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Evaluation of supercritical CO_2 extraction on color stability and antioxidant activity of nanoencapsulated anthocyanin extract of pomegranate peel powder

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ABSTRACT

In this study, anthocyanin and phenolic compounds were extracted from pomegranate peel powder with supercritical CO_2 and *Lepidium perfoliatum* seed gum and maltodextrin were used as the nanoencapsulation wall. The nanoencapsulation extract was dried with a freeze dryer. Color stability and antioxidant activity of nanoencapsulation extract were evaluated. The levels of anthocyanin and phenolic compounds were evaluated by differential pH and Folin-Ciocalteu method, respectively. Particle size, nanoencapsulation efficiency of anthocyanin, nanoencapsulation phenol, color stability at different temperatures and pH as well as antioxidant activity were evaluated by DPPH. The amount of anthocyanin and phenol extracted in this method was 3.943±0.133 mg/g of pomegranate peel powder and 504.521±2.537 mg/g of gallic acid per 100 g, respectively. Nanoencapsulation efficiency was higher for anthocyanin than phenols. The release of anthocyanin at different pHs was different, so that anthocyanin behaved differently at different pHs. Polymerization and color increased with increasing temperature. The antioxidant activity of the fine-grained extract by DPPH method increased with increasing concentration and its IC50 was equivalent to 1.092± 0.024 m /ml.

1. Introduction

Pomegranate (*Punica granatum L.*) is a fruit belonging to the *Punicaceae* family, it is adapted to a wide range of weather conditions and is a native fruit of Iran. This fruit can be consumed fresh or as juice and also as a drink with other food products (jam and jelly) [1]. Pomegranate is a rich source for extracting biologically active or bioactive substances such as phenolic compounds, tannins, and anthocyanins. These compounds have various biological activities such as antioxidant, antimicrobial, and anticancer properties. However, extracting and purifying such compounds from different parts of pomegranate in the same efficient manner is a challenging task [2]. In addition to the edible part, the health-enhancing effect of pomegranate fruit also includes its non-edible parts (especially the peel, which contains more biologically active compounds than the edible part [1]. Pomegranate peel is about 30-40% of Pomegranate fruit is included and remains as a byproduct after extracting water [3]. Pomegranate peel has more phenolic compounds than flesh and seeds and can be a good source for the production of antioxidants with a high [4]. In China, pomegranate peel has the highest antioxidant activity among peel, fruit pulp and plant seeds [5]. The most important parameters affecting the extraction of bioactive compounds from plant sources include properties of the matrix of the plant part, type of solvent, temperature, pressure, time [6], solvent concentration, and liquid/solid ratio [7]. Today, there is an increasing demand for the development of green extraction processes, with reduced operating time, better results, and significantly reduced use of organic solvents, ultrasound extraction, microwave extraction, and liquid extraction Super-critical, are considered part of the green method [8].
In supercritical extraction, the extraction device is in its supercritical state. In this method, temperature and pressure are higher than their critical values. This method is suitable for extracting compounds in a short period with higher efficiency [6]. Carbon dioxide (CO_2) is the most widely used as a supercritical fluid to recover bioactive compounds from plant and animal matrices [9]. The low polarity of CO_2 has been successfully solved by using solvents such as ethanol, methanol, water, and acetone [6]. Encapsulation is widely used in food industries to control the release of aroma, flavoring, and especially bioactive substances and to produce food containing probiotics [10]. Encapsulating is a method for coating materials (such as natural colors) in the form of micro and nanoparticles [11]. The efficiency and stability of encapsulating mainly depend on the components of the encapsulating material. The encapsulating agent can act as a barrier and protect the core against environmental factors such as oxygen, water, light, and other substances [12]. Among the important features of the encapsulating agent are: high solubility in water, stability, and creating a dense network during drying [13], and therefore the properties and composition of the wall and core materials play an important role in efficiency [14] and They have the stability of encapsulatings [12]. Maltodextrin plays an important role in increasing the stability of capsulated food against oxidation [15]. Due to the use and role of hydrocolloids in the food industry and the high price of these compounds, the use of native gums has expanded and in Iran, due to the abundance of plant resources, researchers are thinking of replacing the gum of native seeds, including the gum of Qadumeh Shahri (*Lepidium perfoliatum*) from *Brassicaceae* family are commercial examples [16]. Choosing the right method for nanoencapsulating of anthocyanins and phenolic compounds is very important because it affects the physical and chemical properties as well as the release mechanism of the capsulated core [12]. In this research, supercritical CO_2 was used to extract anthocyanin and phenolic compounds of pomegranate peel powder. Considering the lack of stability of anthocyanin extract in temperature, pH, and storage time, In this study, the combined effect of Gadumeh Shahri seed gum and maltodextrin is investigated as a coating material for anthocyanin extract and phenolic compounds obtained from the extraction of pomegranate peel powder was used and the color stability and antioxidant activity of the nanoencapsulated extract were evaluated.

