Crocin attenuates cisplatin-induced renal oxidative stress in rats, *Food Chem. Toxicol.* 48 (2010) 2650–2655.
- [25] K.N. Nam, Y.-M. Park, H.-J. Jung, J.Y. Lee, B.D. Min, S.-U. Park, W.-S. Jung, K.-H. Cho, J.-H. Park, I. Kang, J.-W. Hong, E.H. Lee, Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells, *Eur. J. Pharmacol.* 648 (2010) 110–116.
- [26] B.M. Razavi, H. Hosseinzadeh, A.R. Movassaghi, M. Imenshahidi, K. Abnous, Protective effect of crocin on diazinon induced cardiotoxicity in rats in subchronic exposure, *Chem. Biol. Interact.* 203 (2013) 547–555.
- [27] E. Tamaddonfard, A.-A. Farshid, K. Eghdami, F. Samadi, A. Erfanparast, Comparison of the effects of crocin, safranal and diclofenac on local inflammation and inflammatory pain responses induced by carrageenan in rats, *Pharmacol. Rep.* 65 (2013) 1272–1280.
- [28] Y.-Q. Zheng, J.-X. Liu, J.-N. Wang, L. Xu, Effects of crocin on reperfusion-induced oxidative/nitritative injury to cerebral microvessels after global cerebral ischemia, *Brain Res.* 1138 (2007) 86–94.
- [29] M. Tsimidou, C.G. Biliaderis, Kinetic Studies of Saffron (*Crocus sativus* L.) Quality Deterioration, *J. Agric. Food Chem.* 45 (1997) 2890–2898.
- [30] M. Tsimidou, E. Tsatsaroni, Stability of saffron pigments in aqueous extracts, *J. Food Sci.* 58 (1993) 1073–1075.
- [31] K. Mahdavee Khazaei, S.M. Jafari, M. Ghorbani, A. Hemmati Kakhki, Application of maltodextrin and gum Arabic in microencapsulation of saffron petal's anthocyanins and evaluating their storage stability and color, *Carbohydr. Polym.* 105 (2014) 57–62.
- [32] K. Mahdavee Khazaei, S.M. Jafari, A. Hemmati Kakhki, Microencapsulation of saffron petal anthocyanins with cross seed gum compared with Arabic gum through freeze drying, *Carbohydr. Polym.* (2015), <http://dx.doi.org/10.1016/j.carbpol.2015.11.079> (in press).
- [33] H. Rajabi, M. Ghorbani, S.M. Jafari, A. Sadeghi Mahoonak, G. Rajabzadeh, Retention of saffron bioactive components by spray drying encapsulation using maltodextrin, gum arabic and gelatin as wall materials, *Food Hydrocolloids* 51 (2015) 327–337.
- [34] A.F. Esfanjani, S.M. Jafari, E. Assadpoor, A. Mohammadi, Nano-encapsulation of saffron extract through double-layered multiple emulsions of pectin and whey protein concentrate, *J. Food Eng.* 165 (2015) 149–155.
- [35] A. Mohammadi, S.M. Jafari, A.F. Esfanjani, S. Akhavan, Application of nano-encapsulated olive leaf extract in controlling the oxidative stability of soybean oil, *Int. J. Biol. Macromol.* (2015) 025, <http://dx.doi.org/10.1016/j.ijbiomac.2015.10.025>.
- [36] E. Dickinson, Double emulsions stabilized by food biopolymers, *Food Biophys.* 6 (2011) 1–11.
- [37] S. Iqbal, M.K. Baloch, G. Hameed, D.J. McClements, Controlling W/O/W multiple emulsion microstructure by osmotic swelling and internal protein gelation, *Food Res. Int.* 54 (2013) 1613–1620.
- [38] N.P. Aditya, S. Aditya, H. Yang, H.W. Kim, S.O. Park, S. Ko, Co-delivery of hydrophobic curcumin and hydrophilic catechin by a water-in-oil-in-water double emulsion, *Food Chem.* 173 (2015) 7–13.
