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Improved Stability of Butterfly Pea Anthocyanins with Biopolymeric Walls

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Synopsis

Butterfly pea (*Clitoria ternatea*) anthocyanins are important natural food colorants. However, the instability hinders industrial applications. The butterfly pea anthocyanin extract was prepared and mixed with biopolymeric wall systems such as maltodextrin (MD) and gum arabic (GA), MD and gelatin (GE), and MD and guar gum at 1/4 and 1/5 ratios with or without acidified condition, and assessed using the accelerated stability test. The total anthocyanin content (TAC) and color were reassessed. The biopolymeric walls of MD and GA (1/5) under acidified condition exhibited best stability enhancement in comparison with the unprotected one (12.04% ± 4.49% and 85.37% ± 0.22% TAC reduction, respectively). *a** and *b* shifts of the protected system were 4.76% ± 0.00% and 0.28% ± 0.00%, respectively. The particle size of this system was 95.44 ± 1.57 μm. This stabilized anthocyanin extract can, therefore, be used in food, pharmaceutical, and cosmetic industries.

INTRODUCTION

Butterfly pea (*Clitoria ternatea* L.) has long been used as a traditional medicine (1) because of its pharmacologically active constituents, of which anthocyanins are the key molecules (2) contributing to their therapeutic effects (3) as per their utilization as a natural additive in food, pharmaceuticals, and cosmetics (4). Anthocyanins are highly reactive and easily degraded following air, light, high temperature, and humidity exposures, which diminish their application in health promotion products. Accordingly, stabilization of anthocyanins is the main focus of several researchers; using these versatile biological molecules in health promotion products (5), which protects the anthocyanins against adverse environmental conditions that downregulate the stability by means of encapsulation in polymeric wall materials. This is regarded as a feasible solution (6) and fit with the industrial aspects.

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Biopolymers are one of the important macromolecules with a variety of health-beneficial effects, including cosmetic benefits (7,8), in addition to their suitability to be used as a protecting wall system for the protection of anthocyanins (9). Most of the studies are devoted for loading and encapsulation efficacies as well as releasing profiles (10). Maltodextrin (MD) is commonly used as the main biopolymeric material because of its high water solubility, low viscosity, and the characteristic to afford a colorless media that interfere less with the color of anthocyanins (11). In addition, a combination system of MD and gum Arabic (GA), MD and gelatin (GE), and MD and guar gum (GG) in 3/1 ratio had been shown to be the optimized mixture of biopolymeric wall system for blackberry (12) and barberry anthocyanin protection (6). Nevertheless, stability enhancement of butterfly pea anthocyanin (BP) extract is less explored, especially with the biopolymeric wall encapsulation.

The objective of this study was to enhance the stability of BP extract by encapsulation in three different biopolymeric wall systems: MD and GA, MD and GE, and MD and GG (3/1) in a ratio between extract and walls of 1/4 and 1/5 with or without acidified conditions. Total anthocyanins content and color parameters of each protecting condition at the baseline and following accelerated stability test was examined. The improved stability of BP extract presented herein would be, therefore, available for a variety applications in the health-beneficial anthocyanins with the retention of quality feasible for the industrial practices, especially for the products in the powder dosage form, e.g., makeup cosmetics. In addition, application of this stable form of natural colorant would meet the consumers' awareness and preference on natural cosmetics.

MATERIALS AND METHODS

Butterfly pea was purchased from the chained supermarket (Makro, Chiang Rai). MD, GA, GE, and GG were food grades. Other chemicals and solvents used in this study were of analytical grades unless otherwise stated.

BP EXTRACTION

BP extract was prepared by the modified method from the literature (6). In short, the coarse-ground petals of butterfly pea were macerated in a mixture of water (30 ml) and 95% ethanol (70 ml) and shaken for 1 h (150 rpm, 50°C). The mixture was filtrated and concentrated to 15° brix (Hand-Held refractometer *N*-1E Brix 0–32%, ATAGO, Tokyo, Japan) by a rotary evaporator (Eyela, Tokyo, Japan) and the sample was stored in a light- and air-tight container at −4°C. Another batch of extraction was carried out using the same procedure, but the filtrated extract was lyophilized for total anthocyanin content (TAC) and stability assessments in a comparison with those stabilized in the biopolymeric walls.

