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Original research article

Encapsulation of Betalain from *Basella rubra* Fruit Using Drum Drying and Its Application in Goat Milk Yogurt

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ABSTRACT

Betalain of mature *Basella rubra* fruit can be exploited as a natural food colorant; however, its instability restricts its use in food processing. This study aimed to determine the physicochemical properties of *B. rubra* fruit extract (BFE) encapsulated with 5%, 10%, and 15% (w/v) maltodextrin (MD) and investigate its application as a colorant in goat milk yogurt. The production of encapsulated BFE was conducted by drum drying at 100 °C. The results showed that 10% MD was the optimum concentration for encapsulation yield (86.93 ± 1.62%), betalain content (184.74 ± 3.56 mg/100 g of dry weight), moisture content (1.26 ± 0.30%), and water activity (0.16 ± 0.00). Concerning morphology, 10% MD exhibited a broken glass shape with the finest particles of all the preparations studied here. Regarding the goat milk yogurt, the addition of encapsulated BFE preserved color, betalain, and phenolic content more than non-encapsulated BFE during refrigerated storage for 21 days. Hence, drum-drying encapsulation could be used to improve the stability of BFE. Encapsulated BFE is a potential candidate as a functional colorant in goat milk yogurt.

Keywords: *Basella rubra* fruit; Betalain; Drum drying technique; Goat milk yogurt; Maltodextrin

1. Introduction

Color is a critical attribute for consumers' perception of food quality. Nowadays, the concern of consumers regarding the safety of artificial colorants in food has increased. Betalains are nitrogen-containing water-soluble natural pigments, including red-violet betacyanins and yellow betaxanthins. Beetroot (*Beta vulgaris*) is a primary source of edible betalain; nonetheless, it has limited application due to its earthy smell and high nitrate level [1]. Thus, novel sources of natural betalains need to be explored.

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Usually, the mature red-purple fruit of *B. rubra* is non-consumable and regarded as waste. Nevertheless, it is a crucial source of betalain pigments and phenolics exhibiting several health benefits, including antioxidant, anti-cancer, and antimicrobial activity [2, 3]. Moreover, *B. rubra* fruit betalains produce a color similar to those found in beetroot [3], giving it the potential for use as a natural colorant in the food industry. However, the stability of betalain is less than ideal and breakdown can occur due to many factors including temperature, light, moisture content, water activity, pH, and oxygen [4].

Encapsulation technology has been implemented as it increases betalain stability and preserves its quality, overcoming the stability obstacle. This process aims to entrap the core material inside the wall material. Also, encapsulation can be achieved through several different drying methods. Spray drying and freeze drying are employed to produce betalain colorant powder. However, freeze drying is a costly and time-consuming process. Thus, it is typically utilized only to dry thermally sensitive and high-value products [5]. On the other hand, spray drying is a rapid and less expensive technique. The product is transformed into droplets in a drying chamber with circulating hot air; however, the high surface area exposed to air increases degradation [6]. Drum drying is the cheapest method used in the food industry to produce many food products [7]. It is suitable for thermally sensitive products, has a high drying rate, is cost-effective, uses less energy, and is easy to handle [8]. To the best of our knowledge, it has yet to be used to encapsulate betalain pigments from *B. rubra* fruit.

Maltodextrin (MD) is a polysaccharide produced by partial hydrolysis of starch and has a dextrose equivalent (DE) value of less than 20, with a neutral taste. MD can reduce stickiness and improve the stability of bioactive compounds by protecting them from oxidation [9].

Goat milk has received substantial attention as an alternative milk source thanks to its high content of short- and medium-chain fatty acids, easy digestibility and absorption in the human body, lower risk of inducing allergic reactions, and lower lactose content than cow milk [10]. A previous study reported that goat milk is appropriate for yogurt production due to its composition and considerable health benefits [11]. Recently, using natural colorants in yogurt has gained a lot of interest. Their incorporation into yogurt has enhanced final product quality and appearance, and further, possesses health benefits such as antioxidant activity. For instance, the addition of betalains from cactus pear extract improves the antioxidant power of cow milk yogurt [12]. However, little knowledge exists on the practical application of encapsulated betalain from *B. rubra* fruit as a color additive in goat milk yogurt.

