Bioactivity and Stability Studies of Betalain-Containing Extracts from *Beta vulgaris* L.

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Abstract: *Beta vulgaris* or beet root contained high pigment of betalains that are used as food colorants and food additives due to their health promoting properties. The extraction of natural colorant compound from beet root under chemical- based extraction was identified as an alternative source of commercial synthetic colorant. Beet root is generally processed before consumption which influences the stability of betalains as well as affects the acceptability and health properties. Therefore, this study aimed to investigate the antioxidant activities of betalain-containing extracts and the influence of UV irradiation and heat on the betalain pigment at different concentrations, temperature and period of time. The impact of these factors was evaluated with spectrophotometric absorbance value on the basis of betalain chromaticity. The results of the DPPH antioxidant test revealed that the scavenging activity of betalain increasing proportional to its concentration and the highest 50% inhibition activity was recorded at 77.48%. At 3.0 g/l betalain concentration, the highest chromaticity value was recorded whereas temperature between 10 to 30°C was the most stable betalain pigment against heat stress. However continuous exposure of betalain pigment towards UV irradiation was found to cause discoloration of the samples.

Keywords: Betalain, natural colorant, chromaticity, pigment stability, antioxidant.

INTRODUCTION

Betalains are water-soluble vacuolar chromoalkaloids found in plants [1,2] and this pigments can be grouped into betacyanins (red–violet) or betaxanthins (yellow). Betacyanins are derivatives of betanidin, an iminium adducts of betalamic acid and cyclo-DOPA [3], whereas betaxanthins result from the condensation of α-amino acids or amines with betalamic acid. Naturally, betalains occur predominantly in fruits and flowers [4], and nearly seventy natural derivatives have been identified so far [5]. The isolation of large amounts of betalain is difficult due to its instability [6]. Although betalains are natural antioxidants, these pigments are used in the food industry exclusively as colourants [7]. Beet root pigment is used commercially as a food dye. The use of bio-colorants may show benefits over synthetic colours. Natural dyes are less toxic, less polluting, less health hazardous, non-carcinogenic and non-poisonous and prevent chronic diseases such as prostate cancer [8]. Beet root is regarded as the popular natural source of betalain pigment for colorant betalains and are natural water-soluble nitrogen-containing pigments. Beet root contain a significant amount of vitamins A and C and also calcium, iron, phosphorus, potassium, protein and carbohydrates [9-11]. They have antimicrobial and antiviral effects and also can inhibit the cell proliferation of human tumor cells [12]. Interestingly, beet root is the only natural betalain source of colorant approved by EU and USA government and labelled as E-126. The advantages of using natural colorants are, to name a few; free from harmful toxics, poisons and health hazardous, eco-friendly, non-carcinogenic and protect from chronic ailments such as cancers as it rich in antioxidants. In food applications, the stability of colorants towards lights, temperature, pH, and oxygen is intolerable as betalains extract are very sensitive to these environmental conditions. The effect from these exposure is discoloration of the colorants which might change the appearance of the products. The heat intolerance of beet root extract explains why the colorants is only applied in low temperature-manufactured confectionaries such as ice-cream and sweets [3,13,14]. Therefore, this study aimed to investigate the stability of betalain pigment towards different temperature and UV lights and to determine the scavenging activity of this antioxidant from beet root.

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MATERIALS AND METHODS

Sample Preparation

Approximately 500-550 g of *Beta vulgaris* (beet root) samples were freeze-dried for 72 hours, after which the samples were ground into fine powder and kept at -20°C until further analysis.

Extraction of Betalain

5.0 g of powdered sample was rehydrated with distilled water with pH adjusted at 2.0 and extracted with methanol at room temperature until colourless. The crude extracted was then centrifuged for 5 min at 10 000 g and stored at 4ºC in the dark prior to analysis. The solution was then allowed to separate and the upper layer containing the betalain was collected. The combined upper phase was then concentrated using rotary evaporator and dried to completion under a gentle stream of oxygen-free nitrogen.

Determination of Antioxidant Activity using DPPH Scavenging Assay

DPPH Solution Preparation

To prepare 0.2 mM of DPPH solution, 0.0788 g was needed to be dissolved in 1 ml of solvent. The solution of DPPH was prepared according to previous method by [15]. This test required around 25 ml of the DPPH solution, therefore 2.00 g of DPPH was weighed and mixed with 25 ml of MeOH to produce 0.2 mM DPPH solution.

