Iranian Food Science and Technology Research Journal
Vol. 13, No. 3, Aug. Sep. 2017, p. 14-27

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد 13، شماره 3، مرداد - شهریور 1396، ص. 27-14

Microencapsulation of anthocyanin pigments obtained from seedless barberry (*berberis vulgaris* L.) fruit using freeze drying

Hassan Mirhojati[1], Parvin Sharayei[2*], Reihaneh Ahmadzadeh Ghavidel[3]

Received: 2016.09.05
Accepted: 2017.07.01

Abstract

The acidified ethanol extracts of dried barberry which have a relatively high anthocyanin content (376.28± 1.45 mg c3g/Kg dmp) were freeze dried using maltodextrin (MDX), polyvinyl-pyrrolidone (PVP) and mixture of MDX and calcium alginate (MDX-CaAlg) as a carrier and coating agents. The qualitative attributes of the powders were characterized by their productively encapsulation efficiency, moisture content, bulk density, colour values (L*, a*, b*, C and H°), particle size, total phenolic compounds (TPC), free radical scavenging activity of DPPH (RSA), ferric reducing-antioxidant power (FRAP) and minimized 50% of radical- scavenging activity (IC_{50}). Scanning electron microscope was used for monitoring the structures of the powders. To determine the stability and half- life period of microencapsulated pigments, samples were stored under different storage temperatures (4°C and 25°C) at relative humidity 75%. Results showed that the encapsulated powder containing PVP 8% as wall material represented the best powder quality (p<0.05). The total anthocyanin content of microcapsules decreased during storage at different temperatures, but encapsulated powder containing PVP 8% had the lowest rate of their decrease. Finally, the obtained results showed that microencapsulation by freeze drying could be recommended as a suitable method for stabilizing anthocyanins of barberries' extract.

Keywords: Anthocyanins, Antioxidant activity, Barberry, Carrier agents, Encapsulation, Freeze drier, Stability.

Introduction

The colour is one of the most important qualitative properties of food that has a significant impact on the popular market. Therefore, the application of colour-producing agents in the food industry has a specific position. Since consumers' desire for red foods is more than any others, therefore more attention has been paid to this colour and its sources. Most of the synthetic red colours which are used in the food industry, including Azorubine (also called Carmoisine), Ponceau 4R (also called Brilliant Scarlet 4R or Cochineal Red A) and Allura Red AC have chemical sources with harmful health effects (Anon, 1995). Today, there are more tendencies to use natural colorants instead of synthetic ones, because of their anticancer and antioxidant properties (Andersen *et al*., 2010). Fruits, vegetables, herbs, nuts, spices are known as potential sources of natural pigments.

Seedless barberry (*Berberis vulgaris* L.) is one of the economical sources of anthocyanin pigments (Fallahi et al., 2010). Barberry is a dicotyledonous, perennial species and well-known medicinal shrub in Iran (Shamsa *et al.*, 1999; Rezvani Moghaddam *et al.*, 2007; Ebadi *et al.*, 2010). It is cultivated as a domestic plant in Southern Khorasan province in the eastern part of Iran since 200 years ago (Kafi and Balandari, 2004; Rezvani Moghaddam *et al*., 2007; Aghbashlo *et al.*, 2008; Fallahi *et al.*, 2010). Currently, barberry is cultivated in more than 11000 hectares with annual production about 9200 tons dry fruit in this region (Radmehr, 2010).

Anthocyanins, a large group of water - soluble pigments, are responsible for red to

1. Former MSc Student, Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran.
2. Assistant Professor, Agricultural Engineering Research Department, Khorasan Razavi Agricultural and Natural Resources Research and Education Center, AREEO Mashhad, Iran.
3. Assistant Professor, Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran
(*Corresponding Author: parvin_sharayei@yahoo.com)
DOI: 10.22067/ifstrj.v1396i3.58661

blue colours of fruits and vegetables, are commonly used in acidic solutions as a red pigment in soft drinks, jams, and confectionary and bakery products. Attractive colour and functional properties such as antimicrobial properties, antiemetic, antipyretic, anti-itch, anti-inflammatory, hypertensive, antiarrhythmic (irregular heartbeats), sedative, analgesic and antioxidant properties of anthocyanins make them a good substitute for synthetic pigments in the food industry (Castaoveda- Ovando *et al*., 2009;Khosrokh-avar *et al*., 2010). The stability is an important aspect to consider for use of anthocyanins as antioxidants and colourants in foods. The stability of anthocyanins has been studied by several researchers and it has been shown that they are affected by pH, temperature, light, oxygen, ascorbic acid, enzymes, sugars, degradation products and metallicions (Patras *et al*., 2010; Bakowska *et al*., 2003).

Encapsulation of anthocyanin extracts may enhance their stability for the use as food colorants (Ersus and Yurdagel, 2007). Microencapsulation is a technique by which the sensitive ingredients are packed within a coating or wall material. The wall material protects the sensitive ingredients against adverse reaction and controls release of the ingredients. In addition, microencapsulation can convert liquids into powders, which are easy to handle. Numerous wall materials or encapsulating agents are available for food application (Shahidi and Han, 1993; Desai and Park, 2005; Pu, 2010). Different kinds of carbohydrates (starch, maltodextrin, dextrin, sucrose and annular dextrin) cellulose (carboxymethyl cellulose, methyl cellulose, ethyl cellulose, nitrocellulose and acetate cellulose), gums (acacia, sodium alginate and carrageenan), fats (wax, paraffin, beeswax, diglyceride, monoglyceride and oils), proteins (gluten, casein, gelatine, albumin, haemoglobin and peptides) and polymers (polypropylene and polyvinyl acetate) are commonly used as wall materials or carrier substances (Kaushik and Roos, 2007). Maltodextrins with different dextrose equivalent (DE) are used as wall material mainly due to their high solubility in water, low viscosity, bland flavor, and colorless solutions (Gibbs *et al*., 1999; Ersus and Yurdagel, 2007; Saénz *et al*., 2009; Avaltroni *et al*., 2004). Polyvinylpyrrolidone (PVP) is a light flaky hygroscopic powder, readily absorbing up to 40% of its weight, has excellent wetting properties and readily forms film. These make it good as a coating, or an additive, or a wall material in encapsulation processes. Furthermore, PVP has amorphous properties and does not form crystals in encapsulated particles (Selim *et al*., 2000). Its safety and biocompatibility have been reported in general biological studies (Xu *et al*., 2010).

