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Characterization of physical properties of red beet pigments

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Abstract

This work aimed to extract betalain pigments from red beet (*Beta vulgaris* L.) by different extraction methods: water and citric acid (2%) solvents to select the optimal method for extraction. In addition, the study determined total betalains, and effect of pH, time and temperature on betalains stability. The results showed that, pH, temperature and time of extraction did not have effect on the stability of Vulgaxanthin-1 pigment extracted using water and citric acid solvents. The concentration of betalain pigments extracted by water was higher than pigments extracted by citric acid solvent. Water extract of betanin pigments was more stable at temperatures between 25-60 ˚C, with maximum concentration of betanin pigment at 50˚C extracted for 5 to15 minutes. Also, the pigment was stable at pH ranged between 2 to 6. The highest concentration was in pH 6 with water extract, while, the highest concentration of betanin pigment was in pH 2 with citric acid extract. Therefore, water is the optimal method for extraction of pigments from red beet.

Keywords: Red Pigment, Betalain, Betanin, Red Beet *(Beta Vulgaris,* L.), Ph-Values, Temperature, Vulgaxanthin-1, Water Extract, Citric Acid Extract.

Introduction

Recently, the interest of the researchers about natural colorants and plants' pigments have been increased, due to the good apparent, lack of toxicity and eco-friendly characteristics. Especially, the food industry uses pigments as additives or supplements. Synthetic food colors in many food formulas may be harmful to the human body, and lead to increase the exposure to toxic and carcinogenic compounds. Fruits and vegetables are rich sources of natural pigments, which include a variety of components such as chlorophylls, carotenoids, anthocyanins, and betalains (Gandia-Herrero *et al*., 2010).

Fruits, flowers and Red beet are rich sources of betalain pigments, especially red and yellow beetroot *(Beta vulgaris L.* ssp. *vulgaris),* colored Swiss chard (*Beta vulgaris L.* ssp. *cicla*), grain or leafy amaranth (*Amaranthus* sp.) and cactus fruits *(Opuntia* and *Hylocereus* genera) (Henriette, 2009).

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Until now, red beet *(Beta vulgaris* L.*)* is the first betalains source exploited for use as natural food coloring, yielding various shades of red and violet colors (Stintzing *et al.*, 2002). Betalains in red beet have two type of pigments, the betanin with red-violet color and vulgaxanthin with yellowish color (George, 2017).

Betalains pigments are sensitive to several conditions, particularly pH, Temperature, light, moisture, oxygen, and sulfur dioxide, especially in systems with high water activity, which has an interactive effect, and pigments can quickly discolor (Nottingham, 2004 and Junqueira-Goncalves *et al.*, 2011).

Betalains pigments are used to enhance the red color in different food products such as strawberry ice-cream, yoghurt, sausages, cooked ham, sauces, jams, biscuits, creams, a range of dessert products (Stintzing and Carle, 2007), jellies, marzipan, dry powder beverages and sugar confectionery (Nottingham, 2004). Lately, betalains received an increasing attention due to their antioxidant, antiviral and antimicrobial effects. In addition, the stability of pigments is still a big issue in the food industry, and have drawn attention of scientists.

Materials and Methods

Materials

Red beet was purchased from local market in Jeddah, Saudi Arabia, filter papers (whatman no.1) from Sigma Aldrich, Germany, citric acid and pectin from Laboratory Reagents and Fine Chemicals, Mumbai, HCL from Scharlau, Spain, NaoH from C.O.O, Germany, Na2HPO4 from BDH Laboratory Supplies, England, and KH2PO from BDH Chemicals. Ttd, England.

Analytical Methods

Extraction of betalains pigments from red beet.

Red beet pigments extracted by using two different solvents, citric acid solution (2%) and water (Du and Francis, 1975; Delgado-vargas *et al.*, 2000). Red beets (50 g) were washed and cut into cubes, blended with (150 ml) of solvent either water or citric acid for (1 min), then filtered by using Whatman no.1 filter paper (Worlstad *et al.*, 2005). Each time for experiments, the extraction was made freshly.

