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Optimization of betalain extraction from *Salicornia fruticosa* and its encapsulation

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Abstract

Betalain use in food industry is limited mostly because it's low stability. The aim of this work was maximization of betalain extraction from *S. fruticosa* as a new source and improving its stability by encapsulation with various matrices consisting of gum arabic along with maltodextrin of DE 4−7 (M_{4-7}) or with maltodextrin of DE 16.5−19.5 ($M_{16.5-19.5}$).Also the microcapsules prepared were further characterized on encapsulation efficiency, particle size distribution, *in vitro* release and storage stability at 60 °C for 8 weeks. Using aqueous 20% ethanol and 0.5% citric acid solution as a solvent, isolation period of 30 min, temperature of 40°C and solvent to solid ratio of 20:1 get the highest betalain extraction yield. Microcapsules consisted of $M_{16.5-19.5}$ and gum arabic had comparably smaller size with lower encapsulation efficiency, but they showed higher release of betalain throughout gastric or intestinal digestion and had higher thermal stability of betalain during the storage.

Keywords: *Salicornia fruticosa,* betalain, micro-encapsulation, betalain stability

1. Introduction

Betalain is a group of dyes containing water-dissolvable nitrogen, which are integrated from tyrosine. Betalains are divided into two groups depending on the structural groups: the red-violet betacyanins, such as betanin (Fig.1), and the yellow-orange betaxanthins. The communal chromophore compound of all betalain pigments is betalamic acid [1].

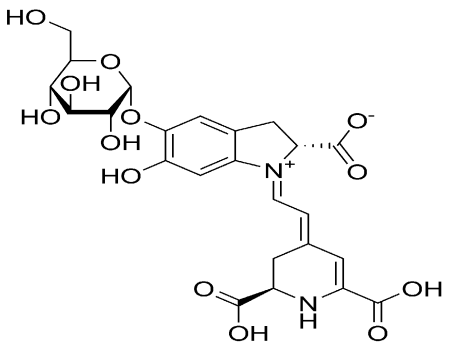

Figure 1. An example of betalain pigments, betanin

Betalain pigments are largely used as food colorants, for example, candy, dry blends, dairy and meat products [2]. Betalain can be found in plants belonging to family *Chenopodiaceae* [3]. *Salicornia fruticosa* L. (also known as glasswort) is fleshy stemmed annual plant of *Chenopodiaceae* family and one of the most popular halophyte herbs. It is found in saline soils and this family in Egypt represented 25 genera and about 300 species [4]. In *S. fruticose*, salt stress plays an important role in betalain synthesis [5], proposed that betalain may act like osmolytes in order to support physiological operations.

Isolation of natural dyes, such as betalain, is usually performed by solid-liquid soaking method. Plant tissues are crushed or soaked in order to make pigment deffusion in extraction liquid more simplify [6]. From previous studies, many factors such as solvent type, extraction time, extraction temperature [7] and solvent to solid ratio [8], amongst other factors, may have a significant effect on the

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isolation efficiency of different natural compounds [9].

Recently, there is mounting attention in the expansion of naturalistic pigments, at most because of they are safe, don't cause environmental pollution and also has been promoted by a powerful consumer desire for naturalistic products [8]. Natural pigments are also nutritional-physiological antioxidants and their existence in food can diminish the risk of heart disease, cancer, and maladies related to ageing stage [10]. Although betalain may be one of the promising natural colorants, it can be deteriorated via heat, basal or highly acidic pH, lighting, air (oxygen), and elevated water activity [11]. The stability of betalain could be improved using microencapsulation prepared from spray-drying [12].

