Encapsulation of Bioactive Compounds of Saffron in Gelatin Using Electrospinning and Freeze-drying Techniques

By

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DEDICATED TO MY FAMILY AND SUPERVISOR

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ABBREVIATIONS

%	Percent
cm	Centimeter
Da	Dalton
DSC	Differential Scanning Calorimetry
EE	Encapsulation efficiency
etc	Et cetera
g	Gram
Ge	Gelatin
h	Hour
ISO	International Standards Organization
K	Reaction rate constant
kV	Kilovolt
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
nm	Nanometer
°C	Degree Celsius
Pa	Pascals
RH	Relative humidity
rpm	Revolutions per minute
S	Second
S	Saffron
SEM	Scanning electron microscope
T_d	Denaturation temperature
T_m	Melting temperature
um	Micrometer
V/V	Volume/volume
W/W	Weight/weight
ΔH	Enthalpy
µm	Micrometer

ABSTRACT

Saffron is the dried stigma of the plant *Crocus sativus Linnaeus*, which is perhaps the most expensive spice in the world. The quality of saffron depends on its color, taste, and aroma. The three major constituents of saffron are crocin (color), picrocrocin (bitter flavor), and safranal (aroma). These compounds are highly sensitive and unstable to environmental conditions such as oxygen, pH, light, and thermal treatment. Therefore, it is important to protect the bioactive compounds of saffron through the encapsulation process in order to exploit its industrial uses.

The main objective of this study was to encapsulate saffron extract powders (5, 10, and 15% w/w) in gelatin as a wall material using electrospinning and freeze-drying techniques. The encapsulation efficiency, thermal properties, storage stability, morphology, and diameter distribution of the encapsulated saffron extract were evaluated.

Test results demonstrated that saffron extracts could be successfully encapsulated in gelatin by both techniques and the highest encapsulation efficiency was associated with 15% saffron extract concentration. The encapsulation efficiency of safranal was lower than crocin and picrocrocin, which could be related to its volatility and instability. Differential scanning calorimetry analysis showed that denaturation temperature and melting temperature of control samples increased, while their enthalpy decreased by the addition of saffron extract. The results also revealed that the encapsulation efficiency, storage stability, and thermal properties of encapsulated samples improved significantly ($p < 0.05$) by increasing the concentration of saffron extract in different treatments. Crocin degradation followed first-order reaction kinetics. Crocin content of encapsulated saffron extract decreased with increasing storage temperature from 4 to 35 °C. Overall, encapsulated saffron extract in gelatin demonstrated superior retention of crocin compared to un-encapsulated one. Encapsulated saffron extract at identical concentrations showed the lower reaction rate constant *(k)* and higher half-life period ($t_{1/2}$) during 42 days storage at 4 °C compared to 24 and 35 °C. In addition, by increasing the concentration of saffron extract in gelatin, reaction rate constant decreased and half-life increased. The highest retention of encapsulated crocin was observed at 15% saffron extract concentration in electrospun gelatin fibers at 4 °C storage temperature with a half-life ($t_{1/2}$) of 138 days, and the reaction rate constant *(k)* of $0.5*10^{-2}$ min^{-1}. Scanning electron microscopy (SEM) images showed no differences between the morphology of the encapsulated fibers and encapsulated particles compared with their controls.

Results of this study revealed that the encapsulation of bioactive compounds of saffron is an effective way to protect them against harsh environmental conditions due to the protection from the wall material obtained through the encapsulation process. The results also indicated that many factors such as type of encapsulation techniques, concentration of saffron extract, storage temperature, and time all play important roles on the stability of the coloring strength of saffron during storage. The electrospinning was found to be the better encapsulation technique with higher encapsulation efficiency, half-life period, and retention of crocin content during storage compared to the freeze-drying technique.

RÉSUMÉ

Le safran est le stigmate séché de la plante *Crocus sativus Linnaeus*, qui est peut-être l'épice la plus chère du monde. La qualité du safran dépend de sa couleur, de son goût et de son arôme. La crocine (couleur), la picrocrocine (saveur amère) et le safranal (arôme) sont les trois principaux composants du safran. Ces composés sont très sensibles et instables aux conditions environnementales tels que l'oxygène, le pH, la lumière et les traitements thermiques. Par conséquent, il est important de protéger les composés bioactifs du safran par le processus d'encapsulation pour en développer ses utilisations industrielles.

L'objectif principal de cette étude était d'encapsuler l'extrait de safran (5, 10 et 15% w/w) dans de la gélatine en tant que matériau de paroi à l'aide de techniques d'électrofilage et de lyophilisation. L'efficacité d'encapsulation, les propriétés thermiques, la stabilité au stockage, la morphologie et la distribution en diamètre de l'extrait de safran encapsulé ont été évaluées. Les résultats des tests ont démontré que les extraits de safran pouvaient être encapsulés avec succès dans de la gélatine par les deux techniques et que l'efficacité d'encapsulation la plus élevée était associée à une concentration de 15% d'extrait de safran. L'efficacité d'encapsulation du safranal était inférieure à celle de la crocine et de la picrocrocine, ce qui pourrait être lié à sa volatilité et à son instabilité. Une analyse calorimétrique différentielle à balayage a montré que la température de dénaturation et la température de fusion des fibres de gélatine et des poudres de gélatine augmentaient avec l'addition d'extrait de safran, tandis que leur enthalpie diminuait par addition d'extrait de safran. Les résultats ont également révélé que l'efficacité d'encapsulation, la stabilité au stockage et les propriétés thermiques des échantillons encapsulés étaient considérablement améliorées ($p < 0.05$) en augmentant la concentration d'extrait de safran dans les fibres de gélatine ou les particules de gélatine. La dégradation de la crocine suit une cinétique de réaction de premier ordre. La teneur en crocine de l'extrait de safran encapsulé a diminué avec l'augmentation de la température de stockage de 4 à 35 °C. Dans l'ensemble, l'extrait de safran encapsulé dans de la gélatine a démontré une rétention supérieure de la crocine par rapport à celle non encapsulée. Un extrait de safran encapsulé à des concentrations identiques a montré une constante de vitesse de réaction plus basse *(k)* et une demi-vie plus longue ($t_{1/2}$) pendant 42 jours de stockage à 4 °C par rapport à 24 et 35 °C. De plus, en augmentant la concentration d'extrait de safran dans la gélatine, la vitesse de réaction constante a diminué et la demi-vie a augmenté. La plus forte rétention

de crocine encapsulée a été observée à une concentration de 15% d'extrait de safran dans des fibres de gélatine électrofilées à une température de stockage de 4 °C avec une demi-vie ($t_{1/2}$) de 138 jours et une vitesse de réaction constante (k) de $0.5*10^{-2}$ min^{-1}. Les images de microscopie électronique à balayage (MEB) n'ont montré aucune différence entre la morphologie des fibres encapsulées et celle des particules encapsulées par rapport à leurs témoins. Cependant, de plus grandes fibres électrofilées et des particules lyophilisées ont été produites à une concentration plus élevée en extrait de safran.

Les résultats de cette étude ont révélé que l'encapsulation de composés bioactifs de safran constituait un moyen efficace de les protéger contre les conditions environnementales difficiles grâce à la protection d'un matériau de paroi stable obtenu par le processus d'encapsulation. Les résultats ont également indiqué que de nombreux facteurs tels que le type de technique d'encapsulation, la concentration d'extrait de safran, la température de stockage et la durée jouent un rôle important dans la stabilité de la force de coloration du safran pendant le stockage. L'électrofilage s'est avéré être la meilleure technique d'encapsulation avec une efficacité d'encapsulation, une période de demi-vie et une rétention du contenu en crocine plus élevées par rapport à la technique de lyophilisation.

CONTRIBUTION OF AUTHORS

A part of this thesis research presented at NABEC (The Northeast Agricultural and Biological Engineering) conference, Quebec City, 16-19 June 2019 and food processing symposium, McGill, 23 July 2019. This thesis has been written in a manuscript style to suitably edit chapters and highlighting research for publication. Authors involved in the thesis work and their contributions to the various articles are as follows:

Fatemeh Golpira is the MSc. candidate who planned and conducted all the experiments, on the advice and guidance of her supervisor, gathered and analyzed the results and prepared drafts for the thesis and the manuscripts for scientific presentations and publications.

Dr. Hosahalli S. Ramaswamy is the thesis supervisor, under whose guidance the research was performed. He coordinated and supervised the candidate in planning and conducting the research, as well as in correcting, reviewing and editing the thesis.

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CHAPTER 1

INTRODUCTION

One of the most important appearance properties of food is color, which is used as the first criterion for their quality assessment and acceptance (De Azeredo, 2009). Artificial colors have been widely used in different food industries; however, their use is still a big issue due to their health risks in children (allergies and hyperactivity) and adults (cancer) (Sasaki et al., 2002; Inomata et al., 2006; Mizutani, 2009). Therefore, consumer demand has been ever increasing for the use of natural colors as an alternative to artificial colors (Rutkowska & Stolyhwo, 2009).

Saffron, a natural source of red to orange pigments, obtained from the dried red stigma of *Crocus sativus* L., is one of the most expensive spices in the world. Saffron is cultivated in a mild and dry climate. The main producers of this plant are Iran, Greece, India, Spain, Turkey, Morocco, Italy, China, Turkey, Azerbaijan, Egypt, and Pakistan (Fernandez, 2004). Saffron is used extensively in different industries such as food, cosmetics, and pharmaceutical due to its coloring, taste, aroma, antioxidant, and therapeutic properties (anti-cancer, anti-depression, anti-spasmodic, carminative). Three major compounds of saffron are crocin, picrocrocin, and safranal which are responsible for its color, aroma, and flavor, respectively (Basker & Negbi, 1983; Zeng et al., 2003). Compounds of saffron are predominantly unstable and can be affected by the final processing condition, moisture, proteins, metallic ions, pH, light, enzymes, oxygen, and storage temperature (Patras et al., 2010). To solve this problem, bioactive compounds contained in saffron (crocin, picrocrocin, and safranal) should be protected.

Encapsulation is a technique to protect sensitive materials (flavors, enzymes, essential oils, and cells) against heat, moisture, and other stress conditions to improve their stability for successful incorporations into other finished products. Through the encapsulation process, the sensitive compounds (core) are entrapped into another material (wall), which leads to a reduction in the evaporation rate and loss of volatile components (Kriegel et al., 2008). Different methods have been used for encapsulation of bioactive materials in the food industry including spray drying (Esfanjani et al., 2015) and freeze-drying (Khazaei et al., 2014). These encapsulation methods produce spherical capsules and beads between several microns to a millimeter in diameter (Gibbs et al., 1999; Yuliani et al., 2004).

Among the many different encapsulation techniques, electrospinning is one of the most effective techniques. Electrospinning is a method to produce fibers with nano to micrometer size by using electrostatic forces to shape a polymer solution into a fibre format. Electrospun fibers show unique properties including a large surface area to volume ratio and high porosity. This property provides a great capacity to entrap the bioactive compounds with high encapsulation efficiency and also to improve the stability of encapsulated compounds (Bhardwaj & Kundu, 2010). This method can be influenced by polymer solution properties (surface tension, viscosity, and electrical conductivity), processing parameters (applied voltage, the distance between the needle and the collector, different types of needle and collector) and environmental conditions (temperature, moisture, relative humidity). Furthermore, there are other methods to produce nanofibers including surface grafting polymerization, polymer phase separation, atomic layer deposition in addition to electrospinning (Kayaci et al., 2012; Yu et al., 2016; Niu et al., 2017). Electrospinning technique has some advantages over the other techniques such as low start-up cost, absence of heat during the spinning process, and simplicity of the instrument setup (Huang et al., 2003; Mirjalili & Zohoori, 2016). Recently, electrospun fibers have gained strong commercial and academia attentions and have been used effectively in many applications including tissue engineering, air and water filtration membrane, protective clothing, artificial organs, thermal energy storage, and drug delivery (Huang et al., 2003; Mirjalili & Zohoori, 2016). However, the electrospinning technique is relaticely new in terms of encapsulation of bioactive materials and their applications in different food industries. Electrospun fibers are an excellent choice as a carrier to encapsulate bioactive compounds because of their small diameter and organized structure (Alborzi et al., 2013). So, in the area of food, electrospun fiber has a strong potential to be used in packaging, encapsulation, and controlled release of food ingredients (Bhushani & Anandharamakrishnan, 2014).

It was recommended to use natural polymers for encapsulation of bioactive compounds in the food industry due to their biocompatibility and non-toxicity, compared to synthetic polymers (Aceituno-Medina et al., 2013; Moomand & Lim, 2014). Electrospun fibers have been successfully produced from natural polymer solutions such as gelatin, pectin, alginate, tragacanth, chitosan, pullulan, amaranth protein, and zein for encapsulation of various compounds (Alborzi et al., 2013; Aceituno-Medina et al., 2015; Horuz & Belibağlı, 2018; Niu et al., 2017). Furthermore, food-derived and food-approved solvents should be used for encapsulation of materials with food-related applications.

During the fiber generation, it is generally assumed that the solvent is mostly evaporated (Aceituno-Medina et al., 2015). Encapsulation of carotenoid extracted from tomato peel was studied using polymeric fiber by electrospinning to improve its thermal and storage stability (Horuz & Belibağlı, 2018).

Freeze-drying is another technique of encapsulation that is suitable to encapsulate heat sensitive materials. Rulkens and Thijssen (1972) reported that stability of volatile compounds during freeze-drying was affected by the composition of wall and core material. Kopelman et al. (1977) also studied the encapsulation of citrus aroma using freeze-drying method to use in soft drink dry mixes.

Since bioactive compounds of saffron are susceptible to degradation at high temperature and high relative humidity, they need to be preserved against harsh environmental conditions during processing and storage. Gelatin is used extensively as a wall material for encapsulation of bioactive compounds due to its unique properties such as hydration (solubility and swelling), gelling (texturizing, gel formation, water binding capacity, and thickening) and surface behavior (film-forming capacity, emulsion formation and stabilization). Other advantages of gelatin are its relatively low cost, availability, and acceptance by the food industry (Gómez-Guillén et al., 2011).

Research Objectives

The main objective of this thesis research is to evaluate the encapsulation of bioactive compounds of saffron extract by using two different encapsulation techniques for their possible incorporation into different food matrices. Encapsulation efficiency, thermal properties, storage stability, and morphology of encapsulated and un-encapsulated samples are parameters that were used in determining the suitability of encapsulation.

The specific objectives of this study are:

1. To evaluate the use of electrospinning method for encapsulation of bioactive compounds of saffron extract powders (5, 10, and 15% w/w) into gelatin fibers to improve its thermal and storage stability at different temperatures (4, 24, and 35 °C) during 42 days of storage.
2. To study the possibility of encapsulation of bioactive compounds of saffron extract powders (5, 10, and 15% w/w) into gelatin powders through freeze drying technique and also monitor the stability of crocin in encapsulated saffron extract during 42 days of storage at different temperatures (4, 24, and 35 °C).

CHAPTER 2

LITERATURE REVIEW

2.1 Saffron

One of the most important characteristics of food products is color that indicates their quality. Artificial colorants are widely used in food industries for purpose of improving the appearance. However, the consumption of chemical food additives such as artificial colorants has remained a problem in the food industries because of their toxicological potential on human health with increased risk of cancer (Sasaki et al., 2002) and allergies in children (Mizutani, 2009; Inomata et al., 2006). Therefore, consumers prefer to use natural pigments with functional properties instead of chemical colorants. This is also supported by positive effects of natural colorant on human health (Moreira et al., 2012).

Saffron is one of the most important natural biocolorants (Figure 2.1). It can be planted in Mediterranean climate with different range of soils from sandy to clay loams (Sarfarazi et al., 2015). Iran is the biggest producer and exporter of saffron in the world with around 94% of the world production (Rajabi et al., 2015). Recently, saffron has got the attention of pharmaceutical industry because of its unique medical properties such as anti-depressant, antitussive, anti-carcinogenic, anti-nociceptive, anti-thrombotic, anti-inflammatory, and anti-oxidant properties (Atefi et al., 2017). Saffron is being used in the range of 1-260 ppm in different food applications including dairy, bakery, meat processing, and beverage (alcoholic and non-alcoholic) (Chranioti et al., 2015). The stability and shelf life of saffron are greatly influenced by its pigments. Since compounds of saffron are highly unsaturated, they are very unstable and affected by different parameters during processing and storage (Patras et al., 2010). Oxidation and isomerization reactions lead to the loss of nutritive value and coloring strength (Tsimidou & Biliaderis, 1997). Accordingly, a proper encapsulation technique is required to protect natural bioactive compounds of saffron in different food products (Chranioti et al., 2015).

Figure 2.1. Dried stigmas of saffron (Ordoudi & Tsimidou, 2004)

2.2. Saffron chemistry

Many researchers have studied the chemical composition of the dried stigma of saffron since the 19th century (Sampathu et al., 1984). Chemical analyses of saffron shows that this plant contains about 12% protein, 10-12% moisture, 5-8% fat, 5% crude fiber, 5% minerals, and 63% carbohydrates containing pectin, starch, dextrin, pentosans, and reducing sugars (w/w%). Also, a small amount of riboflavin (3.4–5.6 μg/g), thiamin (0.3–0.4 μg/g), and fatty acids (palmitic, stearic, oleic, linoleic, and linolenic acid) are also found in saffron (Melnyk et al. 2010). Proximate composition of dried of saffron stigma is shown in Table 2.1. The quality and commercial value of saffron depends on the aroma, bitter taste, and coloring strength. The most important compounds of saffron are described below.

Table 2.1. The proximate compounds of saffron (Ríos et al., 1996)

Parameter	Saffron petals (% w/w)
Protein	10.20
Fiber	8.80
Ash	7.0
Fat	5.3

2.2.1. Pigments

The three major compounds of saffron which provide the unique color, taste and aroma to the saffron stigma are crocin, picrocrocin, and safranal, respectively (Esfanjani et al., 2015). The quality of saffron depends on the age and origin of the saffron, as well as the amount of crocin, picrocrocin, and safranal (Manzo et al., 2015). The concentration of these three different compounds of saffron is affected by the origin of the plant, storage length, and the overall processing conditions (Ordoudi & Tsimidou, 2004).