BIOPOLYMERIC WALL PREPARATION

The polymeric walls were prepared by the modified method from the literatures (12,13). Briefly, the mixture of biopolymeric walls, which were MD and GA, MD and GE, and

MD and GG at a ratio of 3/1 (w/w) were prepared in a 40% total solid form and kept in a refrigerator for complete hydrating.

ENCAPSULATION OF BP

The prepared BP extract was mixed with the prepared three biopolymeric wall systems with ratio between the anthocyanins extract and wall of 1/4 and 1/5, with or without acidified condition to pH 2 using 1.5 N HCl. The mixtures were mixed (100 rpm, 20 min) and lyophilized (13–15). The obtained dried solid particles were grounded and sieved (30 mesh) to give a smaller size less than 600 μm.

TOTAL ANTHOCYANIN (TAC) ANALYSIS

TAC was assessed by means of a pH differential method modified from Wrolstad (16) in KCl (0.2 M, pH 1.0) and $C_2H_3O_2Na$ (1 M, pH 4.5). Absorbance of the sample (10 mg in 1 ml of deionized (DI) water) in each buffer was recorded at 520 and 700 nm using a microplate reader (ASYS, Biochrome UVM340, Cambridge, UK). TAC was calculated with the extinction coefficient for cyanidin-3-glucoside as Absorbance = $(A_{520nm}–A_{700nm})$pH1.0 – $(A_{520nm}–A_{700nm})$pH 4.5 and expressed as mg cyanidin-3-glucoside equivalent per gram sample (mg Cyn-3-glu/g sample). The assay was undertaken in triplicates.

COLOR PARAMETER ANALYSIS

Colorimetric analysis of the samples (0.5 g) was performed using CR-400 Chroma Meter (Konica Minolta, Tokyo, Japan) for L^*, a^*, b^*, C^*, and h in triplicates.

ACCELERATED STABILITY TEST

The accelerated stability test using high temperature and low temperature for seven cycles (heating 45°C, 24 h and cooling 4°C, 24 h) was undertaken (4). TAC and color were reassessed, and reduction (%) of these parameters was calculated.

PARTICLE SIZE ANALYSIS

Particle size distribution (PSD) of the selected biopolymeric wall–encapsulated BP extract was examined. The encapsulated system (0.2 g) in DI water (10 ml) was analyzed by Mastersizer 2000 (Malvern, Cambridge, UK) using the wet method.

STATISTICAL ANALYSIS

Data are presented as the mean ± SD. Statistical analysis was performed using the SPSS program version 16.0 for Windows (IBM, New York, NY). The parameters were compared and analyzed using one-sample t-test and analysis of variance test with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

PREPARATION OF BP EXTRACT AND BIOPOLYERMIC WALLS

BP extract was prepared to be encapsulated in the bipolymeric walls. The anthocyanin extract was quality controlled to 15° brix giving a dark blue color, and some amount of the extract was lyophilized to be used as the reference BP. The macromolecules used to prepare the walls are quite different in color. MD is white, GA is pale brown, GE is pale yellow, and GG is pale beige. Nevertheless, once the macromolecules were mixed and prepared into the biopolymeric walls, their colors were indifferent by the visual assessment, all of them were in off-white tone as exhibited in Figure 1A. Thereafter, the examined colorimetric parameters of BP, macromolecules, and biopolymeric walls were compared as shown in Table I. Color was recorded in terms of L^* (0–100; black to white), a^* (−60 to +60; green to red), and b^* (−60 to +60; blue to yellow), as well as chroma or C^*, saturation of color, in addition to h. h is one of the important values that are widely used to describe the shade of anthocyanins. These parameters were, therefore, analyzed to characterize the color of the studied systems. BP extract was more reddish than the biopolymeric walls as per the bluish shade regarding a^* and b^*, which corresponds with h and C^*.

STABILIZATION OF BP EXTRACT IN BIOPOLYMERIC WALLS

Stabilization of BP was challenged by encapsulation in different biopolymeric walls at the 1/4 and 1/5 ratio, with or without acidified condition to pH 2. Acidified condition was applied in this study as anthocyanins were reported to be better stabilized and the reddish pink color of anthocyanins will be obtained under the acidified condition (15).