Ascorbic Acid Standard Preparation

100 µl of MeOH was pipetted using multi pipettor (6 tips) into holes B1-B6, C1-C6, until H1-H6 of 96- well micro plate. Then, 200 µl of ascorbic acid with 1 mg/ml concentration dissolved in MeOH were pipetted into holes A1-A6. The samples were then diluted using serial dilution technique by pipetting 100 µl of ascorbic acid samples from holes A1-A6 and transferring into holes B1-B6. The step was repeated from holes B1-B6 to the next holes until holes H1-H6. 100 µl of the solution from the last holes were discarded. The test was followed by pipetting 100 µl of the prepared DPPH solution into holes A4-A6 until F4-F6, and 100 µl MeOH into holes A1-A3 until H1-H3. The microplate was covered and placed in a dark cabinet at room temperature for 40 minutes before the absorbance analysis was done using UV-Vis spectrophotometer at 517 nm.

Betalain Sample Preparation

100 µl of MeOH was pipetted using multi pipettor (6 tips) into holes B1-B6, C1-C6, until H1-H6. Then, 200 µl of betalain solution with 1 mg/ml concentration dissolved in MeOH were pipetted into holes A1-A6. The samples were then diluted using serial dilution technique by pipetting 100 µl of betalain solution samples from holes A1-A6 and transferring into holes B1- B6. The step was repeated from holes B1-B6 to the next holes until holes H1-H6. 100 µl from the last holes were discarded. The test was followed by pipetting 100 µl of the prepared DPPH solution into holes A4-A6 until F4-F6, and 100 µl MeOH into holes A1-A3 until H1-H3.

Calculation on DPPH Inhibition Percentage

Inhibition (%) = $[1-(Abs_{sample}/Abs_{DPPH})] \times 100$

*Abs_{sample} = Absorbance (sample + DPPH) – Absorbance (sample + MeOH)

Betalain Pigment Preparation for Stability Test

Heat Test

Betalain sample at concentration 1, 2 and 3 g/l have been prepared accordingly. All samples are incubated in the oven at eight points of temperature (10, 20, 30, 40, 50, 60 and 70°C) for 1 hour. The samples were prepared in triplicate and analysed hourly to monitor their stability. The changes of colour were measured in term of absorbance at 537 nm using the Varian Cary 50 Conc UV-Visible Spectrophotometer.

UV Irradiation Test

Three concentrations comprised of 1, 2, and 3 g/l of betalain has been prepared by dissolving in methanol. All glass tubes containing samples of three different concentrations have been placed in a laminar hood and exposed under UV-A (long wavelength = 365 nm, intensity of 5500 lux) and UV-B (middle wavelength = 312 nm, intensity of 2900 lux) perpendicularly 10 cm below the light source for 8 hours at room temperature. The samples are prepared in triplicate and analysed every 2-hour interval to monitor their stability. Any changes of colour before and after experiment were measured accordingly in term of absorbance using the Varian Cary 50 Conc UV-Visible Spectrophotometer at 537 nm.

RESULTS AND DISCUSSION

Antioxidant Activities

Results of the free radical scavenging activity of betalain and ascorbic acid as a standard are presented in Table **1**. The data established that the DPPH radical scavenging activity in betalain is decreasing with

reduce concentration of the DPPH from 77.48% of 50% inhibition activity to 56. 82%. Meanwhile, the inhibition activity of ascorbic acid is decreasing from 84.3% to 82.62%, with the reducing amount in the concentration of DPPH. Besides, the ability of DPPH as a stable free radical to decolorize in the presence of antioxidants explained its antioxidant assay. From this test, it showed that the dark purple colour of the sample changed into yellow colour, which proved the interaction of antioxidant founds in the compound interact with free radicals [16]. DPPH is now accepted an electron to make it stable diamagnetic molecule [17,18]. Other study also supported the existence of antioxidant in beetroot [7,19]. [20] added that the ingestion of a single dose of red beet juice resulted in an increase of antioxidant compounds including betalains in urinary excretion. Betalains and other phenolic compounds presented in red beet decreases oxidative damage of lipids and improves antioxidant status in humans. Antioxidant activity in red beet is associated involvement of antioxidants in the scavenging of free radicals and consequently in the prevention of diseases like cancer, cardiovascular diseases [3, 21]. Antioxidant activity was also reported to enrich human low density lipoproteins by betalains which increase resistance to oxidation [22]. It can be concluded that the free radical scavenging activity of methanol extract was confirmed in this experiment by having this percentage of inhibition activity value and by discoloration of sample from purple to yellow.