2.2.1.1. Crocins

The main biologically active compounds of saffron that are responsible for its color are a group of crocins. Crocin is a group of red-colored and water-soluble carotenoids which are glycosyl esters of 8,8-diapocarotene-8,8-dioic acid (Tarantilis et al., 1995). Chemical structure of crocin is shown in Figure 2.2. Crocin is widely used as a food colorant due to its high solubility in water, which give an orange-red solution (Ordoudi & Tsimidou, 2004). The UV absorption for crocin in distilled water is at about 440 nm (ISO/TS 3632 procedure, 2003).

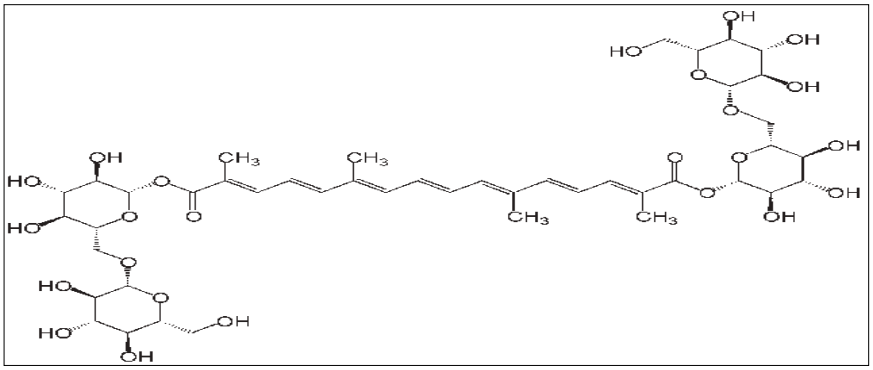


Figure 2.2. Chemical structures of crocin in *C. sativus* (Ordoudi & Tsimidou, 2004)

2.2.1.2. Picrocrocin

The main bitter compound of saffron is the colourless glycoside picrocrocin ($C_{16}H_{26}O_7$, 4 - (β-D-glucopyranosyloxy) - 2, 6, 6 - trimethyl – 1 – cyclohexene - 1 carboxaldehyde) (Figure 2.3). The maximum absorbance of picrocrocin is at 254 nm (Alonso et al., 1999). Picrocrocin is produced by the degradation of the carotenoid zeaxanthin. During the processing of saffron (drying and storage) and even flower development, picrocrocin can easily hydrolyze into safranal and release the aglycone due to the presence of β-glucosidase enzymes. So, a decrease in picrocrocin content leads to an increase in the volatile compound. In 1992, Iborra and his colleagues (Iborra et al., 1992) suggested a method for inactivation of this enzyme. Alonso et al. (2001) found that the concentration of picrocrocin in Spanish saffron was 0.79–12.94%, followed by Indian (1.07–2.16%) and Iranian (2.18–6.15%).

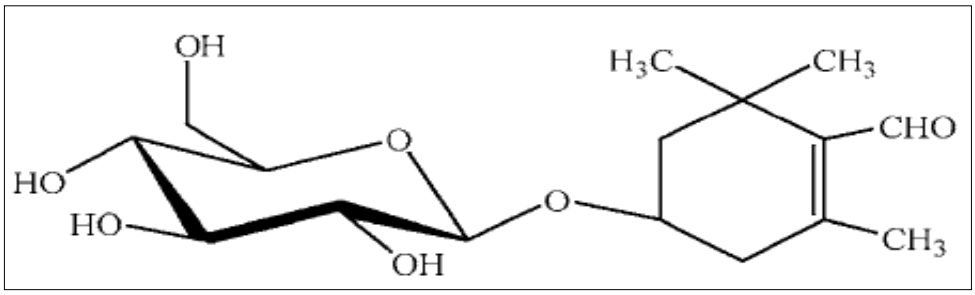


Figure 2.3. Chemical structure of picrocrocin in *C. sativus* (Ordoudi & Tsimidou, 2004)

2.2.1.3. Safranal

Safranal ($C_{10}H_{14}O$) is a cyclical terpenic aldehyde that is obtained from picrocrocin and contains the major volatile oil (60–70%) of saffron (Kulkarni et al., 2014) (Figure 2.4). However, Tarantilis et al. (1992) found that saffron contained more than 23 different volatile components. Safranal is responsible for the aroma of saffron and its level can be influenced by processing and storage conditions, as well as the methods for saffron analysis. Nonetheless, Tarantilis et al. (1997) reported that safranal was the highest volatile component of saffron (> 60% of essential oil) in all samples that were processed and analyzed. Some volatile compounds of saffron are generated from the degradation of non-volatile materials (carotenoids), since they are exposed to light, heat, oxygen, and enzyme (Straubinger et al., 1998). The aroma of saffron may be affected by the presence of minor volatile compounds.

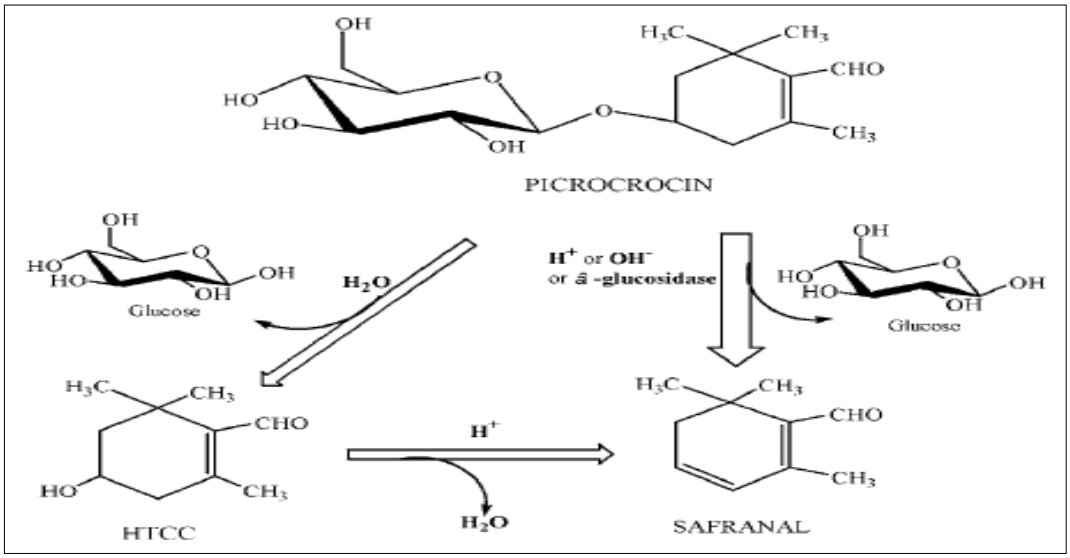


Figure 2.4. Schematic representation of picrocrocin hydrolysis into safranal

2.3. Encapsulation

Encapsulation is a technique which coats or entraps one material or a mixture of materials within another material or system (Ray et al., 2016). Through encapsulation procedure, particles are formed in the macro-meter (macro-encapsulation, >5000 um), micro-meter (micro-encapsulation, 1.0-5000 um) or nano-meter (nano-encapsulation, <1.0 um) sizes (Lakkis, 2007). The encapsulated compound is called the core, active, fill, payload phase or internal. While, the material that encapsulates the core material can be called the shell, membrane, coating, external phase, matrix, capsule or carrier and wall material (Fang et al., 2010). For the first time, encapsulation process was used in the 1950s to prepare carbonless copying paper (Madene et al., 2006). The encapsulation technique is used in cosmetic, pharmaceutical, chemical, biotechnology, agriculture and food (Augustin et al., 2001). In the food industry, enzymes, vitamins, minerals, colorants, oils, aroma and other sensitive food ingredients have been encapsulated within a matrix to protect them against environmental parameters (free radicals, light, oxygen, moisture, heat) to increase their shelf life (Desai & Park, 2005; Shahidi & Han, 1993). A schematic illustration of the encapsulation of active material is shown in Figure 2.5.

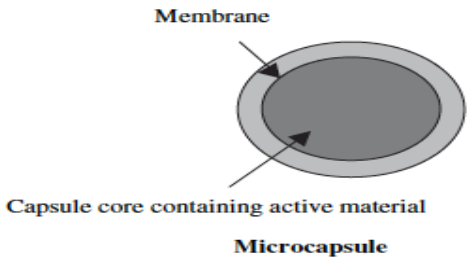


Figure. 2.5. Encapsulation of active material (Madene et al., 2006)

Volatile compounds (flavors) are encapsulated for a variety of reasons including (Tari & Singhal, 2002):

1) To preserve the flavors in food products during storage,
2) To protect the flavors from degradation and undesirable interactions with food, light and oxygen,
3) To reduce flavor–flavor interactions,
4) To increase flavors shelf-life,
5) To control the release of flavors till the right place.

2.3.1. Properties of wall materials

Encapsulation efficiency and storage stability of microcapsules can be affected by wall material characteristics. So, the correct choice of wall material is a very important issue. Some important properties of wall materials are mentioned below (Desai & Park, 2005):

1) To dissolve properly in different solution (e.g., water, and ethanol).
2) To provide a good barrier between the core material and environmental conditions (e.g., oxygen, heat, light, and humidity).
3) To trap and retain the core material within its structure during processing and storage.
4) To release the solvent and other chemical materials used during the encapsulation process under drying condition.
5) To distribute the core material and stabilize the prepared emulsion.
6) Materials with good rheological properties and easy to use at high concentration.
7) No chemical reaction with core materials during the encapsulation procedure.

2.3.1.1. Gelatin

Proteins have got more attention than other food-grade biopolymers for encapsulation of materials due to their unique functional properties. Gelatin is a colorless, odorless, brittle and flavorless substance, extracted from bones, cartilage and skin of pig and pork (46%), and cattle (52.5%) (Gómez-Guillén et al., 2011). Gelatin is a high molecular weight (52000-420000 Da) water-soluble protein containing 20 amino acids linked together with peptide bonds. This protein includes different amino acids compositions such as glycine (26-34%), proline (10-18%) and hydroxyproline (7-15%). There are two different types of gelatin-based on gelatin extraction methods. Type A gelatin is prepared in acid treatment with the isotonic point of 7 to 9. While gelatin type-B is created by alkaline treatment with an isotonic point between 4.7 and 5.2 (Gómez-Guillén et al., 2009). The important factors, which affect the physical and chemical properties of the gelatin are extraction method, animal age, collagen type, and amino acid composition (Gómez-Guillén et al., 2002). Gelatin can be joined to other polymers by hydrogen bonds, hydrophobic interactions, electrostatic interactions, and covalent bonds (Duconseille et al., 2015). Gelatin is widely used in the food industries to improve stability, elasticity, and consistency of food products (Okutan et al., 2014). Besides, it is used in the pharmaceutical industries to make soft and hard capsules to protect drugs against external factors (oxygen, light, etc.) and control their release (Roussenova et al., 2012). Since gelatin makes a gel in water at room temperature, electrospinning and electrospraying cannot be done at ambient temperature (Huang et al., 2004). Various solvents such as diluted carboxylic acid (Songchotikunpan et al., 2008), ethyl acetate (Song et al., 2008), formic acid (Ki et al., 2005), and fluoroalcohol (Huang et al., 2004) have been suggested as alternatives for electrospinning and electrospraying techniques with good results. However, the high toxicity of fluoroalcohol limits its usage in food industries. Previously, Ki et al. (2005) successfully prepared electrospun gelatin fibers by using gelatin–formic acid dope solution. Also, electrospun bovine gelatin (type B) fibers were produced by dissolving gelatin in a non-toxic solvent such as acetic acid (Erencia et al., 2014; Okutan et al., 2014). Figure 2.6 shows the chemical structure of gelatin.

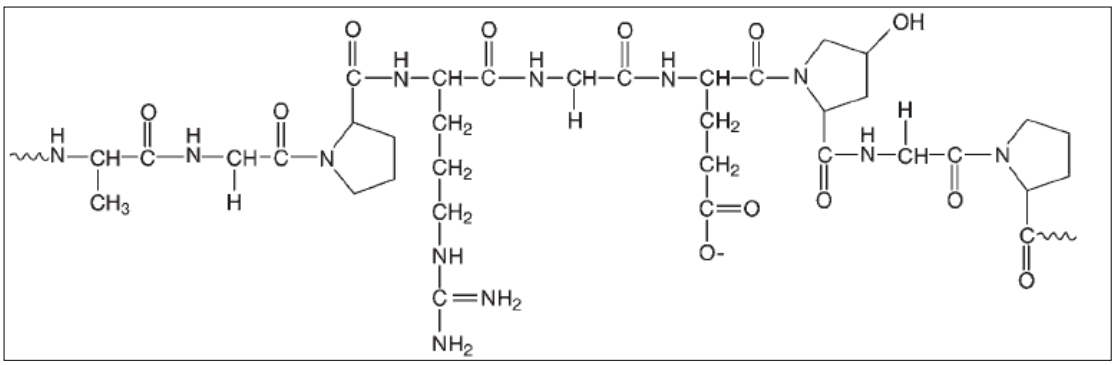


Figure 2.6. Chemical structure of gelatin (Gomez-Mascaraque et al., 2015)

Gelatin is one of the most important proteins, which is widely used for encapsulation of bioactive compounds in different industries. Functional properties of proteins make gelatin a desirable choice for a wall material such as (Jafari et al., 2008):

1) Solubility,
2) Gelling properties,
3) Foaming properties,
4) Film formation,
5) Biodegradability and electrical properties,
6) Emulsification and stabilization of emulsion droplets,
7) Ability to form thermoreversible hydrogels in water,
8) Commercially available at a low cost.

Gomez-Mascaraque et al. (2015) found that gelatin is a great wall material (effectiveness=100%) for encapsulation of green tea polyphenols (catechins) with the electrospraying technique. Also, Wang et al. (2008a) successfully encapsulated capsaicin in gelatin with the simple coacervation method and drying in a vacuum oven.

2.4. Different types of encapsulation techniques

There are different methods for encapsulation of bioactive compounds in the food industry such as physical methods (freeze-drying, spray drying, spray cooling and chilling, fluidized-bed coating, co-crystallization, and extrusion), chemical methods (molecular inclusion and interfacial polymerization) and physicochemical

methods (organic phase separation, coacervation, and liposome entrapment) (Jafari et al., 2008).

2.4.1. Spray drying

Spray drying is the oldest and most popular method for encapsulation of food flavors, oils, nutraceutical, and micronutrients compounds. The spray drying procedure is affordable, flexible, and continuous. But the most important properties of this technique are availability and simplicity of use of the equipment and production of powders with high quality. The three main groups of wall materials which have been used for spray drying encapsulation are proteins (gelatin, casein, milk serum, soy, and wheat), polysaccharides (starches, maltodextrins, corn syrups, and arabic gum), and lipids (stearic acid, mono and diglycerides) (Saenz et al., 2009). With spray-drying, excellent stabilization, protection, solubility and controlled release of the bioactive compounds can be obtained (Zuidam & Heinrich, 2010). In spray drying, proper wall material should be rehydrated in the water at first. Then, the core material is added to the solution to form a stable emulsion, suspension, or solution by using high-speed mixture followed by high-pressure. The mixture is pumped to the spray dryer and atomized in a heated air by atomizers (high-pressure nozzle or centrifugal wheel). When the atomized liquid falls through the drying chamber of the spray drier with the hot air, water is evaporated quickly from the atomized droplets because of the large surface area and spherical shape is formed (Reineccius et al., 2001). The spray-dried encapsulated powders come to the bottom of the drying chamber and collected (Gibbs et al., 1999; Gharsallaoui et al., 2007). Since encapsulated materials such as flavors may contain different components with various boiling points may be lost some aromatics compounds with low boiling points during the drying process. The final solid particles have a very small particle size mostly less than 10 μm.

2.4.2. Lyophilization or freeze drying

One of the most suitable techniques for encapsulation of all thermosensitive materials that are unstable in aqueous solution is freeze-drying (Desai & Park, 2005). Freeze-drying is a drying method to preserve heat-sensitive food for a long time based on the sublimation. This procedure has four different stages including freezing, sublimation, desorption and finally storage (Mascarenhas et al., 1997). The freeze-

drying method can protect the properties of the material such as dimensions, appearance, shape, color, taste, texture, and flavor (Ceballos & Giraldo, 2012). Maltodextrin, gum arabic, whey protein, and emulsifying starches are used extensively in freeze-drying for encapsulations of food ingredients. This encapsulation technique is not extensively used compared to others due to the long processing time, high-energy input, and storage (Ray et al., 2016). Buffo and Reineccius (2001) reported that the final product of freeze-drying showed the most desirable properties compared to spray drying, drum drying, and tray drying. Zuidam and Shimoni (2010) explained that during the freeze-drying process, a high-porous barrier was formed between the active compound and its surrounding environment.

2.5. Nanofibers

Fibers with diameter below 100 nm are defined as nanofibers. There are different techniques to generate nanofibers such as drawing, phase separation, self-assembly, and template synthesis (Ondarcuhu & Joachim. 1998). Recently, the electrospinning process has gained more industrial, scientific, and technological attention for the production of nanofibers due to its outstanding properties such as very large surface to volume ratios, small pore size, high porosity, flexibility in surface functionalities, great mechanical properties (e.g. tensile strength and stiffness), high gas permeability, biodegradability (Huang et al., 2003). Figure 2.7 showed the diameter of an electrospun sodium alginate-pectin/poly (ethylene oxide) fibers (~120 μm).

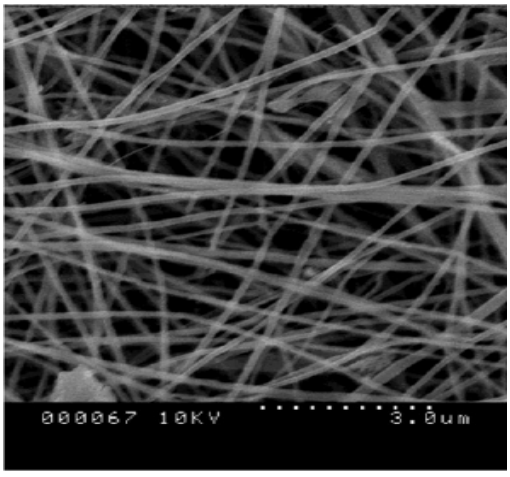


Figure 2.7. Sodium alginate-pectin/PEO electrospun fibers (Alborzi et al., 2013)

2.6. Electrospinning

Electrospinning is a simple and versatile technique to produce thin and solid fibers with tens to hundreds of nanometers in diameter from a large variety of materials such as polymers, composites and ceramics (Dzenis, 2004). Electrospinning is the electrostatic and non-mechanical process that works by applying a high voltage electrostatic field to a polymer solution to cause the ejection of a liquid jet from the spinneret. Some advantages of the electro-spinning technique are mentioned below (Doshi & Reneker, 1995):

1) Low-cost method,
2) High production rate,
3) Ability to produce fibers from different materials,
4) Easy, simple, and repeatable method.