The completely dried anthocyanin–encapsulated systems following lyophilization were sieved to control the particle size of smaller than 600 μm, which afforded the qualitative yields (82.17–95.58%). BPs encapsulated in the biopolymeric walls were purple-blue

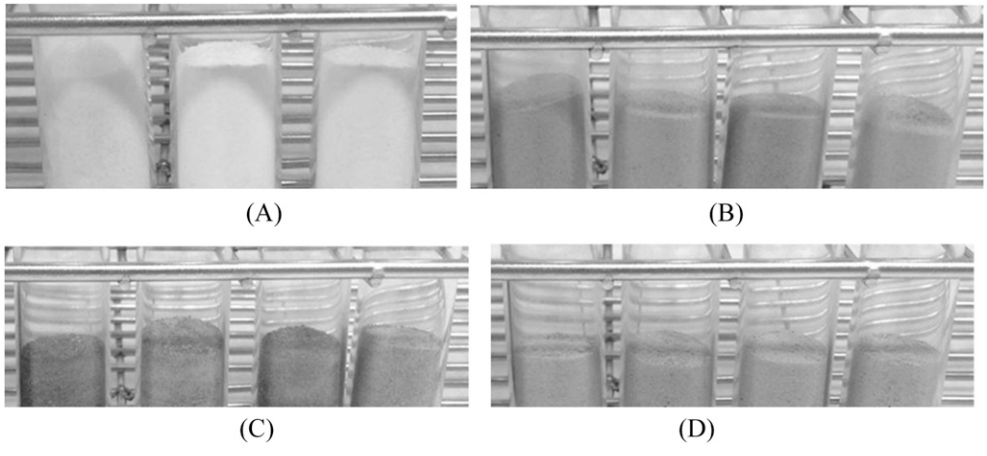

Figure 1. (A) Biopolymeric walls: MD and GA, MD and GE, and MD and GG, (B) BP extract in MD and GA, (C) MD and GE, and (D) MD and GG walls at conditions 1/4 without acidified, 1/4 with acidified, and 1/5 without acidified, 1/5 with acidified, respectively.

Table I
Color of Butterfly Pea Anthocyanin Extract and Biopolymeric Walls

Sample	Color parameters				
	*L**	*a**	*b**	*C**	*h*
BP	30.74 ± 0.04	1.29 ± 0.03	0.48 ± 0.01	1.38 ± 0.02	19.55 ± 0.01
MD	78.70 ± 0.07	−0.63 ± 0.01	1.30 ± 0.02	1.45 ± 0.02	115.68 ± 0.29
GA	69.35 ± 0.11	3.11 ± 0.04	11.08 ± 0.04	11.51 ± 0.05	74.31 ± 0.14
GE	72.72 ± 0.11	−0.43 ± 0.02	12.62 ± 0.03	12.63 ± 0.03	91.94 ± 0.09
GG	72.86 ± 0.03	−0.68 ± 0.01	10.80 ± 0.02	10.82 ± 0.01	93.61 ± 0.06
MD + GA	70.64 ± 0.20	−0.03 ± 0.01	4.65 ± 0.02	4.65 ± 0.02	90.07 ± 0.41
MD + GE	73.40 ± 0.28	−1.07 ± 0.02	2.47 ± 0.08	2.69 ± 0.08	113.48 ± 0.77
MD + GG	76.94 ± 0.26	−126 ± 0.00	4.71 ± 0.02	4.88 ± 0.02	104.97 ± 0.04

and presented in red-pink under the acidified condition (Figure 1). Colorimetric analysis of the BP extract was undertaken in comparison with the biopolymeric wall–stabilized systems as shown in Table II. Most of the encapsulated BPs were increased in *h* and visually notified in red shade except some systems of the MD + GE (1/4, A and 1/5, A). In addition, TAC of the natural extract and their stabilized forms were examined at the baseline (initial) as exhibited in Figures 2 and 3.

