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Microencapsulation of saffron petal anthocyanins with cress seed gum compared with Arabic gum through freeze drying

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ABSTRACT

In this research, encapsulation efficiency of cress seed gum (CSG) as a native hydrocolloid was compared with Arabic gum (AG) and maltodextrin (dextrose equivalent of 20 (M20), and 7 (M7)) for saffron (*Crocus sativus*) petal's extract by freeze drying method. Combinations of CSG-M20, AG-M20, and M7-M20 with ratios of 50:50 and M20 alone (100%) were used as wall materials. A mixture of 1:5 (based on dry matter) between core (concentrated anthocyanin extract of saffron petal) and wall materials were freeze dried and stability of encapsulated anthocyanins along with color parameters (a^* , b^* , L^* , C , H° and TCD) of final powders were measured during 10 weeks of storage (at 35 °C as an accelerated method). Total anthocyanins were determined through pH differential method every week. Four prepared formulations of encapsulated powders didn't show any significant differences ($P > 0.01$) in terms of total anthocyanin content measured immediately after production and after 10 weeks storage. AG-M20 mixture and M20 alone showed the highest and lowest TCD, respectively. The mixture of CSG-M20 in comparison with AG-M20 and M20 had the same protecting effect ($P < 0.01$) but showed a relatively high TCD (9.33).

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1. Introduction

Most colors used in the food industry have chemical and synthetic sources and because of their harmful effects, there is a more tendency toward applying natural dyes. Anthocyanins are appropriate alternatives for synthetic dyes due to their antimicrobial and anticancer properties; they are bright and attractive colors (Anderson et al., 2010). Anthocyanins which are isolated from food resources are very vulnerable and their stability is influenced by various factors such as their chemical structure, concentration, pH, storage temperature, oxygen, presence of light, presence of enzymes, solvents, flavonoids, proteins, and metal ions (Stintzing & Carle, 2004). Anthocyanins are found in all plant tissues including leaves, stems, flowers and fruits and in products such as eggplant, etc. (Ignat, Volf, & Popa, 2012; Andersen & Jordheim, 2010).

Saffron (*Crocus sativus*) is the most expensive spice of the world. Only 12 g of dried saffron is obtained from each kilogram of its flowers (Sarfarazi, Jafari, & Rajabzadeh, 2015; Mehrnia, Jafaria, Makhmal-Zadeh, & Maghsoudlou, 2016). On the other hand, an average 86.4% of wet weight or 96.4% of dry weight of saffron

flowers is related to petals (Hemmati, 2001). Saffron petals usually do not have a commercial value but contain large amounts of anthocyanins, flavonoids and glycosides. Thus, these petals can be a good source of natural dyes applicable in pharmaceuticals, confectionery, and soft drinks (Kafi et al., 2006; Khazaei, Jafari, Ghorbani, Hemmati Kakhki, & Sarfarazi, 2015). Microencapsulation has defined as a process for capturing active substances within other materials to produce particles with dimensions of a few micrometers to a few nanometers (Jafari, Assadpour, He, & Bhandari, 2008). In microencapsulation using freeze-drying (which was used in this study), core and wall material mixtures are homogenized and then freeze dried for producing microencapsulated ingredients with favorable properties. Despite the long necessary time for freeze-drying (around 20 h), this technique is simple and suitable for microencapsulation of sensitive natural oils, colors, and aromas, such as water-soluble bioactive components (Fang & Bhandari, 2011). Some researchers have introduced freeze drying microencapsulation method as an efficient way for protecting heat-sensitive compounds such as anthocyanins (Akhavan, Jafari, Ghorbani, & Assadpour, 2014).