2- Materials and methods

2-1- Raw materials

5 kg of red-peeled Saveh pomegranate fruits were prepared. The pomegranate peel was separated from the fruit, then it was completely dried for 4 days at room temperature away from sunlight. Then it was ground and sieved (18 mesh) to prepare the powder and stored in vacuum nylon in the refrigerator until use. Gadumeh Shahri seeds were purchased from Atark Company. All chemicals were purchased from Merck, Germany, and Sigma-Aldrich.

2-2- Extraction with supercritical CO_2

3 g of pomegranate peel powder were placed in the supercritical apparatus at a certain temperature (45°C) for extraction. Pressure (200 bar) and solvent were adjusted for better extraction (20% ethanol). The intensity of the flow for CO_2 was fixed at 2 ml /min, then the extraction was done for 30 min. It was stored at -20°C to prevent the reduction of extract performance [17].

2-3- Extraction efficiency

Extraction efficiency was measured by dividing the dry weight of the extract by the g of dry pomegranate peel powder multiplied by 100.

$$\text{Extraction efficiency} = \frac{\text{Extract weight}}{\text{Pomegranate peel powder weight}} \times 100$$

2-4- determination of total anthocyanin content (TAC)

Determination of total monomeric anthocyanin content was calculated from the differential pH method described by kirca et al. [17]. 1 ml of the extract was mixed with 9 ml of distilled water and then added to the buffer solution of KCl and $C_2H_3NaO_2$ (pH = 4.5 and 1) at a ratio of 4:1. After 20 min, using a UV-VIS spectrophotometer (model 1502), the absorbance was read at λ=520, 700 nm and if necessary, dilution was done [18].

A = absorbance difference between pH 1.0 ($A_{520\,nm} - A_{700nm}$) and pH 4.5 ($A_{520\,nm} - A_{700nm}$), MW = molecular weight of cyanidin 3-glucoside (449.2 g/mol), DF = dilution factor, V = extraction volume (ml), ε = molar absorption coefficient of cyanidin 3-glucoside (29600 l/mol.cm), L = spectrophotometer cell length (cm), M = weight of pomegranate peel powder (g), TAC = total anthocyanin content (mg/g).

$A = (A_{520\,nm} - A_{700nm})\ pH_{1.0} - (A_{520\,nm} - A_{700nm})\ pH_{4.5}$

$$TAC = \frac{A \times MW \times DF \times 1000 \times V}{\varepsilon \times L \times M}$$

2-5- Determination of total phenolic compounds

The measurement of phenolic compounds was carried out using Folin Ciocalto method according to the method of Singh et al [19]. First, a concentration of 1000 ppm was prepared from the sample and 0.5 ml of it was mixed with Folin Phenol reagent diluted 10 times. 2 ml of 7.5% sodium carbonate was added. It was placed at room temperature for 30 min and absorption was measured at a wavelength of 760 nm by spectrophotometry. The final results of mg of gallic acid / 100 g of sample were calculated from the opposite equation [19].