Many applications of electrospun nanofibers have been investigated in different industries such as drug delivery (Okuda et al., 2010), water filtration (Veleirinho & Lopes-da-Silva, 2009), energy generation, regenerative medicine (Koh et al., 2008), protective clothing for the military, affinity membrane, tissue engineering (Vasita & Katti, 2006), wound dressing, cosmetics (Shen et al., 2010), enzyme immobilization, and biosensors. However, encapsulation of different compounds in electrospun nanofibers for applying in food industries is quite new, and there are only few studies related to this field. Alborzi et al. (2013) used electrospun alginate-pectin fibers to increase the stability of folic acid under different storage conditions. Also, Aceituno-Medina et al. (2015) encapsulated quercetin and ferulic acid in electrospun protein isolate-pullulan fiber by electrospinning method. Furthermore, Horuz et al. (2018) encapsulated tomato peel extract (lycopene and β-carotene) into gelatin nanofibers using the electrospinning technique to improve its solubility, thermal and storage stability, and antioxidant activity.

2.6.1. Electrospinning process

A typical bench top laboratory setup for electrospinning contains a syringe (to hold a polymer solution), infusion pump (to deliver a solution), a high voltage source, and a collector (Figure. 2.8). The syringe is connected to a spinneret, while the spinneret is connected to the positive electrode. Finally, a collector is attached to the electrode to collect produced fibers. During the electrospinning process, a high-

voltage power supply creates an electric field between a droplet of a polymer solution held at the end of the needle tip and a collector. With increasing voltage, the hemispherical shape of pendant droplet at the end of the needle tip changes into a conical shape, which is called the Taylor cone (Alborzi et al., 2013). The applied critical voltage overcomes the surface tension of the droplet due to the accumulated charges in the polymer droplet. Therefore, a charged jet of the polymer solution, which is controlled by the electric field, is ejected from the spinneret tip to the collector. When the polymer jet travels from the needle to the collector through the air, the solvent evaporates and the jet solidifies, resulting in a polymer fiber mat on the collector (Doshi & Reneker, 1995; Alborzi et al., 2013; Okutan et al., 2014). Figure 2.8 shows the schematic diagram for the production of nanofibers by electrospinning method.

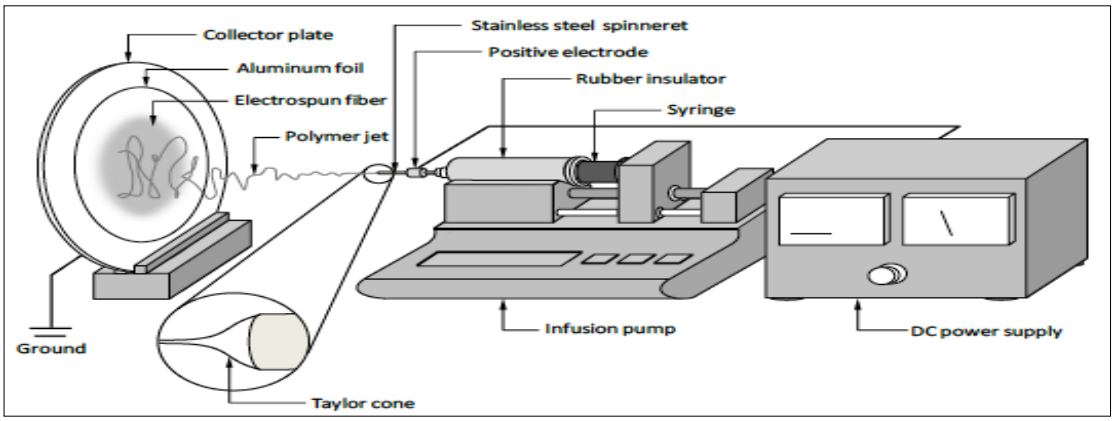


Figure 2.8. Schematic of a typical electrospinning instrument (Huang et al., 2003)

2.7. Parameters affecting the electrospinning process

Electrospinning process and the resulting electrospun fiber morphologies are influenced by solution properties such as viscosity, concentration, surface tension, dipole moment, polymer molecular weight, electrical conductivity, dielectric constant, elasticity, and dielectric properties. It is quite difficult to study the effect of the solution properties on the electrospinning process since changing one factor can influence other solution properties (Pham et al., 2006). Moreover, processing conditions (controlled variables) include the applied voltage, flow rate, needle tip diameter, type of collector, distance between tip and collector, temperature, humidity, and air velocity also affect the electrospinning process (Pham et al., 2006; Bhardwaj & Kundu, 2010; Alborzi et al., 2013).

2.7.1. Viscosity

One of the most important factors, which influences the spinnability in the electrospinning process, is the viscosity of the solution. The solution viscosity is due to the extent of molecules chain entanglement within the solution. The viscosity of the polymer solution is affected by polymer concentration and molecular weight (Huang et al., 2003). Fong et al. (1999) reported that the solution viscosity can affect the size and morphology of produced fibers by electrospinning technique. In 1995, Doshi found that solution with high viscosity produced uniform, bead-free and continuous fiber with large diameter (Figure 2.9a). In contrast, Simon et al. (1966) demonstrated that low viscous solutions result in a shorter and finer fiber with beads due to the absence of polymer chain entanglement that leads to stabilizing the polymer jet as it travels in the air (Figure 2.9b). Therefore, this process under these specific circumstances is more electrospraying rather than electrospinning (Figure 2.10). Deitzel et al. (2001) explained that the presence of junctions and bundles showed the fibers were not fully dried when reaching the collector.

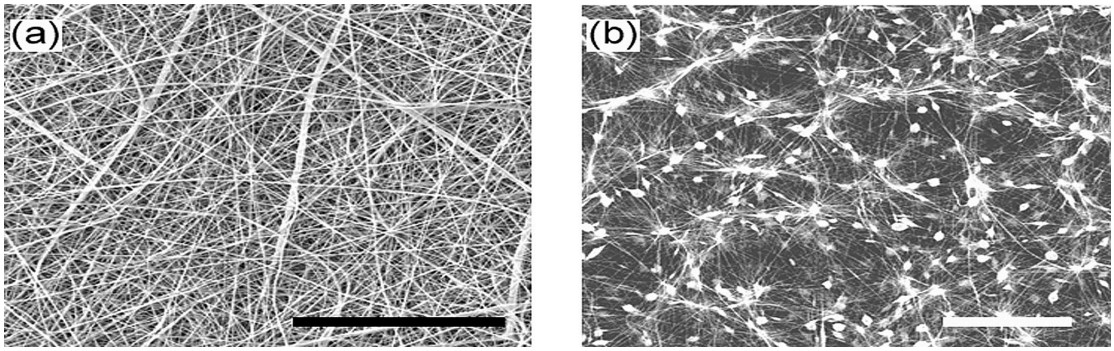


Figure 2.9. SEM images of electrospun fiber formed by electrospinning; a) 9% poly (epsilon-caprolactone), b) 5% poly (epsilon-caprolactone) (Li et al., 2003)

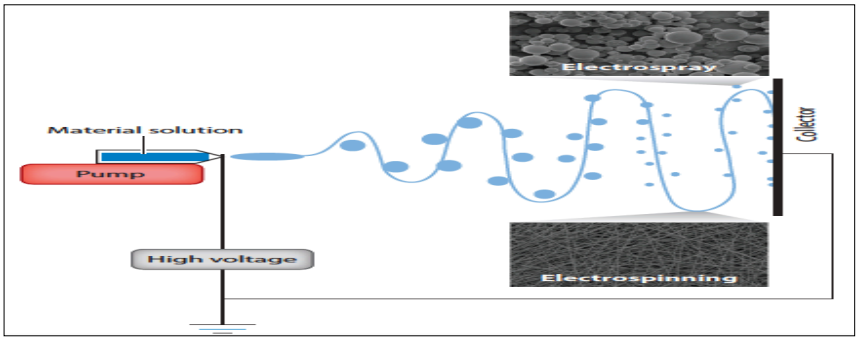


Figure 2.10. Schematic illustration of electrospinning vs. electrospray (Jacobsen et al., 2018)

2.7.2. Surface tension

In the electrospinning process, the polymer solution is retained at the end of the capillary tube by its surface tension. The surface tension can be influenced by the polymer concentration and the solvent composition (Pham et al., 2006). Some solutions cannot be used in the electrospinning process due to high surface tension. Ramakrishna et al. (2005) described that polymer solutions with high surface tension need higher voltage to start the jetting process and the polymer jet is quite unstable and makes shorter fibers. Zuo et al. (2005) also found that beading was affected by the surface tension of the polymer solution. In 1995, Doshi recognized that bead-free fibers could be formed by decreasing the surface tension of a polymer solution.

2.7.3. Electrical conductivity

Electrical conductivity can influence the formation of nanofibers in the electrospinning technique. Many researchers observed that by increasing the electrical conductivity of the solution, uniformity and beading of produced fiber increased and decreased, respectively. The solvent type, availability of ionizable salts, and polymer type may affect the solution conductivity (Bhardwaj & Kundu, 2010). For instance, the addition of salt or alcohol to the polymer solution resulted in the formation of smoother fibers with fewer beads (Zuo et al., 2005). Ramakrishna et al. (2005) found that by increasing the electrical conductivity of the solution, fiber diameter decreased due to the stretching of the solution during the fiber generation. Son et al. (2004) also reported that cationic and anionic polyelectrolytes enhanced the conductivity of a solution that resulted in decreasing the fiber diameter. In 2005, Jarusuwannapoom and his colleagues explained that some solvents with low electrical conductivity did not produce electrospun fibers through electrospinning technique.

2.7.4. Electrical voltage

Voltage or field strength is one of the most important controlled parameters in electrospinning technique. Electrospinning method requires a given level to start fiber production. When the critical voltage is applied, the electrostatic force overcomes the surface tension of the polymer solution and the jet is originated (Reneker & Chun, 1996). The applied voltage can affect the diameter of the fiber (microns to nanometers) and stability of the Taylor cone (Alborzi et al., 2013). At low voltage, a drop is hanged at the end of the needle tip and ejected jet from the Taylor cone

resulting in the production of bead-free electrospun fiber (Deitzel et al., 2001). Ramakrishna et al. (2005) found that by increasing the applied voltage, more beads were observed on the collector due to the reduction of drop volume that leads to instability and decrease of the Taylor cone at the needle tip. Also, at higher voltage, an increased charge leads to greater stretching of the solution and formation of fibers with smaller diameter (Deitzel et al., 2001).

2.7.5. Composition and geometry of collectors

In the electrospinning technique, different types of materials and geometries (Figure 2.11) are used to collect the electrospun fibers. Morphology of the electrospun mats can be affected by types of collectors. Aluminum foil, wire mesh, conductive paper, liquid non-solvent coagulation bath, water reservoir, and wire mesh have been used as a collector in this method. However, aluminum foil is more popular than other collectors (Ki et al., 2007; Wang et al., 2005). In 2005, Kim et al. compared the effect of three different collectors (a water reservoir, methanol, and metal collector) on the morphology of produced fibers. They reported that metal collector produced smooth fibers, while collection on the surface of water and methanol caused fibers to shrink and swell, respectively. Electrical conductivity and movement of the collector can also influence the electrospinning process. Non-conductive collector generates fiber with lower density compared to an electrically conducting collector.

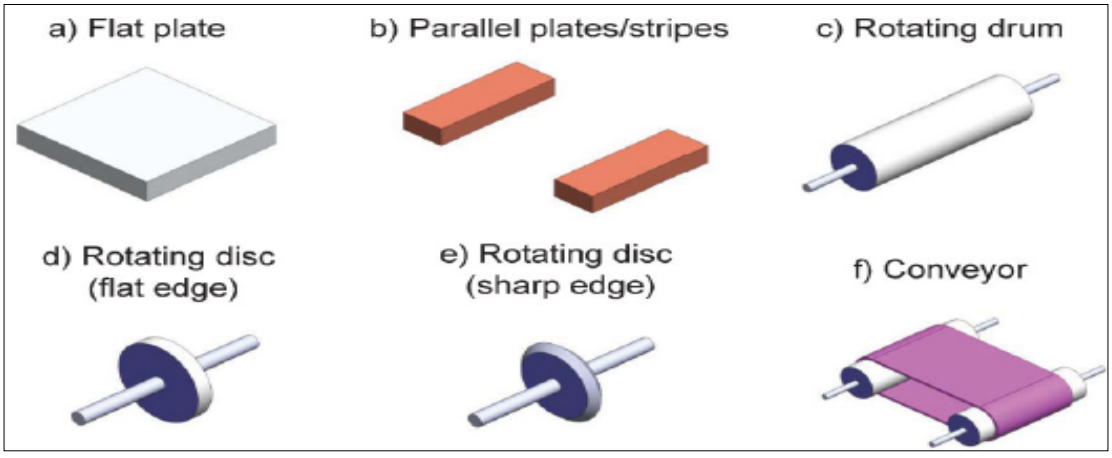


Figure 2.11. Different types of electrospinning collectors (Persano et al., 2013)

2.7.6. Flow rate

Many studies have shown that the flow rate of the polymer solution in the electrospinning process can affect the size and morphology of the produced fiber (Zong et al., 2002; Zhang et al., 2005). The results demonstrated that a high flow rate produced fibers with beads since fibers did not have enough time to dry before collecting on the collector. Also, it was reported that low flow rate resulted in small diameters (Wannatong et al., 2004; Yuan et al., 2004).

2.7.7. Distance between tip and collector

In the electrospinning process, a certain distance between the needle and the collector is required for drying of produced electrospun fiber before reaching the collector. Beading was observed since fibers were produced at the improper distance between tip and collector (either too close or too far) (Pham et al., 2006). Researchers found that the distance between the tip and collector did not influence on the fiber size and morphology of gelatin, chitosan, poly (vinylidene fluoride), and polyvinyl alcohol electrospun fiber (Alborzi et al., 2013; Sriyanti et el., 2017).

2.7.8. Needle tip design

Different types of needle tips for production of fiber in electrospinning technique are shown in Figure 2.12. Li and Xia (2004) designed a co-axial spinneret (two-capillary) to generate hollow nanofibers by using two immiscible liquids. Theron et al. (2005) used the multi-needle spinneret to increase the yield and production rate of electrospinning.

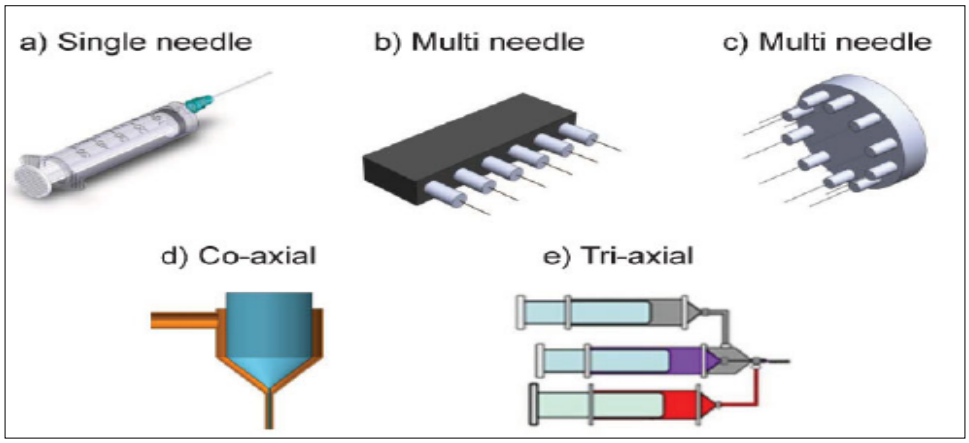


Figure 2.12. Different types of electrospinning spinneret (Persano et al., 2013)

2.7.9. Operating parameters

Very few studies have been carried out on the effects of ambient parameters such as temperature and humidity on the electrospinning process. Mit-Uppatham et al. (2004) found that increasing the temperature from 25 to 60 °C caused a decrease in fiber diameter due to the reduction of polymer solutions viscosity at high temperature. In 2004, Casper and his colleagues (Casper et al., 2004) reported that increasing the humidity resulted in the appearance of circular pores on the surface of the fibers. Table 2.2 briefly shows the effect of different parameters on the morphology of electrospun fiber.

Table 2.2. Effect of different parameters of electrospinnig on the final fiber morphology (Pham et al., 2006)

Process parameter	*Effect on fiber morphology*
Viscosity/concentration	• Low concentrations/viscosities yielded defects in the form of beads and junctions; increasing concentration/viscosity reduced the defects • Fiber diameters increased with increasing concentration/viscosity
Conductivity/solution charge density	• Increasing the conductivity aided in the production of uniform bead-free fibers • Higher conductivities yielded smaller fibers in general (exceptions were PAA and polyamide-6)
Surface tension	• No conclusive link established between surface tension and fiber morphology
Polymer molecular weight	• Increasing molecular weight reduced the number of beads and droplets
Dipole moment and dielectric constant	• Successful spinning occurred in solvents with a high dielectric constant
Flow rate	• Lower flow rates yielded fibers with smaller diameters • High flow rates produced fibers that were not dry upon reaching the collector
Field strength/voltage	• At too high voltage, beading was observed • Correlation between voltage and fiber diameter was ambiguous
Distance between tip and collector	• A minimum distance was required to obtain dried fibers • At distances either too close or too far, beading was observed
Needle tip design	• Using a coaxial, 2-capillary spinneret, hollow fibers were produced • Multiple needle tips were employed to increase throughput
Collector composition and geometry	• Smoother fibers resulted from metal collectors; more porous fiber structure was obtained using porous collectors • Aligned fibers were obtained using a conductive frame, rotating drum, or a wheel-like bobbin collector • Yarns and braided fibers were also obtained
Ambient parameters	• Increased temperature caused a decrease in solution viscosity, resulting in smaller fibers • Increasing humidity resulted in the appearance of circular pores on the fibers

The present study evaluated the encapsulation of bioactive compounds of saffron using two different encapsulation techniques, namely freeze-drying and electrospinning techniques for their possible incorporation into different food matrices. Encapsulation efficiency, thermal properties, storage stability, and morphology of encapsulated and un-encapsulated samples are parameters that were used in determining the suitability of encapsulation.