Various wall materials have been applied depending on the type and nature of core materials, targeted usage of microencapsulated ingredients, and also type of microencapsulation technique, including microencapsulation of saffron petals extract with maltodextrin and Arabic gum by freeze drying method (Khazaei, Jafari, Ghorbani,

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& Hemmati Khakki, 2014), microencapsulation of *Morinda citrifolia* L. with κ -carrageenan and maltodextrin by spray drying method (Krishnaiah, Sarbatly, & Nithyanandam, 2011), limonene microencapsulation with Arabic gum, sucrose and gelatin by freeze drying method (Kaushik and Roos, 2007), microencapsulation of ethanolic extract of *Vaccinium macrocarpon* with mesquite gum as wall material by spray drying (Jimenez-Aguilar et al., 2011), and microencapsulation of calyces of *Hibiscus sabdariffa* extract and blue berry with glucan and pullulan gels, respectively by freeze drying (Xiong, Melton, Easteal, & Siew, 2006). Recently, Rajabi, Ghorbani, Jafari, Sadeghi, and Rajabzadeh (2015) and Faridi Esfanjani, Jafari, Assadpoor, and Mohammadi (2015) worked on spray drying microencapsulation and nanoencapsulation of saffron extract, respectively. Their results revealed that it is possible to extend the shelf-life of saffron bioactive components through encapsulation.

Arabic Gum (AG), maltodextrin with different DEs, and some proteins are the most common wall materials used in microencapsulation (Shahidi & Han, 1993). So far, generally AG compared with other wall materials has been used further in spray-drying microencapsulation method which could be due to its low cost, high solubility, low viscosity, no color or smell, its facilitated usage, and inhibition of oxidation reactions which makes it a suitable wall material to protect sensitive compounds after encapsulation. In recent years due to the high price of AG, its inaccessibility and impurities, application of AG has been decreased. Researchers are trying to use AG in combination with other wall materials or completely replace it with suitable and novel biopolymers (Jafari, Assadpoor, He, & Bhandari, 2008).

Cress with scientific name of *Lipidum sativum* is an annual plant which abundantly grows in Middle East and America. Cress seeds adsorb water rapidly and produces large amounts of mucilage or resinous with high molecular weights (540 kD). Its chemical analysis approved that cress seed gum (CSG) has a high ratio of mannose to galactose (8:2). CSG can be used as an alternative for some hydrocolloids while it has some advantages such as health benefits and natural origin (Karazhiyan, Razavi, & Phillips, 2012). In addition to lower costs and its suitable rheological properties for food processing, CSG is able to form stable gels which is a basic feature for foods production. To the best of our knowledge, there have been no studies on the microencapsulation properties of cress seed gum.

Therefore, in this study in order to evaluate encapsulation efficiency of CSG compared with AG, and maltodextrin in microencapsulation of saffron petals extract, at first concentrated anthocyanins were mixed with combinations of these wall materials with determined ratios and then powdered using freeze-drying method. Finally, physical properties, color features and stability of microencapsulated anthocyanins were studied during storage over 10 weeks at 35 °C.

2. Materials and methods

Materials used consisted of maltodextrin with a high dextrose equivalent (DE = 16.5–19.5) and low DE (DE = 4–7) manufactured by Sigma-Aldrich company in USA, and AG made by Samchun Chemicals Company in Korea. Other chemicals and solvents were analytical grade with high purity and purchased from local companies.

2.1. Saffron petals and their physico-chemical characteristics

Saffron flowers were collected in November 2013 from a specified field in Torbat-E-Heydarieh located in Razavi Khorasan, Iran. Flowers were completely dried after separating stamens and pistils

in a dark and warm room in front of fan. Then, they were ground and sieved (16 mesh) and kept in dark bottles with tight lids in a refrigerator until next tests. Some physico-chemical characteristics of the petals were evaluated such as moisture, fat, protein, total ash, (AOAC, 2005), total fiber (Butterfly, 1385) total sugar (AOAC, 2006) and total monomeric anthocyanins with pH differential method (Giusti & Wrolstad, 2001).

2.2. Preparation of concentrated anthocyanins

To provide core material, 20 g of dried petals (0.01 g) was weighed and mixed with 300 ml of 50% acidified ethanol and kept in a dark place at ambient temperature (25 °C). After 24 h, it was filtered by vacuum filtration. For complete extraction of anthocyanins, remaining petals on filter paper washed with the same volume of solvent. Before concentration, pH of the extract reduced to 3.5 ± 0.2 (with 0.1 N HCL) to decrease destructive effect of heat on anthocyanins. Acidified extract was concentrated by a rotary evaporator (Buchi—Switzerland) for 30 min at 40 °C up to 10 percent concentration of solid materials.