Table 1: DPPH Antioxidant Test on Different Concentration of Betalain

Betalain concentration (mg/ml)	Ascorbic acid (standard)	Inhibition (%)
1.0	84.30	77.48
0.5	84.11	71.59
0.25	83.74	65.05
0.125	82.80	60.65
0.063	83.18	58.32
0.032	82.62	56.82

Betalain Pigment Stability Test

Stability of betalain pigment chromaticity at different concentration of 1,2 and 3 g/l in response to different temperature of 10, 20, 30, 40, 50, 60 and 70°C were analysed in term of absorbance value. Examination of the summarised data in Figure **1** revealed that the temperature had a major effect on the stability of betalain pigment chromaticity. Temperature is regarded as the important factor to effect the stability of the colorant since it is related with the activation energy [14, 23]. In addition, comparison of betalain pigment chromaticity value ranging from 1.0 to 3.0 g/l in seven different temperatures also demonstrated tremendous fluctuation and variation. For example, 3.0 g/l contained relatively high value of chromaticity compared to 1.0 and 2.0 g/l betalain pigment concentration as well as from 10 to 70°C, but temperature from 10 to 30°C contained relatively more stable betalain pigment chromaticity value with slightly decreased for all concentrations of betalain pigment. These data suggest that different concentration of betalain as well as heat had an effect on the chromaticity value and stability on the betalain pigment. [13] reported that higher temperature will degrades the stability of the colorant. This is because betalain pigment slowly damages as a result of increasing temperature. During control state of temperature, the data showed that the chromaticity value increased with the increment of pigment concentration from 1.0 to 3.0 g/l. When treated under 10°C until 30°C, the absorbance reading increased in line with the addition of the concentration. However, the reading of all betalain pigment concentrations slowly decreased significantly starting from temperature 40°C until 70°C. In other words, the highest peak of absorbance reading is during temperature 30°C for three different readings of the concentrations. Similar result was also discovered by [24,25], however previous finding stated that the stability of betalains was recorded only at 20°C [9]. Another report manifested that the optimum temperature for the extraction is 40°C, and stated that betacyanins and betaxanthin yields drops following boiling and roasting methods [26].

Figure **2** represents the stability of betalain pigment at different concentration of 1,2 and 3 g/l exposed under UV-A (365 nm) and UV-B (312 nm) light, at room temperature, measured at every 2-hour for 8 hours. Large differences were detected when betalain pigment exposed to UV irradiation after 2 hours and this proved that the longer the exposure to UV irradiation resulted in continuous discoloration of the samples. The data is supported by the finding of betalain light stability which confirmed the color degradation when treated under light treatment for several times [27]. This is due to the excitement of the electron in betalain chromophores to a more energetic state that cause activation energy of the molecules becomes higher or lower in reactivity [13]. Besides, the deterioration of betalain stability due to light has been discussed widely. In agreement with that, [23] stated that determination of stability of quality

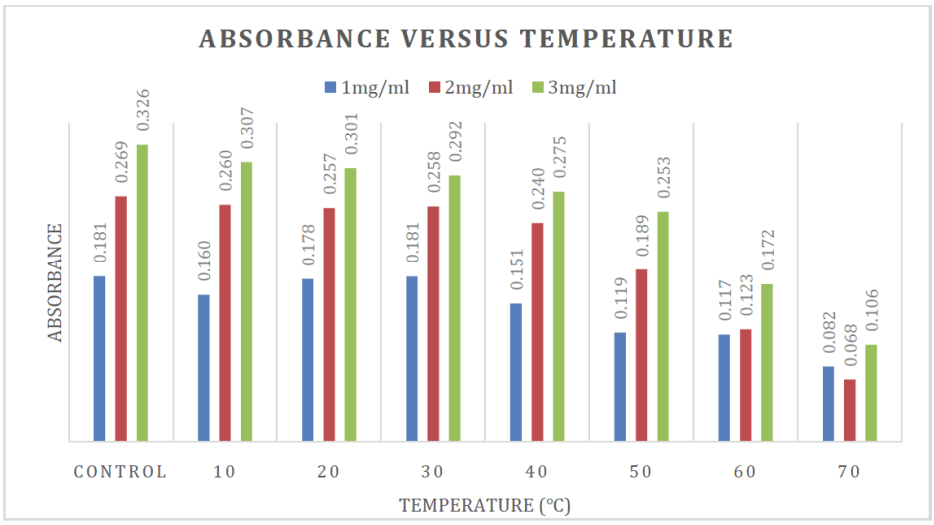

Figure 1: Stability of betalain pigment at different concentration of 1,2 and 3 g/l in response to different temperature of 10, 20, 30, 40, 50, 60 and 70°C.