The objectives of this study were to evaluate the feasibility of producing encapsulated BFE using a drum-drying technique, to determine the optimum MD concentration for encapsulation, and finally, to investigate the betalain, total phenolic content (TPC), and antioxidant activity (AA) of goat milk yogurt fortified with encapsulated BFE.

2. Materials and Methods

2.1 Chemicals and reagents

MD (DE 10-12) was obtained from Jining Xuli Chemical Co., Ltd. (China). Folin- Ciocalteu phenol reagent was purchased from Loba Chemie (Mumbai, India). 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) and gallic acid were obtained from Sigma-Aldrich (MO, USA). All chemicals were of analytical grade.

2.2 Preparation of *B. rubra* fruit extract (BFE)

Fresh mature red-violet *B. rubra* fruits (8 weeks after flowering) were obtained from Chonburi, Thailand. The fruits were

extracted with distilled water at a ratio of 1:10 (w/v) using an electric blender (Panasonic MX-GM1011, Japan) for a maximum duration of 60 sec. The samples were centrifuged at 8,000 g for 20 min at 25°C (Sorvall Biofuge primo R centrifuge, USA) and filtered through Whatman no. 1 filter paper. The aqueous extract of *B. rubra* fruit was transferred into 500 mL PET bottles, protected from light, and stored at -20°C until use.

2.3 Encapsulation of BFE

MD was added to BFE, giving concentrations of 5%, 10%, and 15% (w/v) and then homogenized using a homogenizer (Ultra-Turrax T25, IKA, Germany) at 13,500 rpm for 15 min. Then, each mixture was dried using a single drum dryer (OFM, Thailand) at 100°C at a rotation speed of 5 rpm producing encapsulated BFE powder. The dried powder was finely ground using an ultra-centrifuge mill with a 0.25 mm ring sieve (Retsch, Germany), packed in aluminum foiled-PE bags, and stored in a drying cabinet at room temperature until further analysis.

2.4 Quantification of betalain content

Betacyanin (*BC*) and betaxanthin (*BX*) contents were determined using a UV-VIS spectrophotometer (Shimadzu, Japan) at 538 nm and 480 nm, respectively [13]. The contents of *BC* and *BX* (mg/100g) were calculated using the following equation:

$$BC \text{ or } BX = \frac{A \times V \times DF \times MW}{\varepsilon L W} \times 100,$$

where A = absorbance at 538 nm for *BC* and 480 nm for *BX*, V = extract volume (mL), DF = dilution factor, ε = molar extinction coefficient at 60,000 L $mol^{-1}cm^{-1}$ for *BC* and 48,000 L $mol^{-1}cm^{-1}$ for *BX*, L (path length) = 1.0 cm, W = weight of powder (g), and MW = molecular weight at 550 g/mol for *BC* and 308 g/mol for *BX*.

2.5 Determination of surface betalain content

Encapsulated powder (100 mg) was dispersed in a 2 mL ethanol: methanol (1:1) solution using a vortex mixer for 1 min and then centrifuged at 8,000 g for 15 min [14]. The surface betalain content was determined using a spectrophotometer, as described in Section 2.4.

2.6 Determination of total betalain content

Encapsulated powder (100 mg) was dispersed in 2-mL water using a vortex mixer for 1 min and then centrifuged at 8,000 g for 15 min [14]. The total betalain content was determined using a spectrophotometer, as described in Section 2.4.

2.7 Encapsulation efficiency

The encapsulation efficiency (EE) was calculated using the following equation:

$$\text{EE}(\%) = \frac{\text{Total betalain} - \text{Surface betalain}}{\text{Total betalain}} \times 100.$$

2.8 Encapsulation yield

The encapsulation yield (EY) was calculated using the following equation:

$$\text{EY}(\%) = \frac{\text{Total mass of powder after encapsulation}}{\text{Total mass of powder before encapsulation}} \times 100.$$

2.9 Color measurement

The color of encapsulated powder was determined using a colorimeter (CM-3500d, Konica Minolta, Japan). Color values were expressed as lightness (CIE L* - value), redness (CIE a*-value), yellowness (CIE b*-value), and chroma $\left[C^* = \left(a^* 2 + b^* 2 \right) \frac{1}{2} \right]$. Color retention (CR) was calculated using the following equation:

$$\text{CR}(\%) = \frac{C^* \text{ value at particular storage time}}{C^* \text{ value at zero storage time}} \times 100.$$

2.10 Determination of moisture content and water activity (a_w)

A moisture analyzer (OHAUS, USA) was used to analyze moisture content. A Water Activity Meter (AQUALAB, USA) was used to measure a_w.