$Y = 1.6507X+0.0494$ $R^2=0.990$ Y= absorption X = sample concentration

2-6- Nanoencapsulating method

2-6-1- The method of extracting gum from the seeds of Qadumeh Shahri

First, the impurity present in the seed of Qadumeh Shahri was separated, and then its gum was extracted by the method of Kochaki et al. [20] (the ratio of water to seed is 30:1 at 48 °C and with a pH of 8 in a water bath). The pH of distilled water was adjusted with NaOH alkaline solution and placed in a water bath at 48°C. After the bath reached the desired temperature, the seeds were added to it and stirred continuously (for 2 h) to increase the absorption of water by the seeds. A laboratory juicer was used to extract gum. The resulting extract was dried in an oven at 70°C. Finally, the gum powder after grinding and sieving

(mesh 40) was stored in closed containers in the refrigerator for use in Nanoencapsulating [20].

2-6-2- Nanoencapsulating method of pomegranate peel anthocyanin extract

For nanoencapsulating of anthocyanin extract, maltodextrin (DE = 18) and gum of Qadumeh Shahri seeds were used. In this experiment, for every 9 grams of maltodextrin, 1 gram of Qadumeh Shahri gum powder was used as wall covering material. Maltodextrin was mixed with the gum powder of Qadumeh Shahri seed, then it was slowly added to distilled water using a magnetic stirrer (40 rpm) for better dissolution for 45 min at room temperature to reach a solid content of 15%. The sample was kept in the refrigerator for 12 h to make the texture uniform. Then, the anthocyanin extract was added to the coating material (dry weight) with a ratio of 1 to 2 (w/w) and homogenized using a DRAGONLAB ultrathorax (homogenizer-500D) at a speed of 15,000 rpm at 10°C for 6 min. Next, an ultrasound prop device (model F250 KS-) was used for 6 min (the time of each cycle is 30 s and the rest time is 15 s between cycles) to reduce the size of particles. Then it was frozen in the freezer for 24 h at -18°C and dried for 48 h by a freeze dryer [21].

2-7- Nanoencapsulating properties test

2-7-1- Particle size
Nanoencapsulated compound particle size was measured by Scatroscope 1 (manufactured by Qudix, South Korea)[23].

2-7-2- Nanoencapsulating efficiency of anthocyanin compounds

First, 1 ml of distilled water was added to 100 mg of nanoencapsulated powder, the solution was mixed with a magnetic stirrer (speed 60 rpm) for 5 min, and then 9 ml of ethanol was added to it and the powder was completely dissolved. Then it was filtered with Whatman No. 42 paper. The amount of total anthocyanin (TA) was calculated using the differential pH method [23]**.**

$A= (A_{520\ nm} \_ A_{700nm})\ pH_{1.0} \_ (A_{520\ nm} \_ A_{700nm})\ pH_{4.5}$

$$TAC = \frac{A \times MW \times DF \times 1000 \times V}{\varepsilon \times L \times M}$$

A = absorbance difference between pH 1.0 ($A_{520\ nm} \_ A_{700nm}$) and pH 4.5 ($A_{520\ nm} \_ A_{700nm}$), MW = molecular weight of cyanidin 3-glucoside (449.2 g/mol), DF = dilution factor, V = extraction volume (ml), ε = molar absorption coefficient of cyanidin 3-glucoside (29600 l/mol.cm), L = spectrophotometer cell length (cm), M = weight of pomegranate peel powder (g), TAC = total anthocyanin content (mg/g).

In order to calculate non- nanoencapsulated anthocyanin (SA), 10 ml of ethanol was added to 100 mg of nanoencapsulated powder. Then it was stirred for 2 minutes with a magnetic stirrer and centrifuged for 10 min at 20°C with a speed of 3000 rpm and the supernatant was separated and SA was calculated as TA.

The encapsulating efficiency of anthocyanin (EEA) was calculated according to the method of Barbosa et al. [24] with the following formula.

$$\%EEA = \frac{TA - SA}{TA} \times 100$$

2-7-4- Nanoencapsulating efficiency of phenolic compounds

Nanoencapsulating efficiency of phenolic compounds was performed according to the method described by Kadrides et al. [5]. To measure the coated nanofine phenol (TP), first, 10 mg of the sample was mixed with 10 ml of distilled water, then it was stirred for 10 minutes with a magnetic stirrer, then it was filtered with Whatman No. 42 paper. Then it was calculated by Folin Ciocalto method.

