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Extraction and Preservation of Betacyanin pigment from the Peel of White Dragon Fruit (*Hylocereus Undatus*)

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Abstract: The aim of this study was to examine the effects of extracting and preserving conditions on the betacyanin pigment from the peel of white dragon fruit. The investigated factors for the extraction process include: the ratio of solvent and material, the extraction temperature, the time of extraction, the number of extraction and color stability of the extract under different storage conditions. The results showed that the highest betacyanin content was obtained by double extraction with distilled water at the extraction temperature (40^oC), the time of extraction (8 hours) and the ratio of solvent and material is 20/1 (v/w). After 7 days of storage at 4^oC in the dark, the color of the extract was almost unchanged. However, the crude extract lost red color rapidly when stored at room temperature (RT) under natural light. Betacyanin has potential applications as a natural colorant for foods, medicines and cosmetics.

Keywords: Betacyanin, extraction, the betacyanin content, the time of extraction, the extraction temperature

1. Introduction

In recent years, the trend of using natural colorants to replace synthetic colorants in foods, cosmetics and medicines is more popular due to their beautiful colors and safe for human health [1] [2]. Besides anthocyanin, betacyanin is currently attracting lots of the attention of scientists both at home and abroad because it has the ability to create color. Moreover, betacyanin is also a compound with many valuable biological activities for human health such as being able to fight stress-related disorders (for example: cardiovascular, cancer, aging) and have antioxidant capacity through exterminating free radicals [3] [4] [5] [6] [7]. Betacyanin is a natural colorant with red, contains many polar functional groups (-OH, -COOH, -NH) in its molecule, well soluble in water or ethanol solution. Betacyanin exists in many kinds of fruits and vegetables. Many studies on the extraction process of betacyanin from bougainvillea, beetroot, red radish and dragon fruit have been carried out [8] [9]. Among them, dragon fruit is attracting lots of the attention of scientists because of the abundance and popularity of raw materials.

White dragon fruit (*Hylocereus undatus*) is a tropical fruit belonging to the Cactaceae family from the genus Hylocereus, Caryophyllales order. They are widely grown in many countries such as Mexico, Colombia, Australia, Vietnam, Taiwan, Thailand, Malaysia, Philippines,...[10]. Many studies on dragon fruits in the world have shown their great benefits: ripe dragon fruit contains high contents of vitamins, organic acids, proteins, minerals and antioxidant compounds [8] [9] [11]. Especially, in the peel of dragon fruit also contains a large amount of betacyanin pigment [12] [13].

In Vietnam, white dragon fruit is grown very popularly in the southern provinces. Consumption of dragon fruit will release a large amount of peel that contain betacyanin. So the use of dragon fruit peel to extract betacyanin for using as a natural colorant will contribute to the value of dragon fruit trees, increase income for dragon fruit growers and diversify products.

However, during extraction and storage process, betacyanin will gradually decompose over time under the influence of temperature, oxygen, humidity, pH and light. These degrade the betacyanin content in the product [14]. Therefore, this study was carried out to determine the appropriate conditions in the process of extracting and preserving betacyanin from the peel of white dragon fruit in order to obtain high betacyanin content with good color stability.

2. Materials and Methods

2.1. Materials

The white dragon fruit (*Hylocereus undatus*) which used in this study was purchased from dragon fruit farms in Lagi town, Binh Thuan province, Vietnam. The study was carried out on freshly ripe fruits, pink or red pods, not diseases with an average weight of 500 grams/fruit.

2.1.1. Preparation of white dragon fruit peel

Fresh dragon fruits were rinsed. The peel were seperated from the flesh by a stainless steel knife. The fruit peel was cut off all the non-red parts (stem, head, green ears), scraped the colorless part inside the skin, cut into small pieces (2 -3 cm) and stored at -20^{o}C until extraction.

2.1.2. Extraction of betacyanin pigment from white dragon fruit peel

After being defrosted, the dragon fruit peel was contained in a 250 mL beaker, distilled water was added at the ratio of solvent and material 20/1 (v/w). Using a thermostatic bath for extraction at 30^{o}C for 2 hours. The extract was then filtrated by using filter paper no 41. Filtrate was stored in a refrigerator for further analysis.