- [39] S. Khoee, M. Yaghoobian, An investigation into the role of surfactants in controlling particle size of polymeric nanocapsules containing penicillin-G in double emulsion, *Eur. J. Med. Chem.* 44 (2009) 2392–2399.
- [40] A. Márquez, G. Palazolo, J. Wagner, Water in oil (w/o) and double (w/o/w) emulsions prepared with spans: microstructure, stability, and rheology, *Colloid Polym. Sci.* 285 (2007) 1119–1128.
- [41] M. Matos, G. Gutiérrez, J. Coca, C. Pazos, Preparation of water-in-oil-in-water (W1/O/W2) double emulsions containing trans-resveratrol, *Colloids Surf. A* 442 (2014) 69–79.
- [42] M. Bonnet, M. Cansell, F. Placin, J. Monteil, M. Anton, F. Leal-Calderon, Influence of the oil globule fraction on the release rate profiles from multiple W/O/W emulsions, *Colloids Surf. B: Biointerfaces* 78 (2010) 44–52.
- [43] F. Leal-Calderon, S. Homer, A. Goh, L. Lundin, W/O/W emulsions with high internal droplet volume fraction, *Food Hydrocolloids* 27 (2012) 30–41.
- [44] S. van der Graaf, C.G.P.H. Schroën, R.M. Boom, Preparation of double emulsions by membrane emulsification—a review, *J. Membr. Sci.* 251 (2005) 7–15.
- [45] A. Benichou, A. Aserin, N. Garti, W/O/W double emulsions stabilized with WPI–polysaccharide complexes, *Colloids Surf. A* 294 (2007) 20–32.
- [46] A. Fechner, A. Knoth, I. Scherze, G. Muschiolik, Stability and release properties of double-emulsions stabilised by caseinate–dextran conjugates, *Food Hydrocoll.* 21 (2007) 943–952.
- [47] M.M. Murillo-Martínez, R. Pedroza-Islas, C. Lobato-Calleros, A. Martínez-Ferez, E.J. Vernon-Carter, Designing W1/O/W2 double emulsions stabilized by protein–polysaccharide complexes for producing edible films: Rheological, mechanical and water vapour properties, *Food Hydrocoll.* 25 (2011) 577–585.
- [48] J. O'Regan, D.M. Mulvihill, Sodium caseinate–maltodextrin conjugate stabilized double emulsions: Encapsulation and stability, *Food Res. Int.* 43 (2010) 224–231.
- [49] A. Schuch, C. Helfenritter, M. Funck, H.P. Schuchmann, Observations on the influence of different biopolymers on coalescence of inner water droplets in W/O/W (water-in-oil-in-water) double emulsions, *Colloids Surf. A* (2014) <http://dx.doi.org/10.1016/j.colsurfa.2014.06.012>.
- [50] A. Mohammadi, S.M. Jafari, A.F. Esfanjani, S. Akhavan, Application of nano-encapsulated olive leaf extract in controlling the oxidative stability of soybean oil, *Food Chem.* 190 (2016) 513–519.
- [51] D.J. McClements, Edible nanoemulsions: fabrication, properties, and functional performance, *Soft Matter* 7 (2011) 2297–2316.
- [52] D.J. McClements, Nanoemulsions versus microemulsions: terminology, differences, and similarities, *Soft Matter* 8 (2012) 1719–1729.
- [53] D.J. McClements, J. Rao, Food-Grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity, *Crit. Rev. Food Sci. Nutr.* 51 (2011) 285–330.
- [54] O. Shamsara, Z.K. Muhidinov, S.M. Jafari, J. Bobokalonov, A. Jonmurodov, M. Taghvaei, M. Kumpugdee-Vollrath, Effect of ultrasonication, pH and heating on stability of apricot gum–lactoglobulin two layer nanoemulsions, *Int. J. Biol. Macromol.* 81 (2015) 1019–1025.