In various experiments on encapsulation of anthocyanins, different materials and compounds have been used in spray drying (Cai and Corke, 2000; Robert *et al*., 2010, Mahdavi *et al*., 2016 a and b) or freeze drying techniques such as glucan gel (Xiong *et al*., 2006), maltodextrins with different DEs (Ersus and Yurdagel, 2007; Fang and Bhandari, 2011; Saenz *et al*., 2009, Laine *et al*., 2008; Coralia *et al*., 2010; Nayak *et al*., 2010, Robert *et al*., 2010), maltodextrin and inulin (Bakowska-Barczak and Kolodziejczyk, 2010), maltodextrin, ascorbic acid and mesquite gum (Kandansamy and Somasundaram, 2012), maltodextrin, Arabic gum, and tapioca starch (Tonon *et al*., 2010) and PVP (Xiaoyi *et al*., 2010; Selim *et al*., 2000). But, encapsulation of barberry anthocyanins by freeze drying has not been investigated yet. Therefore, encapsulation of barberry anthocyanins with freeze drying technique through wall materials of maltodextrins and Polyvinylpyrrolidone and then, evaluating the stability of anthocyanin and colour of encapsulated powders during storage could be an initial step to produce natural and highly stable food colorants.

Material and methods

Raw materials

Barberry fruit (dried with moisture content of 12.37 ± 0.85%) were purchased from a local market in Mashhad. After cleaning, samples were packed in low-density polyethylene film with a thickness of 140 microns. Samples were

kept in a freezer at -18°C for further analyses. Polyvinylpyrrolidone 40 (molecular weight of 40,000 Daltons), maltodextrin (dextrose equivalent 16.5-19.5), Calcium alginate salt and other materials were purchased from Merck, Sigma- Aldrich, and Caledon companies.

Extraction and concentration of anthocyanins

Ethanolic extracts of anthocyanins were prepared as follows: frozen fruits were ground with Armfield ball grinder without thawing. Four volume of 96% ethanol 1:1.5 N HCl (85:15 v/v) blend was added to barberries to extract anthocyanins and then were subjected to ultrasonic waves (Hielscher, Germany, UP400S, 24 KHz) for 10 min with 20% intensity. After 24 h stiring at ambient temperature (25± 1◦C), samples were filtered through a filter paper Whatman grade 1. Extraction solvent was evaporated at 45°C under vacuum by the rotary evaporator (Laborota 4000 efficient, Germany) until a level of 8± 0.3% soluble solids was reached (Ersus & Yurdagel, 2007).

Preparation of microencapsulated powders

Wall materials maltodextrin (MDX) 20% (w/v), polyvinylpyrrolidone (PVP) 8 and 15% (w/v) and mixture of maltodextrin 10% and calcium alginate (MDX-CaAlg) 0.1% (w/v) dissolved in distilled water at ambient temperature (25± 1°C). The solution was kept in the refrigerator for complete hydration in 24 h. Then, extract of anthocyanins from barberry and wall materials were mixed in a weight ratio (w/w) of 1:5 (extract: wall material), then mixed with a rotor-stator (120 rpm, 30 min), (Wang *et al.,* 2013). Total anthocyanins of samples were measured through differential pH method (Giusti and Wrolstad, 2001) before drying. The solutions were dried in a freeze dryer (Operon- Korea) for 48 h (−55°C, 0.15mm Hg pressure). Dried materials were ground using a pestle and mortar and passed through a 0.71mm mesh and stored in brown glass bottles with screwed caps in a freezer (−18◦C) until usage. For the preparation of the blank sample, the concentrated extract (8± 0.3% soluble solids) without wall materials freeze- dried in similar conditions with other samples.

Physical and chemical analyses

Moisture content

Moisture of the samples was determined using appropriate device working by infrared (MX-50, Japan) heating at 105± 1°C until a constant weight (Kaushik and Roos, 2007).

Bulk density

The bulk density of powders was measured by weighing 20g of samples and pouring them into a 20ml graduated cylinder. The bulk density was calculated by dividing the powder mass by the volume occupied in the cylinder (g/cm^3), (Tonon *et al*., 2010).

Colour measurement

The color of encapsulated powders was measured using computer vision method adapted from Koocheki *et al.,* (2009) with some modifications. The indices of Hue angle ($H^o= \tan^{-1}(b/a)$), Chroma ($C= (a^2+ b^2)^{1/2}$) was calculated and the mean of three replicates were reported.

Total anthocyanin content

Total anthocyanin content (TAC) was evaluated by pH differential method (Giusti and Wrolstad, 2001). 0.2g of each sample was dissolved in 10 ml of distilled water in a volume flask far from the light. Absorbance was measured at 510 nm and 700nm. Total anthocyanin pigment concentration in the powder (TAC) was calculated according to the following equations:

$$TAC(mg/kg): DA \times Mw \times D_f \times 1000/(M_a \times L) \quad (1)$$

$$DA=(A_{510}\_A_{700})\,pH_{1.0}\,\_(A_{510}\_A_{700})\,H_{4.0} \quad (2)$$

With DA: difference of absorbance; Mw: molecular weight for cyaniding-3-glucoside (449.2 g mol^{-1}); D_f: the dilution factor of the samples; Ma: molar absorptive of cyaniding-3-glucoside (26,900 $Lcm^{-1}mg^{-1}$); and L: Diameter of spectrophotometer cell (cm). Results were expressed as mg of Cyaniding-3-

glucoside Equivalents per Kg of dry matter of powder (mg c3g/Kg dmp).

Encapsulation efficiency

Encapsulation efficiency was calculated using following equation (Najaf Najafi, 2010).

$$\text{Encapsulation efficiency}(\%) = \frac{\text{Total mass of the capsules obtained before microencapsulation}}{\text{Total mass of solids obtained after microencapsulation}} \times 100 \quad (3)$$

Particles size

Laser diffraction particle size analyser (SAL, D-2101, Shimadzu, Japan) was used to measure particle size in terms of diameter. The samples were dispersed in hexane using ultrasonic waves (24 kHz and 20% intensity) for 2 min and then particles size was directly determined (Parrarud and Pranee, 2010).

Scanning electron microscopy

Particle structures of the encapsulated powders were evaluated by scanning electron microscope (LEO-1450, Germany). Powders were attached to SEM stubs using a 2-sided adhesive tape and left in desiccators containing phosphorous pentoxide for 48h. Samples were coated with 200˚A gold under vacuum before the examination. SEM was operated at an accelerating voltage of 10 kV.