2.2. Methods

2.2.1. Effect of the extraction conditions on the betacyanin content

The extraction of betacyanin was carried out by extraction method in distilled water. The investigated factors include the ratio of solvent and material (10/1, 20/1, 30/1, 40/1, 50/1 (v/w)), the extraction temperature (30^{o}C, 40^{o}C, 50^{o}C, 60^{o}C, 70^{o}C), the time of extraction (2h, 4h, 6h, 8h, 10h, 12 h) and the number of extraction (1, 2, 3, 4, 5 times). The extract was filtrated by using filter paper no 41. Filtrate was measured the absorbance at wavelength 538 nm on a spectrophotometer UV-VIS in order to determine the betacyanin content. For each study, triplicate samples were simultaneously carried out, and independently repeated at least three times.

The experiment was arranged in a randomized design with a variable factor, the remaining factors were fixed during the experiment. The optimal results of the previous experiment were used as the basis for the following experiments.

2.2.2. Determination of color stability of the extracts

The extracts were evaluated for color durability under different storage conditions. Preparation fourteen 100 mL conical flask. 20 mL of the extract was placed in each of 100 mL conical flask. Seven flasks were stored at 4^{o}C in the dark. Seven remain flasks were stored at room temperature (RT) under natural light. The color of extract was monitored each 24 hours for 7 days. Measure the absorbance of the extract after 7 days of storage to determine the remaining betacyanin content.

2.2.3. Determination of the betacyanin content in the extracts

The content of betacyanin in the extract was determined by spectroscopy method according to Lambert-Beer law. 20 mL of distilled water was placed in 250 mL beaker that contains 1 - 2 g chopped dragon fruit peel. Extracting at suitable temperature and time. The extract was filtrated by using filter paper no 41. Filtrate was then transfered to a V mL volumetric flask and make up to the mark with distilled water. The absorbance of sample was determined at 538 nm, 1 cm cuvet on a spectrophotometer UV-VIS, using distilled water as reference solution.

The content of betacyanin (mg/100g) in the analyzed sample was determined according to the formula (1):

$$BC\,(mg\ /100\ g) = \frac{A.V.F.M.100}{\varepsilon.d.W} \qquad (1)$$

Where,
BC: betacyanin content (mg/100g)
A: absorbance at wavelength 538 nm
V: volume of volumetric flask (mL)
F: dilution factor
M: molecular weight of betacyanin (550 g/mol)
ε: absorption coefficient of betacyanin in water (60000 L/mol.cm)
d: thickness of cuvette (1 cm)
W: fresh weight of extracting material (g)

Tests were conducted and detemined at room temperature on a spectrophotometer UV-VIS Shimadzu - Japan at wavelength 538 nm at Quality Assurance and Testing Centre 2 (Quatest 2, Danang, Vietnam).

For each study, triplicate samples were simultaneously carried out, and independently repeated at least three times.

3. Results and Discussion

3.1. Effect of the ratio of solvent and material on betacyanin content

In this study, the betacyanin extraction process from the peel of white dragon fruit was carried out with distilled water as solvent at 30^{o}C for 2 hours. The ratio of solvent and material was 5/1, 10/1, 15/1, 20/1, 25/1 and 30/1 (v/w). From the crude extract, the betacyanin content were determined and presented in table 1 and figure 1.

Table 1. Extraction yield (betacyanin content) from the peel of white dragon fruit of different the ratio of solvent and material extraction methods.

The solvent/material ratio (v/w)	*Weight of extracting material W (g)*	*Absorbance A*	*Betacyanin content (mg/100g)*
5/1	1.027	0.412	1.84 ± 0.18
10/1	1.031	0.385	3.42 ± 0.20
15/1	1.037	0.331	4.39 ± 0.16
20/1	1.062	0.301	5.20 ± 0.19
25/1	1.077	0.244	5.19 ± 0.18
30/1	1.042	0.185	4.88 ± 0.15