2.3. Cress seed gum extraction

Preparation of CSG powder was performed according to the research conducted by Karazhiyan et al. (2012). Cleaned cress seeds were soaked in 40 °C distilled water at a ratio of 1:37. The mixture was adjusted to pH = 7 (by NaOH 0.1 M). Seeds and water were mixed for 18 min during soaking. Hydrocolloid of swollen grains was removed from the surface of seeds passing through the extractor equipped with a rotating plate. Extracted gum was filtered and dried in 60 °C in an oven (Mettler, Germany). Dried gum was milled and sieved (18 mesh), packed and stored in a cool and dry place.

2.4. Microencapsulation of saffron anthocyanins

Wall material used in this study were maltodextrin with DE 16.5–19.5 (M20), maltodextrin with DE 4–7 (M7), cress seed gum (CSG), and Arabic gum (AG). All wall materials were dissolved completely in distilled water to make a 40% TS solution. For complete hydration, solutions were kept in a refrigerator for 24 h before encapsulation. Wall materials and the core (saffron anthocyanins) were mixed together based on the proportion of solid materials with a 5:1 ratio. Wall materials were mixed according to four formulations presented in Table 1. The mixtures were mixed on a magnetic stirrer (IKA, Germany) for 20 min at 120 rpm. Also pH was reduced to 2 with 1.5 N hydrochloric acid before freeze drying (Berg, Bretz, Hubbermann, & Schwarz, 2012). A freeze dryer (–86 °C, Operan—Korea) at 5 mm Hg pressure for 42 h was used. Produced porous solid materials were crashed in a pestle and mortar and passed through a 25 mesh sieve and immediately transferred into brown glass containers with screwed caps then stored in a freezer (–18 °C).

In this study, concentrated anthocyanins (core materials without encapsulation) were also freeze dried and used as control sample. Their preparation and freeze drying conditions were exactly the same as encapsulated ones.

2.5. Determination of physical properties of final powders

The moisture content of the powders was determined by vacuum oven drying (Schutzart, Germany) at 70 °C and 500 mbar for 24 h, followed by cooling to room temperature in desiccators, in the presence of excess amount of silica gel (Fang & Bhandari, 2011). Hygroscopic moisture of every 2 g powder samples was measured under saturated solutions of Na₂SO₄. After 1 week, hygroscopic

Table 1
Different formulations of microencapsulated saffron anthocyanins.

Formulation no.	Abbreviation	Type of wall materials and their content (%) in formula			
		Maltodextrin DE = 20	Maltodextrin DE = 7	Arabic gum	Cress seed gum
1	MD7-MD20	50	50	0	0
2	MD20	100	0	0	0
3	AG-MD20	50	0	50	0
4	CSG-MD20	50	0	0	50

moisture was expressed as g of moisture per 100g dry solids (g/100g) to determine hygroscopicity (Ersus & Yurdagel, 2007). Water activity of powders was determined using a Lab master water activity meter (Minova Sina, Switzerland). Temperature was maintained at 25 ± 0.1 °C during the tests. Bulk density (ρ_{bulk}) of powders was measured by weighing 10 g of samples and pouring them into a 10 ml graduated cylinder. The bulk density was calculated through dividing the powder mass by the volume occupied in the cylinder (g/cm³) (Tonon, Brabet, & Hubinger, 2010).

2.6. Measurement of total anthocyanins content of freeze dried powders

0.2 ± 0.01 g of each powder was weighed and mixed in a volumetric flask (10 ml) with distilled water. Total anthocyanin content (TAC) was measured using differential pH method (Fang & Bhandari, 2011) and reported based on mg of cyanidin 3-glucoside per gram dry matter of powder.

