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# Nano-encapsulation of saffron extract through double-layered multiple emulsions of pectin and whey protein concentrate

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ABSTRACT

In this study, nano-particles of saffron extract (<100 nm) were encapsulated by spray drying. For this objective, the primary saffron water extract-in-oil (W/O) micro-emulsion containing 10% (w/w) saffron extract was re-emulsified in order to prepare W/O/W multiple emulsions, with a dispersed mass fraction of 0.25, and stabilized using protein (whey protein concentrate (WPC))/polysaccharide (pectin). Also, the encapsulation efficiency of crocin, picrocrocin and saffranal as core materials and surface characteristics of spray dried powders were investigated. Our results revealed that W/O/W multiple emulsions stabilized by sequential adsorption of WPC/pectin was the most efficient technique resulting in the better encapsulated efficiency for crocin, picrocrocin and saffranal, low yellow color (b*) surface and, smooth surface in final powders, mainly due to fabrication of stable wall materials obtained by sequential adsorption of WPC and pectin.



## 1. Introduction

Saffron has been used in a wide variety of industries, including food, pharmaceutical and, cosmetics due to its natural colorant, antioxidant and therapeutic properties. Crocin, picrocrocin, and saffranal are the major compounds of saffron responsible for its color, aroma, and flavor, respectively (Basker and Negbi, 1983; Sampathu et al., 1984; Zeng et al., 2003). These compounds are rather unstable and influenced by the final processing temperature, storage temperature, pH, light, oxygen, enzymes, proteins and metallic ions (Patras et al., 2010).

Microencapsulation is a technique for coating of bioactive materials (like crocin, picrocrocin, and saffranal) in the form of micro- and nano-particles and, providing protection or controlling the release of the entrapped ingredients. There are different methods for encapsulation in the food industry and spray drying is a common and affordable way to do this (Gouin, 2004; Jafari et al., 2008a; Bhandari, 2004).

Improving the encapsulation efficiency during spray drying, which is preventing volatile losses and extending the shelf-life of the products by minimizing the amount of unencapsulated material at the surface of powder particles, is the major emphasis for microencapsulation of food flavors and oils (Jafari et al., 2008b).

Infeed model food systems (water, carrier, and flavor) play a key role in optimising the encapsulation efficiency. These systems can be prepared by different models including maltodextrins, protein or polysaccharide based systems, and emulsion systems.

The W/O/W multiple emulsion stabilized by biopolymers is a major food system for creating spherical spray dried powder particles in which hydrophilic ingredients are encapsulated in the inner aqueous phase (Rodríduez-Huezo et al., 2004; Mlalila et al., 2014).

The choice of resistant wall materials can affect the encapsulation efficiency of entrapped compounds within W/O/W multiple emulsions. Therefore, applying double-layer techniques (oil droplets coated by double-layered interfacial membranes) for producing W/O/W multiple emulsions, can efficiently coat oil particles during emulsification and result in improved stability to environmental stresses of encapsulated ingredients (Bouyer et al., 2012; Giroux et al., 2013).

Rodríduez-Huezo et al. (2004) revealed powders obtained by spray-drying of double-layer W/O/W multiple emulsions showed the best morphology, highest microencapsulation efficiency, and highest total carotenoids retention and a high biopolymer blend (gum Arabic, mesquite gum and maltodextrin) to primary emulsion ratio also produced a high microencapsulation efficiency.

Rajabi et al. (2015) working on saffron extract microencapsulation observed that a mixture with 40% TS consisting of

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maltodextrin, gum Arabic and gelatin in the weight ratio of 0.94:0.05:0.01 retained the highest amount of picrocrocin, saffranal and crocin, by retention values of 90.06%, 80.37%, and 91.03%, respectively. Serfert et al. (2013) investigated the oxidative stability of encapsulated fish oil by spray drying and showed O/W interface produced with Beta-lactoglobulin and low methoxylated pectin gave the best protection of the oil. Recently, Raei et al. (2015) found that higher alginate concentration resulted in higher lactoferrin encapsulation efficiency and nanocapsuls prepared with thermal treatment had a higher efficiency (almost 100%) along with smaller particle sizes (mostly < 100 nm).

As far as we know, there is no available scientific literature about nano-encapsulation of saffron extract via W/O/W multiple emulsions and spray drying. Consequently, the objective of this study was to evaluate different types of model food systems (including maltodextrin, whey protein based systems, and double layer or single layer W/O/W multiple emulsion systems) and propose a new technique for producing spray dried powders containing saffron extract in order to have a higher encapsulation efficiency.

## 2. Materials and methods

Saffron was provided from Torbat heydariyeh farms, Khorasan-e-razavi, Iran. Sunflower oil and sodium azide was purchased from FRICO (Sirjan, Iran) and Sigma–Aldrich (St. Louis, USA), respectively. Maltodextrin was obtained from Qinhuangdao starch Co. (DE 16-20, China) and citrus pectin with a degree of methyl esterification of 71.1% and Galacturonic acid >65% was purchased from MP biomedical (Netherland). Whey protein concentrate (80% protein) and sorbitan monooleate (Span 80) was obtained from Sapoto cheese (USA) and Merck (Germany), respectively. All other general chemicals used in this study were of analytical grade.

