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Microencapsulation of saffron pollen extract by spray drying to preserve its nutritional properties

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A B S T R A C T

The present study reports the microencapsulation of extracts of saffron pollens containing polyphenolic compounds. Saffron pollen exhibit varying properties such as antioxidant and sensory properties and is also a source of many nutrients and bioactive compounds. In order to protect the polyphenols, the saffron pollen extract was microencapsulated by the spray drying method with maltodextrin as a carrier. Prior to encapsulation, the unbound water, total polyphenols contents and antioxidant activity of the extract was determined. The effect of inlet temperatures on the content of bioactive compounds, powder production yield, solubility and wettability was considered to study the spray drying process. The SEM images reveal that the produced microcapsules are spherical in shape. The results showed that the total polyphenol content of saffron pollen extract was found to be 620 ± 4.50, 470 ± 2.65, 420 ± 2.18 mg gallic acid per g, for 140, 160 and 180 ℃, respectively and spraying drying could significantly maintain the polyphenolic content as well as the antioxidant capacity of extracts. The IC_{50} results for saffron pollen extract was found to be 0.177 ± 0.08, 0.191 ± 0.09, 0.182 ± 0.08 g per 100 g, for 140, 160 and 180 ℃, respectively. The best temperature for this purpose with the highest solubility and wettability of the powder is the inlet temperature of 140 ℃ and Outlet temperature of 85 ℃, thus have potential applications in food and pharmaceutical products.

Keywords: Saffron; Pollen; Microcapsules; Polyphenols; Antioxidant capacity

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1. Introduction

The food industry produces large volumes of solid by-products, which results in the production of significant amounts of food waste, causing to shrinkage, handling, and high disposal costs. The organic food by-products are usually rich in biologically active compounds and they can be used for noble purposes (Torbica et al., 2016; Viuda-Martos et al., 2014). To find a solution for the effective management of food industry by-products, many researchers have investigated the possible use of industrial products to include them in the human diet (Belović et al., 2016; Herrera et al., 2010).

Iran is one of the richest spices sources especially saffron in terms of quantity in the world. Due to its exceptional colour-producing ability, aroma and taste, also ssaffron has many applications in the preparation of food as a colour and flavour and also in the pharmaceutical industry in the production of various drugs. Despite the discovery of many nutritional and medicinal uses of saffron, there is a need for more extensive research to identify all the ingredients and properties in other parts of it, including the saffron pollen that is currently being discarded. Saffron is the scientific name of *crocus sativus* L. The saffron flower contains parts of the three-branched stigma with the style attached to it, three stamens, and six coloured sepals and petals. Saffron stamen and pollen are one of the By-products of the saffron production process that are wasted today. Considering the unique characteristics of the saffron plant, it is possible to make maximum use of this valuable herb by finding out the compounds and properties of its stamen (Abrishami, 2008). There are several reports on the constituents of pollen grains and stamen of saffron (Jadouali et al., 2019; Sani et al., 2013). But the most important

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characteristic of saffron stamen and pollen appears to be their high content of polyphenolic compounds and its antioxidant properties (Moradi, 2016). In addition, rare compounds such as furanocoumarins, hydroxyl amines, naphthoquinones, acetyl fluoro glucinols, and sterols are also distributed among these parts of saffron (Saroya, 2011).

Currently, research on the use of herbal supplements and herbal-phytochemicals has received much attention and popularity (Chen et al., 2007). Medicinal plants are widely used in the food, pharmaceutical and cosmetic industries in various forms. "However, the convenient and widely utilised forms are powders" (Wang & Weller, 2006). There are several encapsulation methods that can improve the stability of plant extracts such as freezing, spray drying, vacuum drying, and electrostatic extrusion and so on. (Isailović et al., 2012; Kalušević et al., 2012a; Kalušević et al., 2012b; Nedovic et al., 2011). Spray drying is the most common and widely used method for drying pharmaceutical products as well as producing powder with the desired specifications. This is done continuously, as almost any pumpable liquid can be converted into a free-flowing powder. Spray drying is widely used to dry heat-sensitive products such as enzymes and proteins. This practice with the least amount of activity loss in the production of drugs with better solubility, nanoparticle drying, microencapsulation, granulation and coating beyond its known background has many applications in drying products in liquid form (Adibkia et al., 2012). However, spray drying is the most common method of microencapsulation due to its ease of use and effectiveness. Microencapsulation technology has a high production rate, lower operating costs compared to others, stable powders and easy to use (Nedović et al., 2013). Compared to other methods of drying plant extracts, powder dried by spray drying method due to the use of some carriers, can have a higher production performance of powder and polyphenol materials (Belščak-Cvitanović et al., 2014). Each of the carrier compounds has its own advantages and disadvantages. But in general, maltodextrin with high solubility, low viscosity, low price, neutral taste and aroma, is a good substance to protect oxidation-sensitive compounds, by facilitating the spray drying process (Goula et al., 2012). "Maltodextrin is a hydrolysed starch, and is known as a popular capsule substance and is used as a carrier in this method". These benefits include low cost, neutral flavour, low adhesion, protection against oxidation, etc.(Đorđević et al., 2015).