Stability of the extracts was assessed by means of an accelerated heating-cooling (HC) test, which could predict the shelf life for 18–24 months; this method is found more applicable in health promotion product industries, including cosmetics (17). BP extract was obviously low in stability with a dramatically decreased TAC with color fading (Figures 2 and 3, Table II). Retention of the encapsulated systems was shown to be 60–90% because of the benefit of using a mixture of biological macromolecules for wall preparation. Of these mixtures, the biopolymeric wall system of MD + GA (1/4; with or without acidified condition) retained TAC that insignificantly differed between the baseline and following the stability test ($p = 0.060$ and 0.074). On the other hand, TAC of BP extract changed insignificantly once protected in MD + GE wall at the 1/4 ratio, acidified, and 1/5 ratio, without acidified conditions ($p = 0.063$ and 0.065, respectively). The MD + GG system was able to protect TAC from degrading at the 1/5 ratio, acidified condition ($p = 0.085$). Acidified condition could better enhance stability of anthocyanins in terms of TAC. In addition, different pH varieties with different color shades of anthocyanins would widen the choice of application (15). Percentage reduction of TAC has been shown in Figure 4, where BP extract was the most reduced (85.37% ± 0.22%), whereas the MD + GA wall at 1/5 ratio, acidified condition, was the least reduced (12.04% ± 4.49%). The sense of MD and GA exacerbating the protection efficacy of anthocyanins was in harmony with the previous reports studying different sources of anthocyanins (6,12).

The color of BP extract protected or unprotected in the biopolymeric walls was examined as shown in Table II. All of the color parameters of the MD + GA at the ratio 1/4, with or without acidified condition were insignificantly shifted ($p > 0.05$) similarly to the MD + GA at the ratio 1/5, acidified condition.

Taken into account with TAC reduction, the best condition for BP protection would be the biopolymeric wall system of MD + GA at 1/5 ratio that protected the natural colorant extract under a acidified condition. PSD of this system was, therefore, analyzed and it showed to be 95.44 ± 1.57 μm.

Table II
Color of BP Extract and the Stabilized Forms at the Base Line (Int.) and Following Accelerated Stability Test (HC)

			Color parameters									
			Int.					HC				
Sample	Condition		*L**	*a**	*b**	*C**	*h*	*L**	*a**	*b**	*C**	*h*
BP			30.74 ± 0.04	1.29 ± 0.03	0.48 ± 0.01	1.37 ± 0.02	19.55 ± 0.41	32.61 ± 0.05	1.31 ± 0.01	0.64 ± 0.02	1.46 ± 0.01	25.77 ± 0.94
MD + GA	1/4	—	49.44 ± 2.47	7.28 ± 0.81	−9.74 ± 0.99	12.70 ± 3.00	306.97 ± 0.57	50.73 ± 0.47	7.94 ± 1.46	−9.69 ± 1.66	12.53 ± 2.21	309.30 ± 0.44
		A	51.73 ± 3.56	19.36 ± 5.34	−0.86 ± 0.76	22.24 ± 0.66	356.67 ± 0.82	48.70 ± 0.05	20.77 ± 0.05	−1.04 ± 0.02	20.79 ± 0.04	357.11 ± 0.04
MD + GE	¼	—	38.03 ± 1.20	6.21 ± 1.62	−10.26 ± 1.95	12.00 ± 2.51	300.90 ± 2.11	37.33 ± 0.23	3.09 ± 0.35	−5.10 ± 0.38	5.97 ± 0.51	301.19 ± 0.96
		A	48.07 ± 0.04	20.42 ± 0.17	2.07 ± 0.04	20.52 ± 0.17	5.78 ± 0.06	42.02 ± 0.02	26.93 ± 0.06	2.91 ± 0.01	27.09 ± 0.05	6.16 ± 0.01
MD + GG	¼	—	51.07 ± 0.02	5.66 ± 0.02	−9.28 ± 0.05	10.87 ± 0.06	301.41 ± 0.10	51.75 ± 0.08	4.65 ± 0.07	−7.61 ± 0.08	8.92 ± 0.10	301.42 ± 0.10
		A	50.78 ± 0.09	17.93 ± 0.66	−5.44 ± 0.27	18.74 ± 0.71	343.12 ± 0.32	45.83 ± 0.01	14.22 ± 0.03	−4.21 ± 0.03	14.83 ± 0.03	343.52 ± 0.02
MD + GA	1/5	—	47.45 ± 1.86	6.23 ± 0.49	−7.93 ± 0.67	14.13 ± 0.58	308.97 ± 0.49	50.42 ± 0.38	9.96 ± 0.40	−11.95 ± 0.30	15.55 ± 0.47	309.80 ± 0.56
		A	55.89 ± 5.63	20.83 ± 2.53	−1.41 ± 0.31	21.79 ± 1.86	355.94 ± 0.41	55.55 ± 0.27	21.89 ± 1.21	−1.18 ± 0.14	21.92 ± 1.21	356.92 ± 0.28
MD + GE	1/5	—	40.22 ± 0.61	6.90 ± 0.89	−11.95 ± 2.57	13.81 ± 2.67	300.30 ± 2.32	40.25 ± 0.16	4.22 ± 0.26	−7.44 ± 0.33	8.55 ± 0.42	299.53 ± 0.46
		A	48.31 ± 0.27	16.55 ± 0.22	1.73 ± 0.04	16.64 ± 0.23	5.97 ± 0.11	43.82 ± 0.08	22.63 ± 0.39	2.44 ± 0.04	22.76 ± 0.39	6.17 ± 0.03
MD + GG	1/5	—	53.46 ± 0.20	6.59 ± 1.53	−10.79 ± 2.27	12.65 ± 2.74	301.34 ± 0.60	47.63 ± 0.04	5.55 ± 0.07	−8.66 ± 0.09	10.29 ± 0.12	302.75 ± 0.05
		A	52.23 ± 0.09	13.83 ± 0.12	−2.98 ± 0.04	14.14 ± 0.13	347.84 ± 0.05	51.50 ± 0.03	13.90 ± 0.04	−3.95 ± 0.05	14.45 ± 0.04	344.15 ± 0.16

