Quantification of total sugars and reducing sugars of dragon fruit-derived sugar-samples by UV-Vis spectrophotometric method

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Abstract. In the present work, the phenol-sulfuric acid method and the 3,5-dinitrosalicylic acid (DNS) method were developed with the aim to quantitatively analyze total sugars and reducing sugars, respectively. In regard with the phenol-sulfuric acid assay, 1.0 mL of sample was treated with 1.0 mL of 5% phenol, 5.0 mL of concentrated H₂SO₄ and measured at 485 nm, with the linearity range of 10–100 ppm for total sugars. The DNS method was performed on 2.0 mL of sample, using 1.5 mL of DNS at 80 °C for 10 minutes and measured at 510 nm, with the linearity range of 50–400 ppm for reducing sugars. The sugar contents of white dragon fruit-derived sugar-samples (extracted from species in Binh Thuan province, Vietnam) were also estimated by the above measured methods, exhibiting the total sugars of above 90% and the reducing sugars of above 5%. The methods were well-performed with the acceptable relative standard deviations of repeatability in accordance with the Association of Official Analytical Chemists (AOAC).

1. Introduction
Dragon fruits (Hylocereus, the Cactaceae family) are beneficial to human health thanks to its essential nutrients such as vitamins, minerals, carbohydrates, fibers and antioxidants. In particular, a large amount of betacyanin extracted from various species of dragon fruits have been widely applied as a red/purple pigment with antioxidant properties in food productions [1]. Without a doubt, dragon fruit-derived high value-added products have been increasingly studied in order to promote the local cultivation.

Among other nutrients, there is a lack of researches on total sugars and reducing sugars extracted from dragon fruits in Vietnam. The main sugars contents were discovered involving glucose and fructose (2–6 g / 100 g); besides, a low amount of sorbitol was also present (0.3 g / 100 g), meanwhile sucrose and maltose were not detected [2, 3]. The optimal extraction conditions were established using 80% (w/v) ethanol with a solvent-to-sample ratio of 2/1 at ambient temperature (28 ± 2 °C), permitting to achieve the sugars contents of 86.2 and 89.6 g/kg for white and red dragon fruits, respectively. The
oligosaccharides content in the extract was 27.4% relative to the sugars content, with the molecular weights of 716, 700, 490 and 474 Da and the degree of polymerization of 3–4. Oligosaccharides have prebiotic properties, such as resistance to acidity in the human stomach, partial resistance to human salivary α-amylase, and the ability to stimulate the growth of lactobacilli and bifidobacteria in human health. Therefore, dragon fruit is a potential source of prebiotics which can be used as an ingredient in functional foods and nutritional products [4].

In this study, total sugars and reducing sugars of white dragon fruit-derived sugar-samples (extracted from species in Binh Thuan province, Vietnam) were estimated by the simple and rapid colorimetric methods based on phenol-sulfuric acid and 3,5-dinitrosalicylic acid (DNS), respectively. In particular, reducing sugars (with free aldehyde or ketone functional groups) possess some predominant features for food productions such as high solubility, a mild sweet taste, involving in Maillard reactions with amines to improve the flavors and colors [5].

2. Experimental

2.1. Quantification of total sugars by the phenol-sulfuric acid method

Determination of the maximum absorption wavelength ($\lambda_{\text{max}}$): 1.0 mL of 100 ppm D-glucose, 1.0 mL of 5% phenol and 1.0 mL of distilled water were mixed for 1 minute; 5.0 mL of concentrated H$_2$SO$_4$ was then added, shaken for 3 minutes. The resulting solution was settled down for 30 minutes and cooled by water for 20 minutes before being measured at 400–800 nm by Ultraviolet-visible spectrophotometry (UV-Vis). The blank sample was prepared in the same procedure without D-glucose.

After affording the wavelength of maximum absorbance, the measured conditions were optimized involving doses of concentrated H$_2$SO$_4$, doses of 5% phenol and complex durability.

Dragon fruits were collected from Binh Thuan province (Vietnam) and their flesh was grinded and then extracted by ethanol (ethanol/H$_2$O of 1/1, v/v) at room temperature with the ratio of dragon fruit’s flesh / solvent of 1/2 (w/v). After filtering by filter paper, the solvent was removed by rotary evaporator under vacuum to achieve the sugars granules. The sugar-samples were dehumidified using moisture-proof granules and stored in refrigerator. As a typical experiment, 0.25 g of sugar-sample was dissolved in distilled water to make 100.0 mL and a 2.0 mL portion of the solution was diluted to 100.0 mL. After filtering, 1.0 mL portion of the diluted solution was mixed with 1.0 mL of 5% phenol and 1.0 mL of distilled water for 1 minute; 5.0 mL of concentrated H$_2$SO$_4$ was then added, shaken for 3 minutes. After settling down for 30 minutes, the resulting solution was cooled by water for 20 minutes and then measured at the wavelength of maximum absorbance by UV-Vis technique. The blank sample was prepared in the same procedure.