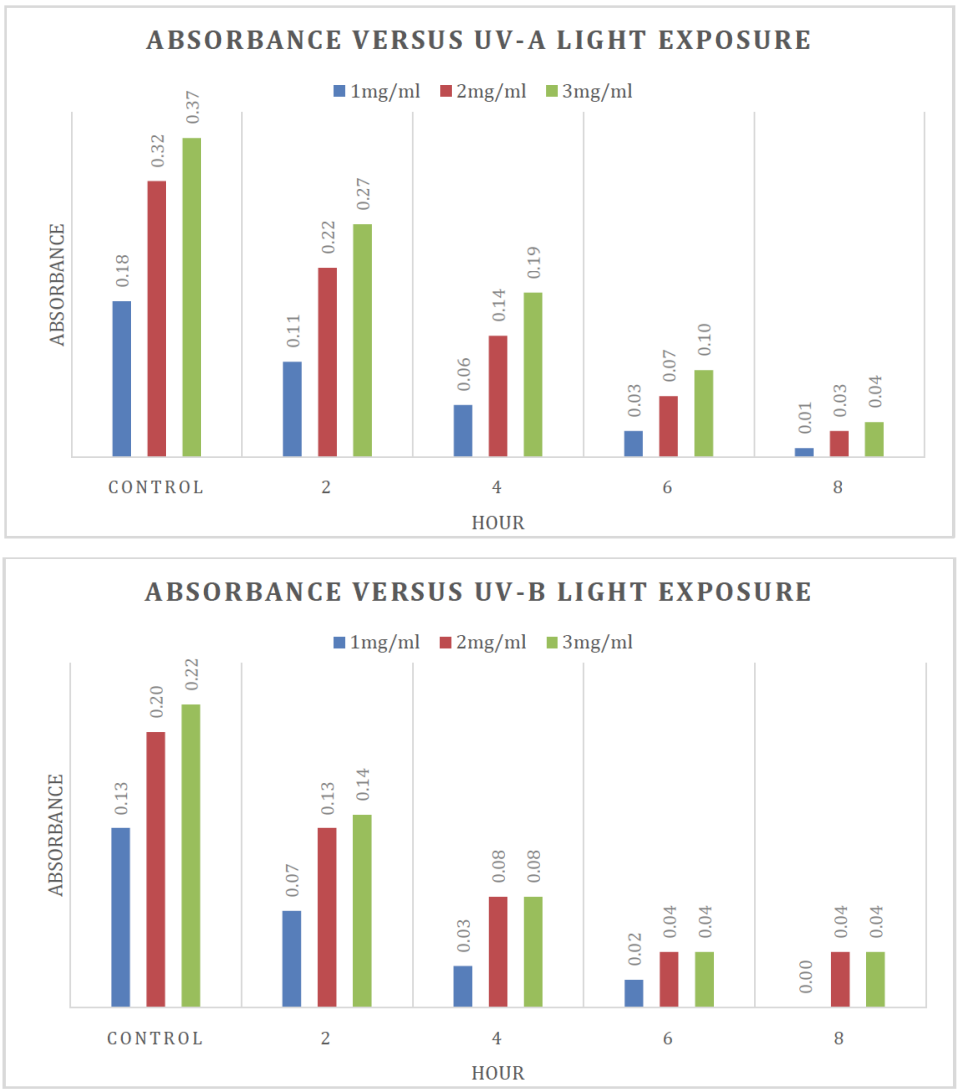

Figure 2: Stability of betalain pigment at different concentration of 1,2 and 3 g/l exposed under UV-A (365 nm) and UV-B (312 nm) light, at room temperature, measured at every 2-hour for 8 hours.

factors in food requires an understanding of rate orders and the parameters that influence them. Colour plays an important role in visual recognition and assessment of the surface and the subsurface properties of the object. It has a great influence on the appearance, processing and acceptance of food materials. The degradation of betanin was reported to follow first order reaction kinetics. It is reported that in the presence of excess of oxygen, betanin degradation follows pseudo first order reaction. Betanins are reported to have some antioxidant activity and are found to be effective in inhibiting lipid peroxidation.

CONCLUSION

Stability test performed explained that this natural pigment is dependent on the temperature and concentration to maintain the colour from degrades. The best temperature to maintain the stability of the colorant is below 40ºC, higher temperature will cause the colour to degrade. In addition, light sensitivity of betalains limits its ability to be used in food industry as the pigment will decolorize when expose to the UV irradiation for a longer period of time. Nevertheless, beet root is a form of functional foods proven by its high percentage of scavenging activity.

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