* —: not acidified; A: acidified.

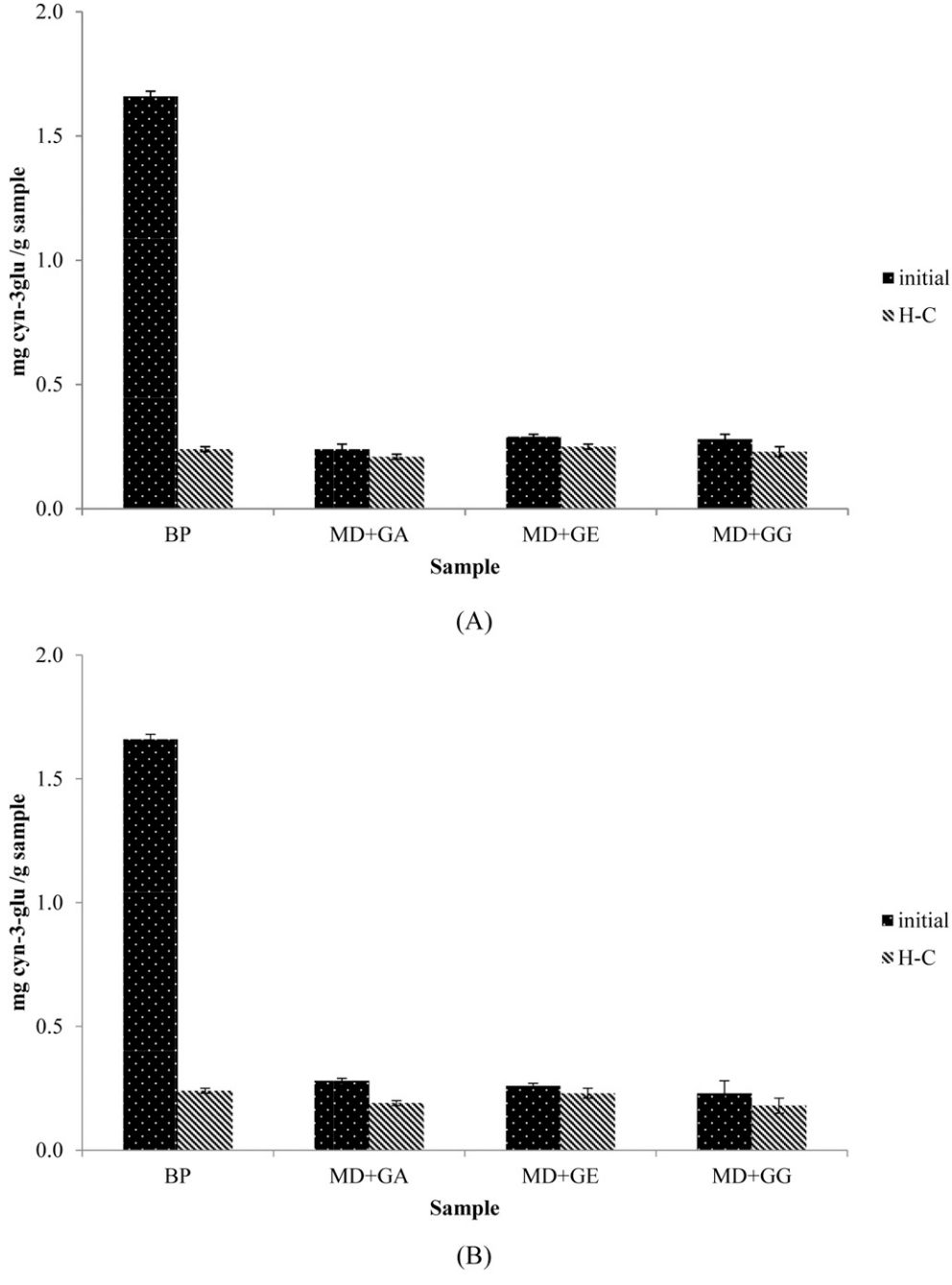

Figure 2. TAC of the BP extract in biopolymeric walls (1/4) (A) without and (B) with acidified conditions.

CONCLUSIONS

Stabilization of the natural anthocyanin extract derived from butterfly pea was successively carried out by using the biopolymeric wall system comprising MD and GA at 3/1 ratio. The most optimized stabilization procedure was mixing the anthocyanin extract