Naturally, the presence of polyphenolic compounds is important due to its bioactive and antioxidant properties. Antioxidants in the diet are important in terms of protecting the body against oxidative stress and maintaining health. But on the other hand, these compounds are very volatile. Are and under certain conditions such as decomposition can lose their bioactive properties. Encapsulation is one of the simplest ways that can protect and improve the stability of these compounds, as well as reduce these bitter taste and unpleasant odours. One of the advantages of high-speed encapsulation is that due to the very short drying time, the nutritional value and quality of the product are largely preserved. Microencapsulation is very important for each carrier compound in the food and pharmaceutical industries (Fang et al., 2012). Also, one of the applied modifications is the use of carrier powders, such as Arabic gum, various starches and their derivatives such as maltodextrin and various hydrocolloids. The use of these carriers to reduce the adhesion of products to the walls of the dryer, which during the process reduces productivity is the reduction of quality and consequently the product remains in the dryer or burns it and causes a lot of economic losses (Santhalakshmy et al., 2015). The aim of this study is to compare the different operating temperature of the spray drying process using maltodextrin as a carrier in terms of antioxidant capacity and polyphenol content of saffron pollen extract powders.

2. Material and Methods

2.1. Instrumentation

The B-191 Mini Spray dryer (BUCHI), equipped with a 0.9 mm nozzle spray with a maximum temperature of 220 ºC, was used to dry the emulsion and prepare the powder. A magnetic stirrer was used to make the extract. The SEM analysis was performed using a TESCAN MIRA 3 LMU (Tescan USA Inc., Warrendale, PA, USA) Variable Pressure Schottky Field Emission Scanning Microscope. The SEM is equipped with both a backscattered electron detector (BSE) and an INCA X-max 80 mm^2 silicon drift detector (SDD) energy dispersive spectrometer (EDS) which are able to provide qualitative elemental/phase information and semi-quantitative elemental information, respectively about the sample being analyzed. A pure cobalt plate was used as a standard to calibrate the beam for analysis.

2.2. Chemicals

Sodium carbonate, gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydrochloride acid and Folin–Ciocalteau phenol reagent were purchased from Sigma–Aldrich GmbH (Sternheim, Germany). Reagent-grade methanol and ethanol were procured from Panreac (Barcelona, Spain). All other chemicals and solvents were of the analytical grade and obtained from Merck (Darmstadt, Germany).

2.3. Plant material

Saffron flowers have been collected manually from a local farmer which have been cultivated free of any chemical treatments in the region of Gonabad (Khorasan Razavi, Iran) in October 2019. The different parts of the flower were separated and each separated parts of the flower were dried at room temperature (23 ± 2 °C) in the dark place. Then the dried part of the flower was powdered using electric mill and stored in a refrigerator at 4 °C until ready for extraction.

2.4. Herbal extract preparation

The air-dried pollen were cut into small pieces and placed in a glass bottle. They were then mixed with warm water (80 °C) in a ratio of 1:30, and mixture of water and pollen was shaken by the shaker for 24 h in dark. The extract was then filtered using filter paper and then the solvent concentrated using a rotary evaporator apparatus.

2.5. Feed mixture preparation and encapsulation by spray drying

The feed mixture was prepared by mixing maltodextrin to the plant extract. The mixture was stirred continuously at room temperature (23 ± 2 °C) until all the 10% (w/v) of the maltodextrin content was added to the extract. The feed mixture was fed into a mini-spray dryer with a flow rate of 8 mL/min and the inlet temperature of the spray dryer was operated at three different temperatures (140, 160 and 180 °C) to study the effect of temperature.