Micro-encapsulation process has been successfully applied in the food processing to protect bioactive compounds which are oversensitive to heat, lighting, oxygen, and humidity, in order to minimize the transfer average from the core to the environment in which the capsules is stored and to modulate the physical properties of the substance, and simplify handling [13]. Freeze-drying, also known as lyophilization or cryodesiccation, has been assured of the superior and simplest technique in encapsulating water-dissolvable substances and naturalistic aromas or real estate [14]. During this procedure, carrier materials such as maltodextrin, gum arabic, modified starch, etc and matrix solutions are homogenized and then co-lyophilized to obtain microcapsules [14]. Maltodextrins composed of α-D-glucose units linked mainly via glycosidic bonds (1→4) and are ordinarily categorized according to their dextrose equivalency (DE). The DE of a maltodextrin is an indicator for its reducing capability and is inversely associated with molecular weight average [15]. Arabic gum, a naturalistic plant polysaccharide exudate from *acacia*, is one of the most important carriers and coating agents used as efficacious shell material for numerous years and still a perfect option because of its high emulsifying capability and a minimum viscosity in aquatic solution. In addition, this technic offers high volatile compounds retention and provides efficacious protection versus oxidation [15].

This study is the first attempt to isolate betalain as natural colorant with a wide range of uses in foods from *S. fruticosa* cultivated in Egypt. Therefore, the first target is to figure out the optimum conditions for maximum isolation of betalain, where the second one is to encapsulate the betalain with two types of maltodextrins in micro size by freeze dying, to investigate the physicochemical properties of capsules, their digestibility in simulated gastric and intestinal fluid and their storage stability.

2. Materials and Methods

2.1. Material

Glasswort (*S. fruticosa*) was compiled from the international seaboard road nearby El-Boruls City, Kafr El-Sheikh governorate, Egypt (latitude 32 35`N and longitude31 16`E) during January 2012. Gum arabic, citric acid, ascorbic acid and all solvents (HPLC spectral grade) applied in this research were acquired from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Maltodextrins, DE 4−7 (M_{4-7}) and DE 16.5−19.5 ($M_{16.5-19.5}$), pepsin from porcine gastric mucosa and pancreatin from porcine pancreas were obtained from Sigma-Aldrich Co., (MO, USA).

2.2. Extraction of betalain

Betalain pigments were extracted from the air parts of *S. fruticosa* as stated in the method briefed by Maran and Manikandan [9]. First, *S. fruticosa* air parts were snipped into short parts. Then, one gram of these pieces placed in 50 ml Erlenmeyer flasks and adds a different composition of solvent (a combination of distilled water, 0.2−1 % citric acid, 0.1% ascorbic acid and 20−50% ethanol) with different ratio of sample to solvent. Every flask was wrapped with a plastic enwrap pending the extraction in order to prohibit the solvent loss, incubated at different temperatures (25−40 °C) in a water bath (Toshiba water bath TIC-4000N, India), and the extractors were taken at various period intervals (10−30 min). After the extraction, the mixture has been centrifuged (2500 rpm for 15 min) (Kubota KN-70Centrifuge, Japan) while the supernatant fluid was compiled for pigment and color determinations. If ethanol was added in the extraction solvent, ethanol was removed using a rotary evaporator (Eyela rotary evaporator N-1000, Japan) and then the extracts thus obtained were lyophilized(Labconco, USA) and stocked at 4 °C until usage.

2.3. Betalain quantification

Betalain quantification was done spectrophotometrically using the procedure described by Moßhammer, Stintzing [16]. The absorbance of extractors was measured via a UV / Vis Spectrophotometer (U-2810 spectrophotometer, Hitachi, Japan) at wavelengths of 535 and 480 nm. The contents of betalain are expressed as the total amounts of betacyanins and betaxanthins calculated from the following equations:

$$\text{betacyanins or betaxanthins (mg/g)} = [(A \times D \times V \times Mw) / \varepsilon]$$

where A is the absorption value at the λ_{max} of 535 nm for betacyanins and 480 nm for betaxanthins, D is the dilution factor, V is the final volume (mL) of the extracts, Mw is the molecular weight (betaxanthins = 308 g/mol and betacyanins = 550 g/mol [17].