2.7. Evaluating the stability of encapsulated anthocyanins during storage

To investigate effect of microencapsulation and different wall materials on stability of anthocyanin pigments through storage, produced powders along with control sample were poured in the same weight to brown glass bottles with dimensions of 35 × 80 mm with screwed caps and tight lids. Then, they were kept in an incubator with constant temperature of 35 ± 1 °C (as an accelerated method proposed by Tonon et al., 2010). TAC of powders was measured every week for 10 weeks.

2.8. Color analysis of samples

To evaluate the effect of storage on color features of microencapsulated powders, color of anthocyanin samples were measured immediately after production and after 10 weeks storage. Measurement was according to proposed method by et al. Hoseini, Jafari, Mirzaei, Asghari, and Akhavan (2015) with some modifications. A digital camera (Canon A550, Kuala Lumpur, Malaysia), an imaging box and image analysis software (Clemex Vision Professional, PE4, Longueuil, Canada) were used to evaluate color features. To prepare liquid samples, 0.5 g of each powder was dissolved thoroughly in 25 ml of deionized water. Constant volume of the solution was poured in glass petri dish with a small diameter and the photographs were taken. Hue angle value ($H^\circ = \tan^{-1}(b/a)$), chroma ($C = \sqrt{a^2 + b^2}$) and total color difference ($\text{TCD} = [(L_0 - L)^2 + a^2 + b^2]$) were calculated as mean of three replicates in each sample.

2.9. Statistical analysis

General linear model in SPSS software (version 16, 2007) was used to analyze variance and determine the difference between average data. Confidence levels of $P \leq 0.01$ and $P \leq 0.05$ were applied for evaluating statistical significance of anthocyanins

stability and color characteristics, respectively. Excel software (Microsoft Office, 2007) was used for drawing graphs. Least significant difference test (LSD) and Duncan's test were used for significant difference between the average data and to analyze scattering of data, respectively. All tests were performed in duplicate and the results were based on average of data.

3. Results and discussion

3.1. Physico-chemical characteristics

Some physico-chemical properties of saffron petals are given in Fig. 1. TAC in petals extract was 1712.19 mg per liter of extract based on cyanidin-3-glucoside. Anthocyanin content (as measured by the pH differential method based on cyaniding-3-glucoside) in other natural resources, has been reported for cranberry juice equal to 13.6, red wine 201.6, natural colors 640.8, strawberry extract 63.6, raspberry juice 336.7, and elderberry 3006.8 mg/l of extract (Kirca et al., 2007). Also, Algarrá et al. (2014) reported that TAC of black carrot was equal to 1750 mg per kg of fresh carrot. As it can be seen, saffron petals compared with other anthocyanins sources had a high anthocyanin content and higher than many other natural color resources (Hemmati, 2001).

3.2. Physical properties of encapsulated anthocyanin powders

Table 2 summarizes the results for physical properties of four encapsulated formulations with different wall materials. As it can be seen, highest bulk density was related to the sample with 50% CSG. Particle size, texture, and flow properties of freeze dried powders can influence their bulk density. Tonon et al. (2010) revealed that bulk density of spray dried tapioca starch powders was higher compared with maltodextrin (DE=10 and 20) and Arabic gum. Higher molecular weight of starch makes particles to better movement in empty areas then the total volume decrease and the bulk density increases.

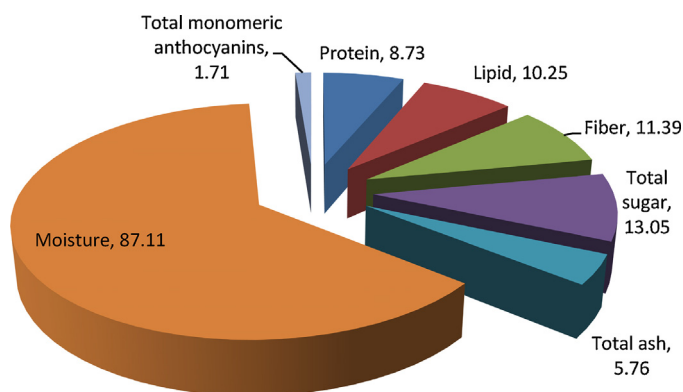


Fig. 1. Physicochemical composition of saffron petals. Measured units was g/100g dry matter except for total monomeric anthocyanins which was in g/l extract. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Physical properties for encapsulated anthocyanin powders.