2.6. Polyphenols determination

Folin-Ciocalteau method measures a reducing activity of samples. Folin-Ciocalteau is a method for measuring the concentration of polyphenol compounds, the result of this test can be used to evaluate the antioxidant features of a sample. For the assay, aliquots (250 µL) of diluted extracts of samples and gallic acid standards were taken in test tubes. Then 1.25 mL of Folin-Ciocalteau phenol reagent (diluted in distilled water at a ratio of 1:10) was added sequentially in each tube. The solution was thoroughly mixed with a vortex and then incubated at room temperature (23 ± 2 °C) for 5 min. Then the mixture was alkalinized with 1 mL of Na_2CO_3 (75 g/L). After that, the test tubes were placed for 60 min in dark place and the absorbance was recorded at 760 nm against the reagent blank. The concentration of total polyphenols was calculated using the calibration curve (100-500 mg/L) of gallic acid with equation of y=0.0054x-0.0048 and finally the results were reported as mg gallic acid equivalents in 100 g dry weight of the sample. Each experiment was repeated triple and the mean values were reported.

2.7. Antioxidant activities

DPPH is one of the finest methods to determine the antioxidant capacity of the plant extracts. The antioxidant capacity of the samples that were dried under different temperatures selected in this study was estimated by DPPH assay. When the stable free radical DPPH is reduced in the presence of a hydrogen-donating antioxidant, a non-radical form of DPPH-H is formed. This non-radical DPPH turns the colour from violet to yellow which was monitored at 517 nm using UV-Visible spectrophotometer (Singh et al., 2002). Calculating a 50% inhibition concentration (IC_{50}) is a good way to compare antioxidant activity. In this study, the IC_{50} of saffron pollen extract was calculated by plotting the percentage of inhibition curve against the extract concentration. The results are summarized in Table 1. All experiments were performed for triple and the mean results were reported.

2.8. Morphology consideration

For morphology consideration, a specimen of nanocapsules suspension was poured into an aluminium foil and dried at 60 °C, and later gold sputtered for SEM analysis.

2.9. The yielded powder, solubility, wettability and water activity measurement

For Production yield was calculated as the ratio of the weight of the powder obtained to the mass of the total solid in the feed (in terms of dry matter). In this study, the powder collected in the bottom glass of the spray dryer was used as the main product to calculate the process efficiency and perform powder tests.

The solubility of saffron pollen powders was measured. For this purpose, 1 g of powder (w_1) was cautiously suspended to 100 mL of distilled water, and mixed with a magnetic stirrer at 700 rpm for 4 min. The resulting solution was centrifuged for 4 min. The volume of 25 mL was separated from the above solution and transferred to a pre-weighed petri dish and dried in an oven at 105 °C for 5 h (w_2). The solubility was calculated according to Eq. 1:

$$Solubility\ (g/100g) = \frac{w_2}{w_1} \times 4 \tag{1}$$

To determine the wettability of powders, the method of Fuchs et al. (2006) was used with some modifications. The powder (0.2 g) was poured at room temperature (23 ± 2 °C) on the surface of 100 mL of distilled water without stirring. The time taken for the particles to settle from the water surface so that no particles were left on the surface was recorded to calculate the wettability index. The water activity of microencapsulates also was determined by a_w meter. The water activity of the samples was measured for the powder obtained in three temperatures of 140, 160 and 180 °C.

2.10. Powder production efficiency

Product yield was calculated as the ratio of the weight of the powder obtained to the mass of the total solid in the feed (in terms of dry matter). In this study, the powder collected in the end glass of the device was used as the main product to calculate the process efficiency.

2.11. Statistical analysis

All the experiments were triplicated and the statistical analysis was performed using STATISTICA (Data Analysis Software System), v. 12, and Excel 2013 software. Differences were significant at $p \leq 0.05$.