2.4. Micro-encapsulation

Preparation of coating materials

Maltodextrins, $M_{4\text{-}7}$ or $M_{16.5–19.5}$, were impregnated with distilled water and maintained at 27 ° C overnight in a shaking water bath (70 rpm) to obtain solutions at a concentration of 12% (w / w). Solutions of gum arabic with a total solids content of 2% (w/w) were prepared. The solutions were blended using a magnetic stirrer(Advantec SR 200, Japan) at 1250 rpm to give a total solids content of 10% (w/w) with the weight ratio of 8:2 (maltodextrins to gum arabic) [18].

Preparation of microcapsules

The betalain extract and coating materials were mixed in a weight ratio of 1:5 (extract to coating material). The mix was magnetically stirred at 3000 rpm for 15 min. Finally, the solution was dehydrated in a freeze dryer for 42 h. Dried materials were ground via a pestle and mortar, passed out of a 0.71 mesh, and stocked in dark glass bottles with screwed caps in a freezer (−25 °C) until usage [19].

2.5. Encapsulation efficiencies

With regard to estimating the effectiveness of micro-encapsulation, total and surface betalain content in the microcapsules were measured after freeze drying.

In order to total betalain content measurement, 1.00 g of encapsulated power was weighed into an amber glass with a screw cap. Then, samples were manually crushed to demolish the microcapsule membrane by the addition of distilled water (1.0 mL). Further 9.0 mL distilled water was added and stood for 5 min followed by centrifugation for 10 min at 3,000 rpm. After finishing the separation step, the pure supernatant was removed, filtered out of a 0.4-micron polytetrafluoroethylene─(PTFE) syringe filter, and the extraction process is duplicated twice to assure maximum extraction yield of betalain.

For determination of surface betalain, the sample (1.00 g) was directly extracted with distilled water (10.0 mL) using a Vortex mixer for 30 seconds, followed by centrifugation for 10 min at 3,000 rpm. After finishing the separation step, the pure supernatant was removed, filtered out of a 0.4-micron PTFE syringe filter. Total and surface betalain contents of the microcapsule sample were quantified spectrophotometrically with the same method described byMo hammer, Stintzing [20]. Encapsulation efficiencies (EE) were calculated via the equation stated by *Barbosa, Borsarelli* [21] as follow:

$$\text{Encapsulation efficiencies (\%)} = [(\text{total betalain} - \text{surface betalain})/(\text{total betalain}) \times 100]$$

2.6. Flow particle image analysis

Betalain microcapsules were studied via a flow particle image analyzer (FPIA, Sysmex FPIA-3000, Japan). Polystyrene latex particle with 2 µm in diameter (polymer microspheres 5200A; Duke scientific corporation, Fremont, CA) was used as the focus adjusting particle before testing the samples. A 10-mg sample aliquot was mixed with 10 ml of distilled water. Five mL of the dispersion was analysed by the FPIA. Size and the circularity of each particle were determined. A statistical distribution was calculated and combined in scatter gram by using IA software (FPIA-3000 part 11).

2.7. In vitro release

Preparation of gastric and intestinal fluids

In direct methods were applied to assess the stabilization of the prepared microcapsules under stomach and intestine conditions. The simulated-gastrointestinal solutions were prepared as described by *Bouayed, Hoffmann [22]*.

Digestion in simulated gastric and intestinal fluids

For the stomach digestion test, 1.4 mL of the simulated gastric fluid (SGF) was placed in a 10 ml tube which included100 mg of the microcapsules. Tight tubes were put in a shaking water bath at 37 ° C for 2 hours with continued shaking at 80 rpm.

For the intestinal digestion test, 2.4 mL of the simulated intestinal fluid (SIF) was placed in a 10 ml tube which included100 mg of the microcapsules. Tight tubes were put in a water bath at 36.6 °C for 2 hours without shaking [23].