PREFACE TO CHAPTER 3

Electrospinning is a new technique that can be used for encapsulation of various compounds in the food industry. The focus of Chapter 3 is to study the effect of electrospinning method on encapsulation of saffron extract in gelatin as a wall material. The main objective of this chapter was to evaluate the use of electrospinning for encapsulation of saffron extract at three different concentrations (5, 10, and 15%) into electrospun gelatin fibers to study its storage stability (4, 24, and 35 °C) and thermal properties. The encapsulation efficiency and morphology of all samples were also evaluated.

All experimental work and data analysis were conducted by the candidate under the supervision of Dr. H. S. Ramaswamy. A part of this thesis research presented at NABEC (The Northeast Agricultural and Biological Engineering) conference, Quebec City, 16-19 June 2019 and food processing symposium, McGill, 23 July 2019.

Golpira, F., Ramaswamy, H.S., 2019. Encapsulation of saffron extract in a gelatin fiber matrix via electro-'spinning' technique to improve its stability. NABEC (The Northeast Agricultural and Biological Engineering) conference, Quebec City, 16-19 June 2019.

Golpira, F., Ramaswamy, H.S., 2019. Encapsulation of saffron extract in gelatin as a wall material using electro-spinning method to improve its stability and thermal properties. Food processing symposium, McGill, 23 July 2019.

CHAPTER 3

ENCAPSULATION OF BIOACTIVE COMPOUNDS OF SAFFRON IN GELATIN USING ELECTROSPINNING TECHNIQUE

3.1. Abstract

In this work, encapsulation of saffron extract into gelatin was studied by electrospinning technique to improve its stability and thermal properties in comparison with un-encapsulated control. The aqueous saffron extract powder at three different concentrations (5, 10, and 15 w/w% – based on the weight of gelatin) was encapsulated in electrospun gelatin fibers. The morphology, fiber diameter, and thermal properties of encapsulated and un-encapsulated saffron extract, as well as the encapsulation efficiency and storage stability were evaluated. The storage stability and half-life period of encapsulated crocin in electrospun gelatin fibers were studied at three different temperatures (4, 24, and 35 °C) during 42 days storage period. Saffron extracts were successfully encapsulated in gelatin fibers by electrospinning technique and the highest encapsulation efficiency was observed at 15% saffron extract concentration. SEM analysis demonstrated that the saffron-gelatin fibers had smooth, bead-free and homogeneously distributed morphology. Differential scanning calorimetry (DSC) characterizations demonstrated that saffron compounds were appropriately encapsulated in the fibers matrix with high thermal stability. Crocin showed first-order degradation kinetics. The encapsulated bioactive compounds of saffron inside gelatin fibers had higher stability compared to un-encapsulated extract during the 42 days of storage. The highest retention of encapsulated crocin was observed at 15% saffron extract concentration at 4 °C storage temperature with a half-life period ($t_{1/2}$) of 138 days, and the reaction rate constant (k) of 0.5 x 10^{-2} min^{-1}. The results of this study revealed that encapsulation of bioactive compounds of saffron by electrospinning is an effective technique resulting in the acceptable encapsulation efficiency and stabilization of crocin mainly due to the fabrication of stable wall material obtained by electrospun gelatin fibers. Therefore, they can be used in various food-related products including desserts, chewing gum and tea bag.

3.2. Introduction

Saffron has been used in different industry (food, pharmaceutical, and cosmetic) due to its coloring strength (crocin), flavor (safranal) and aroma (picrocrocin). This flavoring spice is quite expensive; however, its consumption is increasing because of its functional properties and biologically active compounds. Degradation rate of crocin, picrocrocin, and safranal could be affected by moisture content, oxygen, light, temperature, water activity, and presence of antioxidants. Therefore, bioactive compounds of saffron should be appropriately controlled against all these factors to minimize the loss of compounds during processing and storage (Patras et al., 2010).

Encapsulation is a protective technique that entraps bioactive compounds within a shell (Ahmed et al., 2010). Encapsulation of bioactive ingredients decreases the loss of volatile components and the rate of evaporation against undesirable conditions during storage. Also, encapsulation is an effective method to improve solubility and storage stability of bioactive compounds (Dehcheshmeh & Fathi, 2019).

Encapsulation of saffron extract by electrospinning technique has been less explored, and there is just one study related to this field that was published recently. Dehcheshmeh and Fathi (2019) used electrospun zein and tragacanth to improve the thermal properties and stability of safranal that is the main volatile oil of saffron. But in addition to safranal, saffron consists of crocin and picrocrocin. Therefore, encapsulation of saffron extract, which includes three different bioactive compounds, is a complex procedure. Esfanjani et al. (2015) and Rajabi et al. (2015) evaluated different types of wall materials for encapsulation of three different bioactive compounds of saffron by spray drying method to have higher encapsulation efficiency. Based on the literature, electrospinning has been used for encapsulation of other carotenoids as well. For instance, Horuz and Belibağlı (2018) demonstrated the electrospinning technique for encapsulation of total carotenoids such as lycopene and β-carotene extracted from tomato peel to stabilize them into gelatin nanofibers.

The objective of this reaearch was to investigate the use of electrospinning technique for encapsulation of bioactive compounds of saffron extract (crocin, picrocrocin, and safranal) into gelatin fibers to improve its storage stability and thermal properties. Also, the morphology, encapsulation efficiency, and diameter of produced fibers were studied.

3.3. Materials and methods

3.3.1. Materials

Saffron was obtained from a Persian supermarket in Montreal, Canada. Type A gelatin powder from bovine skin, formic acid and magnesium chloride were obtained from Sigma–Aldrich (St. Louis, Mo., USA).

3.3.2. Preparation of saffron extracts

For extraction of saffron bioactive compounds, the procedure of Selim et al. (2008) was followed with some modifications (Figure 3.1). 12 g of saffron powder was mixed with 500 ml water in a dark colored bottle under continuous shaking in an incubator (Gravity Convention Incubator, Precision Scientific, Inc.) at 25 °C for 24 h. The extract was centrifuged (IEC-Centra® CL2, USA) at 2000 x g for 15 min followed by filtration under vacuum. The prepared extract solution was freeze-dried at -30 °C (freeze-drier VirTis Co., NY, USA) for 6 days. The freeze-dried powders were kept in dark colored bottle at -18 °C until used.

a) b) c)

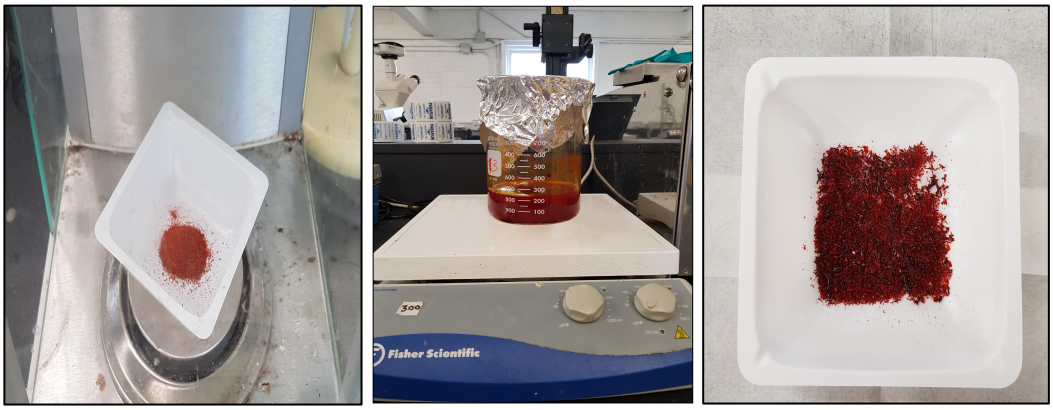


Figure 3.1. Extraction of bioactive compounds of saffron; a) saffron powder, b) saffron solution, c) saffron extract

3.3.3. Preparation of polymer solution

Gelatin solution (25% w/w) was prepared by dissolving gelatin powder in formic acid followed by addition of three different concentrations (5, 10, 15% w/w) of freeze-dried saffron extract powder to the gelatin-formic acid solution (Figure 3.2). These solutions were named Ge-S5%, Ge-S10%, and Ge-S15%, respectively. A gelatin solution without saffron extract was also prepared for the production of gelatin fiber as a control sample. All solutions were stirred with a magnetic stirrer (120 rpm) for about 1 h at room temperature (Horuz & Belibağlı, 2018).

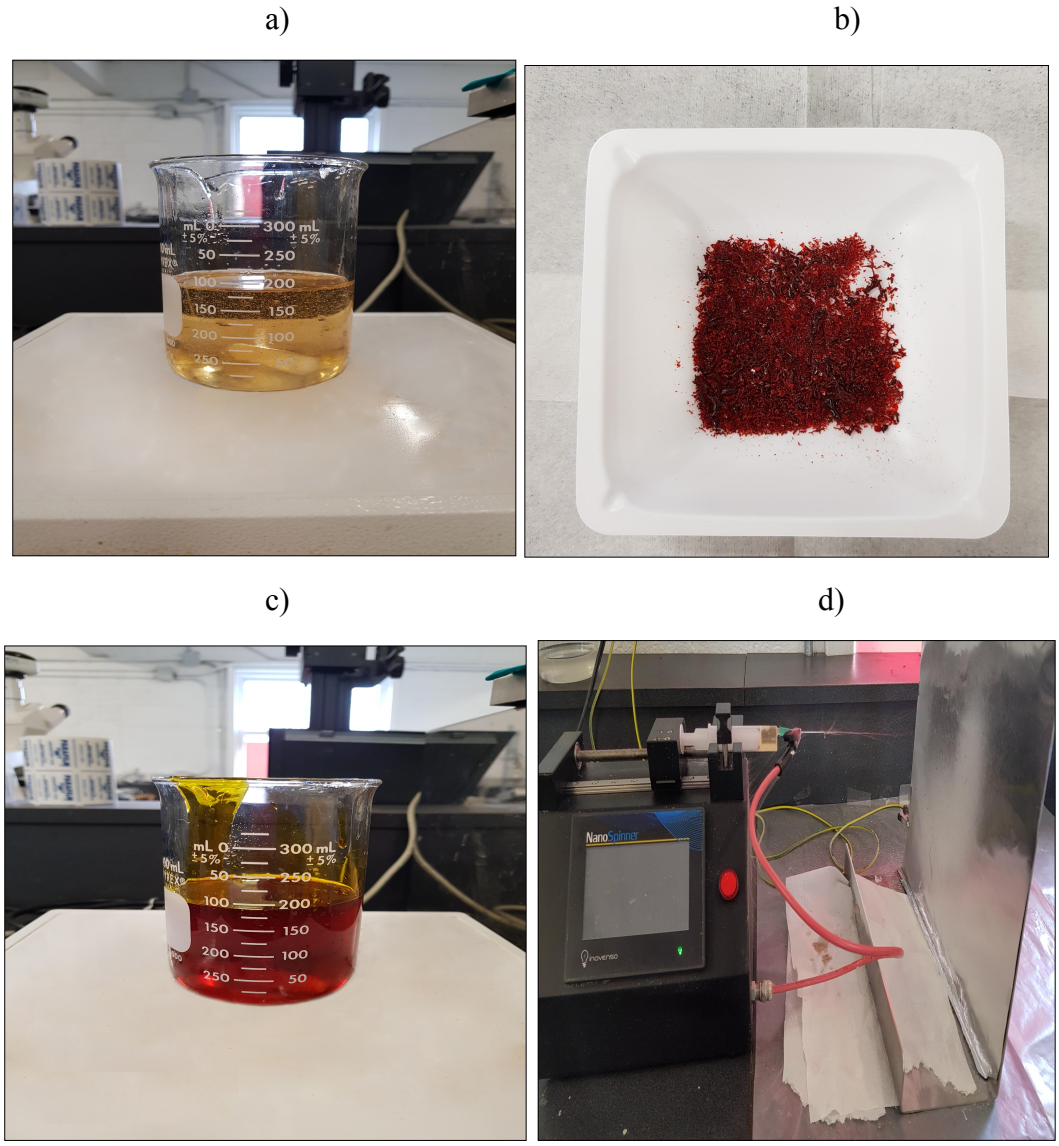


Figure 3.2. Solution preparation; a) gelatin-formic acid solution; b) saffron extract; c) gelatin-formic acid-saffron extract solution; d) electrospinning process

3.3.4. Characterization of the polymer solutions

3.3.4.1. Viscosity

The viscosity of the polymer solutions was measured by using a controlled strain cone and plate geometry rheometer (TA instrument, New Castel, DE, USA) at 25 °C ± 1. The plate diameter and the cone angle were 40 mm and 2°, respectively. The shear rate ranged from 1 to 100 s^{-1} in 60 s. The shear stress (τ) and shear rate ($\dot{\gamma}$) data were collected (Maftoonazad et al., 2019). Apparent viscosity was determined using equation 1 for different shear rates:

$$\eta = k\,(\dot{\gamma})^{n-1} \qquad (1)$$

where η is apparent viscosity (Pa·s).

3.3.5. Electrospinning

Polymer solutions were electrospun using an electrospinning instrument (Invenso Inc., Istanbul, Turkey) as detailed in Maftoonazad et al. (2019). Briefly, a 10 ml disposable plastic syringe containing the polymer solution was connected to the stainless steel needle with an internal diameter of 16 μm, and the needle was directed toward the collector. The plastic syringe was put horizontally on a digitally controlled syringe pump that equipped with a variable high voltage 0–30 kV power supply. The positive electrode of power supply was attached to a stainless-steel needle, while the ground electrode was connected to the steel collector covered with non-stick aluminum foil (Figure 3.2d). The polymer jet was generated from the Taylor cone that travelled in the air and collected on the stainless steel collector plate. Electrospinning parameters (feed rate, applied voltage, and tip to collector distance) were fixed after doing a preliminary test. Therefore, the feed rate, applied voltage, and tip to collector distance were maintained at 2 ml/min, 18 kV, and 15 cm, respectively. The electrospinning process was done at 25 ± 1 °C under 40% relative humidity in the laboratory. After electrospinning, fibers were removed and kept at -18 °C until use for characterization analysis.

3.3.6. Characterization of electrospun fibers

3.3.6.1. Determination of encapsulation efficiency

Encapsulation efficiency (EE) was determined according to the methods described by Esfanjani et al. (2015), Aceituno-Medina et al. (2015), and Horuz et al. (2018) with some modifications, which is based on the difference in light density of soluble bioactive compounds of saffron extract at three different wavelengths. For calculation of the amount of encapsulated crocin, picrocrocin, and safranal in gelatin fibers or gelatin particles (C_t), approximately 3 mg of sample was added into 5 ml of ethanol-hexane mixture (50:50, v/v) and stirred at 12 x g during 10 min to remove the free saffron extract from the surface of encapsulated fibers or encapsulated particles. Then the mixture was centrifuged at ~2000 x g during 10 min at room temperature to separate the sample from the solution. The precipitated sample was collected and placed in a beaker with 5 ml distilled water. Then, it was stirred for 10 min at 12 x g to dissolve the fibers or particles in order to release the encapsulated bioactive compounds of saffron from the gelatin membrane. The absorbance of this solution was read at 440, 330, and 257 nm by using UV–Vis spectrophotometer (SP-3000nano, OPTIMA, Japan) that equipped with quartz cells to measure the amount of encapsulated crocin, safranal, and picrocrocin in gelatin fiber or particle, respectively. Absorption measurements were done in triplicate for each compound. The results were expressed as $A_{1cm}^{1\%}(\lambda \max)$ according to equations 2 and 3 for the calculation of EE of different bioactive compounds of saffron (Esfanjani et al., 2015).

ISO/TS 3632 procedure (2003) was followed to measure the amount of crocin, safranal, and picrocrocin in saffron extract (C_0). Saffron extract solution (1% w/v) was prepared by dissolving 1 g saffron extract in 100 ml distilled water at room temperature. The mixture was stirred continuously with mechanical stirring at 12x g for 15 min until the saffron powder completely dissolved. The amount of crocin, safranal, and picrocrocin in saffron extract was measured by reading the absorbance of prepared saffron extract solution through UV–Vis spectrophotometry at 440, 330, and 257 nm, respectively. The results were calculated based on equation 3.

$$EE\ (\%) = (C_t/C_0) \times 100 \quad (2)$$

$$C_0 \text{ and } C_t = A_{1cm}^{1\%}(\lambda \max) = (A \times 10000)/(m \times 100 - (H)) \quad (3)$$

where:

C_t is the amount of encapsulated crocin, picrocrocin, and safranal in gelatin fiber or gelatin particle.

C_0 is the initial amount of crocin, picrocrocin, and safranal in saffron extract.

λ max is the maximum absorbance of crocin, safranal, and picrocrocin at 440, 330, and 257 nm, respectively.

A is the absorbance value of picrocrocin, safranal and crocin at the specific wavelength, m is the sample weight (g), H is the moisture content of sample.

3.3.6.2. Stability of encapsulated saffron under accelerated storage condition

Encapsulated saffron extract powder into gelatin fibers by electrospinning technique and un-encapsulated one as a control sample were transferred to plastic centrifuge tubes and kept in sealed desiccators with saturated salt solutions of magnesium chloride at 33% relative humidity. Then, desiccators were stored in the fridge (4 °C), room (24 °C), and oven (35 °C) in the absence of light for 42 days to study the effect of temperature on the storage stability of crocin in the saffron extract (Table 3.1).

To measure the loss of encapsulated and un-encapsulated crocins during storage, same procedure was followed for sample preparation as explained in section 3.3.6.1 of this chapter. The test was done after 7, 14, 21, 28, 35, 42 days storage. The UV–Vis spectrophotometer (SP-3000nano, OPTIMA, Japan) was used to measure the change in the retention of encapsulated and un-encapsulated crocin. Infact, crocin is expressed based on changes in the light density of this compound at 440 nm (ISO/TS 3632 procedure, 2003). The amount of this compound was calculated by the equation 3.

Table 3.1. Matrix of experimental design of storage stability study

Sample	Temperature	Relative humidity (%)
Un-encapsulated saffron (control)	4	33
Ge-S5%	4	33
Ge-S10%	4	33
Ge-S15%	4	33
Un-encapsulated saffron (control)	24	33
Ge-S5%	24	33
Ge-S10%	24	33
Ge-S15%	24	33
Un-encapsulated saffron (control)	35	33
Ge-S5%	35	33
Ge-S10%	35	33
Ge-S15%	35	33

Treatment abbreviations: Ge (gelatin), and S (saffron).