To measure non- nanoencapsulated phenolic compounds, first pour 10 mg of the sample in 10 ml of distilled water and quickly remove from the top surface of the mixture, and to measure uncoated nanofine phenolic compounds (SP) using Folin Ciocalto method was used. Calculation of the encapsulating efficiency of phenolic compounds (EEP) was performed according to the following formula .[۵]

$$\%EEP = \frac{TP - SP}{TP} \times 100$$

2-8- Assays of color stability and antioxidant activity

2-8-1- pH effect on anthocyanin release

The nanoencapsulated extracts of pomegranate peel powder were placed in aqueous solutions with different pH (2/0, 5/0 and 7/0) for 2 h and then the absorption spectrum of the solution was measured at different times at 520 and 700nm wavelengths was measured by a spectrophotometer and the amount of color was calculated [25].

$AT=A_{520nm}-A_{700nm}$

$$\%R = \frac{AT}{AMAX} \times 1000$$

AT = absorbance difference between wavelength $A_{520\ nm}$ - $A_{700\ nm}$ in time, R = rate of anthocyanin release, Amax = maximum absorbance among different pH of the sample, A_{520} nm = absorbance at wavelength 520 nm, $A_{700\ nm}$ = absorbance at wavelength 700 nm

2-8-2-The effect of temperature on the efficiency of nanoencapsulated anthocyanin color

The efficiency of nanoencapsulated anthocyanin color was measured according to the method of Assous et al. [25]. 200 mg of the sample was poured into 10 ml of acidic solution (pH = 2/0) and placed in a water bath with different temperatures (25 to 100°C). After 30 min, the samples were placed in an ice bath and the absorbance of the solution was measured with a spectrophotometer at the wavelength of λ=520, 700 nm and the percentage of color was measured [25].

$AT=A_{520nm}-A_{700nm}$

$$\%C = \frac{AT}{AT25} \times 100$$

A = absorbance, AT = absorbance difference between wavelengths of 520 and 700 nm at a certain temperature (C°), C = amount of anthocyanin color, AT 25 = absorbance at a temperature of 25°C

2-8-3- Thermal stability of nanoencapsulated anthocyanin and polymerization

100 mg of the sample was added to 10 ml of acidic solution (pH=2.0) and kept at 90°C for 180 min. Sampling was done every 30 min and after cooling in an ice bath, the absorbance of the solution was measured at the wavelength of λ=520, 700 nm and the color percentage was calculated [26].

$AT=A_{520nm}-A_{700nm}$

$$\%C = \frac{AT}{A0} \times 100$$

A = absorbance, AT = absorbance difference between wavelengths of 520 and 700 nm at a certain time, C = amount of anthocyanin color, A0 = absorbance at zero time, $A_{520\ nm}$ = absorbance at 520 nm wavelength, $A_{700\ nm}$ = absorbance at 700 nm wavelength

In order to measure the polymerization of anthocyanin, it was done according to the method of Tien et al. [26]. Every 30 min, 2.8 ml of the sample was added with 0.2 ml of 1N sodium bisulfite, and 0.2 ml of distilled water was added to the other sample. And after 15 min of storage in the environment, the percentage of polymerization (P) was calculated [27].

$CD= (A_{420nm}– A_{700nm}) + (A_{520nm}– A_{700nm})$

$PC= (A_{420nm}– A_{700nm}) + (A_{520nm}– A_{700nm})$

$$\%P = \frac{PC}{CD} \times 100$$

CD = color density (mixed with water), PC = polymerized color (mixed with 1 N sodium bisulfite solution), $A_{520\ nm}$ = absorbance at a wavelength of 520 nm, $A_{700\ nm}$ = absorbance at a wavelength of 700 nm, A_{420nm} = absorption at a wavelength of 420 nm

