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Extended Abstract

The Encapsulation of Saffron Extract in Double Emulsion System and Stability Evaluation of Its Active Constituents using Principal Component Analysis Method during Storage Period

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Abstract

In this study, a double emulsion system was developed based on an optimized initial simple emulsion with the view to increase the stability of effective saffron compounds (crocin, picrocrocin and safranal). To this end the effect of various concentrations of surfactant (10, 12.5 and 15%) and different types of carbohysrates (sucrose, sorbitol, and dextran) on the stability of simple emulsion was investigated. The principal component analysis method was used to identify the relationships between the quantitative changes of the active components and quality attributes of the emulsion. Based on the results, day 7 was recognized as a critical point on which dramatic changes in the quality attributes of the emulsion coincided with the loss of saffron active compounds. In other words, physical changes in the stability of emulshion correlated well with the destruction trend of active compounds, although picrocrocin changes were found to be of independents. Also, it was revealed that the incorporation of 1% w/w sorbitol into the aqueous extract of saffron significantly decreased the loss rate of saffron active compounds in the emulsion. In addition, the double emulsion increased the half-life of saffron active compounds during a 14 day storage period. The results showed that double emulsion system was a suitable method for saffron extract, but the qualitative properties of initial emulsion also had a great influence on their stability. Multivariate analysis methods can also be used to clarify the relationships between the qualitative properties of the emulsion and the active compounds entrapped within it.

Keywords: Active ingredients, Double emulsion, Principal component analysis, Saffron

Introduction

The major bioactive compounds in saffron are crocin, picrocrocin and safranal all contributing not only to the sensory profile of saffron (color, taste and aroma, respectively) but also to the health-promoting properties. All of these compounds lose their desirable properties exposure to destruction of these compounds. Emulsions are heterogeneous colloidal systems consisting of at least two immiscible liquids (Anton, Benoit, & Saulnier, 2008). Also, these systems are capable to develop multiple emulsions (Tadros, 2004).The formation and stability of multiple emulsions has recently been the object of intensive study (Benichou, Aserin, & Garti, 2004; Garti & Bisperink, 1998; Hanson *et al.*, 2008; Okushima, Nisisako, Torii, & Higuchi, 2004). However, the main drawback of double emulsions is their inherent thermodynamic instability. The existence of two oppositely-curved interfaces within the same structure requires the presence of two different emulsifiers; one lipophilic and one hydrophilic. The effect of surfactant concentration is considered as a critical factor in emulsion stability (Nihant, Schugens, Grandfils, Jérôme, & Teyssié, 1994). In this study, a double emulsion system was developed based on an optimized initial simple emulsion with the view to increase the stability of effective saffron components. To this end the effect of various concentrations of surfactant and different types of carbohydrates on the stability of simple emulsion was investigated.

Materials and methods

The required saffron with premium quality obtained from Zaffranchi Toos Company. Saffron extract was prepared according to (Fernandez, 2002) method. A spectrophotometer UV-VIS mod was used to analyze the active component of saffron extract at wavelengths 440, 330 and 257 nanometer (ISO/TS 3632-1, 2003). The Beer-Lambert law implies that both the absorbance and concentration of an absorbing species have linear relationship. Therefore, the absorption rate at each wavelength can be reported as the concentration of each compound. The methods of Dickinson & Ritzoulis (2000) and Jafari, He, & Bhandari (2007) were used to prepare initial emulsion with some modifications. The constituents of the initial emulsions were: Saffron extract as aqueous phase (15%), Oil phase (70, 72.5 and 75% w/w) and surfactants (10, 12.5 and 15 % w/w). A combination of surfactants was prepared from Tween 80 (49%) and Span 80 (51%) which possessed HLB value close to 9.54. Emulsification was performed using ultrasound device (Model VCX750, manufactured by Sonics & Materials, USA) for 3 minutes continuously at maximum power of 750 W and 20 kHz frequency. At the same time, saffron extract was added drop wise to the mixture (Najafi, Kadkhodaee, & Mortazavi, 2011). The macroscopic stability of the emulsion was evaluated based on equation 1 on 7, 14, 21, and 28th day after preparation (Dickinson & Ritzoulis, 2000).

$$s = \frac{h0-ht}{h0} \times 100 \qquad (1)$$

The emulsion droplet size was measured immediately after preparation using a Zeta Sizer (Malvern Model Nano-Zeta sizer, UK) at 25 °C (Najaf Najafi, Hosaini, Mohammadi-Sani, & Koocheki, 2016).

In order to increase the stability of simple emulsions, dextran, sorbitol and sucrose were added to saffron extract at three levels of 0, 1 and 2% w/w. Their effect on the stability and release rate of the active components in the absence of light was evaluated during 14-days period.

In order to obtain double emulsions, the constituents were formulated at a ratio of 1: 3: 3 (w/o emulsion: surfactant: external aqueous phase, respectively) (Jafari *et al.*, 2007; Sapei, Naqvi, & Rousseau, 2012). A combination of surfactants was prepared from Tween 20 (97.5%) and Tween 80 (2.5%) and optimal HLB was 16.56. Emulsification step was the same as previously described (Jafari *et al.*, 2007; Sapei *et al.*, 2012).

In order to investigate the protective effect of each simple and double emulsions on saffron components in exposure to light and darkness conditions, emulsions was divided into two parts and transferred to transparent and dark containers. The light intensity was estimated about 1440 lux. The stability of components was evaluated by spectroscopy on days 0, 7 and 14 after production (Buffo, Reineccius, & Oehlert, 2001).