Glass transition

Samples of freeze-dried pigment powders were equilibrated at 75% RH (NaCl saturated solution) for 1 week. Glass transition temperature (Tg) was determined using a differential scanning calorimeter (DSC1 Mettler Toledo, Switzerland). The temperature range was set from -40°C to 200°C with a heating rate of 10°C/min (Cai and Corke, 2000). Five milligrams powder was weighed directly into a DSC sample pan and sealed. An empty pan was used as a reference.

Total phenolic compounds

The total phenolic content (TPC) of the extracts was determined using the Folin–Ciocalteau method (Singleton *et al.*, 1999). 100 µl of the sample solutions (100 mg in 10 mL of Methanol), 6 ml of twice distilled water and 500 µl of Folin-Ciocalteau reagent were added; after waiting between 8 s and 8 min at room temperature, 1.5 ml of sodium carbonate (20% w/v) were added. The extracts were mixed and allowed to stand for 30 min at room temperature before measuring the absorbance at 765 nm. A mixture of water and reagents was used as blank. Results were reported as mg Gallic acid equivalents per kg, (mg GA/kg). A calibration curve of Gallic acid in methanol was performed in the concentration range of 0.04–0.40 mg per mL.

Determination of antioxidant activity

Total antioxidant activity was estimated by two standard procedures, DPPH and FRAPS assays. In DPPH radical- scavenging assay, various concentrations of the methanolic sample solutions (1 ml) were mixed with 1 ml of methanolic solution containing DPPH radicals (0.006 % w/w). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm (Ramadan *et al.*, 2003). The radical-scavenging activity (%RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\%RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100 \quad (4)$$

Where A_S is the absorbance of the solution when the sample has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. α- Tocopherol was used as the positive reference while methanol was used as negative one.

Ferric reducing-antioxidant power (FRAP) was measured using 2, 4, 6-Tripyridyl-S-triazine (TPTZ) according to Benzie & Strain, (1996). Acetate buffer (0.3 M, pH 3.6) was prepared by dissolving 3.1 g $C_2H_3O_2Na$ -$3H_2O$

and 16 mL of acetic acid in 1 L of distilled water. TPTZ solution was prepared by dissolving 23.4 mg of TPTZ in 7.5 mL of 40 mM HCl solution. Ferric solution (20 mM) was prepared using $FeCl_3$-$6H_2O$. The final working FRAP reagent was prepared freshly by mixing acetate buffer, TPTZ, and ferric solutions at a ratio of 10:1:1. In brief, 900 mL FRAP working reagent was mixed with 90 mL distilled water and was warmed to 37°C in a water bath. The reagent control reading was recorded at 595 nm, followed by adding 30 mL of sample solutions (100 mg in 10 mL of methanol). The absorbance was taken at 595 nm, against the control solution. A standard curve was prepared using different concentrations of $FeSO_4$-$7H_2O$ (200– 2000 µmol per L). All solutions were freshly prepared. The results were expressed in µmol Fe^{2+} per liter.

Storage stability evaluation

Encapsulated powders were stored at controlled temperature and humidity and in the absence of light for 42 days. Samples of 3 g of each powder were transferred to closed low-density polyethylene bags (5cm× 5cm). The samples were placed on sealed desiccator containing saturated NaCl solution to obtain humidity values of 76% and placed at 4 and 25°C temperature (Gradinarua *et al*., 2003).

Statistical analysis

All experiments and measurements were carried out in triplicate, and data were subjected to analysis of variance (ANOVA).

ANOVA and regression analyses were performed according to the MStatC and Excel software. Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

Results and discussion

Physical properties of encapsulated powders

Table 1 summarizes the results for physical properties of samples with different wall materials.

Table 1. Physical properties of microencapsulated powders

Parameters	MDX	MDX -CaAlg	8% PVP	15% PVP
Efficiency (%)	69.26± 1.23d	71.26±0.98c	92.54±0.15a	80.64±1.68b
Moisture (%)	4.77 ±0.12c	7.84±0.16b	8.34±0.59a	4.66±0.72d
Density (kg/m^3)	513 ±1.34c	607±1.87b	654±2.70a	469±1.90d
*L**	54.22 ±0.78b	46.07±1.03c	51.81±1.44b	78.45±0.51d
*a**	29.81 ±0.56a	30.26±1.12a	14.23±0.26b	5.06±0.41c
*b**	23.14± 0.48a	22.10±1.85a	14.60±0.25b	10.21±0.31c
C	37.73± 0.12	37.47±0.18	20.38±0.05	11.40±0.14
H°	37.82 ±0.23	36.14±0.10	45.73±0.28	63.64±0.03
Particles size (µm)	19.71± 0.36d	49.20±0.50a	36.91±1.01c	46.53±0.19b
Glass transition temperature (°C)	23.17±0.69b	10.66±0.78d	19.49±0.82c	46.70±0.92a

Means ± SD (standard deviation) with the same lowercase letters are not significantly different at $P < 0.05$.

The type and concentration of the wall had significant effects on physical characteristics of encapsulated powders. It has been reported that type and concentration of polymers as wall material affect the encapsulation efficiency (Jalil and Nixon, 1990). As can be seen from table 1, unexpectedly, encapsulation efficiency decreased with increasing concentration of PVP from 8 to 15%. It has been proven that increase in encapsulation efficiency (more mass microencapsulation), depends on moisture content. The final moisture content of microencapsulated powders is affected by different factors such as the number of groups linking wall material to water, sealing methods of containers and storage temperature (Najaf Najafi, 2010). This might be due to low moisture content in microencapsulated powder prepared with PVP 15% compared with PVP 8%. In addition, low encapsulation efficiency in microencapsulated powders prepared with MDX compared with

MDX- CaAlg might be due to gelatinous texture of the wall on account of calcium alginate (Belscak *et al*., 2011). Cai and Corke, (2000) in their work on encapsulated anthocyanin extract from Amaranthus plant obtained similar results in the effect of moisture content (1.25 to5.67%) on microencapsulation efficiency.

The bulk density of encapsulated powders had significant differences ($P<0.05$), and varied between 469 (related to the samples with 8% PVP) to 654 g/cm^3 (related to the samples with 15% PVP). Size of the crashed particles, frangibility, and moisture content and flow properties may influence the bulk density of produced powders. The heavier the material, more easily it accommodates into the spaces between the particles, thus occupying less space and resulting in a higher bulk density (Tonon *et al.*, 2010).