The anti-radical activity of the nanoencapsulated extract and TBHQ was performed using stable DPPH radicals according to Saviz et al. method [27]. In this way, 50 µl of nanoencapsulated extract with

different concentrations (100, 300, 500, 700, and 900 μg/ml) were added to 5 ml of 0.004% DPPH solution in methanol and stirred vigorously. After 30 min of heating at 25°C, the light absorption of the samples at a wavelength of 517 nm was witnessed. In this experiment, TBHQ with a concentration of 100 μg/ml was used as a control sample. DPPH free radical inhibition percentage was calculated using the following formula [27].

$$\%I = \frac{\text{A blank} - \text{A sample}}{\text{A blank}} \times 100$$

2-9-Statistical analysis

All statistical methods in this research were analyzed using SAS software version 9.4 by one-way analysis of variance (ANOVA) and to show the minimum significant difference at the confidence level higher than 95% (PValue≤0.05) of Duncan's test was used. The results were shown in the form of average with standard deviation, and in order to reduce the error, all experiments were performed in 3 repetitions. Excel version 2013 software was used to draw the graphs.

3- Results and Discussion

3-1- Extraction efficiency, total anthocyanin content, and total phenol content

The results are shown in Table 1. Pomegranate peel is rich in phenolic and anthocyanin compounds. In research by Maran et al. [28], phenolic and anthocyanin compounds were extracted from fruit pulp with supercritical fluid. By increasing the pressure (200bar), the content of phenolic and anthocyanin compounds increased, so that the maximum amount was 1144 and 231 mg/100g calculated, respectively. Also, in the research of Bossamant et al. [29], who extracted the phenolic compounds of pomegranate peel with supercritical fluid, the highest amount of extracted phenolic compounds was equal to 8.94 mg of gallic acid/g with extraction conditions (20% ethanol, temperature 40°C and pressure 400 bar) reported. They reported the lowest amount of extraction with increasing temperature and decreasing the percentage of ethanol at the same pressure equal to 1.24 mg of gallic acid/ g.

Table 1 Efficiency Extraction, Total Anthocyanin Content and Total Phenolic Content Supercritical CO_2 extract

Extraction	Efficiency Extraction	Total Anthocyanin Content (mg/g)	Total Phenolic Content(mg GA/100g)
Supercritical CO_2	11.499±0.965	3.943±0.133[b]	504.521±2.379[a]

P_{Value}≤0.05

3-2- Assays the properties of nanoencapsulating

3-2-1-The size of nanoencapsulating particles

The size of the nanoencapsulated particles of anthocyanin extract was measured by a Scatteroscope 1 (Qudix Company, South Korea) (Figure 1). As shown in diagram 1, the particle size in the first peak is 179 nm, the distribution percentage of particles in this peak is 34.65%. The particle size of 10% of the sample is less than 100 nm. The average size of nanoencapsulated particles is 308 nm. In a research by Murali et al. [30], they stated that when gum was used as a wall in the microencapsulation of anthocyanin extract, it caused non-uniformity in the distribution of particles.

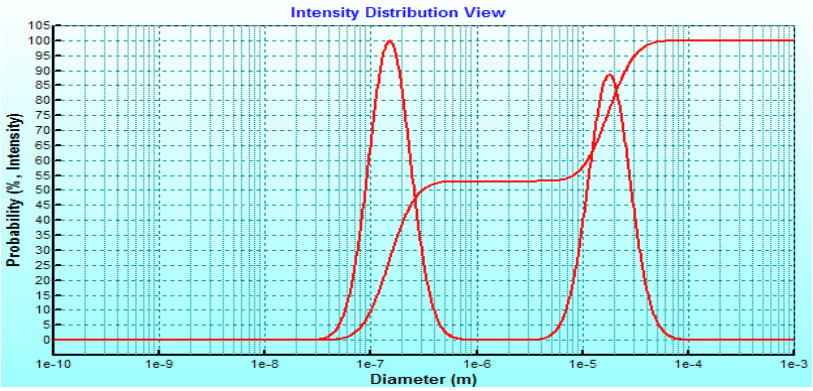

Fig 1 Droplets size distribution of nanoencapsulated extract with *Lepidium perfoliatum* and maltodextrin