Release rate and half-life of each components were calculated according to following equation. (Shu, Yu, Zhao, & Liu, 2006).

$$t_{1/2}=0.693/K \qquad (2)$$

Results and discussion

Simple emulsion (w/o)

The stability evaluation of the initial emulsions showed that the emulsion containing 15% surfactant was completely stable for 28 days and the emulsion containing 10% surfactant, showed phase separation. Evaluation of droplet size changing during storage time showed droplet size and polydispersity index decreased by increasing levels of surfactant. The 7th day was also considered as a critical point in the emulsion stability in all surfactant levels, indicating the beginning of effective interactions between droplets. The emulsion prepared with 15% surfactant had more protective effect on crocin, picrocrocin and safranal. Also, in the evaluation of crocin stability under light conditions, it was found that in the short-term storage conditions (7 days) the emulsification process increased crocin shelf life. These results were also confirmed by (Serrano-Díaz, Sánchez, Maggi, Carmona, & Alonso, 2011). Exposed to fluorescent white light, the trans-crocetin di-ester is isomerized to cis crocetin di-ester and the intensity of dye decreases. Generally in light conditions, Due to the transparency of the emulsion, the inhibitory power of the surfactant membrane against the oxidative effects of light is not high. The results indicated, the stability of picrocrocin exposure to light and darkness conditions is independent of the emulsion conditions (Fig. 1).

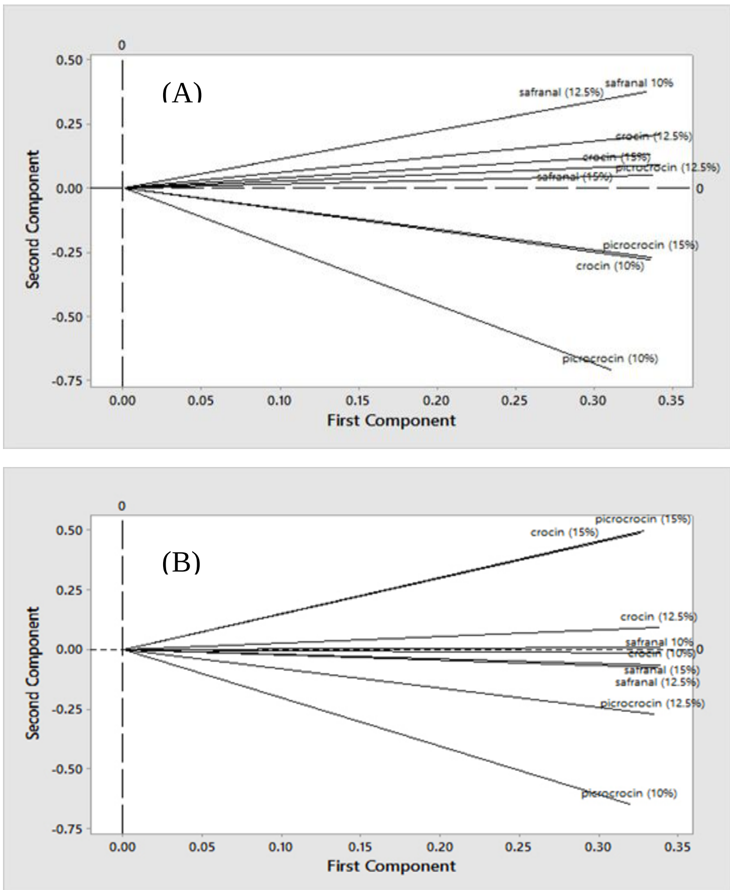

Fig. 1. Relationship between changes of saffron active constituents under the influence of surfactant concentration (A: dark conditions, B: light conditions)

The effect of carbohydrate addition

In order to increase the resistance of saffron compounds to environmental conditions and intensify the protective effects of emulsions sucrose, sorbitol and dextran were added to the aqueous phase (saffron extract). Apparently, all emulsions were completely transparent at shelf life period except at the 2% dextran level. Sorbitol had a higher efficiency in maintaining crocin than sucrose and dextran. But the presence of dextran improved the preservation of safranal. Emulsions containing 1% sorbitol had the greatest effect on the preservation of picrocrocin. So 1% sorbitol level was used as the optimum concentration in the preparation of double emulsion.

Double emulsion (w/o/w)

Based on the results, the initial emulsion containing 15% surfactant and 1% sorbitol was used for preparation of double emulsions. Droplet sizes gradually increased over the time, but the emulsions were stable. As expected, in the presence of light, the degradation of the saffron effective compounds occurred at a high intensity. On the other hand, the results of the half-life of these compounds in the double emulsion in the presence of light and darkness conditions showed in Table (1). In the absence of light, the double emulsion increased the half-life of the saffron effective compounds effectively. This is due to the creation of two layers of oil and water on the aqueous extract of saffron, restricting oxygen from the environment and improving the initial emulsion conditions.

Table 1. Half-life of saffron extract active constituents in light and dark conditions in w/o/w emulsions

Storage condition	Active constituent	Regression equation	R^2	Half-life (day)
Darkness	Crocin	Y= -0.0071x+4.5933	0.94	97.60
	Safranal	Y= -0.0136x+4.5917	0.97	50.95
	Picrocrocin	Y= -0.0114x+4.5933	0.97	60.78
Light	Crocin	Y= -0.01136x+4.6283	0.99	6.1
	Safranal	Y= -0.0157x+4.5767	0.91	44.14
	Picrocrocin	Y= -0.0879x+4.6217	0.99	7.88

Conclusions

In this study, a double emulsion system (w/o/w) was developed as a suitable carrier for preserving saffron effective compounds. The results showed that double emulsion system was a suitable method for saffron extract, but the qualitative properties of initial emulsion also had a great influence on stability of the double emulsion system and its protective effects on the active constituents of saffron.

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