Matrix components	Physical properties of encapsulated powders			
	Moisture (%)	Hygroscopicity (%)	aw	Bulk density (g/ml)
M20, M7 (50, 50)	3.13 ± 0.07 ^d	61.74 ± 2.6 ^c	0.126 ± 0.01 ^a	1.87 ± 0.02 ^a
M20 (100)	1.88 ± 0.06 ^a	46.60 ± 1.61 ^a	0.289 ± 0.03 ^b	1.90 ± 0.02 ^a
M20, AG (50, 50)	2.31 ± 0.04 ^b	47.26 ± 0.41 ^a	0.069 ± 0.02 ^a	2.16 ± 0.01 ^b
M20, CSG (50, 50)	2.89 ± 0.05 ^c	52.49 ± 1.35 ^b	0.082 ± 0.03 ^a	2.85 ± 0.03 ^c

^{a-c}: Means with different letters in column differed significantly ($P < 0.05$) by Duncan's test.

Table 3
Color indices (a^* , b^* , L^* , C , H° and TCD) in different formulas of encapsulated saffron petal's extract after production (0) and after storage at 35 °C for 10 weeks (t).

Matrix components	a^*		b^*		L^*		C		H°		TCD
	a^*_0	a^*_t	b^*_0	b^*_t	L^*_0	L^*_t	C_0	C_t	H°_0	H°_t	
M20, M7 (50, 50)	26.34	26.60	-6.50	-7.15	62.28	57.65	27.13	27.54	-13.86	-15.04	5.11 ^a
M20, GSC (50, 50)	35.02	36.98	-9.45	-10.22	50.25	41.46	36.27	38.37	-15.10	-15.44	9.33 ^c
M20 (100)	37.73	39.91	-8.56	-9.18	56.95	51.82	38.69	40.96	-12.78	-12.95 [*]	5.64 ^b
M20, AG (50, 50)	40.10	25.83	-7.40	-6.69	55.69	58.93	40.79	26.68	-10.46	-14.52	14.61 ^e
Control sample	60.12	61.73	-13.14	-16.25	66.19	56.47	61.54	63.82	-12.33	-14.75	10.33 ^d

^{a-e}: Means with different letters in column differed significantly ($p < 0.05$) by Duncan's test.

The variation of aw in all samples was low (maximum aw was 0.29) and there were no significant differences ($P > 0.01$) among aw of final powders except in 100% M20 sample which had the highest aw (0.29). aw of powders formulated with AG and CSG were lower (0.07 and 0.08, respectively) but close to each other. Differences in hygroscopicity and moisture content were also significant ($P < 0.05$). There were no special trends in moisture content or hygroscopicity of samples.

3.3. Stability of encapsulated anthocyanins during storage

Trend of TAC differences for microencapsulated anthocyanin powders and control sample during 10 weeks storage at 35 °C have been shown in Fig. 2. The results were calculated based on the average of two replicates by comparing each pairs of anthocyanin contents for each powder in every sampling to its original value immediately after production. In all microencapsulated powders, we did not observe a significant difference ($P > 0.01$) between TAC of powders immediately after production and its value in 10th week. Also, LSD tests showed that these changes were not significant at 0.01 levels.

For control sample (un-encapsulated anthocyanins), TAC after 10 weeks storage compared with its initial level showed a 33% reduction from 181.16 ± 12.3 to 120.78 ± 9.7 mg/g anthocyanins. Reduction of TAC for control sample and lack of significant anthocyanin changes in microencapsulated powders revealed the efficiency of microencapsulation in terms of protecting saffron natural pigments during storage conditions and showed encapsulating properties of wall materials on anthocyanins.