2.2. Quantification of reducing sugars by the 3,5-dinitrosalicylic acid (DNS) method

Determination of the maximum absorption wavelength ($\lambda_{\text{max}}$): 4.0 mL of 500 ppm D-glucose, 1.0 mL of DNS solution (1.00 g of DNS in 20.0 mL of 2.0 N NaOH solution and 50.0 mL of distilled water, 30.0 g potassium sodium tartrate were heated using a water bath until completely dissolving and then diluted to 100.0 mL with distilled water) and 7.0 mL of distilled water were shaken for 1 minute and then heated at 80 °C using a water bath for 10 minutes and cooled by water for 20 minutes before being measured at 400–800 nm by Ultraviolet-visible spectrophotometry (UV-Vis). The blank sample was prepared in the same procedure without D-glucose.

After affording the wavelength of maximum absorbance, the measured conditions were optimized involving doses of DNS and complex durability.

Pretreated as previous experiments (in the section of 2.1), 0.25 g of sugar-sample was dissolved in distilled water to make 25.0 mL and a 2.0 mL portion of the solution was diluted to 10.0 mL. After filtering, 2.0 mL portion of the diluted solution was mixed with 1.5 mL of DNS solution and 6.5 mL of distilled water for 1 minute, and then heated at 80 °C using a water bath for 10 minutes and cooled...
by water for 20 minutes. The resulting solution was measured at the wavelength of maximum absorbance by UV-Vis technique. The blank sample was prepared in the same procedure.

3. Results and Discussion

3.1. Quantification of total sugars by the phenol-sulfuric acid method

In principle, carbohydrates (monosaccharides, disaccharides, polysaccharides and their derivatives) can be decomposed in the presence of strong acid, leading to the multiple-step reactions started by dehydration. As a consequence of exothermic processes, furfural derivatives were generated and then condensed with phenol to form yellow compounds that can be analyzed by colorimetric methods [5]. The wavelength of maximum absorbance was observed at 485 nm (Figure 1), being consistent with the previously reported by Wichienchot et al. [4].

![Figure 1. UV-Vis spectrum of resulting solution between D-glucose and phenol-sulfuric acid measured at 400–800 nm.](image1)

At low doses of concentrated H$_2$SO$_4$ (< 3.0 mL), the yellow solutions were not observed and their absorbances were correspondingly extinct, evidencing the complex formation did not occur. In fact, 5.0 mL of concentrated H$_2$SO$_4$ adapted the required doses for furfural derivatives formation without affecting the chemical interferences like burning. On the other hands, the condensation with phenol should be afforded to occur completely by varying the phenol doses. The absorbance increased with the increase of the phenol doses and it kept unchanged at 1.0 mL of 5% phenol. Besides, the yellow complexes were stable during 60 minutes with their constant absorbances (Figure 2). Therefore, the calibration curve for the quantification of total sugars of dragon should be constructed in the optimal conditions, such as using 5.0 mL of concentrated H$_2$SO$_4$, 1.0 mL of 5% phenol and measuring at 485 nm during first 60 minutes.

![Figure 2. Effects of 5% phenol doses (a) and complex durability (b) on absorbance.](image2)
3.2. Quantification of reducing sugars by the 3,5-dinitrosalicylic acid (DNS) method

In principle, aldehyde functional groups of reducing sugars are oxidized to carboxylic acids accompanying the colored reaction with DNS reagent [6]. The color intensity is proportional to concentration of reducing sugars. The orange complex of DNS was measured at 400–800 nm, showing the wavelength of maximum absorbance at 510 nm (Figure 3).

![Figure 3. UV-Vis spectrum of resulting solution between D-glucose and DNS measured at 400–800 nm.](image)

Effect of DNS was observed on both blank and measured samples as evidenced by the increase of absorbances with the increase of DNS doses, although the excess amount of DNS is necessary for the reaction occurring completely. As clearly, the DNS volume of 1.5 mL should be used to earn the maximum magnitude of absorbance subtraction between measured sample and blank sample. The complex durability was also examined during 30 minutes with unchanged absorbances (Figure 4). Therefore, the calibration curve for the quantification of reducing sugars of dragon should be constructed in the optimal conditions, such as using 1.0 mL of DNS and measuring at 510 nm during first 30 minutes.