For all experiments, the sample collected at 0, 30, 60, 90 and 120 min. At the end of the incubation period, the tubes were immediately washed with water to cool and then, they were centrifuged (2000 rpm for 15 min) and filtered. The filtrates were then neutralized via addendum of NaOH solution (0.2 M). The neutralized filtrate was analyzed for determination of betalain content released from microcapsules.

2.8. Storage test

The betalain extracts and the microcapsules were stored in brown bottles at 60 °C by an accelerated method [24]. Degradation of betalain was monitored for 8 weeks and the betalain content was analyzed every two weeks until the study was completed. The stability of betalain was calculated according to the following equation:

Stability of betalain (%) = [(total betalain after the storage)/(total betalain at zero time) × 100]

2.9. Statistical analysis

In order to determine the differences between means, the general linear model SPSS [25] was applied to conduct ANOVA, the level of potential for statistical procedures was considered as $P \leq 0.01$and $P \leq 0.05$ is considered important. All measurements and tests were carried out in triplicate.

3. Results and Discussions

3.1. Optimum conditions for the extraction of betalain from S. fruticosa air parts

Table 1 shows the effects of solvent composition, extraction time and temperature on the extraction yield of betalain from *S. fruticosa* air parts.

The eleven solvent systems were tested at 25ºC for 10 min and the solid to solvent ratio was 1:5.

It could be observed that, the highest amount of isolated betalain was obtained with the solvent of 20% ethanol 0.5% citric acid in water. The addition of citric acid lowers samples pH values and may cause improvement in betalain accumulation and also prohibit pigment decomposition pending the extraction process [26].

Isolation process was carried out at different solvent/sample ratio (1:5, 1:10, 1:15, 1:20, and 1:25, v/w) where the solvent of 20% ethanol 0.5% citric acid in water was used. Upon reaching the ratios ranged from 1:20 to 1:25, the amount of extracted betalain did not significantly differ. As mentioned by Cissé, Bohuon [27] in all the instances, the increment of the solvent amount in the system led to better extraction yields and therefore more efficacious extraction. First of all, the increase in the quantity of solvent causes a positive modification in the final balance case. Secondly, this causes an increment in driving power for the mass transfer, such as the difference in concentration between the solid and the liquid stage.

Within the third experiment, the isolation process was carried out at different periods (10–30 min). The results indicated that, the amount of extracted betalain was gradually increased with extending the extraction period from 10 to 25 minutes. Then, constant rate was recorded thereafter. This means that, the optimum period for maximum protein isolation was 25 min. Similar results were obtained elsewhere [8] who reported that, the betalain amount in the extract was increased with extending extraction period and prolonging the extraction time after reaching the optimum time causes decomposition of active compounds.

The last experiment was carried out at different extraction temperatures (25–40 °C). The results revealed that, the amount of extracted betalain and its color increased with increasing the temperature. Similar observations were reported by Cacace and Mazza [28].

Table 1. Effect of process variables on the extraction of betalainfrom air parts of *S.fruticosa*.

Type of experiment	Solvent type	Ratio (sample/solvent)	Time (min)	Temperature (°C)	Betalain yield (mg/g sample)
Solvent					
	Water	1:5	10	25	71.4 ± 0.2
	1% CA	1:5	10	25	88.6 ± 0.7
	0.5% CA	1:5	10	25	92.3 ± 0.4
	0.2% CA	1:5	10	25	95.1 ± 0.6
	0.1% AsA	1:5	10	25	63.9 ± 0.6
	50% EtOH	1:5	10	25	72.7 ± 0.3
	20% EtOH	1:5	10	25	75.4 ± 0.6
	0.5% CA + 0.1% AsA	1:5	10	25	98.2 ± 0.5
	0.2% CA + 0.1% AsA	1:5	10	25	101.0 ± 0.4
	20% EtOH + 0.1% CA	1:5	10	25	91.7 ± 0.4
	20% EtOH + 0.5% CA	1:5	10	25	106.3 ± 0.3
Sample/solvent ratio					
	20% EtOH + 0.5 %CA	1:10	10	25	110.7 ± 0.3
	20% EtOH + 0.5% CA	1:15	10	25	112.4 ± 0.3
	20% EtOH + 0.5% CA	1:20	10	25	114.0 ± 0.5
Extraction time					
	20% EtOH + 0.5% CA	1:20	15	25	116.3 ± 0.3
	20% EtOH + 0.5% CA	1:20	20	25	117.5 ± 0.4
	20% EtOH + 0.5% CA	1:20	25	25	118.9 ± 0.4
	20% EtOH + 0.5% CA	1:20	30	25	119.0 ± 0.3
Temperature					
	20% EtOH + 0.5% CA	1:20	30	30	121.2 ± 0.3
	20% EtOH + 0.5% CA	1:20	30	35	124.5 ± 0.2
	20% EtOH + 0.5% CA	1:20	30	40	129.9 ± 0.3