3-2-2-Efficiency of nanoencapsulating of phenolic and anthocyanin compounds

The results in Table 2 showed that the combination of Qadumeh Shahri gum and maltodextrin are suitable coatings for anthocyanin compounds obtained from supercritical extraction. In research by Mahdavi et al [31] on microencapsulation of anthocyanin (50%=wall/core) they reported that the microencapsulation efficiency with maltodextrin/gum arabic wall, maltodextrin/gelatin, and maltodextrin was 89%, 87%, and 86% respectively. The efficiency of nanoencapsulating with the same core-to-wall ratio by the gum of the Qadumeh Shahri seed and maltodextrin in supercritical CO_2 extraction was 93.537±0.690%. The results show that the combination of Qadumeh Shahri seed gum with maltodextrin has been bonded with supercritical CO_2 extraction extract compared to other mentioned compounds, and as a result, it has a higher efficiency. Also, the results showed that wall compounds are a more suitable coating for preserving anthocyanin than phenolic compounds. In a report by Bossamant et al. [29], the phenolic compounds of pomegranate peel obtained from supercritical fluid extraction were microcoated with modified starch, and they stated that the highest efficiency of phenolic compounds (ponicalagin) was 72%. In this study, the efficiency of nano-micro coating of phenolic compounds by maltodextrin and Qadumeh Shahri gum was 77.443±0.710%, which is a more suitable coating than modified starch.

Table 2 Nanoencapsulated efficiency of anthocyanin and phenolic compounds of the extract

Sample	Encapsulation Efficiency of Phenol	Encapsulation Efficiency of Anthocyanin
Supercritical CO_2	77.443±0.710^b	93.537±0.690^a

$P_{Value} \leq 0.05$

3-3- Assays of color stability and antioxidant activity

3-3-1- The effect of time and different pH on the release and stability of nanoencapsulated of anthocyanin

The results showed that the release of anthocyanin compounds was affected by pH, and the release of anthocyanin had a significant difference at the 95% level. The release of anthocyanin at pH = 2 was linear. The highest amount of anthocyanin at pH = 2 indicates the stability of anthocyanin in an acidic environment. Researchers stated that in acidic pH, anthocyanin extract is more stable than in alkaline pH, and they also reported a lower amount of anthocyanin with increasing pH [25, 32]. Two important and effective factors in this test are the effectiveness of the wall and anthocyanin about pH. An increase in pH leads to the expansion of the structure of Qadumeh Shahri gum [33], as a result, the release increases with an increase in pH, and anthocyanins are unstable at high pH. As shown in Table 3, the release of anthocyanin at pH = 7 is initially higher than other pHs, but due to its

instability, the release slope decreases, which is an indication of the instability of anthocyanin.

Table 3 Release percentage of anthocyanin compounds of nanoencapsulated extract in 120 minutes at different pH (2.0, 5.0 and 7.0)

	Time(min)					
pH	**0**	**5**	**15**	**30**	**60**	**120**
2	8.852±0.132^{c}	15.844±0.419^{c}	19.751±0.841^{c}	34.336±0.284^{c}	62.383±0.718^{c}	100^{a}
5	10.643±0.227^{a}	24.867±0.475^{b}	57.732±0.899^{a}	73.423±0.938^{a}	84.824±1.352^{a}	87.329±1.293^{c}
7	10.123±0.073^{b}	34.150±0.162^{a}	36.627±0.333^{b}	64.499±0.820^{b}	72.838±1.003^{b}	89.627±0.819^{b}

P_{Value}≤0.05

3-3-2- The effect of temperature on the efficiency of nanoencapsulated anthocyanin color

The results in Table 4 showed that the nanoencapsulated extracts have an increase in color at different temperatures in a period of 30 min. The reason for that is the increase in the release of anthocyanins with increasing temperature, and the highest release occurred at 100℃. Among other factors that cause color, we can mention polymerization with increasing temperature and also the presence of pro-anthocyanidin compounds in the extract. In research by Wang et al [33], they stated that 2.65% of pro-anthocyanidin compounds and 3.92% of flavonoid compounds were extracted from pomegranate peel.