3. Results and Discussion

3.1. Encapsulation by spray drying

Spray drying is one of the simplest and the best methods, which has been adopted in the present study. The spray drying inlet temperature was adjusted to three different temperatures that shows the optimum temperature for best performance. Powder production performance is one of the main indicators and shows the economy, efficiency and also the success rate of the process. The results of this study showed that by increasing the inlet air temperature from 140 to 160 °C, the amount of powder production is significantly reduced. (Table 1) This is probably due to the partial adhesion of the drain to the spray dryer wall at higher temperatures. Production efficiency and other nutritional, physical and chemical properties, drying process of saffron pollen spray at 140 °C showed the best powder production efficiency. Also, powders produced at 140 °C temperature are a great source and rich in phenolic compounds with amazing antioxidant activity.

Depending on the speed, force, angle and time of contact, it may cause the particles to stick (Sarabandi et al., 2018). This finding is also consistent with the results of research by Nadeem et al. (2011). They observed the effect of increasing the inlet air temperature on reducing the production yield of Salvia Miltiorrhiza herb extract powder and black grape juice.

Similar observations has been reported by drying of mountain tea spray at different temperatures (Nadeem et al., 2011). When the concentration of maltodextrin is greater than 10%, the production efficiency is high and thus overcomes low production efficiency due to the material adhering onto the walls of the drain chamber (Sansone et al., 2011). Also, scanning electron images of other powders produced from other foodstuffs showed that when concentrations much higher than 10% maltodextrin (such as when values of 30%) were used, the powder particles produced, were spherical in proportion to the surface area. They were smooth, but some powder particles with irregular shapes and wrinkles were observed on the surfaces. Therefore, in this paper, in order to produce powder particles with more regular shapes and cost savings, a concentration of 10% maltodextrin was used (Sarabandi et al., 2018).

In another study, the production of *S. montana* dry powder with the addition of 10% maltodextrin had a production yield of about 66%. (Vidović et al., 2014). Earlier reports on orange water spray dries was also found to have decreasing yield with increasing temperature (Chegini & Ghobadian, 2007).

3.2. *Measurement of powder production yield, solubility, wettability indices*

Powder solubility is an important functional property of food powders that affects the behavior of the powder when reconstituted in water. This feature is of special importance for producers and consumers (Jayasundera et al., 2011). The results are shown in Table 1. As can be seen, the solubility at 140 °C was better than the other two temperatures and the solubility result was better at 180 °C than 160 °C. Factors such as size, shape, composition, surface properties, particle microstructure and the presence of insoluble additives and compounds, type and composition of raw material, type of feed (solid concentration), drying conditions (temperature used, inlet air velocity, pressure and atomizer round) can be very effective in regenerating powders (Bhandari et al., 2008).

The results of this study in relation to the effect of temperature increase on the solubility of flag powder and saffron pollen, agree with the findings that observed the effect of temperature increase on the solubility of the studied powders.

Cano-Chauca et al. (2005) observed the effect of reducing the solubility of watermelon and mango water powders under the influence of increasing temperature, respectively. Moisture or water absorption of particles/capsules is one of the most important physical properties related to the regenerative properties of powders that are directly affected by molecular reactions between the two phases (Bae et al., 2008). The observed values in dampness indicate the urgency of the damping process in flag and saffron pollen powders produced with maltodextrin. This finding is in agreement with the findings of Santhalakshmy et al. (2015), who observed a decrease in the wetting time of fruit powder due to the increase in inlet air temperature. They attributed this result to the increase in size and spaces between particles and the subsequent facilitation of moisture penetration into the structure of powders. Powder production efficiency is one of the main indicators the amount being economical, efficient as well as being successful the process. Table 1, shows the effect of incoming air temperature on the production efficiency of saffron pollen. The production yield for the temperature of 140, 160 and 180 °C were 45.2, 44 and 10 g/100g, respectively. As can be seen in the table, powder production decreased significantly with increasing temperature from 140 to 180 °C.

At higher temperatures, for production reasons such as the effect of increasing the mass transfer coefficient of mass and heat on the drying process of atomized droplets, the formation of the initial dry layer on the particle surface before reaching the dryer wall and the adhesion of wet and semi-wet droplets, production efficiency is reduced. This finding agrees with the results of the researches of Santhalakshmy et al. (2015), they examined the juice of a kind of Indian blackberry.

3.3. *Measurement of water activity*

Water activity is the amount of unbound water in a sample. Water that is not bound to the ingredients themselves can be used by unwanted microorganisms which could lead to one of the contributing factors for food spoilage. Water activity is based on a scale from 0.0 to 1.0. The water activity in all samples was below 0.30, indicating that the spray dried powders of saffron pollen has features, such as: microbiological stability, shelf-life, food safety and chemical stability (Troller, 1983) (Table 1).