Citric acid (CA), ascorbic acid (AsA), and ethanol (EtOH) was dissolved in distilled water.

3.2. Encapsulation efficiency

Encapsulation efficiency (EE) points to the possibility of the shell substances to hold or enclose the core substances inside of microcapsules. The results in Table 2 revealed that the combination of $M_{4–7}$ with gum arabic has reported the highest EE of betalain (92.30 ± 0.04%), followed by the combination of $M_{16.5\text{-}19.5}$ and gum arabic (EE of betalain, 86.50 ± 0.06%). It has been reported that maltodextrin of DE 5.0−8.0, compared to the DE 18.5, has not only provides high encapsulation efficiency, but it also offers a great stability for bioactive components pending storage period [29]. Thence, $M_{4–7}$ was preferred as a wall material in our microcapsulation.

Table2. Effect of wall material type on the encapsulation efficiency of betalain.

Wall material type	Encapsulation efficiency (%)
$M_{4–7}$ and gum arabic	92.30±0.04
$M_{16.5\text{-}19.5}$ and gum arabic	86.50± 0.06

Where: $M_{4–7}$ is maltodextrins DE4−7 and $M_{16.5–19.5}$ is maltodextrins DE16.5−19.5

3.3. Particle size of microcapsules

Figure 2 shows the particle size distribution of the prepared microcapsules. The diameter of particles in μm at HPF mode was detremined to be 3.03 ± 1.86 μm for microcapsules consist of a combination of $M_{4–7}$ with gum arabic and to be 2.63 ± 1.19 μm for microcapsules consist of a combination of $M_{16.5–19.5}$ with gum arabic. The microcapsules consisted of a maltodextrin with higher DE, $M_{16.5–19.5}$, seems to be smaller in size and narrow in distribution compared with the microcapsules consisted of a maltodextrin with lower DE, $M_{4–7}$.

3.4. *In vitro* release:

Figure 3 shows the release of encapsulated betalain during *in vitro* gastric and intestinal digestions. Generally, the release of betalain from microcapsules was increased gradually with extending the digestion time from zero to 120 min in both of gastric or intestinal digestion. Then, the final amount of betalain released by SGF was 31.8 and 35.0% from microcapsules consisted of $M_{4–7}$ and $M_{16.5–19.5}$, respectively.

On the other hand, the final amount of betalain released by SIF was 78.1 and 85.7 % from microcapsules consisted of $M_{4–7}$ and $M_{16.5–19.5}$, respectively. In addition, the release of betalain from microcapsules by SIF was higher than the release by SGF. This may be due to poor solubility of the coating substance at lower pH. The obtained results revealed that the encapsulated betalain could be efficiently absorbed in the small intestine. This action can be explained by increase in water interaction, solubility and wettability of the microcapsules at the higher pH of the SIF [30].