3.3.6.3. Scanning electron microscopy

The morphology and diameters of the electrospun fiber were analyzed by using a scanning electron microscopy (S-4700 FEG-SEM, Hitachi Ltd., Mississauga, ON, Canada) at room temperature. The small amount of fiber mat (5*5 mm) was placed onto specimen stubs using a two-sided adhesive tape with accelerating voltage of 5 kV and photographed at different magnifications. Fiber diameter distribution was determined by randomly counting 110 parts of fiber per SEM image by using the Image J analysis software (Maftoonazad et al., 2019).

3.3.6.4. Thermal Properties

Thermal behavior of the saffron extract and neat gelatin powder as well as the electrospun gelatin fibers with and without saffron extract was analyzed by differential scanning calorimeter (Pyris DSC-6 Perkin Elmer Ltd., Norwalk, USA). In this experiment, about 6-7 mg of samples were weighed and put in an aluminum pan. The sealed samples were heated over a range from 20 to 200 °C at a scanning rate of 10 °C/min under nitrogen gas flow (Shao et al., 2007). An empty aluminum pan was considered as a reference. Thermal properties measurements of samples were done in triplicate.

3.3.6.5. Kinetics of degradation of crocin in saffron extract

To study the degradation kinetics of encapsulated crocin in gelatin matrix and un-encapsulated crocin in saffron extract powder, samples were stored at different conditions based on the procedure that was explained in detail in section 3.3.6.2. Degradation rate constants (*k*) was determined from the slope of a plot of the logarithm of crocin retention at a given temperature against time. The half-life ($t_{1/2}$) was also calculated from the k value using equations 4 and 5:

$$C_t = C_0 \exp(kt) \quad (4)$$

$$t_{1/2} = -\ln 0.5/k \quad (5)$$

where, C_0 is the initial amount of crocin in saffron extract and C_t is the saffron extract content after time t (day), while *k* is the first-order kinetic constant (Wang & Xu 2007).

3.4. Statistical analysis

The results were analyzed statistically by using SPSS Version 25 (SPSS Inc., CHI, IL, USA). One-way analysis of variance and Tukey's test at the confidence level of $p < 0.05$ was applied to determine the significant difference in the average of encapsulation efficiency, thermal behavior, storage stability, and diameters of electrospun gelatin fibers with and without saffron extract. The results were represented as mean ± standard deviation in the form of figures and tables. Each sample was tested in triplicate.

3.5. Results and discussions

3.5.1. Encapsulation efficiency (EE)

One of the main quality parameters of encapsulation that distinguishes the potential of a wall material to protect the core material inside the capsule is encapsulation efficiency (Mahdavi et al., 2016). Electrospinning technique has been shown to be more effective for encapsulation of bioactive compounds with highest retention of the core material inside the wall material and less quantity on the surface of nanofibers compared to other encapsulation methods such as coacervation dispersion and emulsification (Chronakis, 2005). Important factors that can affect encapsulation efficiency are encapsulation technique as well as core and wall materials properties (Mahdavi et al., 2016). Table 3.2 shows the effects of saffron extract powder concentrations on the encapsulation efficiency of crocin, picrocrocin

and, safranal in electrospun gelatin fibers. Results show that by increasing the concentration of saffron extract, encapsulation efficiency of three major components of saffron into electrospun gelatin fibers increased significantly ($p < 0.05$) (Table 3.2). The encapsulation efficiency of picrocrocin was 71.2 ± 1.36, 79 ± 0.95, and 86 ± 1 at the concentrations of 5, 10, 15% of saffron extract, respectively (Table 3.2). Regarding safranal and crocin, their encapsulation efficiency varied from 63.5 ± 0.55 to 74.2 ± 0.68 and 68 ± 0.78 to 76.3 ± 1.52, respectively. The results showed that during the electrospinning process around 70% of the saffron extract could be encapsulated into electrospun gelatin fibers. The concentration of saffron extract had a positive effect on the encapsulation efficiency. Therefore, by increasing the saffron extract from 5% to 15% in gelatin fiber, the encapsulation efficiency increased significantly ($p < 0.05$). The encapsulation efficiency of picrocrocin was significantly ($p < 0.05$) higher than safranal and crocin at a specific concentration of saffron extract (Tables 3.2 & 3.3).

Results of this study were close to those are reported by Dehcheshmeh and Fathi (2019) for encapsulation of safranal into a zein-tragacanth nanofiber. They also reported that by increasing the concentration of saffron extract from 5 to 10%, EE increased from 68 to 90% in zein-tragacanth nanofiber. Our findings are also in agreement with the results of Horuz and Belibağlı (2008) who found that the higher concentration of carotenoid extract from tomato peel resulted in the higher EE of carotenoid in gelatin nanofiber by electrospinning technique. So, it can be concluded that encapsulation efficiency was largely dependent on core material concentration.

Two-way ANOVA (Table 3.4) showed that the encapsulation efficiency was significantly ($p < 0.05$) affected by concentration and bioactive compounds of saffron through electrospinning procedure. Two-way ANOVA also demonstrated that there is an interaction between the concentration and bioactive compounds of saffron on the encapsulation efficiency. It should be mentioned that the effect of saffron extract concentration on encapsulation efficiency was influenced significantly by different bioactive compounds of saffron.

Table 3.2. Effect of different concentrations of saffron extract on encapsulation efficiency of crocin, picrocrocin, and safranal in electrospun gelatin fibers

Sample	EE% of picrocrocin	EE% of safranal	EE% of crocin
Ge-S5%	71.2 ± 1.36^{aA}	63.5 ± 0.55^{aB}	68 ± 0.78^{aC}
Ge-S10%	79 ± 0.95^{bA}	67.7 ± 1.06^{bC}	71.5 ± 0.66^{bB}
Ge-S15%	86 ± 1^{cA}	74.2 ± 0.68^{cB}	76.3 ± 1.52^{cB}

Small letters within the same column indicate significant differences ($p < 0.05$).
Big letters within the same row indicate significant differences ($p < 0.05$).

Table 3.3. Results of One-way ANOVA analysis for the effect of different concentrations of the saffron extract on EE of crocin, picrocrocin, and safranal in electrospun gelatin fibers

Compounds	Source	SS	df	MS	F-value	P-value
Picrocrocin	T (between treatments)	327.3	2	163	130.3	0.00
	R (within treatments)	7.53	6	1.25		
	Total	334.9	8			
Safranal	T (between treatments)	173.5	2	86.7	137.7	0.00
	R (within treatments)	3.77	6	0.63		
	Total	177.3	8			
Crocin	T (between treatments)	102.8	4	51.4	45.61	0.00
	R (within treatments)	6.76	6	1.12		
	Total	109.6	8			

T: Treatment
R: Residual

Table 3.4. Results of Two-way ANOVA analysis for the effect of different concentrations of saffron extract the on encapsulation efficiency of different bioactive compounds of saffron in electrospun gelatin fibers

Source	SS	df	MS	F-value	Sig.
Corrected Model	1090.45	8	136.3	135.7	0.00
Intercept	144207.3	1	144207.3	143572.39	0.00
Saffron Concentration	568.75	2	284.38	283.12	0.00
Saffron Compound	486.69	2	243.34	242.27	0.00
Saffron Concentration x Saffron Compounds	35	4	8.75	8.71	0.00
Error	18.08	18	1		
Total	145315.84	27			
Corrected Total	1108.53	26			

3.5.2. Thermal analysis

One of the simplest methods for illustrating the compatibility of polymer is differential scanning calorimetry (DSC) (Shao et al., 2018). In this study, DSC was used to investigate the effect of concentration of saffron extract on the thermal behavior of electrospun gelatin fibers. Melting temperature (T_m), denaturation temperature (T_d), and enthalpy (ΔH) of saffron extract and gelatin fibers with and without saffron extract are presented in Table 3.5. All samples showed an endothermic peak due to the breakage of hydrogen bonds (Shao et al., 2018). The denaturation temperature (T_d) measures thermal stability of protein (Meng & Ma, 2001). Therefore, denatured protein shows low T_d, while compact and tight protein molecules in a network structure indicates high T_d (Ma & Harwalkar, 1988). Melting temperature is another thermodynamic property of material. It is related to the beginning of denaturation. Melting temperature measures thermal stability of materials. So, it will be high at high thermal stability (Ma & Harwalkar, 1988).

DSC curve of saffron extract showed an endothermic peak at around 36 °C that may be due to the melting point of natural carotenoid and other compounds of saffron (Dehcheshmeh & Fathi, 2019). The denaturation temperature of electrospun gelatin fibers without saffron extract was at 87.7 °C relating to the helix to coil transition (Tavassoli-Kafrani et al., 2017). Electrospun gelatin fibers without saffron contained the highest denatured protein compared to Ge-S5%, Ge-S10%, and Ge-S15% due to its low T_d. Results showed that T_m and T_d of electrospun gelatin fibers (control) increased by addition of saffron extract. From present work results, electrospun gelatin fibers containing 15% saffron extract had the highest T_m and T_d leading to the highest thermal stability.

The enthalpy shows the needed energy to break covalent bonds, hydrogen bonds, and Van der Waals interactions. It also presents the degree of un-denatured structure of protein, crystallinity or the triple-helical content (Zhang et al., 2006; Jalaja & James, 2015). The higher the ΔH, the less denatured is the protein. It shows more thermal energy is required to complete protein denaturation (Ma & Harwalkar, 1988). The results demonstrated that, enthalpy showed the same trend of T_d and T_m. According to the DSC curves, T_m, T_d, and ΔH of electrospun gelatin fibers increased when the concentration of saffron extract increased from 5 to 15% (Table 3.5). It might be due to the reduction of possible interactions between water and fibers. Shao et al. (2018) reported that the formation of hydrogen bonding and inter-molecular

complexes between bioactive compounds and polysaccharides prevented the interaction of polyelectrolyte and water.

Overall, Table 3.6 shows that different concentrations of saffron extract powders (5, 10, and 15%) had a significant effect ($p < 0.05$) on T_m, T_d, and ΔH of electrospun gelatin fibers. Since there are no secondary peaks or phase segregation in gelatin fiber containing saffron extract, it can be concluded that the saffron extract was encapsulated into the electrospun gelatin fibers homogeneously (Li et al., 2006). Horuz and Belibağlı (2018) explained that the electrospinning technique can change the crystalline structure of saffron compounds to the amorphous state in gelatin fiber that leads to disappearance of the endothermic peaks of saffron extract in the DSC curve of encapsulated saffron extract into gelatin fiber (Dehcheshmeh & Fathi. 2019).

Table 3.5. Thermal properties (melting temperature, denaturation temperature, and enthalpy) of electrospun gelatin fibers with and without saffron extract

Sample	T_m (°C)	T_d (°C)	ΔH (j/g)
Saffron extract	32 ± 2.34^{d}	36.1 ± 3.31^{c}	27 ± 1.66^{d}
Ge fiber	46.4 ± 4.06^{c}	87.6 ± 5.73^{b}	414 ± 8.66^{a}
Ge-S5%	55.3 ± 2.54^{bc}	90.1 ± 6.35^{b}	340 ± 19.86^{c}
Ge-S10%	65.7 ± 5.27^{ab}	99.3 ± 5.72^{ab}	354 ± 10.06^{bc}
Ge-S15%	78.3 ± 7.63^{a}	108 ± 7.31^{a}	385 ± 5.18^{ab}

Small letters within the same column indicate significant differences ($p < 0.05$).

Table 3.6. Results of One-way ANOVA analysis for the effect of different concentrations of the saffron extract on T_m, T_d, and ΔH of electrospun gelatin fibers

TP	Source	SS	df	MS	F-value	P-value
T_m	T (between treatments)	3764.27	4	941.06	41.07	0.00
	R (within treatments)	229.13	10	22.91		
	Total	3993.4	14			
T_d	T (between treatments)	9489.66	4	2370.16	69.47	0.00
	R (within treatments)	341.13	10	34.11		
	Total	9821.79	14			
ΔH	T (between treatments)	298386.64	4	74596.6	508.2	0.00
	R (within treatments)	1467.7	10	146.77		
	Total	299854.34	14			

TP: Thermal Properties
T: Treatment
R: Residual

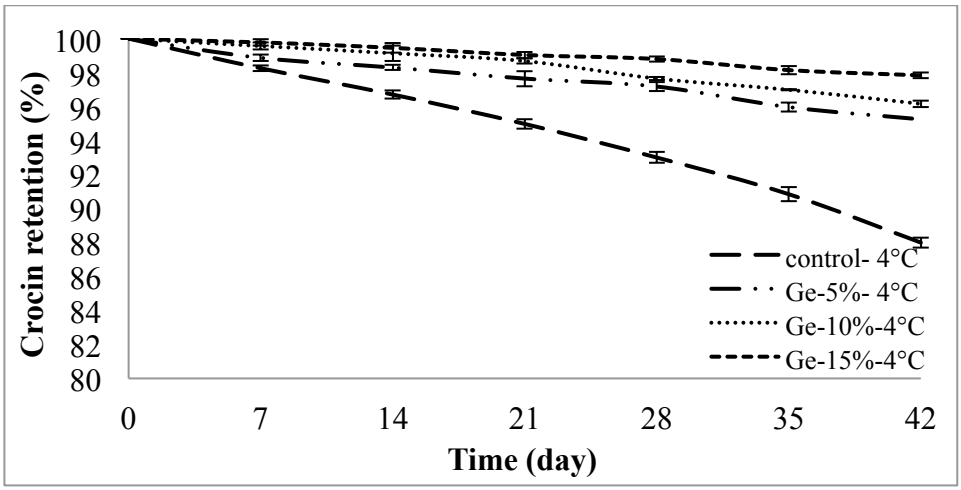
3.5.3. Storage stability of encapsulated saffron extract

The stability of encapsulated saffron extract in electrospun gelatin fibers and un-encapsulated saffron extract as a control sample was evaluated in terms of coloring strength under various storage conditions (4, 24, and 35 °C) during 42 days storage. Figure 3.3 shows the retention of crocin at different temperatures during storage. The degradation of encapsulated and un-encapsulated saffron extract showed a first-order kinetic reaction with a linear reduction in the concentrations of crocin with respect to time (Chranioti et al., 2015). The reaction rate constant (k) and half-life period ($t_{1/2}$) of the encapsulated and un-encapsulated crocin in saffron extract at various temperatures were calculated and shown in Table 3.7. Overall, amount of encapsulated crocin at 15% saffron extract concentration stored at 35, 24 and 4 °C under constant RH (33%) was significantly higher than un-encapsulated saffron extract (control sample) during 6 weeks of storage. Encapsulated samples showed a lower reaction rate constant and consequently higher half-life period during 42 days storage at 4 °C compared to the samples that were stored at 24 and 35 °C at the same concentration of saffron extract. The results showed that the half-life period of crocin was influenced by the concentration of saffron extract and storage temperature. Therefore, by increasing the concentration of saffron extract in electrospun gelatin fibers reaction rate constant and half-life period decreased and increased, respectively. For instance, 15% saffron extract exhibited the highest half-life period ($t_{1/2}$) with values of 138.62 for crocin at 4 °C under 33% RH. Therefore, the results demonstrated that the coloring strength of saffron was significantly ($p < 0.05$) affected by time, storage temperature, and the concentration of saffron extract in electrospun gelatin fibers (Table 3.8).

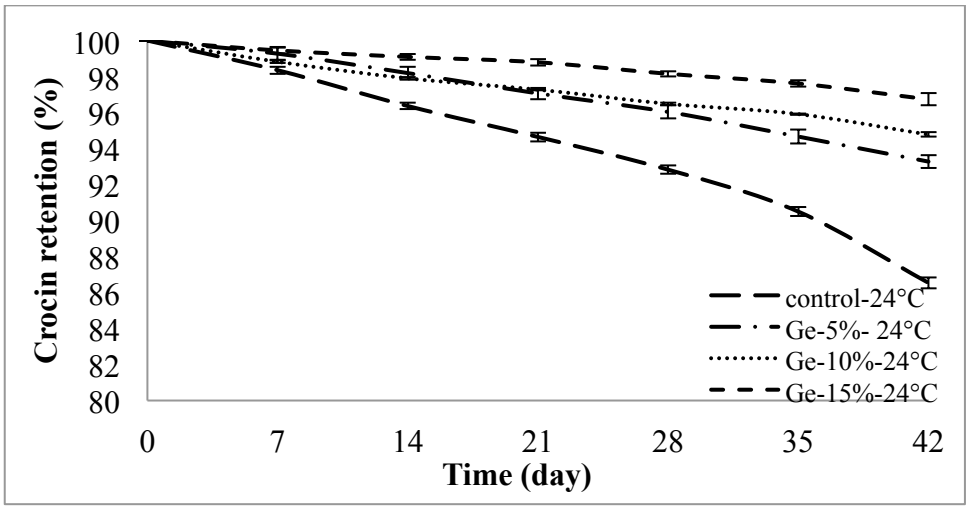
Several researchers also found a first-order kinetic reaction for the degradation of saffron carotenoids such as crocins during storage at different temperatures and water activity conditions (Tsimidou & Tsatsaroni, 1993; Tsimidou & Biliaderis, 1997; Selim et al., 2000; Sánchez et al., 2008; Chranioti et al., 2015). Rodriguez-Amaya (2002) reported that the degradation of carotenoids in food is mainly due to the enzymatic or nonenzymatic oxidation during processing and storage. The color of food can be changed by the isomerization of trans-carotenoids to cis-isomers and is increased by exposure to oxygen, acids, light, moisture, and heat (Rodriguez-Amaya, 2002). Shu et al. (2006) found that by encapsulation of lycopene in sucrose and gelatin as the wall materials using spray-drying technique followed by storage at 0 °C in the presence of light, the amount of lycopene decreased by only 15% after 28 days

storage. Silva et al. (2011) reported that by increasing the concentration of microencapsulated lycopene in gelatin and pectin through the freeze-drying method, the stability of lycopene improved compared to un-encapsulated one during storage, similar to the results observed in this study. Also, they found that by increasing the storage temperature from 10 to 25 °C, the concentration of lycopene in freeze-dried powder decreased. Jafari et al. (2007) concluded that encapsulation techniques protect sensitive ingredients against oxygen, light, heat and moisture since the wall materials act as a barrier and decrease the effect of environmental conditions. Another factor that can influence the stability of encapsulated compounds during storage is moisture content. Tonon et al. (2010) reported that reduction of moisture content of the environment during storage leads to decreasing molecular mobility of the encapsulating agent in the glassy state.