Changes in the color indices (a*, b*, L*, C, and H◦) in microencapsulated powders with different wall materials are shown in table 1. Differences in wall materials had significant effects on *L** values ($p<0.05$). The lightness increased with increasing PVP concentration from 8 to 15% (from 51.81 to 78.45 units). a* and C values of samples with 15% PVP were found to be significantly lower than the samples produced with MDX, MDX-CaAlg, and 8% PVP. The chroma value was proportional to the strength of the colour and indicates its degree of saturation (Maskan, 2001). H° values of PVP (8 and15%) samples were found to be significantly higher than other samples. Higher a* and lower H° indicates bright and purple shade of red color. It is concluded that increasing of PVP, the color of anthocyanins becomes paler.

Table 2 shows the particle size distribution for the powders produced with the different carrier agents. The diameters of encapsulated powders varied from 19 to 49µm. This increase in particle sizes is related to the molecule size of each carrier agent and also longitudinal breakage and sublimation of ice crystals. Our results are in accordance with Zuidam and Shimoni, (2010) and Heinzelmanna *et al*. (2000) findings. These results coincided with the changes in bulk density. The same behavior was observed by. According to Keogh *et al*. (2003) and Shrestha *et al*. (2007), the particles with higher median diameter occupied higher volume because of increasing interstitial air content between particles.

Glass transition temperature (T_g) of PVP15% was the highest. Lower T_g of carrier agents caused higher hygroscopicity of the encapsulated powders. Maltodextrins are the components with low molecular weight and contain shorter chains and more hydrophilic groups. Therefore, powders prepared with carrier agent MDX, MDX- CaAlg had rubbery and soft texture in ambient temperature. In rubbery and soft polymers, core materials tend to release or transfer from wall materials. Also, Glass transition temperature decreased with increasing moisture content.

Chemical properties of encapsulated powders

The Chemical properties of encapsulated powders are given in Table 2.

Table 2. Chemical properties of microencapsulated powders

Parameters	MDX	MDX-CaAlg	8% PVP	15% PVP
Total Anthocyanin content (mg c3g/Kg dmp)	160.85±2.35d	178.30±0.67c	303.50±0.54a	214.40±0.62b
TPC (mg GA/kg)	13.78±0.56b	16.78±0.83a	15.19±0.38ab	13.69±0.11b
RSA (%)	49.83±0.32a	61.98±0.459b	79.96±0.16d	59.75±0.17c
FRAP (Fe^{2+}, µmol/l)	1055.30±3.56d	1727.05±2.45c	3949.20±2.71a	1942.08±1.98b

Means ± SD (standard deviation) with the same lowercase letters are not significantly different at $P < 0.05$.

The highest total anthocyanin content (303.50 mg c3g/Kg dmp) and DPPH radical-scavenging activity (RSA, 79.96%) belonged to the encapsulated powders with 8% PVP carrier. The highest total phenolic compounds with no statistical differences belonged to the encapsulated powders with MDX- CaAlg (61.98 mg GA/kg) and 8% PVP (15.19 mg

GA/kg) as a carrier agents. The direct correlation between antioxidant activity and anthocyanin or phenolic compounds content was observed. Although phenolic compounds have several critical biological activities such as free radical scavenging, are often considered from the antioxidant activity point of view (Aparicio *et al*., 1999; Morello *et al*., 2009). Previous researchers reported antioxidant activity has high correlation with anthocyanin content and total phenolic composition of food materials (Brand-Williams *et al*., 1995; Sanchez *et al*., 2006; Belscak *et al*., 2011; Parejo *et al*., 2000; Camire *et al*., 2002; Moyer *et al*., 2002; Wanget al., 1997).

The FRAP test is a simple, reproducible, rapid, and inexpensive procedure that measures the ability of anti- oxidative compounds to convert the ferric ion Fe^{3+} to ferrous Fe^{2+}, as a measure of total antioxidant capacity (Prior and Cao, 1999). Many similarities were found to exist between the results of the FRAP test and those of the DPPH radical-scavenging activity assay. The encapsulated powders with wall material 8% PVP had a FRAP quantity (3949.20 µmol Fe^{2+} per liter) significantly higher than that of the others.

Microstructure of encapsulated powders

The outer topography of the freeze-dried capsules was affected by wall composition (Fig 1, A-D). All encapsulated powders except for MDX- CaAlg showed amorphous glassy shapes which were formed during dehydration, grounding, and sieving of freeze-dried solid materials. These glassy structures can protect entrapped anthocyanins from exposure to heat and oxygen (Roos, 1995). According to the figure 1, encapsulated powders with MDX are more spherical and have the smooth surface and fewer wrinkles compared with powders with PVP. These differences might be due to the difference between covering power and spatial structure of MDX and PVP. Furthermore, wrinkles might be due to mechanical stresses induced by atomization or drying conditions. Wall composition and drying speed, especially at early stages, affect surface characteristics of encapsulated powders (Lee and Rosenberg, 2000). Encapsulated powders with MDX- CaAlg as a wall material had globular shape, because of gelatinous properties of alginate. Similar results were obtained by Semyonov *et al*. (2010).

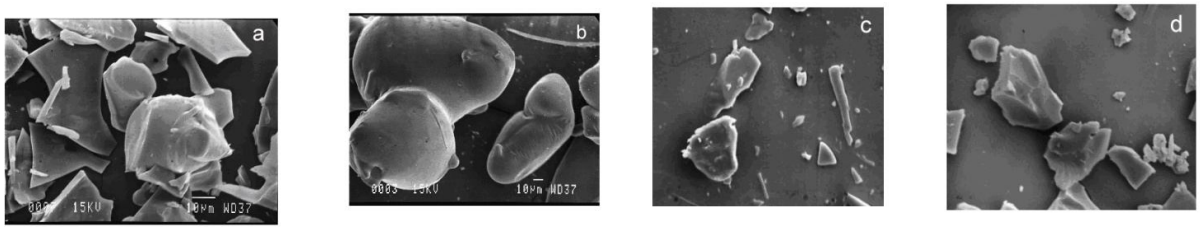

Fig.1. Scanning electron microscopy (5000×), a: MDX, b: MDX-CaAlg c: 8% PVP, d: 15% PVP.