- [55] R.C. Santana, F.A. Perrechil, R.L. Cunha, High- and low-energy emulsifications for food applications: a focus on process parameters, *Food Eng. Rev.* 5 (2013) 107–122.
- [56] N. Anton, T.F. Vandamme, The universality of low-energy nano-emulsification, *Int. J. Pharm.* 377 (2009) 142–147.
- [57] K. Bouchemal, S. Briançon, E. Perrier, H. Fessi, Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation, *Int. J. Pharm.* 280 (2004) 241–251.
- [58] A.H. Saberi, Y. Fang, D.J. McClements, Fabrication of vitamin E-enriched nanoemulsions: factors affecting particle size using spontaneous emulsification, *J. Colloid Interface Sci.* 391 (2013) 95–102.
- [59] J. Santana-Solano, C.M. Quezada, S. Ozuna-Chacón, J.L. Arauz-Lara, Spontaneous emulsification at the water/oil interface, *Colloids Surf. A* 399 (2012) 78–82.
- [60] C.A. Miller, Spontaneous emulsification produced by diffusion — a review, *Colloids Surf.* 29 (1988) 89–102.
- [61] A.H. Saberi, Y. Fang, D.J. McClements, Fabrication of vitamin E-enriched nanoemulsions by spontaneous emulsification: effect of propylene glycol and ethanol on formation, stability, and properties, *Food Res. Int.* 54 (2013) 812–820.
- [62] S.M. Jafari, P. Beheshti, E. Assadpoor, Emulsification properties of a novel hydrocolloid (Angum gum) for d-limonene droplets compared with Arabic gum, *Int. J. Biol. Macromol.* 61 (10) (2013) 182–188.
- [63] J. Komaiko, D.J. McClements, Food-grade nanoemulsion filled hydrogels formed by spontaneous emulsification and gelation: optical properties, rheology, and stability, *Food Hydrocoll.* 46 (2015) 67–75.
- [64] D.J. McClements, Critical review of techniques and methodologies for characterization of emulsion stability, *Crit. Rev. Food Sci. Nutr.* 47 (2007) 611–649.
- [65] F. Hadizadeh, A. Mohajeri Seyed, M. Seifi, Extraction and purification of crocin from saffron stigmas employing a simple and efficient crystallization method, *Pak. J. Biol. Sci.* 31 (2010) 691–698.
- [66] F.Y. Ushikubo, R.L. Cunha, Stability mechanisms of liquid water-in-oil emulsions, *Food Hydrocoll.* 34 (2014) 145–153.
- [67] A.L. Márquez, A. Medrano, L.A. Panizzolo, J.R. Wagner, Effect of calcium salts and surfactant concentration on the stability of water-in-oil (w/o) emulsions prepared with polyglycerol polyricinoleate, *J. Colloid Interface Sci.* 341 (2010) 101–108.

- [68] L. Wang, J. Dong, J. Chen, J. Eastoe, X. Li, Design and optimization of a new self-nanoemulsifying drug delivery system, *J. Colloid Interface Sci.* 330 (2009) 443–448.
- [69] A. Azeem, M. Rizwan, F.J. Ahmad, Z. Iqbal, R.K. Khar, M. Aqil, S. Talegaonkar, Nanoemulsion components screening and selection: a technical note, *AAPS PharmSciTech* 10 (2009) 69–76.
- [70] R. Pal, Rheological behaviour of surfactant-flocculated water-in-oil emulsions, *Colloids Surf. A* 71 (1993) 173–185.
- [71] M.R. Noor El-Din, I.M. El-Gamal, S.H. El-Hamouly, H.M. Mohamed, M.R. Mishrif, A.M. Ragab, Rheological behavior of water-in-diesel fuel nanoemulsions stabilized by mixed surfactants, *Colloids Surf. A* 436 (2013) 318–324.