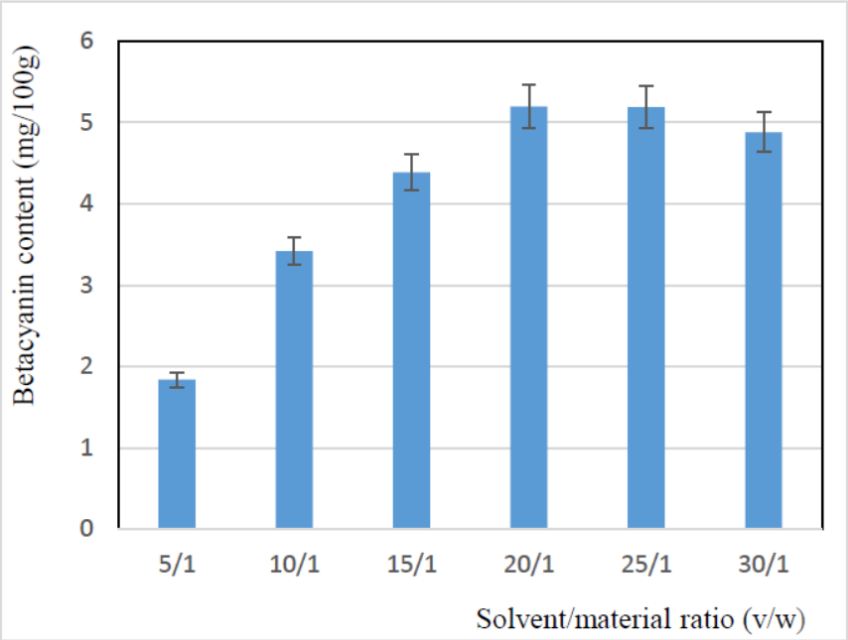

Fig 1. Extraction yield (betacyanin content) from the peel of white dragon fruit of different the ratio of solvent and material extraction methods.

As shown in figure 1, the betacyanin yield was the highest value (5.2mg/100g) when extracting in distilled water at the ratio of solvent and material 20/1 (v/w). The betacyanin content began to decrease slightly at the ratio of solvent and material 25/1, 30/1 (v/w). It has been explained that at the ratio of solvent and material less than 20/1 (v/w), the amount of solvent is not enough to submerge all the material, leading to incomplete extraction of betacyanin. Increase the solvent/material ratio, the whole material is completely submerged in the solvent. At this time, the amount of betacyanin in the raw material will be transferred to the solvent, so the betacyanin content will increase significantly. If the ratio of solvent/material continues to be increased, the betacyanin content will be almost unchanged, but on the contrary, it will cause waste of solvent, energy and time consumption in the later stages of the extraction process. Therefore, the most suitable solvent/material ratio selected for further studies was 20/1 (v/w).

3.2. Effect of the extraction temperature on betacyanin content

To study the effect of the extraction temperature on betacyanin content in the extract, the process of extraction was carried out at varying of temperature: 30^{o}C, 40^{o}C, 50^{o}C, 60^{o}C and 70^{o}C with the fixed

solvent/material ratio 20/1 (v/w). The results of betacyanin content of the extracts were presented in table 2 and figure 2.

Table 2. Extraction yield (betacyanin content) from the peel of white dragon fruit of different the extraction temperature.

The extraction temperature (^oC)	*Weight of extracting material W (g)*	*Absorbance A*	*Betacyanin content (mg/100g)*
30	1.031	0.211	3.75 ± 0.12
40	1.016	0.303	5.47 ± 0.11
50	0.973	0.218	4.11 ± 0.15
60	1.022	0.201	3.61 ± 0.17
70	0.983	0.155	2.89 ± 0.12