Storage stability evaluation of barberry microencapsulated anthocyanins

Stability of anthocyanins in freeze-dried microencapsulated powders was evaluated under various conditions of storage temperature (Figs 2, 3). The increase of temperature led to faster anthocyanin degradation, at the end of 6 weeks storage period, the pink colour of the samples was not changed at 4˚C where it was turned to brown colour at 25˚C, which was expected since these pigments are highly thermosensitive. This negative influence of temperature on anthocyanin stability has been observed by many researchers (Pacheco-Palencia *et al*., 2007; Ersus and Yurdagel, 2007; Tnon *et al*., 2010). The faster anthocyanin degradation at the higher temperature may also be related to the presence of sugars, together with proteins, which can result in the Maillard reaction (non-enzymatic browning), which generally occurs during food processing at high temperatures or during food storage for a long time. According

to Von Elbe and Schwartz (1996), the presence of sugars or products resulting from their degradation can accelerate the anthocyanin degradation, since this reaction rate follows the rate of conversion of sugars to furfural. Furfural, which is a derivative from aldopentoses, as well as hydroxyl methyl furfural, which is a derivative from keto-hexoses, are products resulting from the Maillard reaction that condenses together with the anthocyanins, leading to the formation of compounds with brown coloration. This reaction is highly dependent on temperature, being accelerated by the presence of oxygen and occurring more frequently in fruit juices.

The kinetics of degradation of anthocyanins was monitored over the storage period, rate constants and half- life values of reactions were determined (Table 3). Previous works on anthocyanin degradation showed that reaction followed in first- order degradation kinetics (Calvi and Francis, 1978; Cemerog *et al.*, 1994; Kırca *et al.*, 2003; Markasi, 1974). An increase in storage temperature led to an increase in rate constants.

Rate constants were predicted with the use of equations as:

$$\text{Log}(C_o/C_t) = k \times t \text{ and } t_{1/2} = {}^{-}\ln 0.5/k \quad (5)$$

Where k is the slope, C_o is the initial anthocyanin content, C_t is the anthocyanin content at a specific time and t is time. Half-life ($t_{1/2}$) values were then determined and are given in Table 3.

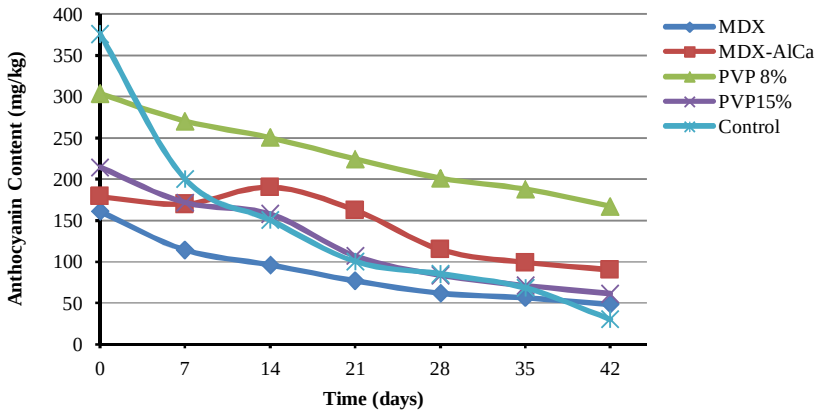

Fig.2 Anthocyanin content of freeze dried microencapsulated powders with different wall materials during storage at 25°C

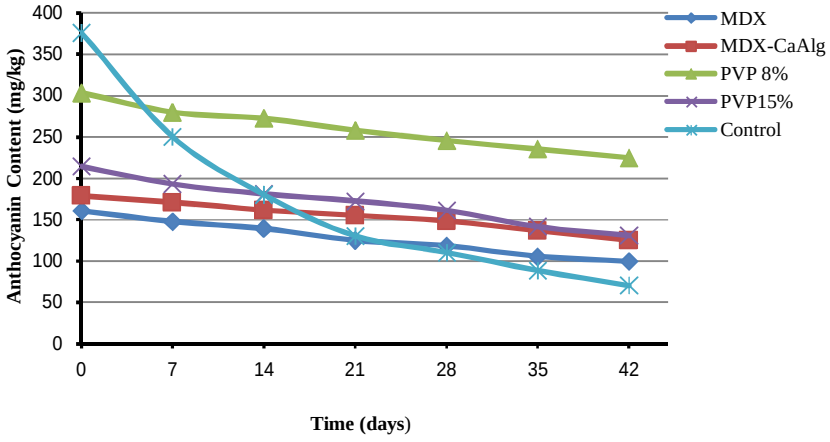

Fig.3 Anthocyanin content of freeze dried microencapsulated powders with different wall materials during storage at 4°C.

Table 3. Kinetic degradation data for freeze - dried microencapsulated powders with different wall materials under different storage conditions

Storage condition	Microencapsulated powders	K (1/days)	Half live(days)	R^2
4°C	MDX	0.011	63	0.99
	MDX-CaAlg	0.008	87	0.97
	PVP8%	0.006	116	0.99
	PVP15%	0.011	63	0.98
	Control	0.039	18	0.98
25°C	MDX	0.027	26	0.97
	MDX-CaAlg	0.018	39	0.83
	PVP8%	0.014	50	0.99
	PVP15%	0.031	22	0.98
	Control	0.052	13	0.97

The half- life of anthocyanins at storage temperature (4°C) was found 63- 115 days where it is 29- 50 days for 25°C storage temperature. Concerning to the different carrier agents used, the particles produced with PVP 8% had the highest half-life, in the conditions studied, followed by those produced with MDX-CaAl. The powders prepared with MDX showed higher degradation rates and, consequently, lower half-lives, with respect to the others

Conclusion

In conclusion, encapsulation of barberries' extract with freeze-drying technique and wall materials MDX, MDX-CaAlg and PVP (with different concentration) could protect anthocyanins during storage. PVP with 8% concentration as a wall material gave the highest anthocyanin content powder at the end of the drying process. Storage at 4°C increased half -life of freeze dried anthocyanin pigments 2.3- 2.8 times according to 25°C storage temperature.

Acknowledgement

The authors would like to thank Khorasan Razavi Agricultural and Natural Resources Research and Education Center and Eksir-e-Gol-e-Sorkh Company for financial support.