Regarding the effect of wall material formulations on anthocyanin stability, there was no significant differences ($P > 0.01$) among four different produced powders. Presence of oxygen in the environment intensifies anthocyanins degradation caused by other factors, as far as removal of oxygen can prevent thermal degradation and presence of both factors together are the most destructive agents for anthocyanins (Jackman, Yada, Tung, & Speers, 1987); factors that sustained anthocyanin extracts through encapsulation were the wall material biopolymers as physical barriers in front of destructive agents. Higher humidity increases the molecular motions and accelerates chemical reactions and physical degradations of anthocyanins. Generally, high viscosity of matrix in glassy form (around 1012 Pa s) doesn't allow phenomena with diffusion-controlled reactions to be occurred. Therefore, glassy form of

encapsulated materials and reducing the moisture content and water activity of the extract could be considered as another factor for improving anthocyanin stability of powders (Tonon et al., 2010).

After 10 weeks of storage at 35 °C away from light and moisture in 4 different formulations made from anthocyanin extracts of saffron petals, no caking or agglomeration phenomena were observed. To conclude, our results of monitoring TAC showed high stability of the pigments during storage. In other words, microencapsulation by freeze drying method was efficient for stabilizing saffron petal's anthocyanins during storage of 10 weeks. In comparing the wall materials stabilizing effect for M20, M7, AG with CSG when constituted 50% of wall compounds, significant differences ($P \leq 0.01$) were not observed and could be concluded that all four wall materials were appropriate for protecting the saffron anthocyanin during storage.

3.4. Color features of encapsulated powders

The results of mean values for colorimetric factors (a^* , b^* , L^* , C , H° and TCD) of microencapsulated powders immediately after production and after 10 weeks has been shown in Table 3. High value of color parameter a^* in all powders was due to the large amount of anthocyanins in extracts of saffron petals (Jimenez-Aguilar et al., 2011). Our data indicated that in samples with 50% gum Arabic, differences of a^* and b^* after storage were significant ($P < 0.05$). For other samples, a decrease in b^* value (increase in blueness) were observed although the differences were not significant ($P > 0.05$). In a research conducted by Skrede (1985) on black grape juice, b^* factor after storage was reduced too. Decline of L^* that was observed in the majority of samples was related to dark and turbid solutions obtained from dissolved powders.

Chroma valued (C factor) after storage were increased except in the case of samples built by AG; in other words, the color of solutions have got more vivid. H° less than 10° indicated the red color of powders (Jimenez-Aguilar et al., 2011) and its significant reduction ($P < 0.05$) after storage indicated that the samples were more blue in color. The least TCD was related to encapsulated powders containing maltodextrin (5.637 and 5.107). Samples composed of AG and CSG, and control sample showed the highest value of TCD (14.609, 9.33 and 10.33, respectively). Maskan (2006) considering TCD of each sample before and after heat treatment concluded more color changes during storage which results in more TCD of samples.