![Figure 4. Effects of DNS doses (a) and complex durability (b) on absorbance.](image)

3.3. Construction of calibration curves and quantification of total sugars and reducing sugars of white dragon fruit-derived sugar-samples

At the optimal conditions on quantification of total sugars by the phenol-sulfuric acid assay and reducing sugars by the 3,5-dinitrosalicylic acid (DNS) assay, the calibration curves were constructed (Figure 5) and their sugars contents of white dragon fruits were also quantitatively analyzed (Table 1). The methods were found to be linear in the measured ranges with the strong correlation coefficients ($R^2 > 0.99$). The total sugars and reducing sugars were performed on 6 white dragon fruit-derived sugar-samples with 2 replicates / sample. The relative standard deviations of repeatability for the phenol-sulfuric acid assay (RSD% = 0.66% < 1.3%) and the 3,5-dinitrosalicylic acid (DNS) assay (RSD% = 2.27% < 2.7%) were well-performed in accordance with the Association of Official
Analytical Chemists (AOAC) [7]. With the high contents of total sugars (above 90%) and reducing sugars (above 5%), white dragon fruit-derived sugar-based products are promising to widen diverse outputs for local cultivation.

![Calibration curves for quantification of total sugars by the phenol-sulfuric acid assay (a) and reducing sugars by the 3,5-dinitrosalicylic acid (DNS) assay (b).](image)

**Figure 5.** Calibration curves for quantification of total sugars by the phenol-sulfuric acid assay (a) and reducing sugars by the 3,5-dinitrosalicylic acid (DNS) assay (b).

**Table 1.** Quantification of total sugars and reducing sugars of white dragon fruit-derived sugar-samples.

| Sample | 1    | 2    | 3    | 4    | 5    | 6    |
|--------|------|------|------|------|------|------|
| $x_{i1}$ | 99.3 | 99.7 | 88.9 | 93.4 | 93.4 | 99.0 |
| $x_{i2}$ | 99.5 | 99.9 | 89.3 | 92.4 | 95.2 | 99.6 |
| $x_{\text{mean}}$ | 99.4 | 99.8 | 89.1 | 92.9 | 94.3 | 99.3 |
| $d_i$ | 0.2  | 0.2  | 0.4  | 1.0  | 1.8  | 0.6  |
| $D_i$ | 0.0020 | 0.0020 | 0.0045 | 0.0108 | 0.0191 | 0.0060 |

**Total sugars (%)**, w/w by the phenol-sulfuric acid assay

$$D_{\text{mean}} = \frac{\sum D_i}{n} = 0.0074$$

$$\text{RSD}\% = \frac{D_{\text{mean}}}{1.118} \times 100 = 0.66\%$$

| Sample | 1    | 2    | 3    | 4    | 5    | 6    |
|--------|------|------|------|------|------|------|
| $x_{i1}$ | 5.61 | 5.68 | 4.48 | 5.90 | 5.68 | 6.16 |
| $x_{i2}$ | 5.69 | 5.94 | 4.52 | 6.20 | 5.52 | 6.20 |
| $x_{\text{mean}}$ | 5.65 | 5.81 | 4.50 | 6.05 | 5.60 | 6.18 |

**Reducing sugars (%)**, w/w by the 3,5-dinitrosalicylic acid (DNS) assay

$$D_{\text{mean}} = \frac{\sum D_i}{n} = 0.0254$$

$$\text{RSD}\% = \frac{D_{\text{mean}}}{1.118} \times 100 = 2.27\%$$
|       | 0.08 | 0.26 | 0.04 | 0.30 | 0.16 | 0.04 |
|-------|------|------|------|------|------|------|
| $D_i$ | 0.0142 | 0.0448 | 0.0089 | 0.0496 | 0.0286 | 0.0065 |

4. Conclusions
The reliable, sensitive, rapid and simple colorimetric methods were developed to apply in the quantification of total sugars and reducing sugars of white dragon fruit-derived sugar-samples, based on phenol-sulfuric acid and 3,5-dinitrosalicylic acid (DNS), respectively. After optimizing measured conditions, such methods exhibited the acceptable relative standard deviations of repeatability in accordance with the Association of Official Analytical Chemists (AOAC). The high contents of total sugars (above 90%) and the reducing sugars (above 5%) promise to widen diverse outputs for local cultivation, in particular white dragon fruit-derived sugar-based products.

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