References

Aghbashlo, M., Kianmehr, M.H., Samimi-Akhijahani, H. 2008. Influence of drying conditions on the effective moisture diffusivity, energy of activation and energy consumption during the thin-layer drying of berberis fruit (*Berberidaceae*). *Energy Conversion and Management* 49: 2865–2871.

Andersen, M., Jordheim M., Editors-in-Chief: Lew, M., & Hung-Wen, L. 2010. 3.16– Chemistry of flavonoid-based colours in plants. Comprehensive natural productsII (pp. 547–614). Oxford: Elsevier.

Anonymous. 1995. Edible colours, Iran National Standard, No. 740. Standards and Industrial Research Institute, Iran.

Anonymous. 2007. Fruit juices, Test methods, Iran National Standard. No. 2685, Standards and Industrial Research Institute, Iran.

Aparicio, R., Roda, L., Albi, M.A., Gutierrez, F. 1999. Effect of various compounds on virgin olive oil stability measured by Rancimat. *Journal of Agricultural Food Chemistry* 47: 4150-4155.

Avaltroni, F., Bouquerand, P.E. Normand, V. 2004. Maltodextrin molecular weight distribution influence on the glass transition temperature and viscosity in aqueous solutions. *Carbohydrate Polymer* 58: 323–324.

Bakowska-Barczak, A. M., Kolodziejczyk, P. P. 2010. Black currant polyphenols: Their storage stability and microencapsulation. *Industrial Crops and Products* 34(2): 1301–1309.

Belscak-Cvitanovic, A., Stojanovic, R., Manojlovic, V., Komes, D., Juranovic, C.I., Nedovic, V., and Bugarski, B. 2011. Encapsulation of polyphenolic antioxidants from medicinal plant extracts in alginate–chitosan system enhanced with ascorbic acid by electrostatic extrusion. *Food Research International* 44: 1094–1101.

Benzie, I.F.F., and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. *Analytical Biochemistry* 239: 70-76.

Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology* 28(1): 25–31.

Cai, Y.Z., and Corke, H. 2000. Production and properties of spray-dried *Amaranthus Betacyanin* pigments. *Journal of* Food *Science* 65**:** 1248-1252**.**

Calvi, P., Francis, F. J. 1978. Stability of concord grape (V. *labrusca*) anthocyanins in model systems. Journal of Food Science 43: 1448–1456.

Camire, M. E., Chaovanalikit, A., Dougherty, M. P., Briggs, J. 2002. Blueberry and grape antocyanins as breakfast cereal colorants. *Journal of Food Science* 67(1): 438-441.

Castaoveda-Ovando, A., Pacheco-Hernondez, M. d. L., Priez-Hernandez, M. E.,Rodriguez, J. A., Galoan-Vidal, C. A. 2009. Chemical studies of anthocyanins: A review. *Food Chemistry* 113(4): 859–871.

Cemeroglu, B., Velioglu, S., Isık, S. 1994. Degradation kinetics of anthocyanins in sour cherry juice and concentrate. *Journal of Food Science* 59(6): 1216–1219.

Coralia, O., Baudilio, A., Silke, H., Jose, C., Peter, W., Alicia, P. 2010. Microencapsulation by spray- drying of anthocyanin pigments from corozo (*Bactris guineensis*) fruit. *Journal of Agricultural Food Chemistry* 58: 6977–6985.

Desai, K.G.H., and Park, H.J. 2005. Recent developments in microencapsulation of food ingredients. *Drying Technology* 23:1361-1394.

Ebadi, A., Rezaei, M., Fatahi, R., 2010. Mechanism of seedlessness in Iranian seedless barberry (*Berberis vulgaris* L. var. *asperm*a). *Scientia Horticulturae* 125: 486–493.

Ersus, S., and Yurdagel, U. 2007. Microencapsulation of anthocyanin pigments of black carrot (Daucus *carota* L.) by spray drier. *Food Engineering* 80: 805-812.

Fallahi, J., Rezvni Moghaddam, P., Nasiri Mohallati, M. 2010. The effect of harvesting date on quantitative and qualitative of barberry (*Berberis vulgaris*). *Iranian Field Crops Research* 8: 225–234.

Fang, Z., and Bhandari, B. 2011. Effect of spray drying and storage on the stability of bayberry polyphenols. *Food Chemistry* 129(3): 1139–1147.

Gibbs, B.F., Kermasha, S., Alli, I., Mulligan, C.N., 1999. Encapsulation in the food industry: a review. *International Journal of Food Science and N*utrition 50: 213–224.

Giusti, M.M., and Wrolstad, R.E. 2001. Anthocyanins characterization and measurement with UV-visible spectroscopy. In: Wrolstad, R.E. (Ed.), Current Protocols in Food Analytical Chemistry, 1st ed. John Wiley & Sons, New York

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. 1999. Analysis of total polyphenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology* 299: 152–178.

Gradinarua G, C.G. Biliaderisb, S. Kallithrakac, P. Kefalasa, C. Garcia-Viguerad. 2003. Thermal stability of Hibiscus sabdariffa L. anthocyanins in solution and in solid state: effects of copigmentation and glass transition. Food Chemistry 83 , 423–436.

Heinzelmanna, K., Frankea, K., Jensenb, B., Haahrb, A. 2000. Protection of fish oil from oxidation by microencapsulation using freeze-drying Techniques. *European Journal of Lipid Science and Technology* 102:114–121.

Jalil, R., and Nixon, J.R. 1990. Microencapsulation using poly (DL-lactic acid). II: Effect of polymer molecular weight on the microcapsule properties. *Journal of Microencapsulation* **7**:

245-54.

Kafi, M., and Balandari, A. 2004. Barberry (*Berberis vulgaris*): Production and Processing. Ferdowsi University Press, Mashhad, Iran.

Kandansamy, K., and Somasundaram, P. D. 2012. Microencapsulation of colours by spray drying – a review. *International Journal of Food Engineering* 8(2):1556-3758.

Kaushik, V., And Roos, Y.H. 2007. Limonene encapsulation in freeze- drying of gum Arabic-sucrose - gelatin systems. *LWT, Food science and. Technology* 40: 1381-1391.

Keogh, M. K., Murray, C. A., OKennedy, B. T. 2003. Effects of ultrafiltration of whole milk on some properties of spray-dried milk powders. *International Dairy Journal* 13(12): 995–1002.