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Table 4 Anthocyanin dye percentage of nanoencapsulated extract at different temperatures (25-100℃) at pH=2.0

	Temperature (℃)							
sample	25	40	50	60	70	80	90	100
Supercritical CO_2 (%)	100^{h}	110.422±0.595^{g}	118.221±1.015^{f}	126.089±0.547^{e}	140.590±0455^{d}	169.290±1.414^{c}	178.175±0.620^{b}	181.360±1.551^{a}

P_{Value}≤0.05

3-3-3-Effect of thermal stability of nanoencapsulated anthocyanin and polymerization

The results (Table 5) showed that heat first leads to an increase in color in the nanoencapsulated
extract. The greatest increase in color was observed at 150 min, and a decrease in color was observed at 180 min. Long-term storage of anthocyanins at high temperatures leads to the destruction of anthocyanins. The degree of polymerization at zero time was 85.636±0.744, which indicates the bonding of anthocyanins in carbon number 4 with other compounds. The highest polymerization was observed after 180 min, 97.694±0.944. In research by Tien et al. [26] on the dry extract of anthocyanin stored in an acidic solution at 45 ℃, they stated that polymerization increased with time and also that anthocyanins are unstable at high temperatures. Tsai et al. [35] reported the highest polymerization rate of 85% at 146 ℃, which confirms the results of this research.

Table 5 Percentage of dye and polymerization of nanoencapsulated anthocyanin extract for 180 minutes at pH=2.0 and temperature 90 ℃

	Heating Time(min)						
Supercritical CO_2	zero	30	60	90	120	150	180
Color (%)	100^{g}	178.175±1.029^{f}	184.789±1.876^{e}	205.061±1.213^{d}	215.796±1.428^{b}	232.152±1.405^{a}	209.938±1.297^{c}
Polymerization (%)	85.636±0.744^{e}	87.384±0.661^{d}	95.812±1.437^{b}	90.384±1.033^{c}	95.814±1.011^{b}	90.635±1.950^{c}	97.694±0.944^{a}

P_{Value}≤0.05

3-3-4-DPPH assays

DPPH is a stable free radical with a purple color that changes to yellow in the presence of antioxidants. In general, the reduction of DPPH free radical capacity is determined by the reduction of its absorbance at 517 nm [36]. By increasing the concentration or degree of hydroxylation of phenolic compounds, DPPH radical inhibition activity also increases and is defined as antioxidant activity [37]. Due to the high sensitivity of free radicals, the whole system is performed in the presence of hydrogen donors in a very small concentration [38]. The inhibitory results of the nanoencapsulated extract obtained by supercritical CO_2 extraction and the synthetic antioxidant TBHQ are shown in Figure 2. The results show that the inhibition percentage of the extract increased with the increase in concentration, and a significant difference was observed between the inhibition the percentage of the nanoencapsulating extract at different concentrations. So IC_{50} = 1.092±0.024 mg/g was reported and also the inhibition percentage of TBHQ in this test with a concentration of 100 ppm was calculated as 60%. As can be seen in Table 4. The antioxidant activity of the extract increased from 100 to 900 ppm. The researchers stated that with the increase in the concentration of pomegranate peel extract and other extracts, the antioxidant activity of the extract increased [39, 40], which is consistent with the results of this study.