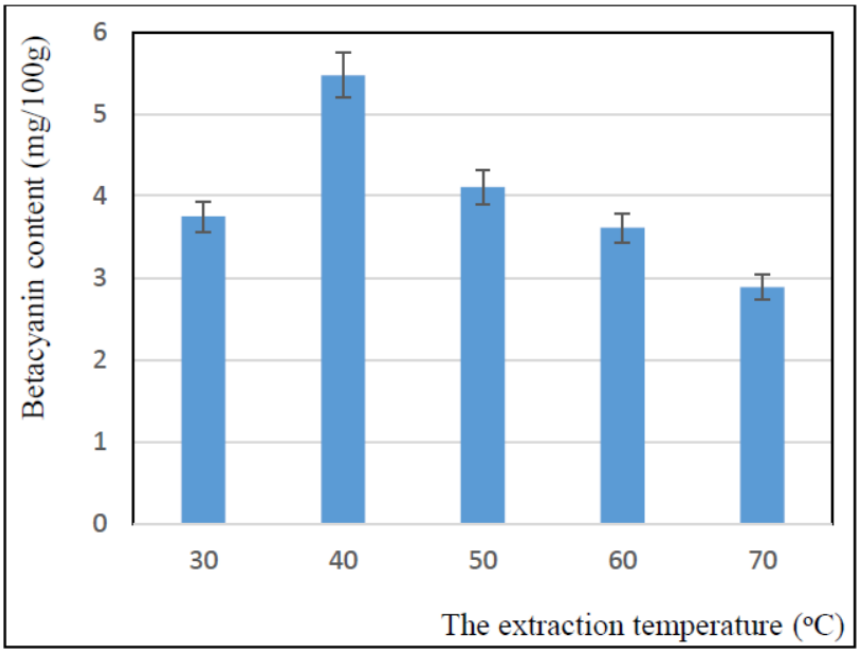

Fig 2. Extraction yield (betacyanin content) from the peel of white dragon fruit of different the temperature of extraction.

The results (figure 2) shown that the extraction temperature had a significant influence on the betacyanin content in the extract. In fact, the betacyanin content in this study was increased from 3.75 mg/100g to 5.47 mg/100g when the extraction temperature increased from 30^{o}C to 40^{o}C. Then, the betacyanin content was reduced gradually with the increasing extraction temperature from 40^{o}C to 70^{o}C.

The temperature increases, the viscosity decreases. So, the diffusion speed increases, this enable betacyanin from the raw materials diffuses into the solvent easily. However, betacyanin is very sensitive to high temperature [16]. The extraction temperature increased, betacyanin is decomposed. This causes betacynin content in the extract reduced. The higher the temperature, the stronger the decomposition of betacyanin. This leads to a decrease in betacynin content with increasing temperature to 50^{o}C, 60^{o}C and 70^{o}C. Therefore, the suitable temperature for betacyanin extraction is 40^{o}C.

3.3. Effect of the time of extraction on betacyanin content

The effect of the time of extraction on betacyanin content was studied by extraction the dragon fruit peels with distilled water at the ratio of solvent and material 20/1 (v/w), at 40^{o}C in the time of extraction 2, 4, 6, 8, 10 and 12 hours. The results of betacyanin content of the extracts obtained are shown in the figure 3 and table 3.

Table 3. Extraction yield (betacyanin content) from the peel of white dragon fruit of different the time of extraction.

The time of extraction (hours)	*Weight of extracting material W(g)*	*Absorbance A*	*Betacyanin content (mg/100g)*
2	1.02	0.185	3.33 ± 0.10
4	1.027	0.259	4.62 ± 0.09
6	0.995	0.277	5.10 ± 0.13
8	1.015	0.298	5.38 ± 0.15
10	1.043	0.267	4.69 ± 0.10
12	1.052	0.231	4.03 ± 0.11

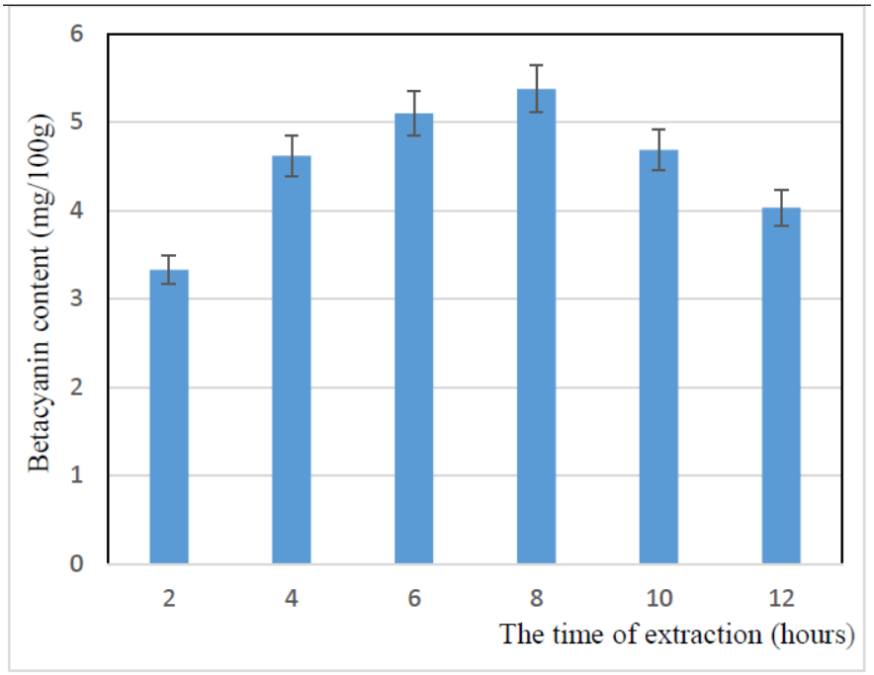

Fig 3. Extraction yield (betacyanin content) from the peel of white dragon fruit of different the time of extraction.