Khosrokhavar, R., Ahmadiani, A., Shamsa, F. 2010. Antihistaminic and anticholinergic activity of metabolic extract of barberry fruit (*Berberis vulgaris*) in the Guinea- Pig Ileum. *Journal of Medicinal Plants Research* 9: 99-105.

Kırca, A., Ozkan, M., Cemeroglu, B. 2003. Thermal stability of black carrot anthocyanins in blond orange juice. In: International Congress of Information Technology in Agriculture, Food and Environment (pp.525–528). 7–10 October, Izmir, Turkey.

Koncic, Z.M., Kermer, D., Kraloric, K., Kosales, I. 2010. Evaluation of antioxidant activities and phenolic content of *Berberis Vulgaris* L. and *Berberis croatica Hor*vat. *Food and Chemical Toxicology* 48: 2176- 2180.

Koocheki, A., Taherian, A. R., Razavi, S. M. A., Bostan, A. 2009. Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from Lepidium perfoliatum seeds. *Food Hydrocolloids* 23(8): 2369–2379.

Laine, P., Petri, K., Heinonen, M., and Jouppila, J. 2008. Storage stability of microencapsulated cloudberry (*Rubus chamaemorus*) phenolics. *Journal of Agricultural Food Chemistry* 56: 11251–11261.

Lee, S.J., and Rosenberg, M. 2000. Preparation and some properties of water-insoluble, whey protein-based microcapsules. *Journal of Microencapsulation* 17: 29-44.

Mahdavi, S.A., Jafari, S.M., Assadpoor, E., Dehnad, D., 2016. Microencapsulation optimization of natural anthocyanins with maltodextrin, gum Arabic and gelatin. International Journal of Biology Macromolecules 85, 379-385.

Mahdavi, S.A., Jafari, S.M., Assadpoor, E., Ghorbani, M. 2016.Storage stability of encapsulated barberry's anthocyanin and its application in jelly formulation. Journal of Food Engineering 181 :59- 66

Markasis, P. 1974. Anthocyanins and their stability in foods. *Critical Reviews in Food Technology* 4(4): 437–456.

Maskan, M. 2001. Kinetics of color change of kiwi fruits during hot air and microwave drying. *Journal of Food Engineering* 48, 169–175.

Morello, J.R., Motilva, M.J., Tovar, M.J., Romero, M.P. 2004. Changes in commercial virgin olive oil (CV *Arbequina*) during storage, with special emphasis on the phenolic fraction. *Food Chem*istry 85:357-364.

Moyer, R. A., Hummer, K. E., Finn, C. E., Frei, B., Wrolstad, R. E. 2002. Anthocyanins, phenolics and antioxidant capacity in diverse small fruits. *Journal of Agricultural and Food Chemistry* 50: 519–525.

Najaf Najafi, M. 2010. Usage of ultrasonic waves in the production of emulsion and microencapsulation of effective compounds of Cardamom Oil. PhD dissertation. Ferdowsi University of Mashhad.

Nayak, C.A., Srinivas, P., Rastogi, N. K. 2010. Characterization of anthocyanins from *Garcinia indica* Choisy. *Food Chem*istry 118: 719-724.

Nogala-Kalucka, M., Korczak, J., Dratwia, M., Lampart-Szczapa, E., Siger, A., Buchowski, M. 2005. Changes in antioxidant activity and free radical scavenging potential of rosemary extract

and tocopherols in isolated rapeseed oil triacylglycerol during accelerated tests. *Food Chem*istry 93: 227–235.

Pacheco-Palencia, L. A., Hawken, P., Talcott, S. T. 2007. Phytochemical, antioxidant and pigment stability of acai (*Euterpe oleracea Mart.*) as affected by clarification, ascorbic acid fortification and storage. *Food Research International* 40(5): 620–628.

Pacheco-Palencia, L. A., Hawken, P., Talcott, S. T. 2007. Phytochemical, antioxidant and pigment stability of acai (*Euterpe oleracea Mart.*) as affected by clarification, ascorbic acid fortification and storage. *Food Research International* 40(5): 620–628.

Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A., Diamantidis, G. 2006. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chem*istry 102: 777-783.

Parrarud, S., and Pranee, A. 2010. Microencapsulation of Zn- Chlorophyll pigment from pandan leaf by spray drying and its characteristic. *International Food Research Journal* 17:1031-1042.

Patras, A., Brunton, N. P., O'Donnell, C., Tiwari, B. K. 2010. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology* 21(1): 3–11.

Prior, R. L., and Cao, G. 1999. In vivo total antioxidant capacity: Comparison of different analytical methods. *Free Radical Biology and Medicine* 27: 1173–1181.

Pu, J. 2010. Development of stable microencapsulated astaxanthin powders using extracted astaxanthin from crawfish and shrimp byproducts. A thesis, 13-15.

Radmehr, A. 2010. Results of Sampling Survey of Garden Products in 2009. Ministry of Agriculture, Tehran, Iran.

Ramadan, M. F., Kroh, L. W., Morsel, J. T. 2003. RSA of black cumin (*Nigella sativa* L.), coriander (Coriandrum *sativum* L.) and niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions. *Journal of Agricultural and Food Chemistry* 51: 6961–6969.

Rezvani Moghaddam, P., Huda, A.K.S., Parvez, Q., Koocheki, A. 2007. Indigenous knowledge in agriculture with particular reference to medicinal crop production in Khorasan, Iran. In: World Association for Sustainable Development (WASD) Conference. Fifth International Conference, Griffith University, Brisbane, Australia.

Robert, P., Gorena, T., Romero, N., Sepulveda, E., Carmen Saenz, G. 2010. Encapsulation of polyphenols and anthocyanins from pomegranate (*Punica granatum*) by spray drying. *International Journal of Food Science & Technology* 45:1386- 1394.

Roos, Y. 1995. Characterization of food polymers using state diagrams. *Journal of Food Engineering* 24(3): 339–360.

Saenz, C., Tapia, S., Chavez, J., Robert, P. 2009. Microencapsulation by spray drying of bioactive compounds from cactus pear (*Opuntia ficus*). *Food Chemistry* 114(2): 616–622.

Saénz, C., Tapia, S., Chvez, J., Robert, P. 2009. Microencapsulation by spray drying of bioactive compounds from cactus pear (Opuntia ficus-indica). *Food Chemistry* 114:616–622.

Sanchez, F.D., Santos lopez, E.M., kerstupp, S.F., Ibarra, R.V., Scheinvar, l. 2006. Colorant extraction from red prickly pear (*Opuntia Lasiacantha*) for food application. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 5: 1330-1337.