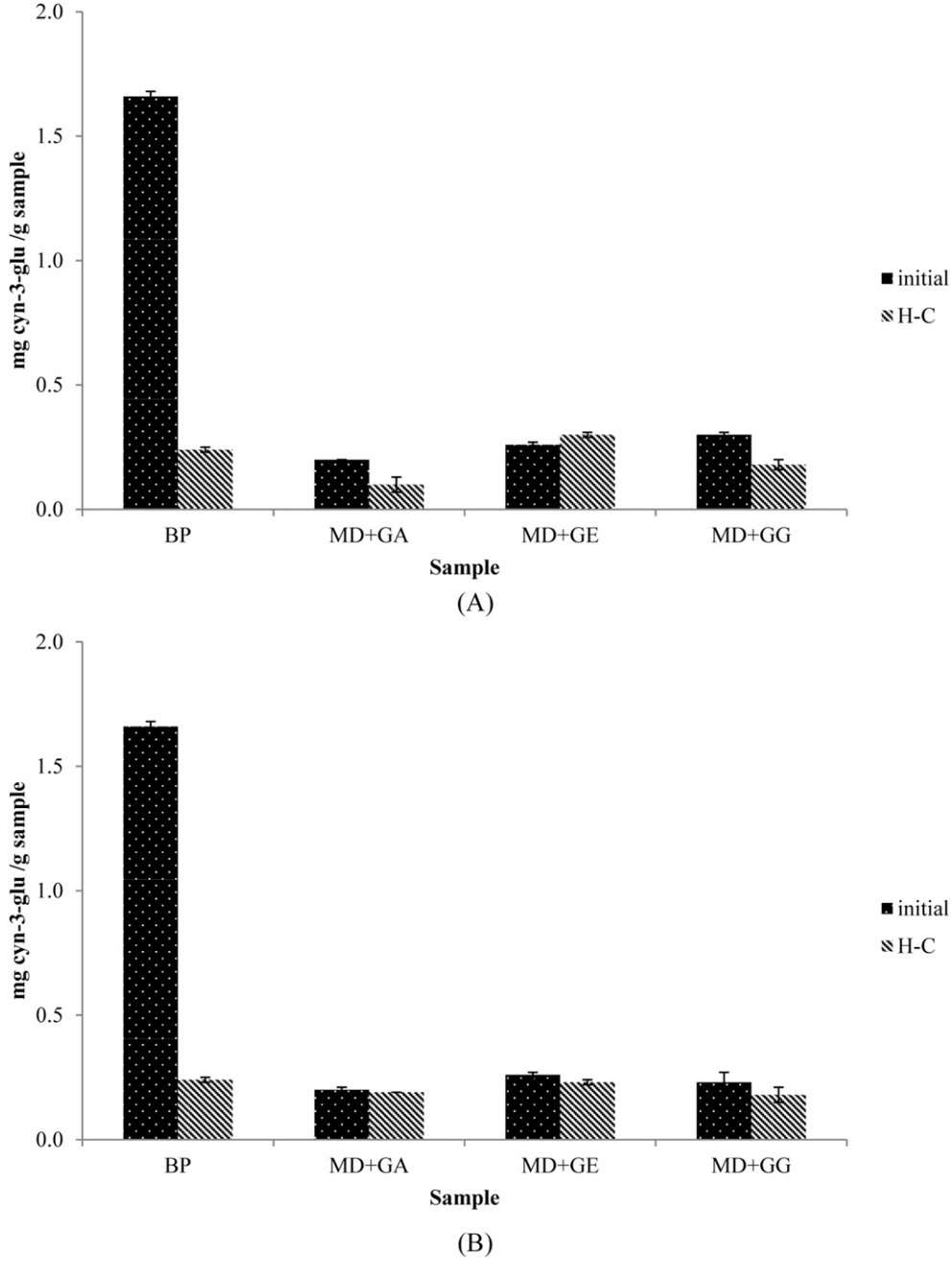

Figure 3. TAC of the BP extract in biopolymeric walls (1/5) (A) without and (B) with acidified conditions.

with the prepared biopolymeric wall at 1/5 ratio under pH 2. Reduction of TAC was suppressed to 12.04% ± 4.49% in comparison with 85.37% ± 0.22% reduction of the unprotected BP extract. The quality was not only retained in terms of TAC but also included color. Most correspondence color parameters of anthocyanins a^* and h were shifted only 4.76% ± 0.00% and 0.28% ± 0.00%, respectively. The natural colorants, anthocyanins,

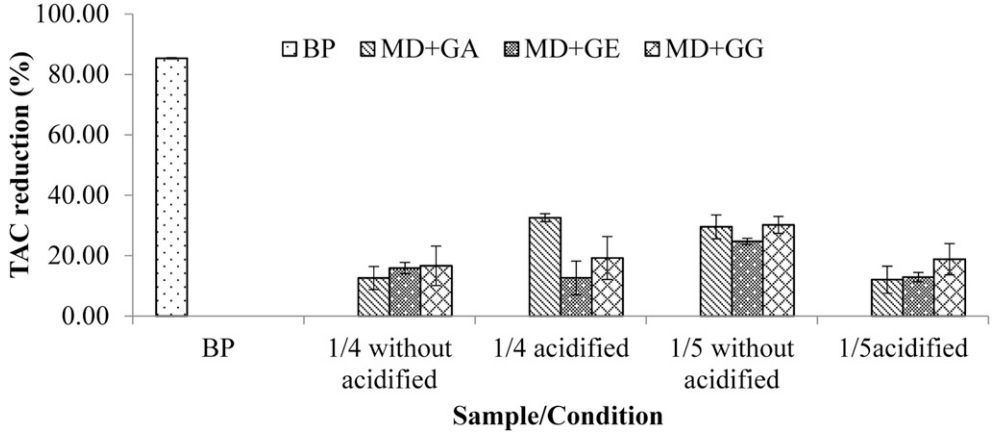

Figure 4. TAC reduction of the protected and unprotected BP extract.

are, therefore, stabilized with the application of the food-grade macromolecules MD and GA. Furthermore, delivery of this BP with this microencapsulation system with PSD of 95.44% ± 1.57 μm would have more benefits in terms of stability and bioavailability. Thus, application of the health-beneficial anthocyanins surplus with the protecting wall would be feasible for food and certain industries, i.e., cosmetics and pharmaceutics, especially those of powder dosage form for instance makeup products.

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