Result of the figure 3 showed that the betacyanin content was increased from 3.33 mg/100g to 5.38 mg/100g when the time of extraction increased from 2 to 8 hours. The highest betacyanin content was observed in peels which extracted for 8 hours. The longer the time, the greater the amount of betacyanin in the dragon fruit peel diffused into the solvent. However, if the extraction time continued to increase, the betacyanin content decreased, only 4.03 mg/100g for 12 hours.

The reduced betacyanin content can be explained when betacyanin (reddish purple) is exposed to water for a long time, it will be hydrolyzed into betanidine (red), betacyanin acid (yellow) and amine (colorless). So, the suitable time for betacyanin extraction selected for further studies is 8 hours.

3.4. Effect of the number of extraction on betacyanin content

After optimized the time of extraction, we continued study the effects of the number of extraction on the betacyanin content in the extracts. The dragon fruit peels were extracted with the number of extraction such as 1, 2, 3, 4 and 5 (times). Then, the betacyanin content of the extracts were determined and presented in table 4 and figure 4.

Table 4. Extraction yield (betacyanin content) from the peel of white dragon fruit of different the number of extraction.

The number of extraction (times)	*Weight of extracting material W(g)*	*Absorbance A*	*Betacyanin content (mg/100g)*
1	1.022	0.307	5.51 ± 0.15
2	1.022	0.195	9.01 ± 0.10
3	1.022	0.032	9.58 ± 0.11

4	1.022	0.009	9.74 ± 0.12
5	1.022	0.005	9.83 ± 0.15

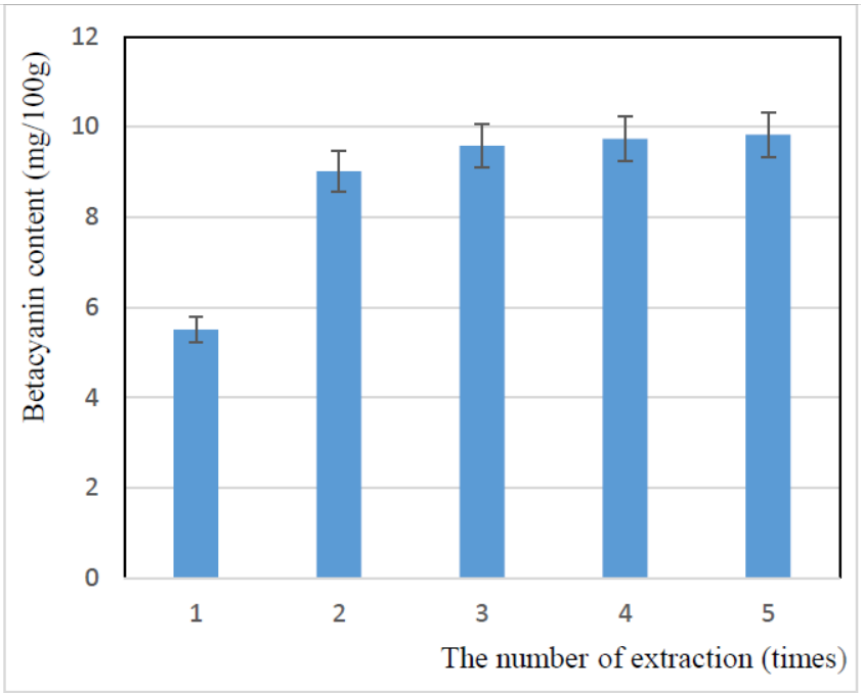

Fig 4. Extraction yield (betacyanin content) from the peel of white dragon fruit of different the number of extraction.