Determination of total Betalains

Pigments content (betanin and vulgaxanthin-1) was measured in double beam UV-15-02 spectrophotometer. Samples were diluted by (0.05 M) phosphate buffer pH (6.5), then measured at 538 nm wavelength to detected betanin, 476 nm to detect vulgaxanthin-1, as well as 600 nm to correct small amounts of impurities. Phosphate buffer (0.05 M) with pH 6.5 was used as solvent blank. The concentrations of betanin and vulgaxanthin-1 were calculated using the following equations:

x=1.095*(a-c), z=a-x and y= (b-z) -(x/3.1), where:

a = light absorption of the sample at wavelength of 538 nm,

b = light absorption of the sample at wavelength of 476 nm,

c = light absorption of the sample at wavelength of 600 nm,

x = light absorption of betanin minus the colored impurities,

y = light absorption of vulgaxanthin-1 corrected for the contribution of betanin and colored impurities, z = light absorption of the impurities (Worlstad *et al*, 2005).

Determination of betalains stability

Effect of pH-value

pH value was determined using adopted Kadian and Sharma method (2013). Drops from the buffer (0.1 M HCl or 0.1 M NaOH) was added gradually until reaching to desired pH (2, 4, 6, 8, 10 and 12) at room temperature. Then concentration of pigments was measured at wavelengths of 538 nm, 476 nm and 600 nm (Kadian and Sharma, 2013).

Effect of temperature

The stability of red pigments at high temperature was measured. 5 ml of samples were held for 5, 10, 15, 20, 25 and 30 minutes in thermostatically controlled water bath at 25, 40, 50, 60, 70 and 80 °C, and cooled immediately in an ice bath. Then, to measure the concentration of pigments, the absorptions at wavelengths of 538 nm, 476 nm and 600 nm were recorded in the spectrophotometer (Kadian and Sharma, 2013).

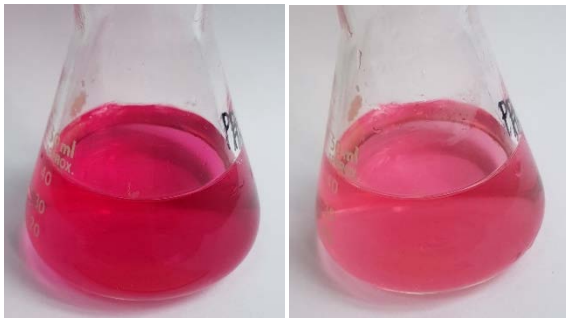
Statistical analysis

SPSS Statistics 20 and analysis of variance (ANOVA) using a tukey-test was used for Statistical analysis.

Results

This chapter conclude the results of total betalains extracted from red beet by using water and citric acid (2%) solvents, also, the results of the effects of pH, time and temperature on betalains' stability.

Figures 1 and 2 show the concentration of betanin pigment in both water and citric beetroot extracts (15.75 mg/100 ml and 13.79 mg/100 ml of fresh weight, respectively), which were higher than vulgaxanthin-1 pigment extracted by water and citric solvents (4.43 mg/100 ml and 3.77 mg/100 ml of fresh weight, respectively).



Water Extract **Citric Acid Extract**

Fig. 1: Red beet pigments extracted by water and citric acid solvents at room temperature.

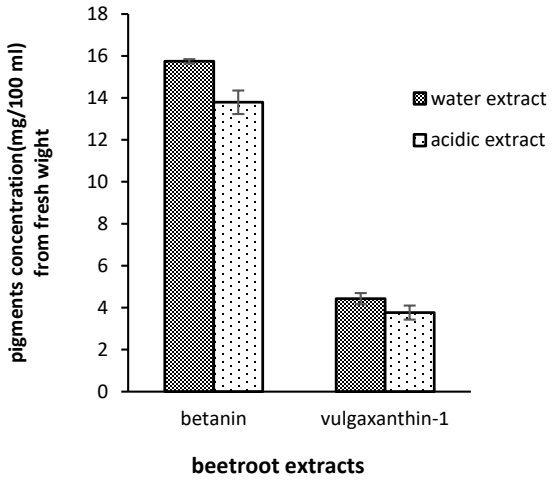


Fig. 2: Concentration of total betalains in red beet water and acidic extracts at 10 minutes in 25 °C.