2.11 Determination of total phenolic content (TPC) and antioxidant activity (AA)

TPC was determined using the Folin-Ciocalteu method from a previous study [15]. First, 100 µl from each sample was mixed with 2.9 mL of ddH_2O and 0.125 mL of Folin-Ciocalteu reagent. Then, 1.25 mL of Na_2CO_3 (7% w/v) was added to the mixture, then incubated at room temperature for 20 min. The absorbance was measured at 765 nm. All data are expressed as mg gallic acid equivalent (GAE)/g of extract.

AA was determined by the ferric reducing antioxidant power activity (FRAP) method as previously described [16]. First, 100 µl of each sample was mixed with 3 mL of FRAP reagent (300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$) and incubated at room temperature for 15 min. Measurements were taken at 593 nm. All data are expressed as mg gallic acid equivalent (GAE)/g of extract.

2.12 Particle morphology

The surface morphology of encapsulated powder was determined using an SEM (LEO 1450 VP, LEO, USA) with an accelerating voltage of 15 kV and a magnification of 500 x.

2.13 Preparation of goat milk yogurt

Fresh raw goat milk was provided by Sam Paow Village farm, Chachoengsao, Thailand, and pasteurized at 85 °C for 15 min. Then, sugar (6% w/v) and whey protein (3% w/v) were added to the pasteurized product. After cooling to 40°C, a commercial yogurt starter culture (4% w/v) (Dutch Mill, Co., Ltd, Thailand) was added to the mixture with or without the BFE colorant. The treatments included: 1) plain goat milk yogurt (control), 2) goat milk yogurt containing 0.8% (v/v) non-encapsulated BFE, and 3) goat milk yogurt containing 2.5% (w/v) encapsulated BFE. Colorant concentration used here was chosen based on sensory evaluations from a previous study investigating the highest scores in color and odor [17]. Goat milk yogurt was incubated at 42°C for 6 h (until the pH reached 4.2) and stored at 4°C for 21 days. Three samples from each treatment were taken on days 1, 7, 14, and 21 to determine color, betalain content, TPC, and AA.

2.14 Statistical analysis

Data were collected in triplicate and are presented as mean ± standard deviation. A one-way ANOVA test and Duncan's Multiple Range Test (DMRT) were conducted to determine significance of data ($p < 0.05$) with SPSS version 17.0.

3. Results and Discussion

3.1 Encapsulation yield (EY) of encapsulated BFE

EY is a critical parameter for industrial processing and is directly related to production cost and efficiency. Table 1 depicts that MD concentrations significantly ($p < 0.05$) impact BFE powder EY. Using 5% MD resulted in the lowest EY (64.00 ± 5.66 %). However, increasing MD concentration to 10% (w/v) substantially increased EY (86.93 ± 1.62 %). However, EY was slightly lower at 15% MD (81.33 ± 1.78%). Therefore, the optimum MD concentration was determined to be 10% (w/v), giving the highest EY. Similarly, reports in other studies have revealed that higher levels of MD increase the yield of powders [18]. Generally, the drum drying method is suitable for slurry samples [19]. In this study, low MD concentration (5% MD) unproductively formed a thin layer or film on the heated drum surface during drying due to its low viscosity.

The EYs of BFE encapsulated with 10-15% (w/v) MD were higher than that of beetroot juice encapsulated by MD using spray drying (58.1-67.4%) [20, 21]. Therefore, these results indicate that drum drying is feasible for producing encapsulated BFE.

3.2 Encapsulation efficiency (EE) of encapsulated BFE

EE indicates the ability to encapsulate the coating materials on active agents [22]. Measured EE values of encapsulated BFE were between 83.59 ± 2.40 and 88.06 ± 0.14, with a decrease seen when using 15% MD (Table 1).