a)



b)



c)

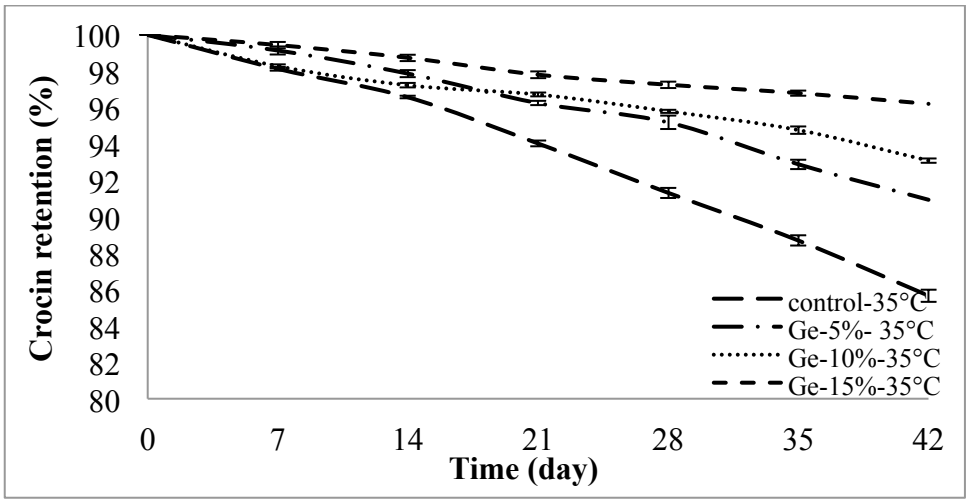


Figure 3.3. Storage stability of crocin in electrospun gelatin fibers under different temperatures; a) 4 °C, b) 24 °C, c) 35 °C

Table 3.7. Regression analysis of bioactive compounds of saffron in electrospun gelatin fiber during storage at different temperatures for 42 days storage

Sample	Temperature	Compound	T^a ½ (day)	(K^b * 10^{-2}) (min^{-1})	R^2
Control	4	Crocin	22.3	3.1	0.98
Ge-S5%	4	Crocin	53.3	1.3	0.94
Ge-S10%	4	Crocin	77	0.9	0.96
Ge-S15%	4	Crocin	138	0.5	0.95
Control	24	Crocin	21	3.3	0.96
Ge-S5%	24	Crocin	40	1.7	0.97
Ge-S10%	24	Crocin	49.5	1.4	0.98
Ge-S15%	24	Crocin	86.6	0.8	0.97
Control	35	Crocin	18.7	3.7	0.98
Ge-S5%	35	Crocin	31.5	2.2	0.96
Ge-S10%	35	Crocin	38.5	1.8	0.97
Ge-S15%	35	Crocin	63	1.1	0.99

Table 3.8. Results of Univariate analysis for the effect of different concentrations of saffron extract on the storage stability of encapsulated crocin in electrospun gelatin fibers during 42 days storage at 4, 24, and 35 °C

Source	SS	df	MS	F-value	Sig.
Corrected Model	39.82	83	0.48	1355.25	0.00
Intercept	40539.81	1	40539.81	114511557	0.00
Day	23.68	6	3.94	11151.21	0.00
Concentration	6.69	3	2.23	6302.36	0.00
Temperature	1.33	2	0.66	1884.86	0.00
Day x Concentration	7.17	18	0.39	1125.92	0.00
Day x Temperature	0.65	12	0.05	154.53	0.00
Concentration x Temperature	0.08	6	0.04	38.25	0.00
Day x Concentration x Temperature	0.19	36	0.00	15.31	0.00
Error	0.08	252	0.00		
Total	40579.72	336			
Corrected Total	39.91	335			

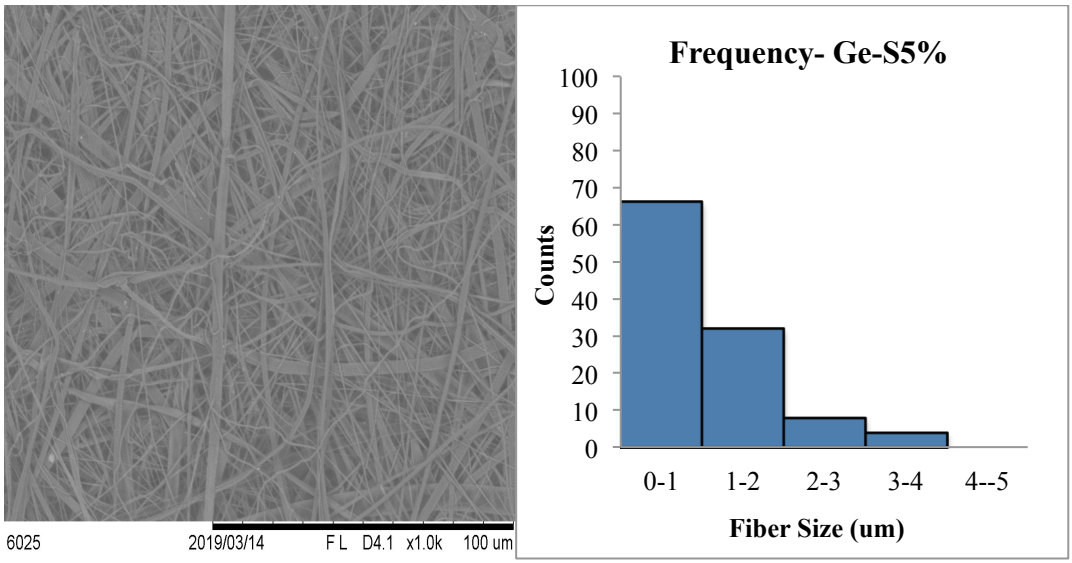
3.5.4. Scanning electron microscopy (SEM)

Morphology and diameter distributions of fibers were studied by scanning electron microscopy. Figure 3.4 shows SEM images of electrospun gelatin fibers with and without the saffron extract. The results showed that during the electrospinning process, the bioactive compounds of saffron dispersed uniformly and did not observe any phase separation. SEM images presented that all fibers were smooth, bead free, and homogeneously distributed. Also, they exhibited that there was no big difference between the morphology of fibers containing saffron extract compared to neat gelatin fibers. Table 3.9 indicated the effect of saffron extract concentration on fibers diameter. Fiber diameter provides great information about the uniformity of the fibers. The results showed that by addition of saffron extract to the neat electrospun gelatin fiber, the diameters of fibers increased significantly ($p < 0.05$). Besides, by increasing the concentration of saffron extract from 5 to 15%, the average diameter of the fibers increased significantly ($p < 0.05$) from 1.05 ± 0.69 µm to 2.04 ± 1.07 µm, respectively. The thicker diameters of electrospun gelatin fibers at higher concentration of saffron extract is likely due to the increased solid contents in the feed solution (Sriyanti et al., 2017).

Same results were also reported by Horuz and Belibağlı (2018). They described that fibers diameters increased significantly by addition of tomato peel extract to the electrospun gelatin fibers. Shao et al. (2018) found that higher concentration of tea polyphenols in pullulan-CMC electrospun fibers resulted in thicker fibers diameters with high uniformity. Suwantong et al. (2007) reported that fibers diameters can be affected by the viscosity of solution. Therefore, the higher viscosity results in the thicker diameter of fiber. The static charges also influence the fiber diameter through electrospinning process (Horuz & Belibağlı, 2018). They affect the whipping motion on a jet surface by a reduction in conductivity of polymer solution that leads to increase in capacity of polymer solutions on the tip of the needle (Wang et al., 2013). So, electrospun fibers with thicker diameter will be produced.

According to Table 3.9 and 3.10 and Figure 3.4 addition of saffron extract to the gelatin solution slightly increased the viscosity of the solution, however no significant differences ($p > 0.05$) were found between different treatments since the formic acid keeps the gelatin solution dilute at room temperature.

a)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Frequency- Ge-S5%
Counts
100
90
80
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0-1
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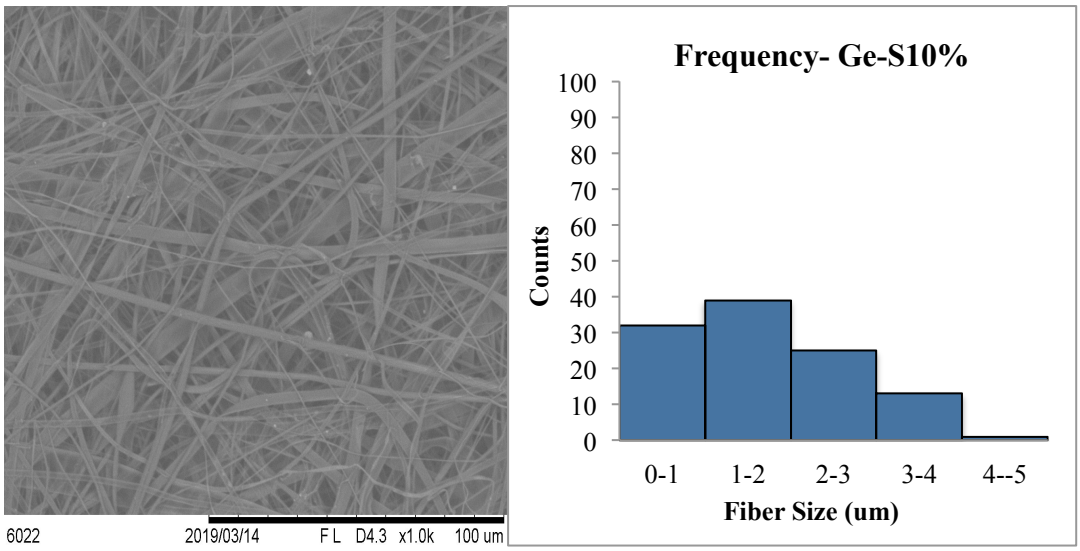
3-4
4--5
Fiber Size (um)

b)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Frequency- Ge-S10%
Counts
100
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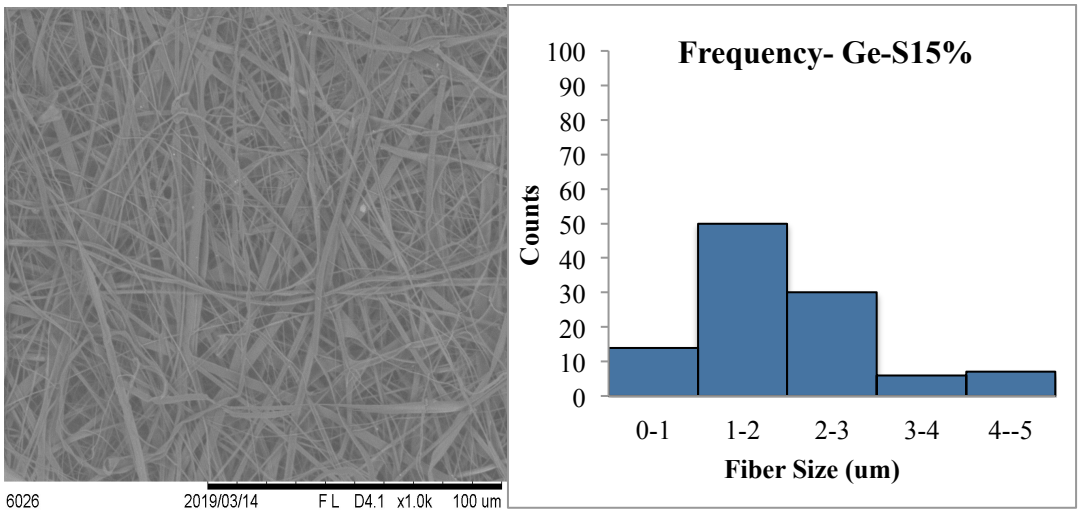
0-1
1-2
2-3
3-4
4--5
Fiber Size (um)

c)


6026 2019/03/14 F L D4.1 x1.0k 100 um
Frequency- Ge-S15%
Counts
100
90
80
70
60
50
40
30
20
10
0
0-1
1-2
2-3
3-4
4--5
Fiber Size (um)

d)

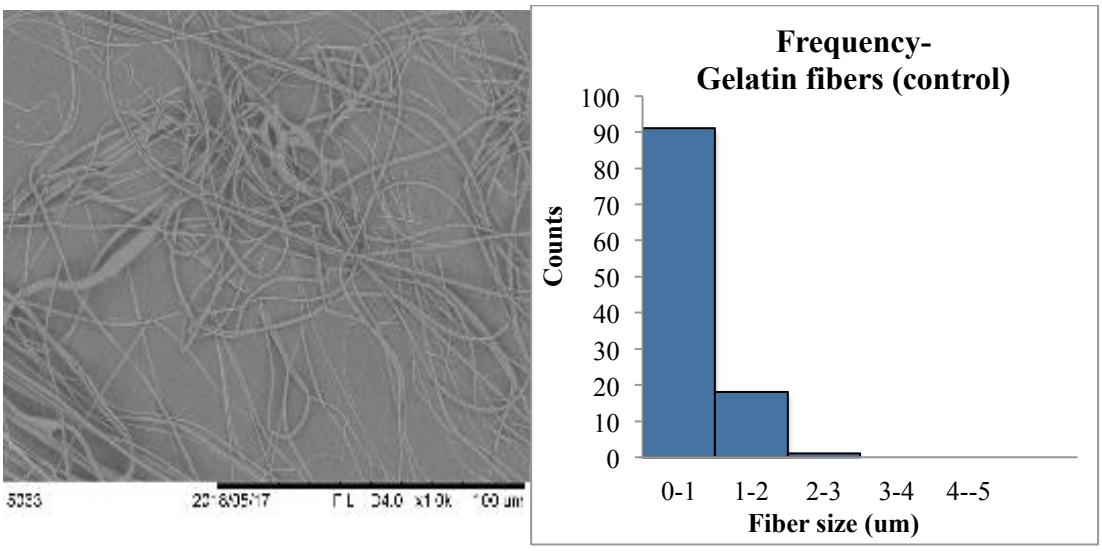


Figure 3.4. SEM images of gelatin fibers containing; a) 5%, b) 10%, c) 15%, and D) 0% of saffron extract (at 2000 * magnification)

Table 3.9. Viscosity of gelatin solutions and gelatin fibers diameters with and without saffron extract

Sample	Diameter (um)	Viscosity (Pa.s)
Ge fiber	0.75 ± 0.34^{d}	1.87 ± 0.02^{a}
Ge-S5%	1.05 ± 0.69^{c}	1.9 ± 0.03^{a}
Ge-S10%	1.7 ± 0.95^{b}	1.98 ± 0.02^{a}
Ge-S15%	2.04 ± 1.07^{a}	1.98 ± 0.05^{a}

Small letters within the same column indicate significant differences ($p < 0.05$).

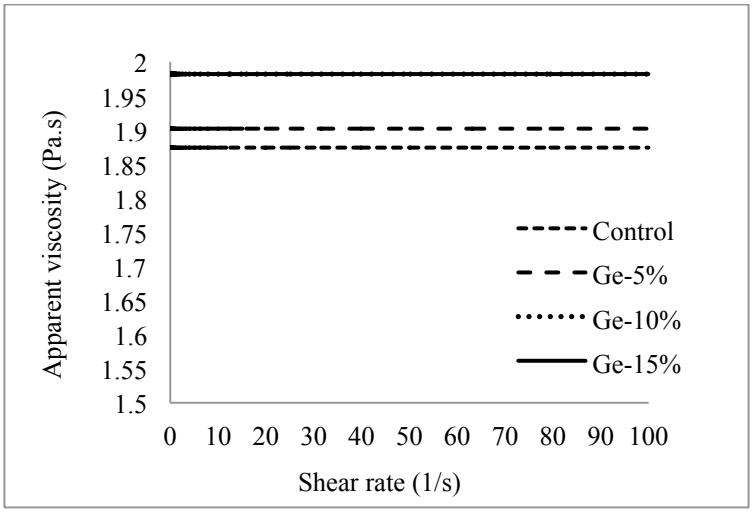


Figure 3.5. Effect of different concentrations of the saffron extract on the apparent viscosity of gelatin solution

Table 3.10. Results of One-way ANOVA analysis for the effect of different concentrations of the saffron extract on diameter and viscosity of electrospun gelatin fibers

Response	Source	SS	df	MS	F-value	P-value
Diameter	T (between treatments)	112.17	3	37.39	56.42	0.00
	R (within treatments)	286.28	432	0.66		
	Total	398.45	435			
Viscosity	T (between treatments)	0.02	3	0.00	1.97	0.19
	R (within treatments)	0.03	8	0.00		
	Total	0.06	11			

T: Treatment
R: Residual

3.5.5. Conclusions

Overall, the results showed that the concentration (5,10, and 15% w/w) of saffron extract in the gelatin fibers significantly ($P < 0.05$) influenced encapsulation efficiency, thermal properties, storage stability, and diameter of fibers. According to the DSC curves, denaturation temperature and melting temperature of neat gelatin fibers as a control sample increased, while the enthalpy decreased by addition of saffron extract. The results also demonstrated that the degradation of crocin was linear at all three different storage temperatures. Storage stability of crocin decreased significantly ($P < 0.05$) by increasing the storage temperature from 4 to 35 °C. Encapsulated saffron extract showed a lower reaction rate constant (k) and consequently higher half-life period ($t_{1/2}$) during 42 days storage at 4 °C compared to 24 and 35 °C at the same concentration of saffron. As a results, 15% saffron extract exhibited the highest half-life period ($t_{1/2}$) with values of 138.62 for crocin at 4 °C under 33% RH. The diameters of gelatin fibers increased significantly ($P < 0.05$) from 1.05 ± 0.69 to 2.04 ± 1.07 μm when the concentration of saffron extract increased from 5 to 15%. It might be due to the greater solid contents in the feed solution. Viscosity of gelatin solution slightly increased by addition of saffron extract, however no significant differences were found between different treatments. This phenomenon is related to formic acid that keeps gelatin solution dilute at room temperature.

PREFACE TO CHAPTER 4

In the previous chapter, three different concentrations of saffron extract powder were encapsulated into gelatin as a wall material using the electrospinning technique. This chapter focuses on the encapsulation of saffron extract powder (5, 10, 15% w/w based on gelatin content) in gelatin with the use of the freeze-drying technique as a common method of encapsulation in food industries. The main objective of this chapter was to study the storage stability of the color of encapsulated saffron extract under different storage conditions (4, 24, and 35 °C). The reaction rate constant (k) and half-life period ($t_{1/2}$) of encapsulated and un-encapsulated saffron extracts were determined. The effect of freeze-drying on encapsulation efficiency, thermal properties, morphology, and particle size distribution of encapsulated saffron extract powder in gelatin was also to be evaluated.