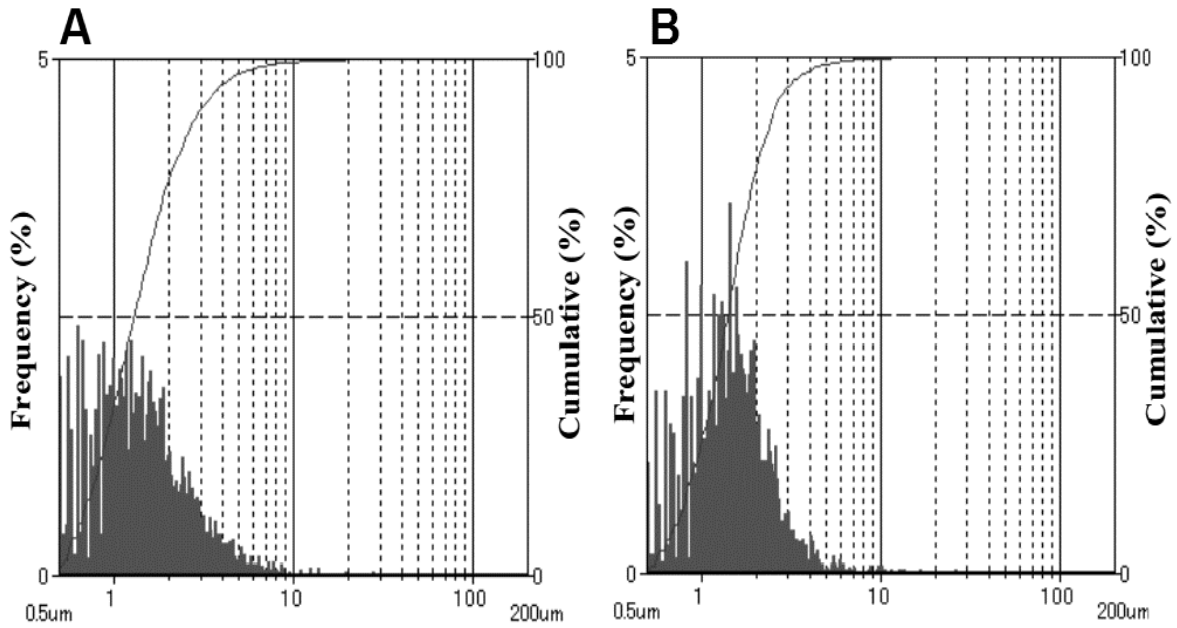

Figure. 2. Particle size distribution at HPF mode of microcapsules prepared from gum arabic (GA) with maltodextrin DE 4−7 (A) or with maltodextrin DE 16.5−19.5 (B).