Selim, K., Tsimidou, M., Biliaderis, C.G. 2000. Kinetic studies of degradation of saffron carotenoids encapsulated amorphous polymer matrices. *Food Chem*istry 71: 199-206.

Semyonov, D., Ramon, O., Kaplun, Z., Levin-Brener, L., Gurevich, N., Shimoni, E. 2010. Microencapsulation of Lactobacillus paracasei by spray freeze drying. *Food Research International* 43: 193-202.

Shahidi, F., and Han, X.-Q. 1993. Encapsulation of food ingredients. *Critical Review of Food Science and Nutrition* 33: 501–547.

Shamsa, F., Ahmadiani, A., Khosrokhavar, R., 1999. Antihistaminic and anticholinergic activity

of barberry fruit (*Berberis vulgaris*) in the guinea-pig ileum. *Journal of Ethnopharmacology* 64: 161–166.

Tavasoli-kafrani, M.H. 2010. Antioxidant properties of skin oil of wild pistachio (*Pistacia atlantica* subsp. mutica). Master thesis, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran.

Tonon, R. V., Brabet, C., Hubinger, M. D. 2010. Anthocyanin stability and antioxidant activity of spray-dried aCai (*Euterpe oleracea Mart*.) juice produced with different carrier agents. *Food Research International* 43(3): 907–914.

Tonon, R. V., Brabet, C., Hubinger, M. D. 2010. Anthocyanin stability and antioxidant activity of spray-dried aCai (*Euterpe oleracea Mart*.) juice produced with different carrier agents. *Food Research International* 43(3): 907–914.

Von Elbe, J. H., Schwartz, S. J. 1996. Colorants. In O. R. Fennema (Ed.), *Food Chemistry* (3rd Ed.). New York: Marcel Dekker.

Wang, H., Cao, G., Prior, R. L. 1997. Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry* 44: 701–705.

Wang,G., Chen, J., Shi, Y. 2013. Preparation of microencapsulated xanthophyll for improving solubility and stability by nanoencapsulation. *Journal of Food Engineering* 117:82–88

Xiong, S., Melton, L. D., Easteal, A. J., Siew, D. 2006. Stability and antioxidant activity of black currant anthocyanins in solution and encapsulated in glucan gel. *Journal of Agricultural and Food Chemistry* 54(17): 6201–6208.

Xu, X., Wang, Q., Choi, H.C., Kim, Y.H. 2010. Encapsulation of iron nanoparticles with pvp nanofibrous membranes to maintain their catalytic activity. *Membrane science* 348:231-237.

Zuidam, N.J., and Shimoni, E. 2010. Overview of microencapsulates for use in food products or processes and methods to make them. In: Encapsulation technologies for active food ingredients and food processing, edited by Zuidam, N.J., Nedovic, V. A. 5,6.

نشریه پژوهشهای علوم و صنایع غذایی ایران
جلد 13، شماره 3، مرداد - شهریور 1396، ص. 27-14

Iranian Food Science and Technology Research Journal
Vol. 13, No. 3, Aug. Sep. 2017, p. 14-27

ریزپوشانی رنگدانهٔ آنتوسیانینی زرشک با استفاده از خشککن انجمادی

حسن میرحجتی[1]- پروین شرایعی*[2]- ریحانه احمدزاده[3]

تاریخ دریافت: 1395/06/15
تاریخ پذیرش: 1396/04/10

چکیده

در این پژوهش، عصارهٔ الکلی اسیدی حاصل از زرشک که حاوی مقدار نسبتا بالایی آنتوسیانین بود (376/26±1/45 بر حسب سیانیدین -3-گلیکوزید بر وزن خشک) با استفاده از دیواره‌های مالتودکسترین، پل‌وینیل‌پیرولیدون و مخلوط مالتو دکسترین و آلژینات‌کلسیم و با روش خشک‌کردن انجمادی ریزپوشانی شدند. خصوصیات ریزکپسول‌های حاصل نظیر راندمان ریزپوشانی، رطوبت، دانسیته‌توده، مولفه‌های‌رنگی، اندازه قطرذرات، مقدارترکیبات‌آنتوسیانینی، مقدار ترکیبات فنلی، مقدار ترکیبات گیرنده رادیکال آزاد، مقدار ترکیبات احیاءکننده‌آهن III و دمای انتقال شیشه‌ای مورد ارزیابی قرار گرفتند. از میکروسکوپ الکترونی نیز برای بررسی ریزساختار ریزکپسول‌ها استفاده شد. همچنین میزان رهایش ترکیبات آنتوسیانینی طی 42 روز نگهداری در دمای 4 و 25 درجه سلسیوس و رطوبت نسبی 75درصد مورد پایش قرار گرفت. نتایج نشان داد که ریزکپسول‌های تهیه شده با مادهٔ دیوارهٔ پلی‌وینیل‌پیرولیدون 8 درصد، دارای خصوصیات کمی و کیفی بهتری نسبت به سایر ریزکپسول‌ها بودند ($p<0/05$). میزان آنتوسیانین کل ریزکپسول‌ها طی مدت نگهداری کاهش یافت، اما ریزکپسول‌های حاوی مادهٔ دیوارهٔ پلی‌وینیل‌پیرولیدون 8 درصد، کمترین میزان کاهش ترکیبات آنتوسیانینی را داشتند. به‌طور کلی نتایج نشان داد ریزپوشانی ترکیبات آنتوسیانینی زرشک با استفاده از خشک‌کن انجمادی، روشی مناسب و قابل توصیه برای حفظ و پایداری این ترکیبات می‌باشد.

واژه‌های کلیدی: ترکیبات دیواره، پایداری، خشک¬کن انجمادی، ریزپوشانی، زرشک، فعالیت آنتی¬اکسیدانی.

1- دانش‌آموخته کارشناسی ارشد، دانشگاه آزاد اسلامی، واحد قوچان، گروه صنایع غذایی.
2- استادیار، پژوهش بخش تحقیقات فنی و مهندسی کشاورزی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان خراسان رضوی، سازمان تحقیقات، آموزش و ترویج کشاورزی، مشهد، ایران.
3- استادیار، گروه صنایع غذایی، دانشگاه آزاد اسلامی، واحد قوچان، قوچان، ایران.
(*- نویسنده مسئول : Email: parvin_sharayei@yahoo.com)