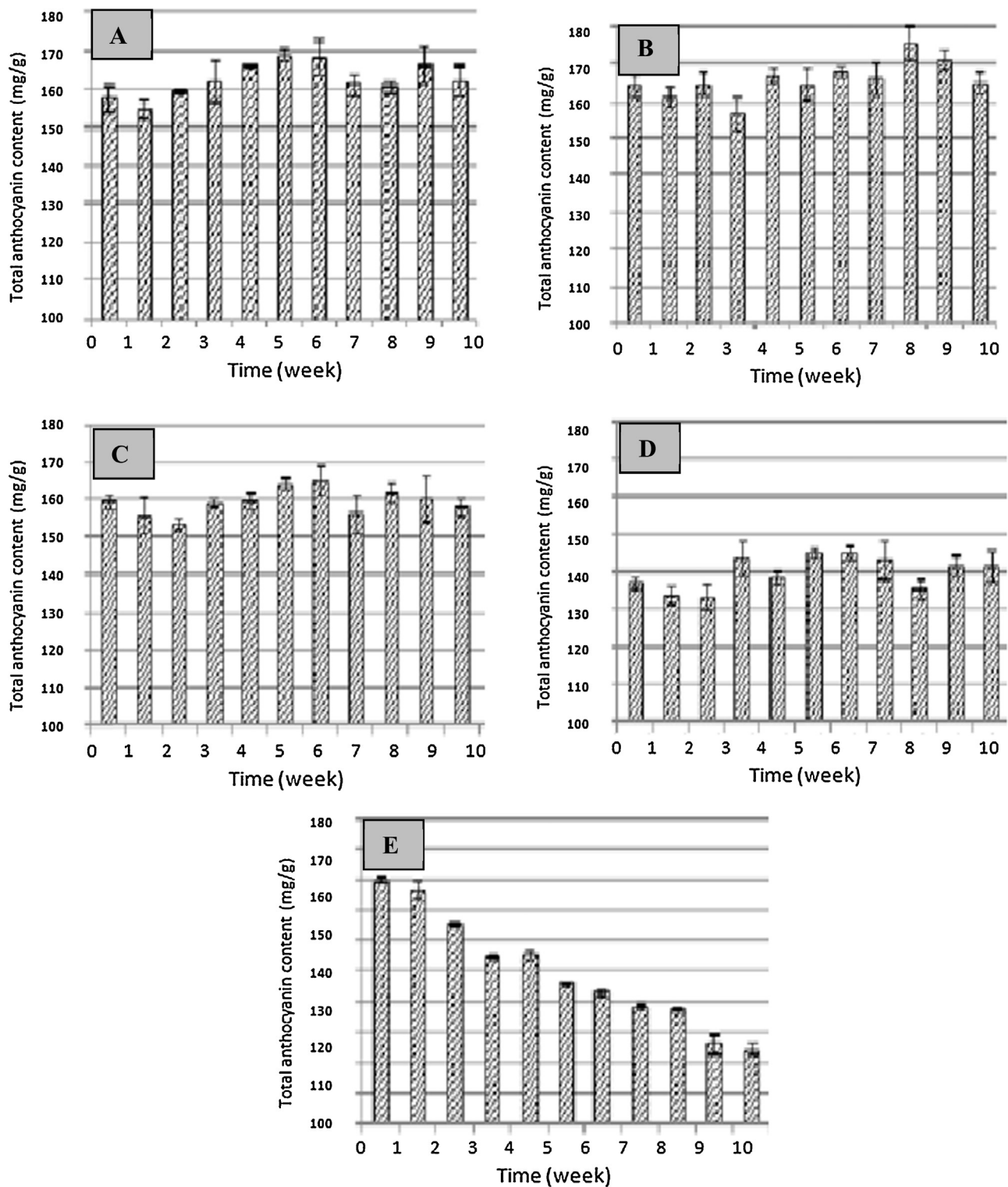


Fig. 2. Anthocyanin content of freeze dried microencapsulated powders with different wall materials during storage at 35 °C: (A) maltodextrin (100% M20), (B) maltodextrin and Arabic gum (50% M20, 50% AG), (C) maltodextrins (50% M20, 50% M7), (D) maltodextrin and Cress seed gum (50% M20, 50% CSG), (E) control sample.

TCDs can be resulted by adverse reactions of wall material with anthocyanins, or different anthocyanins with each other during heat treatment by copigmentation reactions or totally structural changes of the materials (Jimenez-Aguilar et al., 2011). Also Bchir et al. (2012) observed that in pomegranate seeds with rising drying temperature from 69 to 84 °C, H° and L^* values decreased and TCD increased. They reported that the decrease in L^* and increase

in TCD reflected browning pigments during the heating process. In general, lower TCD for most microencapsulated powders compared with the control sample (10.33) represented protective role of the wall materials for anthocyanins against color changes. Compared with maltodextrin, CSG showed higher color variations, but in comparison with the control sample and AG, it showed lower changes.

4. Conclusion

Arabic gum because of its specific characteristics is used widely in microencapsulation and maltodextrins due to their low prices and high covering abilities are also suitable wall materials, microencapsulation by freeze drying method can be used as an effective method for stabilizing the anthocyanins of saffron petals. We found that cress seed gum (CSG) is an appropriate wall material which had a protective effect on microencapsulated anthocyanins. The results of this study showed that although GSC compared with other wall materials didn't show significant differences ($P < 0.05$) in stabilizing encapsulated anthocyanins, but especially when comparing with maltodextrin (M20 and M7), it can't prevent high color changes of powders during storage.

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