The study also examined the effect of different ranges of pH on red beet pigments extracted by different solvents (water and citric acid). Figures 3 and 4 show the effect of pH on betanin and vulgaxanthin-1 pigments concentration extracted by water and citric solvents. The results revealed that, red beet betanin pigment extracted by water and acidified extracts were more stable at pH ranged from 2 to 6. The pH values at pH 2 and pH 6 in water extract and citric extract were 17.5- 14.7 mg/100 ml and 5- 6.1 mg/100 ml, respectively. The maximum stable pH of betanin and vulgaxanthin-1 pigments in red beet extracted by water was pH 2 and values were 17.51 and 10.35 mg/100 ml, respectively. In contrast, the maximum stable pH of betanin and vulgaxanthin-1 pigments in red beet extracted by citric acid was pH 6 and values were 6.09 and 4.17 mg/100 ml, respectively.

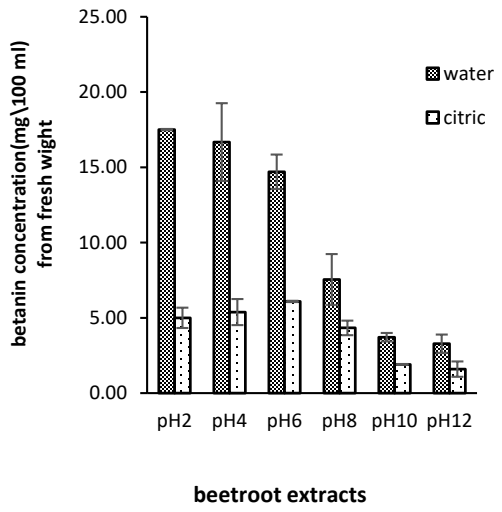


Fig. 3: Effect of pH (2-12) on red beet betanin pigment stability extracted by water and citric acid solvents.

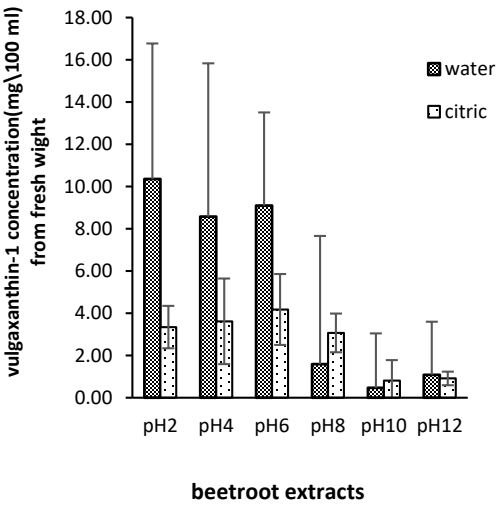


Fig. 4: Effect of pH (2-12) on red beet vulgaxanthin-1 pigment stability extracted by water and citric acid solvents.

The study results were analyzed by using ANOVA, and the results indicated that, there was no correlation between vulgaxanthin-1 pigment concentration extracted from red beet by water and citric solvents, as well as changes in pH values. In addition, there was a negative correlation between pH values with concentration of betanin pigment extracted from red beet by water and citric solvents. There were significant differences between pH 2, pH 4 and pH 6 compared with pH 8, pH 10 and pH 12 ($P = 0.000$, $P \leq 0.05$) and betanin pigment concentration extracted from red beet by water and citric solvents. Also, there was a significant difference between pH 8 in comparison with pH 10 and pH 12 ($P = 0.000$, $P \leq 0.05$) and betanin pigment concentration extracted from red beet by water and citric solvents, which means that the concentration of red pigment reduces by increasing pH values. Also, red beet pigments extracted by water solvent were more stable with increasing pH values compare with red beet pigments extracted by citric acid solvent.

The influence of temperature and time on stability of red beet betalain pigment extracted by water and citric acid solvents results revealed that, betanin pigment extracted by water and acidified extracts were more stable at temperatures ranged between 25 – 60 °C. The maximum stable temperature of betanin pigment in red beet extracted by water solvent was 50 °C with extraction time of 5, 10 and 15 minutes, and values were 21.16, 21.55, and 21.11 mg/100 ml, respectively. In contrast, the maximum stable temperature of betanin pigment extracted from red beet by citric acid solvent was 40 °C with extraction time of 5 minutes and value was 15.62 mg/100 ml. In addition, the maximum stable temperature of vulgaxanthin-1 extracted from red beet by water solvent was 25°C with extraction time of 5 minutes and value was 11.69 mg/100. Furthermore, maximum stable temperature of vulgaxanthin-1 extracted by citric acid solvent was 80°C with extraction time of 15 minutes and value was 9.68 mg/100.