From figure 4, it can be seen that the most suitable number for betacyanin extraction is twice. The betacyanin content was almost unchanged when increasing the number of extraction to 3, 4 and 5 times. After 2 times of extraction, the remaining betacyanin content in the raw materials is very small (only about 2% of the original amount). At this time, increasing the number of extraction not only did not increase the betacyanin content, but also increased the cost.

3.5. Effect of storage conditions on betacyanin content and color stability of the extracts

The results of the color stability of the extracts were given in figure 5.

(a) 4^{o}C, in the dark

(b) RT, under natural light

Fig 5. The color stability of the extracts from the peel of white dragon fruit after 7 days of storage under different conditions.

According the figure 5, the extracts was stored at room temperature under natural light, the red of the extracts gradually faded, then changed to pale yellow and rapidly discolored. Besides, if the crude extract was stored at 4^{o}C, in the dark, the red of betacyanin was light and it has not changed significantly. This study shows that temperature and light have a great influence on the color stability of the extracts. Direct exposure to light, at room temperature for a long time will promote the decomposition of betacyanin (reddish purple) into betanidine (red), betamic acid (yellow) and amines (colorless). As a result, the color of the extract was paled and the betacyanin content decreased during storage.

The results of betacyanin content after 7 days of storage under different conditions shown in table 5 and figure 6 also indicated that betacyanin content was almost unchanged when stored at 4^{o}C, in the dark. Meanwhile, when stored at room temperature, under natural light, the betacyanin content decreased by 31.8%.

Table 5. Extraction yield (betacyanin content) from the peel of white dragon fruit after 7 days of storage under different conditions

Time of storage (days)	*Betacyanin content (mg/100g)*	
	Preserviation (4^{o}C, in the dark)	*Preserviation (RT, natural light)*
Initial	5.38 ± 0.15	5.38 ± 0.15
7 days	5.31 ± 0.17	3.67 ± 0.29

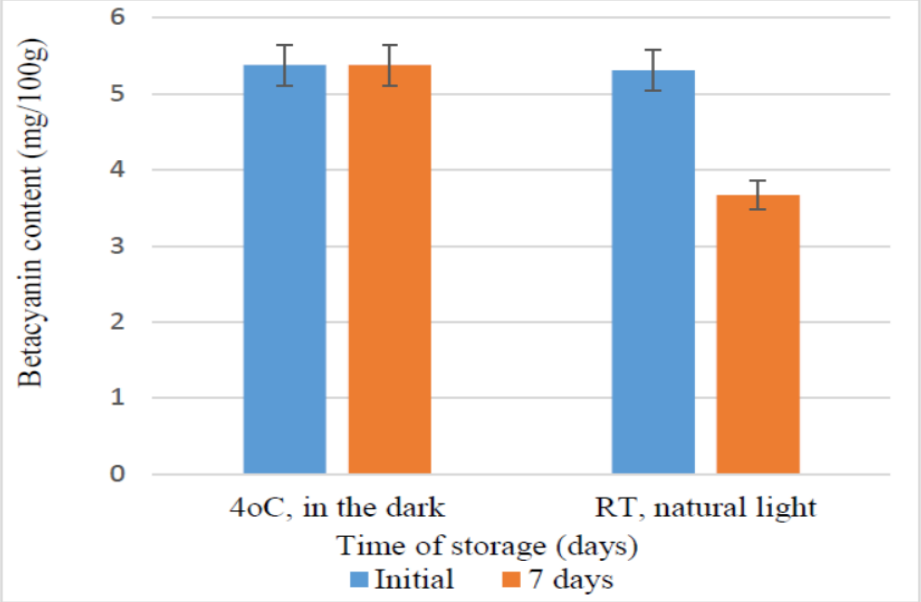

Fig 6. Extraction yield (betacyanin content) from the peel of white dragon fruit after 7 days of storage under different conditions

4. Conclusion

This study allows to extract betacyanin from white flesh dragon fruit peels. The suitable conditions for the extraction of betacyanin are: extraction method in distilled water with the solvent/material ratio 20/1 (v/w) at 40^{o}C for 8 hours. The number of extractions is twice.

The results also showed that the temperature and light of the storage environment have a great influence on the color stability and betacyanin content in the extract. The betacyanin degrades rapidly when stored at room temperature, under natural light, but it is quite stable when stored at low temperature (at 4^{o}C), in the dark. Therefore, betacyanin colorant should be used for refrigerated products.

5. Acknowledgements

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