3.4. *Total polyphenols determination*

Total phenolic content based on gallic acid was calculated by a standard calibration curve. The main purpose of designing the drying of saffron pollen extract with spray is to adjust the process conditions in such a way that high quality and effective powder with high concentration of polyphenol compounds is produced.

The total polyphenol content of saffron pollen extract was found to be 620 ± 4.50, 470 ± 2.65, 420 ± 2.18 mg gallic acid per 100 g, for 140, 160 and 180 °C, respectively (Table 1). The total polyphenol content was found to be high at 140 °C compared to those at higher temperature. High temperatures result in loss of polyphenol contents of the saffron pollen extracts powder. Similar observations has been reported (Nadeem et al., 2011). They reported that its phenolic value decreases with increasing inlet air temperature to 165 °C. Also, with decreasing the inlet air temperature, a slight decrease in the total polyphenols of the total soybean extract dried from the spray was also reported from 11.75 to 10.96 mg/g.

3.5. *Antioxidant activity*

The use of DPPH free radical scavenging function is commonly used as part of various types of experiments, especially in plants (Iwashima et al., 2005). Investigation of the effect of inlet temperature on antioxidant properties showed that with increasing temperature, the antioxidant potential of powders decreases. The same powder has shown that the highest amount of total polyphenols will necessarily have the most antioxidant activity. In any case, the techniques used in the encapsulation may be able to affect the physicochemical properties and, consequently, the quality of the resulting capsules.

So far, there is no report on the antioxidant properties of encapsulated saffron pollen. But the antioxidant properties of other part of saffron flower have been reported (Jadouali et al., 2019; Kosar et al., 2017; Lahmass et al., 2018). The IC_{50} results for saffron pollen extract was found to be 0.177 ± 0.08, 0.191 ± 0.09, 0.182 ± 0.08 g per 100 g, for 140, 160 and 180 °C, respectively. The IC_{50} value of saffron pollen extract for 140 °C was found to be low compared to the other high temperatures. This is another evidence on the retention of polyphenols in the capsules at low temperature spray drying process.

Table 1. Parameters of spray drying process on obtained powders (mean ± SD, n=3).

Inlet Temperature (°C)	Outlet Temperature (°C)	Yield (g/100g)	Water activity (a_w)	IC_{50}* (g/100g)	TPC** (mg GAE/100 g sample)	Wettability (s)	Solubility (g/100g)
140	85	45.2 ± 1.52^a	0.28	0.177 ± 0.08^a	620 ± 4.50^a	13.21±1.45^a	91.72±2.35^a
160	90	44 ± 1.08^a	0.21	0.191 ±0.09^b	470 ± 2.65^b	11.22±0.96^a	72.12±1.98^b
180	90	10 ± 0.54^b	0.26	0.182 ± 0.08^c	420 ± 2.18^c	9.23±0.85^b	80.16±2.15^c