The results of chapter 3 and 4 help us to compare the efficiency of two different encapsulation techniques (electrospinning and freeze-drying) for encapsulation of the bioactive compounds of saffron.

All the experimental work and data analysis were conducted by the candidate under the supervision of Dr. H. S. Ramaswamy.

CHAPTER 4

ENCAPSULATION OF BIOACTIVE COMPOUNDS OF SAFFRON IN GELATIN USING FREEZE-DRYING TECHNIQUE

4.1. Abstract

In this chapter, the freeze dried saffron extract powders was encapsulated in gelatin (5, 10, and 15% w/w based on gelatin content) as a wall material through a freeze-drying method at a temperature of −25 to -55 °C under pressure of 67 (Pa). The encapsulated powders were analyzed for thermal properties, storage stability, surface morphology, particle size distribution as well as the encapsulation efficiency of crocin, picrocrocin and safranal as core materials. Results showed that encapsulation efficiency was positively affected by the concentration of saffron extract. So, by increasing the concentration of saffron extract in gelatin, the EE of three different bioactive compounds of saffron increased significantly ($P < 0.05$). Results also demonstrated that the melting temperature and denaturation temperature of un-encapsulated saffron extract increased significantly ($P < 0.05$) by encapsulation of this compound in gelatin powders. Therefore, encapsulation of saffron extract in gelatin powders improved the thermal stability of this saffron product. SEM analysis revealed that at 15% concentration of saffron extract, the particles of encapsulated powder were larger compared to 5 and 10% saffron extract concentrations. Storage stability of encapsulated and un-encapsulated saffron extract was analyzed by measuring the absorbance of crocin at 440 nm. It was found that encapsulated saffron extract had higher stability than un-encapsulated saffron during the 42 days storage. The highest crocin content was observed in the freeze-dried encapsulated powder containing 15% saffron extract at 4 °C with a half-life ($t_{1/2}$) of 77 days and the reaction rate constant (k) of 0.9 x 10^{-2} min^{-1}.

4.2. Introduction

The chemical constituents of saffron are unstable upon exposure to heat, light, oxygen, and water activity. Degradation of bioactive compounds of saffron leads to loss of nutritive value, colorant power as well as off-flavor development (Rodriguez-Amaya, 1993). Therefore, it is needed to provide an efficient protection from these factors to improve the stability of saffron compounds in different food systems during processing and storage. Freeze-drying is one of the most commonly used

encapsulation techniques. This technique is an effective and suitable method for encapsulation of heat-sensitive and water-soluble compounds in the food industry due to the absence of high temperatures in the process (Murali et al., 2015). The main objective of this chapter is to encapsulate saffron extract using the freeze-drying technique to study the effect of different concentrations of saffron extract powders (5, 10, 15% w/w) on EE, thermal properties, morphology, and particle size distribution of produced encapsulated powder. The storage stability of encapsulated and un-encapsulated saffron extract under accelerate storage condition (4, 24, and 35 °C) was also studied during 42 days storage.

4.3. Materials and methods

4.3.1. Materials

This section was explained in details in Chapter 3, section 3.3.1.

4.3.2. Preparation of saffron extracts

The detailed procedure was explained in Chapter 3, section 3.3.2. Same procedure was followed to prepare saffron extracts.

4.3.3. Preparation of polymer solution

The detailed procedure was explained in Chapter 3, section 3.3.3. Same procedure was followed to prepare polymer solution.

4.3.4. Freeze-drying technique

In this technique, after preparation of gelatin solutions with and without saffron extract, all liquid samples were frozen in a freezer (Top-Freezer Refrigerator Rona Co., Boucherville, QC, Canada) (−18 °C) for 24 h. Then, they were placed in a freeze-dryer chamber at −55 °C under pressure of 67 (Pa) for 5 days to dry completely. After removing the samples from freeze-dryer chamber, dried specimens were grounded by a pestle and mortar, followed by sieving of the powders using a stainless steel sifter with a 0.71 mm mesh. Freeze-dried powders were stored in amber glass jars in a freezer (−18 °C) until further use.

4.3.5. Characterization of encapsulated freeze-dried powder

Encapsulation efficiency, thermal properties, storage stability, morphology, and particle size distribution of all samples were evaluated based on the methods presented in detail in Chapter 3 of this work.

4.4. Statistical analysis

The results were analyzed statistically by SPSS Version 25 (SPSS Inc., CHI, IL, USA). One-way analysis of variance and Tukey's test were applied to determine the significant difference ($p < 0.05$) in the average of encapsulation efficiency, thermal behavior, storage stability, and diameters of freeze-dried powders with and without saffron extract. The results were represented as the mean ± standard deviation in the form of figures and tables. Each sample was tested in triplicate.

4.5. Results and discussions

4.5.1. Encapsulation efficiency

The encapsulation efficiency shows the efficiency of the process to entrap the bioactive compounds into gelatin matrix. EE of crocin, picrocrocin, and safranal in freeze-dried gelatin powder are presented in Table 4.1. Results indicated that 15% had the highest and 5% saffron extract had the lowest encapsulation efficiency ($P < 0.05$). The amount of crocin, picrocrocin, and safranal trapped into the gelatin matrix was 69, 74.6, and 65.8 % at 15% saffron extract, respectively. Gelatin retained the highest ($P < 0.05$) amount of picrocrocin as compared to crocin and safranal independently of the saffron extract concentration. The lowest value (51.6 to 65.8 %) of encapsulated safranal as a volatile compound as compared to crocin and picrocrocin (59 to 69 – 62.6 to 74.6 %) was related to its sensitivity and instability during the encapsulation procedure. The results revealed that the concentration of saffron extract as a core material, different types of bioactive compounds of saffron extract, and their interactions had a significant effect on the EE of saffron within the gelatin capsules (Tables 4.2 & 4.3).

This finding is in agreement with the results reported by Tavassoli-Kafrani et al. (2018). They found that by increasing the concentration of orange essential oil in electrospun gelatin fibers, the encapsulation efficiency increased significantly. Rajabi et al. (2015) reported that the encapsulation efficiency of three different bioactive compounds of saffron increased by increasing the total solids content in the polymer

solution due to the reduction of emulsion droplet size. Ballesteros et al. (2017) demonstrated that encapsulation efficiency was strongly affected by different types of encapsulated compounds. Results clearly showed EE of freeze-drying technique is less than electrospinning technique that may be due to the effect of mechanical stress caused by the grinding of dried samples. Actually, mechanical stress during the grinding procedure can break the wall material (gelatin) that entrapped the bioactive compounds of saffron and it leads to the release of encapsulated compounds.

Table 4.1. Effect of different concentrations of saffron extract on encapsulation efficiency of crocin, picrocrocin, and safranal in freeze-dried gelatin powders

Sample	EE% of picrocrocin	EE% of safranal	EE% of crocin
Ge-S5%	62.5 ± 0.5^{cA}	51.6 ± 1.15^{cC}	59 ± 1^{cB}
Ge-S10%	68.6 ± 0.57^{bA}	59.6 ± 2.08^{bB}	61.6 ± 0.53^{bB}
Ge-S15%	74.6 ± 1.15^{aA}	65.8 ± 0.76^{aC}	69 ± 1^{aB}

Small letters within the same column indicate significant differences ($p < 0.05$).
Big letters within the same row indicate significant differences ($p < 0.05$).

Table 4.2. Results of One-way ANOVA analysis for the effect of different concentrations of the saffron extract on EE of crocin, picrocrocin, and safranal in freeze-dried gelatin powders

Compound	Source	SS	df	MS	F-value	P-value
Picrocrocin	T (between treatments)	220.83	2	110.41	172.52	0.00
	R (within treatments)	3.84	6	0.64		
	Total	224.67	8			
Safranal	T (between treatments)	302.72	2	151.36	72.65	0.00
	R (within treatments)	12.5	6	2.08		
	Total	315.22	8			
Crocin	T (between treatments)	161.45	2	80.72	106.02	0.00
	R (within treatments)	4.56	6	0.76		
	Total	166.02	8			

T: Treatment
R: Residual

Table 4.3. Results of Two-way ANOVA analysis for the effect of different concentrations of saffron extract on encapsulation efficiency of different bioactive compounds of saffron in freeze-dried gelatin powders

Source	SS	df	MS	F-value	Sig.
Corrected Model	1099.29	8	137.41	118.29	0.00
Intercept	109305.52	1	109305.5	94101.5	0.00
Saffron Concentration	660.17	2	330.08	284.17	0.00
Saffron Compound	414.27	2	207.13	178.32	0.00
Saffron Concentration x Saffron Compounds	24.84	4	6.21	5.34	0.00
Error	20.9	18	1.16		
Total	110425.72	27			
Corrected Total	1120.19	26			

4.5.2. Thermal analysis

DSC analysis of pure saffron extract powder and freeze-dried gelatin powder with and without different concentrations of the saffron extract was performed to evaluate the thermal stability of the samples and the results are summarized in Table 4.4. All samples showed endothermic peaks due to the evaporation of unbound water and volatile compounds of saffron (Pillay et al., 2013). The melting temperature, denaturation temperature, and enthalpy of pure saffron extract powder appeared at 32, 36, and 27 °C, respectively. These values were significantly lower than the T_m, T_d, and enthalpy of encapsulated 15% saffron extract in the gelatin matrix. These findings are in agreement with the results of Carmona et al., (2005) and Tsimidou (1997). They studied the thermal properties of different saffron samples and reported approximately the same values for T_d (10 to 40 °C) and enthalpy (16 to 25 °C). Our study also found that melting temperature of all samples increased significantly ($p < 0.05$) from 36.8 °C to 42.0, 57.8, and 65 °C when the concentration of saffron extract increased from 0% to 5, 10, and 15%, respectively. The T_d of freeze-dried gelatin powders without saffron extract observed at 73.9 °C with an enthalpy of 86.22 J/g. In the case of samples containing saffron extract, T_d increased to 79.36, 88.77, and 93.16 °C by addition of 5, 10, and 15% saffron extract, respectively. DSC analysis of the samples demonstrated that the enthalpy of freeze-dried gelatin powder as a control sample decreased from 86.2 to 76.9 by addition of 5% saffron extract however the difference was not statistically significant. Furthermore, the enthalpy of freeze-dried gelatin powders containing saffron extract reduced with the increase of saffron extract concentration. Haroun and Toumy (2010) reported that reduction in enthalpy was related to the breaking of hydrogen bonds and the generation of covalent bonds during the encapsulation process. These results showed that the concentration of encapsulated saffron extract in the freeze-dried gelatin powder could significantly ($p < 0.05$) affect the thermal properties of the final product (Table 4.5).

Table 4.4. Thermal properties of freeze-dried gelatin powders with and without saffron extract

Sample	T_m (°C)	T_d (°C)	ΔH (j/g)
Saffron extract	32 ± 2.34^{d}	36.1 ± 3.31^{c}	27 ± 1.66^{d}
Ge fiber	36.7 ± 3.89^{b}	73.8 ± 3.77^{b}	86 ± 4.55^{a}
Ge-S5%	42.2 ± 5.78^{b}	79 ± 5.65^{ab}	76 ± 4.45^{ab}
Ge-S10%	57.8 ± 6.43^{a}	89.2 ± 10.11^{ab}	66.5 ± 5.22^{bc}
Ge-S15%	65.4 ± 4.62^{a}	93.1 ± 7.25^{a}	61.1 ± 3.47^{c}

Small letters within the same column indicate significant differences ($p < 0.05$).

Table 4.5. Results of One-way ANOVA analysis for the effect of different concentrations of the saffron extract on T_m, T_d, and ΔH of freeze-dried gelatin powders

TP	Source	SS	df	MS	F-value	P-value
T_m	T (between treatments)	2426	4	606.51	25.95	0.00
	R (within treatments)	233.71	10	23.37		
	Total	2659.7	14			
T_d	T (between treatments)	6182.8	4	1545.7	36.43	0.00
	R (within treatments)	424.22	10	42.42		
	Total	6607.1	14			
ΔH	T (between treatments)	6015.4	4	1503.8	90.84	0.00
	R (within treatments)	165.54	10	16.55		
	Total	6181	14			

TP: Thermal properties
T: Treatment
R: Residual

4.5.3. Storage stability of encapsulated saffron extract

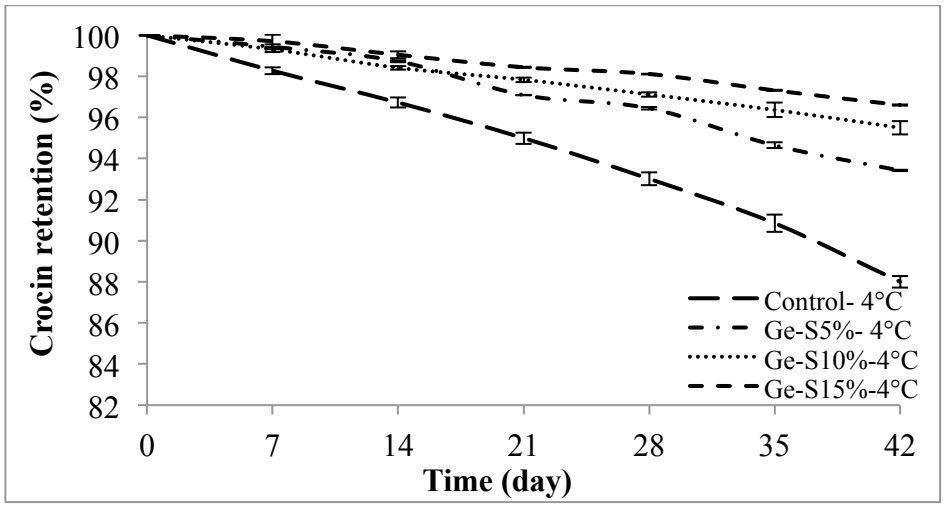
As mentioned in chapter 1 and 2, crocin is responsible for saffron's color. Since color is the main property of this spice, it is important to measure the amount of crocin concnetration in encapsulated and un-encapsulated saffron. Therefore, storage stability of crocin in encapsulated and un-encapsulated (control) saffron extract during 42 days of storage at three different temperatures (4, 24, and 35 °C) is shown in Figure 4.1. All samples (encapsulated and un-encapsulated) showed a significant difference ($P < 0.05$) between crocin content of freeze-dried gelatin powders immediately after production (day 1) and its value at the 6^{th} week (day 42). Crocin content of un-encapsulated saffron extract (control sample) showed a 12, 14, and 15% reduction compared to its initial content at 4, 24, and 35 °C, respectively. The encapsulated saffron extract showed a significantly higher amount of crocin than control sample at all storage conditions. Regarding the effect of temperature on crocin stability during storage, there were significant differences ($P < 0.05$) between the samples stored at three different storage conditions. The results showed that increasing the temperature from 4 to 35 °C caused a significant decrease in crocin content of samples. Temperature plays an important role in the stability of bioactive compounds due to oxygen permeability and thermal degradation processes (Andersen et al., 2000). At higher temperature, molecular interactions increase and molecules have more energy to overcome the activation barrier to start chemical and physical degradations of materials. Therefore, higher crocin content of the encapsulated saffron extract was related to the presence of gelatin as a wall material that made a physical barrier against destructive agents. Azarpazhooh et al. (2018) also reported that the degradation of encapsulated anthocyanin in maltodextrin by freeze-drying technique increased when the storage temperature increased from 4 to 25 °C. The similar results were reported by Fang and Bhandari (2011) on the storage stability of bayberry polyphenols.

The amount of crocin at 5 and 10% encapsulated saffron extract decreased from 100 to 93.4 and 95.5 %, respectively, after 42 days storage at 4 °C. By increasing the concentration of saffron extract from 10 to 15% in gelatin particles, the amount of crocin showed less reduction (3.4%) under the same storage conditions. This result is in agreement with Silva et al. (2012) who studied the storage stability of encapsulated lycopene in gelatin using freeze-drying method and found that the stability of lycopene in wall material improved by increasing the concentration of

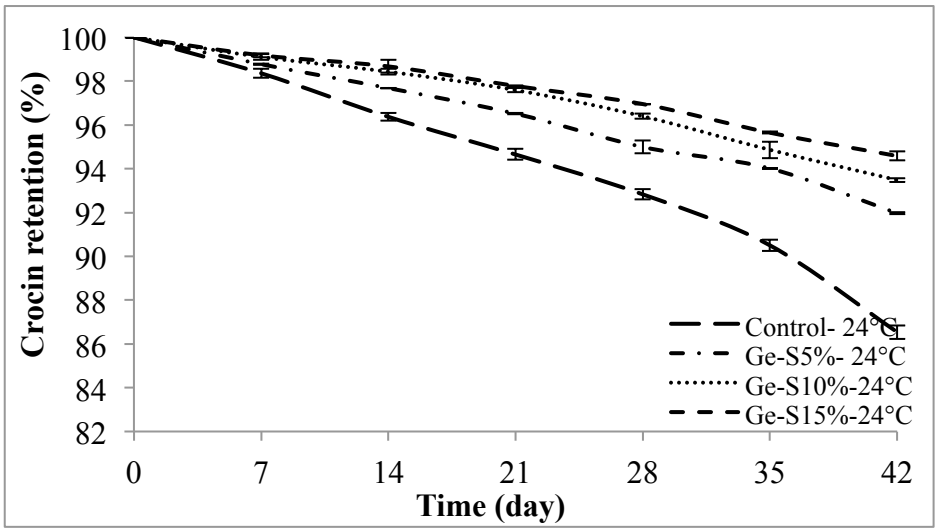
lycopene. Degradation of crocin revealed first-order reaction kinetics with a linear reduction of the ln *(E)* as a function of time. The reaction rate constant *(k)* and half-life period ($t_{1/2}$) of the encapsulated and un-encapsulated saffron extract powders are shown in Table 4.7. The half-life period ($t_{1/2}$) for 15% saffron extract encapsulated in gelatin matrix was 77, 49.5 and 40.7 at 4, 25, and 35 °C, respectively (Table 4.6).