High EE values determined after the drum drying technique could be due to the decreased degradation of betalains and strong interaction between BFE and MD. However, the greater amount of total solids seen in the BFE mixture might explain the relative decrease in EE at 15% MD. A previous study discovered that high viscosity could decrease the EE values [23]. The EE of drum-dried BFE encapsulated by MD was higher than that of both spray drying (64.70%) and freeze drying (42.19%) [24]. Therefore, drum drying was deemed an efficient method of obtaining encapsulated BFE.

3.3 BC, BX, TPC, and AA of encapsulated BFE

Table 1 shows total BC and BX content depending on MD concentration. Increased concentration of MD resulted in significantly ($p < 0.05$) decreased total BC and BX content, with the highest content of BC (206.86 ± 7.93 mg/100 g) and BX (137.64 ± 9.53 mg/100 g) at 5% MD. These results align with a previous study in which spray-dried BFE was encapsulated using the lowest (10%) MD concentration [25].

Likewise, increased MD concentrations led to significantly decreased ($p < 0.05$) TPC and AA values (Table 1) due to the dilution effect. These results agree with a previous study on spray-dried purple cactus pear peel extract encapsulated with MD at a range of concentrations [9].

Table 1. Physicochemical properties of BFE encapsulated with different concentrations of MD.

Parameter	5% MD	10% MD	15% MD
Encapsulation yield (%)	64.00 ± 5.66^{a}	86.93 ± 1.62^{c}	81.33 ± 1.78^{b}
Encapsulation efficiency (%)	88.06 ± 0.14^{b}	87.04 ± 0.34^{b}	83.59 ± 2.40^{a}
Betacyanin (BC) (mg/100 g)	206.86 ± 7.93^{c}	116.19 ± 1.82^{b}	64.85 ± 1.64^{a}
Betaxanthin (BX) (mg/100 g)	137.64 ± 9.53^{c}	66.81 ± 0.57^{b}	45.31 ± 1.38^{a}
Moisture content (%)	1.27 ± 0.15^{a}	1.26 ± 0.30^{a}	1.64 ± 0.18^{a}
a_w	0.22 ± 0.01^{a}	0.23 ± 0.03^{a}	0.25 ± 0.00^{a}
L*	45.23 ± 0.29^{a}	52.08 ± 0.16^{b}	57.13 ± 0.19^{c}
a*	30.36 ± 0.06^{c}	28.37 ± 0.10^{b}	20.77 ± 0.09^{a}
b*	-1.35 ± 0.07^{a}	-2.19 ± 0.02^{b}	-2.00 ± 0.06^{b}
TPC (mg/g extract)	4.15 ± 0.29^{c}	2.16 ± 0.06^{b}	1.69 ± 0.04^{a}
AA (mg/g extract)	1.24 ± 0.09^{c}	0.54 ± 0.01^{b}	0.34 ± 0.02^{a}

Different superscript letters indicate a significant difference ($p < 0.05$) between treatments within a row.

3.4 Moisture content and a_w of encapsulated BFE

Moisture content represents the available water component of food systems. The moisture content ranged from 1.26±0.30% to 1.64±0.18% (Table 1). No significant difference ($p > 0.05$) existed between the moisture content of the samples. This aligns with the results of the encapsulation of beet extract by spray drying with 10 to 15% MD [26]. However, a previous study reported an increased wall material concentration when encapsulating spray-dried black mulberry juice, which has a higher moisture content of [27].

The a_w of encapsulated powders was between 0.22 ± 0.01 and 0.25 ± 0.00. Increased MD concentration did not yield a significant change ($p > 0.05$) in a_w. Usually, food with a_w values of less than 0.6 prevents the growth of microorganisms [28]. Therefore, BFE powders produced by drum drying can be microbiologically stable.

3.5 Color analysis of encapsulated BFE

The MD impacted the final color of the powder due to its white color (Fig.1). Higher concentrations of MD yielded a higher lightness value (Table 1), with the highest value seen when using 15% MD ($p < 0.05$). These results are similar to those of a previous study using beetroot-orange juice powder encapsulated with MD at a range of concentrations [29].

a b c

Fig. 1. Encapsulated powder of *B. rubra* fruit extract with varied MD concentrations at (a) 5% (b) 10% and (c) 15%.