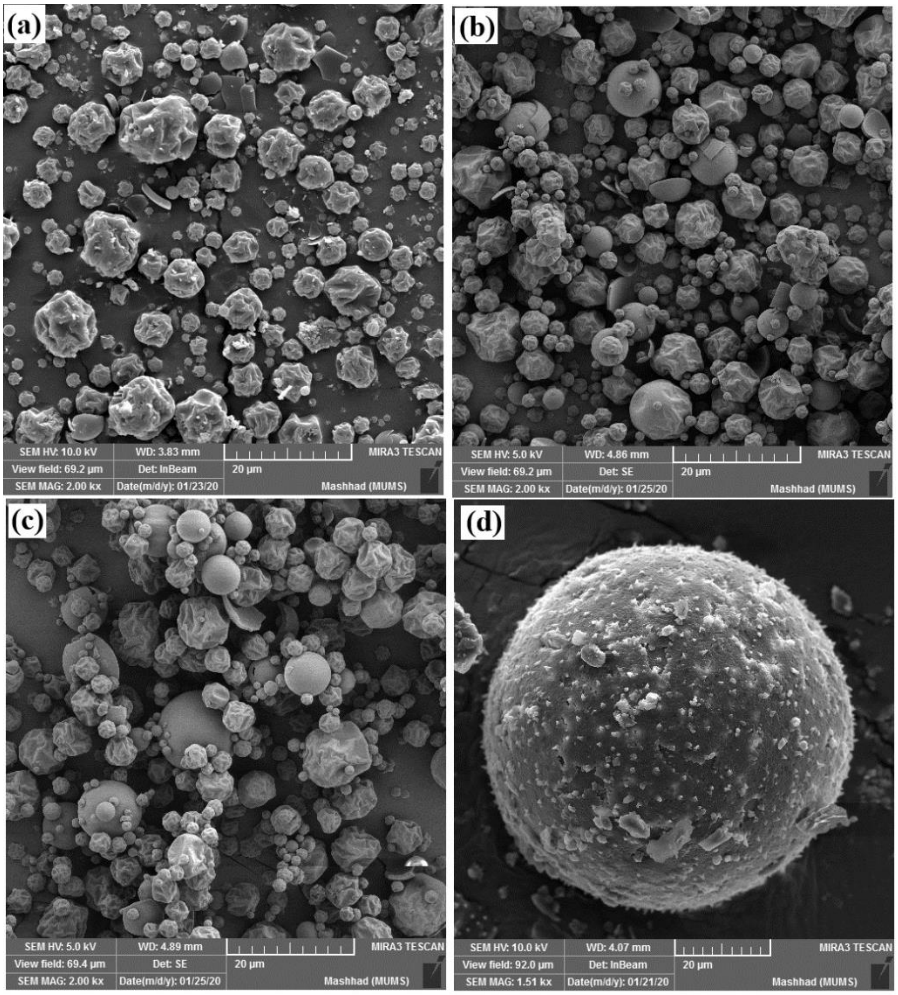

Fig.1. SEM image of saffron pollen capsule powder prepared at 140 ºC (a) 160 ºC (b), 180 ºC (c) and non-capsule (d) (SEM magnification 2.00 kx).

3.6. *Surface morphology analysis*

In this study, a 90:10 carrier treatment (maltodextrin) was selected for the samples at (140, 160 and 180 °C) to evaluate the surface morphology of the particles. The surface morphology of the nanocapsules prepared at three different temperatures is represented in Fig. 1(a-c). The un-encapsulated sample of saffron pollen extracts is represented in Fig. 1d. It was found that all powders produced nanocapsules prepared at different temperatures of saffron pollen extracts. The nanocapsules were spherical in structure and found to have smooth surface but some have cracked surfaces. The formation of serrated surfaces can be due to the shrinkage of high temperature particles during the spray drying process (Wu et al., 2014).

This property is related to high solubility and suitable bulk density. Irregular sizes, irregular surfaces and curved surfaces have also been observed. Some powdered saffron pollens are seen at different temperatures with smaller particles and more immersion than others. In addition, on the surface enclosed with composite wall materials, more serrated surfaces are seen, while on the powder enclosed with single-walled materials, a small number of serrated surfaces are observed (Mohd Nawi et al., 2015). This shape and surface properties may be one of the reasons for the high level of antioxidant compounds. As can be seen in the figures, a temperature of 140 °C has the best level of pollen powder in terms of smoothness and spherically.

4. Conclusion

The present study investigated the microencapsulation of saffron pollen extracts by spray drying process. Maltodextrin has high advantages (such as high solubility, low viscosity, no taste and odour and reasonable price) as a suitable carrier for the production of powder. As results the low adhesion of saffron pollen powder, low carrier values were required to achieve proper production efficiency. All tests related to nutritional, physical and chemical properties including water activity, polyphenol content, antioxidant activity, solubility and high moisture content depended on the inlet air temperature, considering the efficiency and economy of the process. Observations from the scanning electron microscopic images showed that the formed capsules were all spherical in shape and had a uniform surface. As can be seen in the figures, a temperature of 140 ºC has the best level of pollen powder in terms of smoothness and spherically.

On the other hand, the surface structure of the particles and the results of the electron microscope also showed an improvement in the surface properties and uniformity of the particles under the influence of the process air temperature. As to, achieving a product with suitable nutritional, physical, chemical, and functional properties, along with the economics of the process, depends on considering and maintaining optimal drying conditions (both process and feed).Therefore, these powders of extracts of saffron pollen are a very good potential for development of different types of food or drinks with increased functional value.

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Conflict of interest

The authors declare that they have no conflict of interest.

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