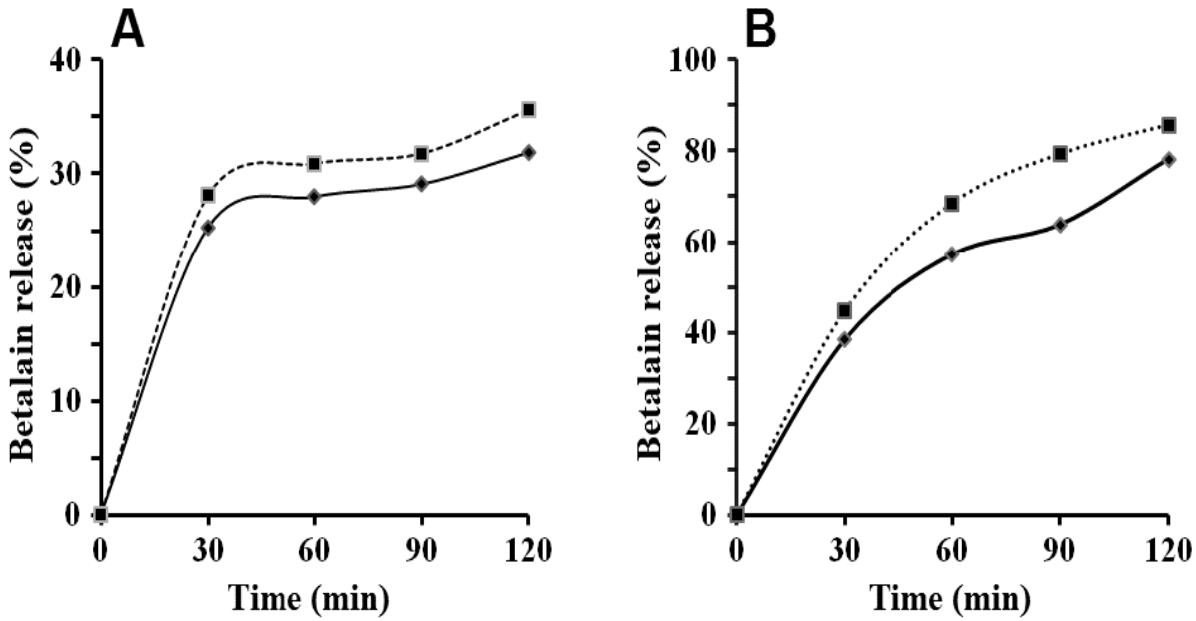

Figure. 3. Release of betalain from microcapsules prepared by maltodextrins of DE 4−7 (◆) or DE 16.5−19.5 (■) using simulated gastric fluid (A) and simulated intestinal fluid (B).

3.5. Storage stability

Stabilities of betalain in the extracts and microcapsules were evaluated for the storage at 60 °C (Fig. 4). In two types of microcapsules, there was no significant divergence in the betalain amounts among the before and after 8 weeks storage ($P < 0.01$). Using a combination of gum arabic with $M_{4\text{-}7}$ or $M_{16.5\text{-}19.5}$ as wall materials did not display any significant differences in betalain stability ($P \leq 0.01$). Meanwhile, in control sample (extracts from *S. fruticosa* air parts), there was significant differences in betalain stability compared to the microcapsule samples.

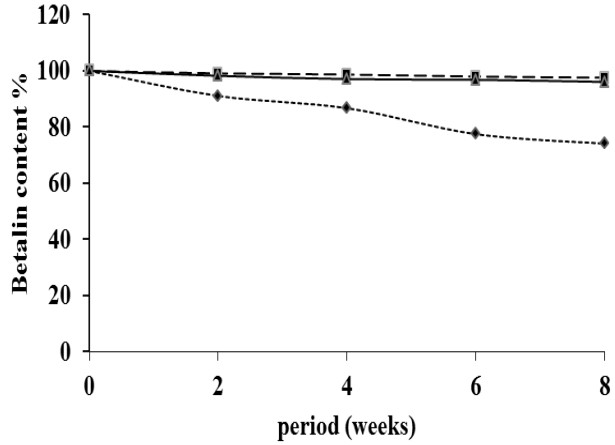

Figure 4. Stabilities of betalains in the lyophilized extracts or the microcapsules during storage.

The lyophilized extracts from *S. fruticosa* air parts (◆), microcalsules prepared by maltodextrins of DE 4−7 (▲) or microcapsules by maltdextrines of DE 16.5–19.5 (■) were stored at 60 °C.

After the 8 weeks storage, 25.87% of the betalain was lost. The result indicates that the encapsulation or wall materials have strong protective effect against the degradation of betalain during the storage. This may be due to the effect of shell materials as physical barriers that can reduce the influence of moisture, heat, light, and oxygen on encapsulated ingredients [31].

4. Conclusion

It can be concluded that *S. fruticosa* air parts was a new source for betalain pigment. The optimal betalain isolation conditions were found to be a solvent of aqueous 20% ethanol with 0.5% citric acid, temperature of 40°C, extraction period of 30 minutes and a solid to solvent ratio of 1:20. Encapsulations of *Salicornia* betalain extract with freeze-drying technique and wall materials of $M_{4–7}$ or $M_{16.5–19.5}$ with gum arabic could protect the degradation of betalain during storage. Our results also showed that, the use of $M_{4–7}$ gave higher encapsulation efficiency than that of $M_{16.5–19.5}$.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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