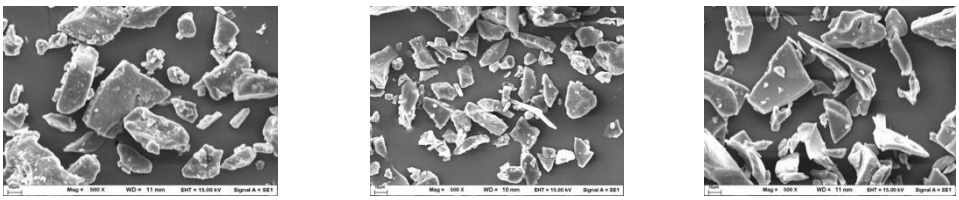
Redness values, representing the red pigments of the encapsulated BFE powder, ranged from 20.77 ± 0.09 to 30.36 ± 0.06, with the lowest values being seen at 15% MD. This decrease is due to the MD's white color.

Yellowness values of all samples were negative, being just slightly below zero, showing that both yellow and blue pigments did not have an impact on the resulting color.

3.6 Powder morphology

Images obtained by SEM of powder particles with 5%, 10%, and 15% MD are shown in Fig. 2. The MD concentration did not affect particle morphology. All powders depicted a broken glass shape, irregular structure, and smooth surfaces. A similar morphology has been reported in a previous study in which the drum drying encapsulation method was used [7]. The finest particles were seen at 10% MD, likely to get better solubility in our study.

Concerning EY, moisture content, a_w, particle morphology, bioactive compounds (betalain and TPC), and AA, BFE encapsulated with 10% MD using the drum drying method was the optimum treatment. Therefore, it was selected for application in goat milk yogurt.



a b c

Fig. 2. Particle morphology imaging by SEM of encapsulated BFE with a) 5% MD b) 10% MD and c) 15% MD at 500x magnification.

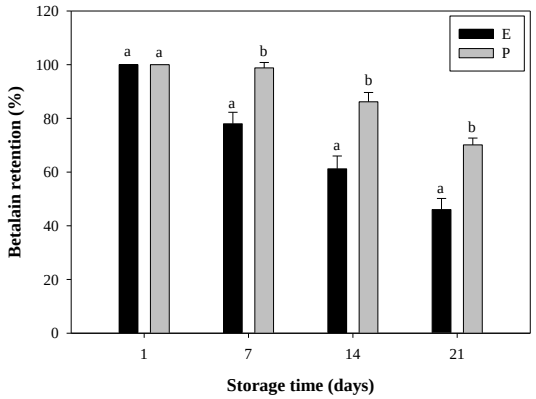


Fig. 3. Betalain retention of goat milk yogurt fortified with non-encapsulated BFE (E) and goat milk yogurt fortified with encapsulated BFE (P) at refrigerated storage for 21 days. Different letters indicate a significant difference ($p < 0.05$) between treatments within a storage time.

3.7 Application of BFE in goat milk yogurt

3.7.1 Betalain content and color retention

Non-encapsulated and encapsulated BFE were incorporated into goat milk yogurt, with samples all having a similar total betalain content of about 4 mg/100 g yogurt. Goat milk yogurt without colorant did not contain any native betalains. The initial storage time for betalain retention of goat milk yogurt revealed no significant difference (Fig. 3). After 7 days of refrigerated storage, the addition of encapsulated BFE resulted in a significantly ($p < 0.05$) higher betalain retention than the non-encapsulated BFE. Improved betalain stability might be owed to the protection from hydrolysis conferred by the encapsulation process [30].

Regarding color retention, loss of color was observed on day 14 of storage in goat milk yogurt, regardless of the BFE form used (Fig. 4). Interestingly, the loss of color was slower than the decrease in betalain content during the storage period. This might be due to the formation of red degradation products (such as hylocerenin and phyllocactin) that help contribute to the red color even though the level of the original 'parent' molecule is decreasing [31]. The encapsulated BFE retained color in goat milk yogurt more than non-encapsulated BFE. Therefore, we conclude that the encapsulation process retards BFE degradation in goat milk yogurt.