### 2.1. Saffron extracts preparation and analysis

For extraction of saffron compounds, the procedure of Kumpati et al. (2003) was adopted by some modifications. 10 g of saffron powder was mixed with 150 ml water in a dark colored bottle and, placed in an incubator with shaker for 24 h. A rotor–stator homogenizer (10,000 rpm for 10 min, Heidolph Silentcrusher, Germany) was used for maximum extraction of saffron bioactive compounds. After homogenization, the extract was filtered under vacuum, and kept in the freezer at −18 °C until doing the tests.

ISO/TS 3632 procedure (2003) was used for the measurement of saffron components. According to ISO, picrocrocin, saffranal and crocins are expressed as direct reading of the absorbance of 1% aqueous solution of dried saffron at 257, 330 and 440, respectively. After the extraction was over, the solution was passed through a Whatman filter paper No. 42. Then, 2.5 ml of the filtrate was transferred to a 50-ml volumetric flask and made to the mark with distilled water. The final concentration of the powdered saffron in water was 0.005% (w/v).

Results are obtained by direct reading of the absorbance, D, at three wavelengths by using the T80+UV/VIS spectrophotometer (PG-Instruments-LTD, USA) equipped with a 1-cm path quartz cell, as follows:

$$E^{\%}_{1cm}(440\,nm): \text{absorbance at } 440\,nm \text{ (maximum absorbance of crocins)};$$
$$E^{\%}_{1cm}(330\,nm): \text{absorbance at } 330\,nm \text{ (maximum absorbance of safranal)};$$
$$E^{\%}_{1cm}(257\,nm): \text{absorbance at } 257\,nm \text{ (maximum absorbance of picrocrocin)}:$$
$$E^{\%}_{1cm} = (D \times 10{,}000)/(m \times (100 - H)) \quad (1)$$

where $D$ is the specific absorbance; $m$ is the mass of the saffron sample, in grams; $H$ is the moisture and volatile content of the sample, expressed as a mass fraction (Sarfarazi et al., 2015).

Crocin content $\left(E^{\%}_{1cm}\,257\right)$, picrocrocin content $\left(E^{\%}_{1cm}\,257\right)$ and, saffranal content $\left(E^{\%}_{1cm}\,257\right)$ in saffron extract were 240.83, 150.6, and, 81.53, respectively.

### 2.2. Biopolymer solution preparation

Hydrated solution of the outer aqueous phase of W/O/W multiple emulsions were prepared by dissolving pectin and maltodextrin powders in buffer solution (phosphate buffer, pH = 6) while stirring at 50 °C for 30 min and kept overnight to warrant a full saturation of the polymer molecules. In the case of Whey protein concentrate (WPC), the WPC powders were dispersed in buffer solution (phosphate buffer, pH = 6). The pH of WPC solutions was adjusted back to pH 6.0 using 1 M HCl if required and kept refrigerated for 24 h for complete hydration; Then the solutions were heat-treated at 70 °C for 20 min, and cooled down quickly. The total concentration of dissolved solids was composed of 27 wt% maltodextrin and 8.2 wt% of emulsifying ingredients (including 8% WPC and 0.2% Pectin). 0.004% of sodium azide was included in the solutions as an antimicrobial substance.

### 2.3. Preparation of infeed aqueous materials

Two model food systems (including matrix of maltodextrin, whey protein based systems and double layer or single layer W/O/W multiple emulsion systems) were prepared for nano-encapsulation of saffron extract:

(a) Maltodextrin and WPC was produced by mixed 8% WPC, and 27% maltodextrin and dissolving in distilled water at ambient temperature (25 ± 1 °C) to obtain 35% total solids concentration. The solution was kept in refrigerator for complete hydration in 24 h. Then, saffron extract and solution of maltodextrin/WPC as wall materials were mixed in a weight ratio (w/w) of 1:3.5 (extract: wall material). The pH of mixtures adjusted on 6.0 with phosphate buffer, and then mixed with a magnetic stirrer (120 rpm, 10 min).
(b) W/O/W multiple emulsion systems were prepared in two different ways; a single layer multiple emulsion stabilized by whey protein (WPC) alone and, a double layer multiple emulsion stabilized by complex of whey protein (WPC) and pectin (simultaneous and sequential adsorption) according to Mohammadi et al. (2016). Briefly, first, W/O micro-emulsions were produced by drop-wise addition of 10% saffron extract into the mixture of 60% sunflower oil and 30% Span 80 while stirring (300 rpm). After each addition, the system was given enough time to become transparent and isotropic.

The coarse W/O/W multiple emulsions were prepared by gradually adding W/O micro-emulsions into the outer aqueous phase while blending by a homogenizer (12,000 rpm for 5 min at 10 °C, Heidolph Silentcrusher, Germany) and then these coarse emulsions were further emulsified using mentioned homogenizer (15,000 rpm for 8 min at 10 °C).

W/O/W multiple emulsions stabilized by sequential (layer by layer) adsorbed WPC and pectin were produced in two stages. First, the primary W/O micro-emulsion was gradually added into aqueous solution of WPC and maltodextrin while blending by homogenizer (12,000 rpm for 5 min at 10 °C, Heidolph Silentcrusher, Germany), and then this emulsion was gradually added into aqueous solution of pectin while homogenization