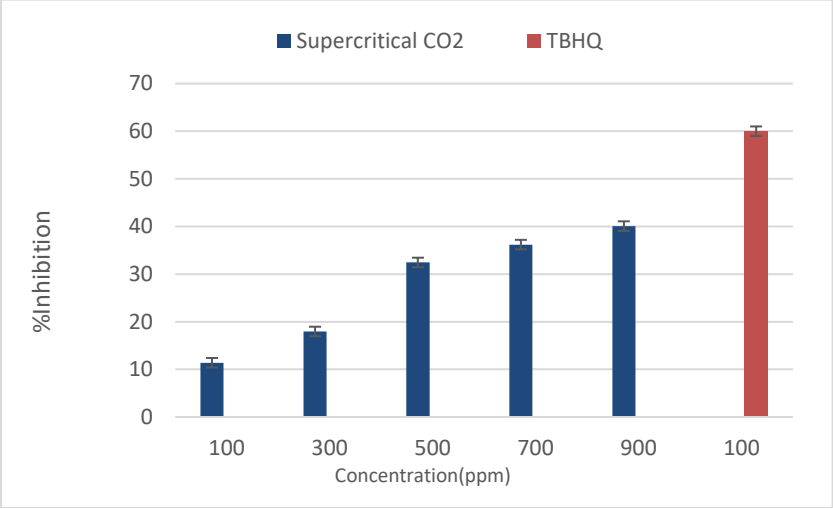

Fig 2 DPPH radical scavenging activity of extract and TBHQ. $P_{Value} \leq 0.05$

4 - Conclusion

In this research, pomegranate peel powder extract was extracted using supercritical CO_2. Ethanol solvent was used to better extract polar compounds, and then it was nanoencapsulated with maltodextrin and the gum of Qodumeh Shahri seed. The results showed that pomegranate peel is rich in phenolic and anthocyanin compounds, and also the combination of the gum of Qodumeh Shahri seed and maltodextrin is considered a suitable encapsulating for anthocyanin and phenolic compounds of pomegranate peel. The extract obtained from supercritical CO_2 extraction had good color stability in acidic pH and temperature and also showed a high radical inhibitory power so the antioxidant activity increased with the increase in the concentration of the extract. Considering the nativeness of pomegranate fruit and the industrial production of pomegranate juice in the country, and as a result of the production of a large amount of pomegranate peel as side waste, which has a

large number of potential antioxidant compounds and anthocyanin pigment, it can be used in the food industry and medicine benefits.

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مقاله علمی_پژوهشی

ارزیابی استخراج CO_2 فوق بحرانی بر پایداری رنگ و فعالیت آنتی‌اکسیدانی عصاره آنتوسیانین نانوریزپوشانی شده پودر پوست انار

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۱-دانشجوی کارشناسی ارشد علوم و مهندسی صنایع غذایی دانشگاه علوم کشاورزی و منابع طبیعی ساری

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چکیده

در این مطالعه، ترکیبات آنتوسیانین و فنولی از پودر پوست انار با CO_2 فوق بحرانی استخراج و از صمغ دانه قدومه شهری و مالتودکسترین به عنوان دیواره نانوریزپوشانی استفاده شد. عصاره نانوریزپوشانی شده با خشک کن انجمادی خشک شد. پایداری رنگ و فعالیت آنتی-اکسیدانی عصاره نانوریزپوشانی ارزیابی گردید. میزان آنتوسیانین و ترکیبات فنولی به ترتیب از طریق pH افتراقی و روش فولین سیوکالتو بررسی شد. اندازه ذرات، راندمان آنتوسیانین نانوریزپوشانی شده، فنول نانورزپوشانی شده، پایداری رنگ در دما و pH مختلف و همچنین فعالیت آنتی‌اکسیدانی از طریق DPPH ارزیابی شد. میزان آنتوسیانین و فنول استخراج شده در این روش به ترتیب۰/۱۳۳± ۳/۹۴۳ میلی‌گرم بر گرم پودر پوست انار، ۲/۳۷۹± ۵۰۴/۵۲۱ میلی‌گرم اسید گالیک در ۱۰۰ گرم پوست انار بود. راندمان نانوریزپوشانی برای آنتوسیانین‌ها بیشتر از فنول‌ها بوده است. رهایش آنتوسیانین در pH مختلف، متفاوت بود به طوری که آنتوسیانین‌ها در pH مختلف رفتار متفاوتی از هم داشتند. با افزایش دما پلیمریزاسیون و رنگ افزایش یافت. فعالیت آنتی‌اکسیدانی عصاره نانوریزپوشانی شده به روش DPPH با افزایش غلظت افزایش یافت و IC50 آن معادل ۰/۰۲۴± ۱/۰۹۲ میلی‌گرم در میلی‌لیتر بود.