Generally, Table 4.7 shows that the storage stability of encapsulated and un-encapsulated crocin was significantly ($p < 0.05$) affected by concentration of saffron extract, time, temperature, and their interactions. This result also demonstrated that the good efficiency of freeze-drying for the protection of natural pigments of saffron during storage as the most common encapsulation technique.

a)



b)



c)

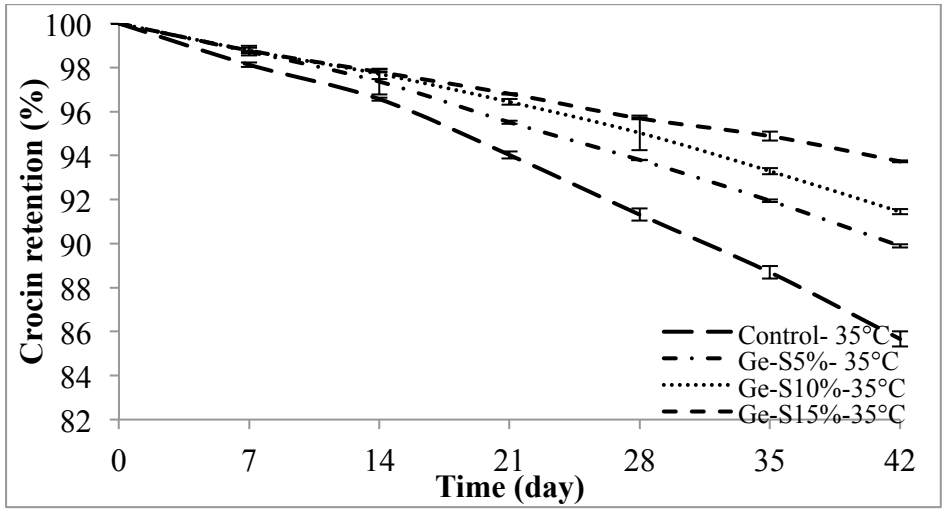


Figure 4.1. Storage stability of crocin in freeze-dried gelatin powders under different temperatures; a) 4 °C, b) 24 °C, c) 35 °C

Table 4.6. Regression analysis of bioactive compounds of saffron in freeze-dried gelatin powders during storage at different temperatures for 42 days storage

Sample	Temperature	Compound	T^a ½ (day)	(K^b * 10^{-2}) (min^{-1})	R^2
Control	4	Crocin	22.3	3.1	0.98
Ge-S5%	4	Crocin	43.3	1.6	0.96
Ge-S10%	4	Crocin	57.7	1.2	0.98
Ge-S15%	4	Crocin	77	0.9	0.97
Control	24	Crocin	21	3.3	0.96
Ge-S5%	24	Crocin	34.6	2	0.97
Ge-S10%	24	Crocin	43.3	1.6	0.97
Ge-S15%	24	Crocin	49.5	1.4	0.97
Control	35	Crocin	18.7	3.7	0.98
Ge-S5%	35	Crocin	27.7	2.5	0.97
Ge-S10%	35	Crocin	33	2.1	0.98
Ge-S15%	35	Crocin	40.7	1.7	0.99

Table 4.7. Results of Univariate analysis for the effect of different concentrations of saffron extract on storage stability of encapsulated crocin in freeze-dried gelatin powder during 42 days storage at 4, 24, and 35 °C

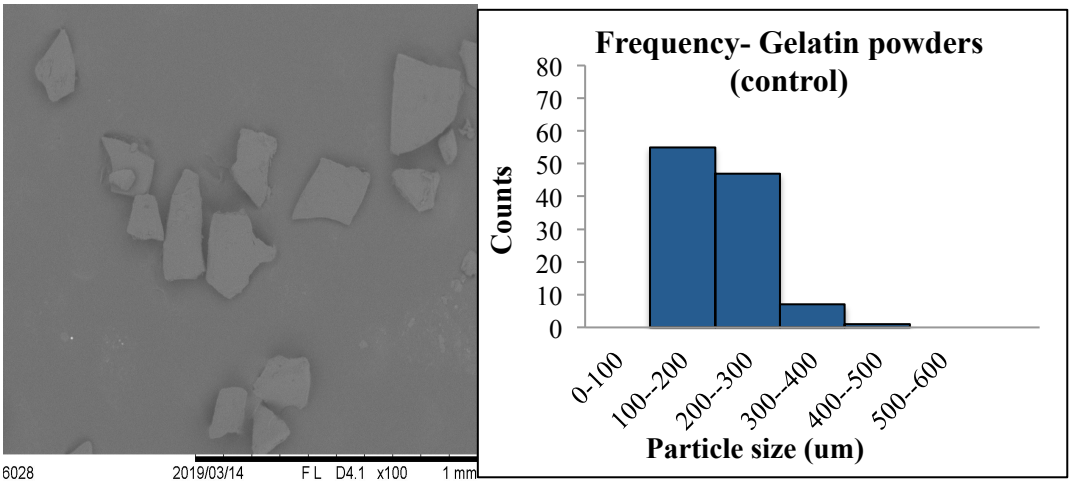
Source	SS	df	MS	F-value	Sig.
Corrected Model	44.68	83	0.53	1369.33	0.00
Intercept	39483.44	1	39483.44	100429625	0.00
Day	32.07	6	5.34	13596.58	0.00
Concentration	5.02	3	1.67	4260.23	0.00
Temperature	1.73	2	0.86	2209.18	0.00
Day x Concentration	4.84	18	0.26	683.87	0.00
Day x Temperature	0.84	12	0.07	177.96	0.00
Concentration x Temperature	0.05	6	0.00	23.73	0.00
Day x Concentration x Temperature	0.11	36	0.00	8	0.00
Error	0.09	252	0.00		
Total	39528.22	336			
Corrected Total	44.78	335			

4.5.4. Scanning electron microscopy

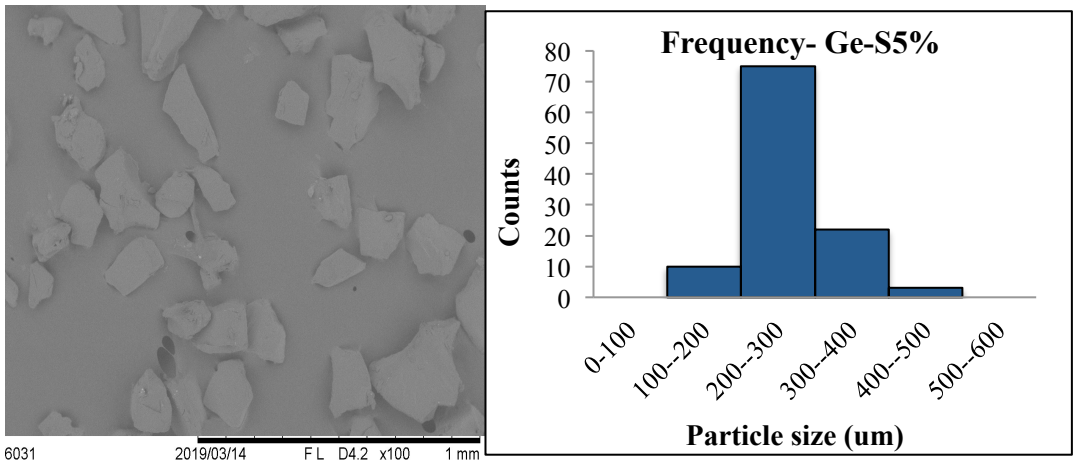
Particle distribution is one of the most important properties of a powder. SEM images and the particle size distribution of gelatin powder with and without saffron extract using freeze-drying technique are shown in Figure 4.2. All samples showed similar morphologies with irregular structure, surface dents, and indentations (Azarpazhooh et al., 2018). The powders demonstrated a very large size range, with particle size distribution ranging from 151 ± 40.6 to 217 ± 86.1 μm (Table 4.8). Overall, the results showed that size of particles increased significantly by addition of saffron extract to the freeze-dried gelatin powder (control) (Table 4.10). Furthermore, by increasing the concentration of saffron extract from 5 to 15%, the size of particles increased significantly ($p < 0.05$). According to Table 4.8, the average particle size for samples containing 15% saffron extract was 217.4 ± 86.11 μm, which was significantly higher than 5% (163 ± 57.2 um) and 0% (151 ± 40.6 μm) saffron extract concentration. Figure 4.2 showed that all samples with saffron extract have breakages, cracks, and holes on the surface of particles due to the mechanical pressure during the grinding process (Azarpazhooh et al., 2018). Kaushik and Roos (2007) reported that the presence of dents and cracks on the surface of freeze-dried particles influenced powder flow-ability, encapsulation efficiency and storage ability of encapsulated compounds. Therefore, any morphological changes can influence the encapsulation properties because of the different surface area of the wall material that leads to the degradation of the encapsulated compounds (Table 4.9) (Ballesteros et al., 2017).

Results showed that shape, size, and morphology of the matrix can be affected by encapsulation techniques, due to the different conditions in each method. Khazaei et al. (2014) reported that freeze-drying can change the original morphology of material due to the lyophilisation process. These results are in agreement with Rajabi et al. (2015) and Hogan et al. (2001) who found that the size of particles increased when the total solids content increased in the polymer solution. This phenomenon can be explained by the viscosity of the solution that increased by the addition of a higher amount of solids (Jafari et al., 2008). Chranioti et al. (2015) encapsulated saffron extract in different polysaccharide gums using freeze-drying method and found that the samples had an amorphous glass-like structure that leads to preserve the encapsulated compounds against oxygen and heat.

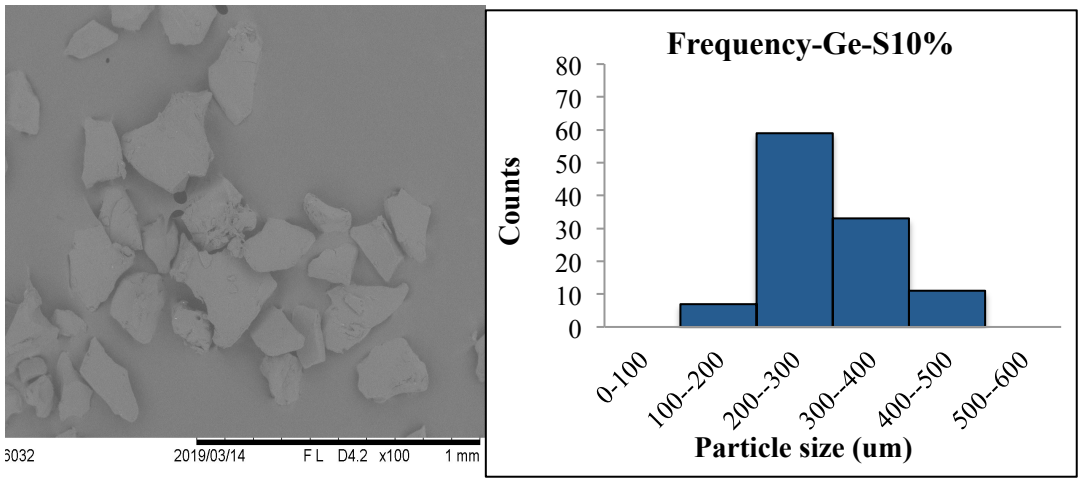
a)


6028 2019/03/14 F L D4.1 x100 1 mm
Frequency- Gelatin powders (control)
Counts
80
70
60
50
40
30
20
10
0
0-100
100--200
200--300
300--400
400--500
500--600
Particle size (um)

b)


6031 2019/03/14 F L D4.2 x100 1 mm
Frequency- Ge-S5%
Counts
80
70
60
50
40
30
20
10
0
0-100
100--200
200--300
300--400
400--500
500--600
Particle size (um)

c)


6032 2019/03/14 F L D4.2 x100 1 mm
Frequency-Ge-S10%
Counts
80
70
60
50
40
30
20
10
0
0-100
100--200
200--300
300--400
400--500
500--600
Particle size (um)

d)

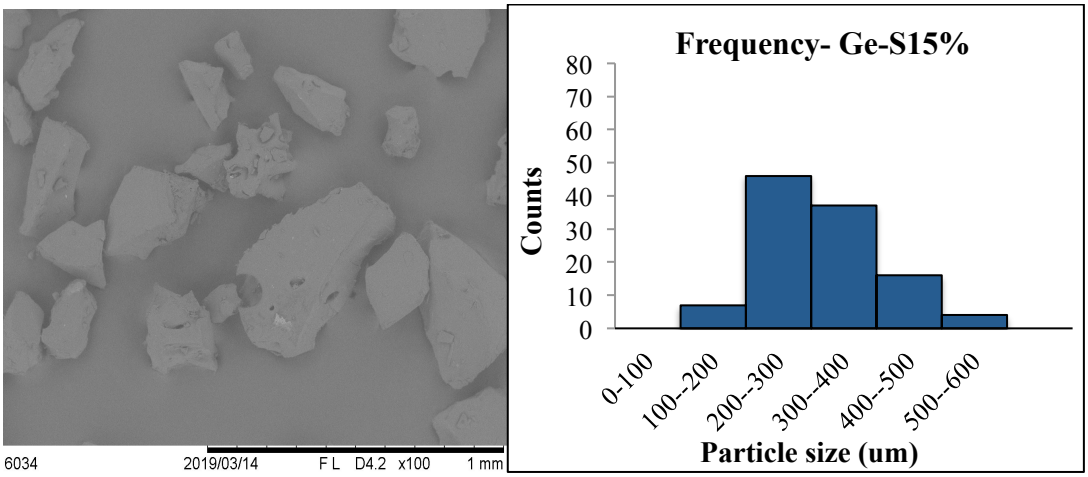


Figure 4.2. SEM images of freeze-dried gelatin powder containing; a) 5%; b) 10%; c) 15%; and D) 0% of saffron extract (at 100 * magnification)

Table 4.8. The particle size distribution of freeze-dried gelatin powders with and without saffron extract

Sample	Diameter (um)
Ge powder	151 ± 40.6^{b}
Ge-S5%	163 ± 57.1^{b}
Ge-S10%	195 ± 68.7^{a}
Ge-S15%	217 ± 86.1^{a}

Small letters within the same column indicate significant differences ($p < 0.05$).

Table 4.9. Results of One-way ANOVA analysis for the effect of different concentrations of the saffron extract on the particle size distribution of freeze-dried gelatin powders

Response	Source	SS	df	MS	F-value	P-value
Diameter	T (between treatments)	296911.46	3	98970.48	23.2	0.00
	R (within treatments)	1859503	436	4264.91		
	Total	2156414.46	439			

T: Treatment
R: Residual

4.6. Conclusions

In this chapter bioactive compounds of saffron were encapsulated in gelatin powders using the freeze-drying technique. The results revealed that encapsulated powder with 15% saffron extract showed the highest value of encapsulation efficiency, melting temperature, denaturation temperature, retention of crocin, and particle size compared to 5 and 10% saffron extract. The highest crocin content was observed in the freeze-dried gelatin powders containing 15% saffron extract at 4 °C during 42 days storage with a half-life ($t_{1/2}$) of 77 days. While encapsulated gelatin powders with 5% saffron extract showed the lowest retention of crocin at 35 °C compared to 10 and 15% saffron with a half-life ($t_{1/2}$) of 27.7 days and the reaction rate constant (k) of 2.5 x 10^{-2} min^{-1}. SEM images demonstrated that particle size of encapsulated powders increased significantly ($p < 0.05$) by increasing the concentration of saffron extract from 5 to 15%. It might be due to the incorporation of saffron extract into the gelatin powders. Overall, the results showed that encapsulated saffron extract in gelatin powders had better thermal properties and storage stability compared to non-encapsulated one.

CHAPTER 5
GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE STUDY

5.1. GENERAL SUMMARY & CONCLUSIONS

In this study electrospinning and freeze-drying techniques were used to encapsulate saffron extract in gelatin fibers and gelatin particles, respectively. The results of this study indicated that bovine gelatin could be used as a wall material for encapsulation of this compound in these two techniques. The results also showed that the viscosity of gelatin solutions did not change significantly with increasing saffron extract concentration from 5 to 15%. Scanning electron microscopy demonstrated that the size of gelatin fibers and gelatin particles increased significantly ($P < 0.05$) with increasing concentration of saffron extract. Encapsulation efficiency determined that saffron extract was successfully encapsulated in fibers and particles through electrospinning and freeze-drying methods with high encapsulation efficiency. Melting temperature, denaturation temperature, and enthalpy of saffron extracts increased significantly ($P < 0.05$) by encapsulation of this spice in gelatin. SEM images presented that the extract-loaded fibers and particles show similar morphology as the neat electrospun gelatin fibers and gelatin particles, respectively. Encapsulated extract in gelatin fibers showed a smooth, bead-free, and homogeneously distributed morphology while; freeze-dried gelatin powders exhibited a very large particles size with irregular structure, indentations, and cracks.

The results revealed that encapsulated saffron extract in gelatin fibers and gelatin particles showed higher retention of crocin compared to un-encapsulated one during 42 days storage at 4, 24, and 35 °C. Moreover, the amount of encapsulated crocin in all samples decreased as the period of storage was increased. Generally, it was observed that increasing the concentration of saffron extract resulted in higher crocin content under all tested conditions during storage. Also, degradation of crocin significantly increased ($P < 0.05$) with an increase in storage temperature. The results showed that crocin degradation followed the first-order kinetics reaction. Encapsulated crocin showed a half-life period ($t_{1/2}$) with values of 138 and 77.x days at 15% saffron extract under 4 °C for electrospinning and freeze-drying techniques, respectively. Overall, among the different concentrations (5, 10, and 15%) of saffron

extract as a core material, 15% saffron extract provided better encapsulation efficiency, thermal properties, and storage stability.

This phenomenon may be due to the breaking of wall materials and losing of the initial structure of gelatin capsules during the freeze-drying and grinding procedure. Therefore, encapsulation by electrospinning could be recommended as a proper technique for stabilizing bioactive compounds of saffron during processing and storage. Accordingly, it can be concluded that encapsulated saffron into fine electrospun fibers can be used in different food such as desserts, dairy products, chewing gums, beverages, and tea bags.

5.2. Recommendation for future study

The current study recommends applying electrospun fibers containing saffron extract in various food applications (saffron dessert, saffron beverage, saffron yogurt, etc.) to evaluate the stability of color, flavor, and aroma of saffron under different storage conditions. Also, different studies are needed to find out, which chemical reactions result in the reduction of various bioactive compounds of saffron during storage. Moreover, it is required to figure out possible physical and chemical interactions between bioactive compounds of saffron extract and gelatin, which lead to the effective encapsulation of saffron extract in gelatin. This study also recommends analyzing the release of encapsulated saffron extract in different media such as hot water and simulated gastro-intestinal conditions.

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