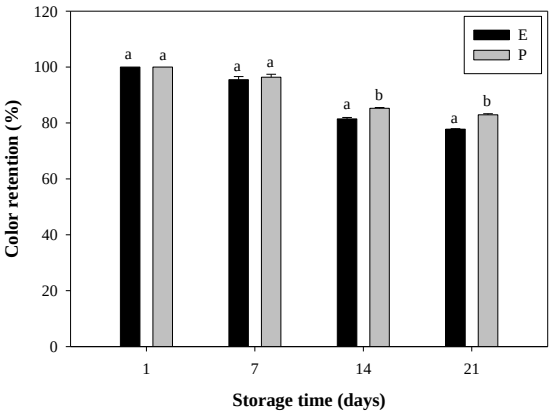


Fig. 4. Color retention of goat milk yogurt fortified with non-encapsulated BFE (E) and goat milk yogurt fortified with encapsulated BFE (P) refrigerated for 21 days. Different letters indicate a significant difference ($p < 0.05$) between treatments within a storage time.

Table 2. TPC and AA of plain goat milk yogurt, goat milk yogurt fortified with non-encapsulated BFE, and goat milk yogurt fortified with encapsulated BFE in refrigerated storage for 21 days.

Parameter	Storage time (days)	Plain goat milk yogurt	Goat milk yogurt fortified with non-encapsulated BFE	Goat milk yogurt fortified with encapsulated BFE
TPC	1	2.52 ± 0.00^{aA}	7.50 ± 0.04^{bC}	7.70 ± 0.06^{bA}
(mg/100 g extract)	7	2.90 ± 0.15^{aA}	8.00 ± 0.09^{bD}	9.16 ± 0.17^{cC}
	14	2.34 ± 0.65^{aA}	7.27 ± 0.10^{bB}	8.54 ± 0.19^{cB}
	21	2.39 ± 0.13^{aA}	6.42 ± 0.15^{bA}	8.25 ± 0.31^{cB}
AA	1	0.42 ± 0.08^{aB}	0.74 ± 0.01^{bD}	0.83 ± 0.03^{bD}
(mg/100 g extract)	7	0.43 ± 0.04^{aB}	0.59 ± 0.01^{bC}	0.62 ± 0.02^{bC}
	14	0.42 ± 0.11^{aB}	0.53 ± 0.02^{bB}	0.53 ± 0.03^{bB}
	21	0.00 ± 0.06^{aA}	0.00 ± 0.02^{bA}	0.04 ± 0.02^{cA}

Different superscript lowercase letters indicate a significant difference ($p < 0.05$) between treatments within a particular storage time.
Different superscript uppercase letters indicate a significant difference ($p < 0.05$) between storage time (days) within a treatment.

3.7.2 TPC and AA

Values of TPC and AA in goat milk yogurt are shown in Table 2. The addition of non- encapsulated and encapsulated BFE significantly ($p < 0.05$) increased TPC in goat milk yogurt more so than control on day 1 of storage. A significant ($p < 0.05$) increase in TPC was observed in goat milk yogurt with encapsulated BFE during the first week of refrigerated storage. This could be due to the proteolysis of milk proteins, likely releasing amino acids with phenolic side chains, such as tyrosine, and reacting with the Folin-Ciocalteu reagent [32]. After storage for 14 days, lower TPC of non-encapsulated and encapsulated BFE was evident in goat milk yogurt. Betalain in BFE may contribute to TPC, via its phenol group [33]. Therefore, the loss of betalain might decrease TPC.

Phenolic compounds and betalain are potent antioxidant compounds [34]. A previous study reported that betacyanin from pitahaya increased the antioxidant property of yogurt [35]. In our study, the non-encapsulated and encapsulated BFE significantly ($p < 0.05$) enhanced AA in goat milk yogurt more than plain yogurt. The presence of betalain and TPC of BFE explains the higher antioxidant activity of goat milk yogurt. However, a decrease in AA was observed starting from day 7 of storage for both treatments. Such an effect might be due to the degradation of betalain and phenolic compounds caused by the hydrogen peroxide production by lactic acid bacteria cultures during storage [36]. These results agree with a previous study on cactus pear betalain in yogurt [12].

4. Conclusion

The production of encapsulated BFE using the drum drying technique was successful, with the optimum formulation being 10% MD as the encapsulating agent. This encapsulation process protects betalain pigment, color alteration, and TPC in goat milk yogurt more than non-encapsulated extract. Therefore, encapsulated pigments from BFE can be incorporated in goat milk yogurt to enhance nutritional value concerning betalain content, TPC, and antioxidant activity.

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