The results of ANOVA indicated that, there was no correlation between vulgaxanthin-1 pigments concentration extracted from red beet by water and citric solvents and changes in extraction temperature or extraction time. In addition, there was a negative correlation between extraction temperature and extraction time with concentration of betanin pigments extracted from red beet by water and citric solvents. There was a significant difference between extraction temperature at 25°C compare with 70 and 80 °C, respectively ($P = 0.000, 0.000, P \leq 0.05$), and betanin pigment concentration extracted from red beet by water and citric solvents. There were significant differences between extraction temperature at 40 and 50 °C ($P = 0.005, 0.000$ and $0.000, P \leq 0.05$) compare with 60, 70 and 80 °C ($P = 0.041, 0.000$ and $0.000, P \leq 0.05$), respectively, and betanin pigment concentration extracted from red beet by water and citric solvents. Moreover, there was a significant difference between betanin pigment concentration extracted from red beet by water and citric solvents and extraction temperature at 60°C compared with 70 and 80°C ($P = 0.000, 0.000, P \leq 0.05$), respectively. There was a significant difference between time of extraction at 5 minutes compared with 25 and 30 minutes ($P = 0.000, 0.000, P \leq 0.05$) and betanin pigment concentration extracted from red beet by water and citric solvents. In addition, there was a significant difference between time of extraction at 10 minutes in comparison with 20, 25 and 30 minutes ($P = 0.053, 0.000$, and $0.000, P \leq 0.05$), respectively, and betanin pigment concentration extracted from red beet by water and citric solvents. Also, there was significant differences between time of extraction at 15 and 20 minutes compared with 30 minutes ($P = 0.010$ and $P = 0.012, P \leq 0.05$), respectively, and betanin pigments concentration extracted from red beet by water and citric solvents.

That means concentration of red pigment extracted from beetroot by water and citric solvents was reduced along with increase in time and temperature of extraction. Red beet pigments extracted by water solvent was more stable with increasing temperature and

time of extraction compared with red beet pigments extracted by citric acid solvent.

Discussion

The results of water and citric acid extraction concluded that betanin (red pigment) concentration was higher than vulgaxanthin-1 (yellow pigment), and water extract yielded more betanin and vulgaxanthin-1 pigments' concentration compared with citric extract.

In another research aimed to extract natural pigments from red cabbage and red beet by using different solvents (water, water with 1% HCL, HCL 1% aqueous: ethanol with ratio 1:1), with extraction time of 16-18 hours at room temperature, no significant difference was found in betanins concentration in both extracts (acidified water with HCL and water-ethanol-HCL). The result of the concentration of betalains extracted from red beet by using water as solvent was 8.8 mg /100 ml of fresh weight, which was below the concentration of the current results (15.75 mg/100 ml; Bruno *et.al*., 2012). Maybe the differences in the concentration results between the previous study and current study is due to longer time of magnetic agitation leading to loss of pigments.

Another work aimed to investigate the effect of type of extraction, temperature time and mass of red beet betalain in the water extraction. The betalain pigments were extracted using red beet samples (0.5 g, 1 g and 1.5 g) with 50 ml of water as solvent with different temperatures (40 to 70 ˚C) and times (30 to 90 minutes). The results revealed that the total betanins was 30.9 mg\100 g and total vulgaxanthin-1 was 16.3 mg\100 g (Swamy *et al*., 2014). The results of betanin and vulgaxanthin-1 quantifications of current study were less than the study performed by Swamy (2014). Maybe in Swamy's work the sample-solvent ratios (0.01, 0.02, 0.03:1) were different with current study (0.33:1), or Box-Behnken design was better method for extraction than solvent extraction method.

The current study showed that, aqueous and acidic extracts of betanin pigment were more stable at pH ranged from 2-6. While, Stintzing et al. (2006) blended four commercial anthocyanin (strawberry, cherry, black carrot and elderberry) with one betalain pigment extracted from red beet by using water as solvent. The purpose of mixing betalain with anthocyanin was to produce new acceptable and high quality color. This color was evaluated over three weeks and pH stability of betalains was determined. The results showed optimum betalains stability at pH 4.5 and pH 5 and high stability at pH 7. The current results agreed with Stintzing and colleagues (2006). Little differences in results of this paper with previous studies maybe due to differences in red beet cultivars in the country.

Another work aimed to measure the effect of specific food additives like citric acid in stability of betalains extracted from red beet juice. This juice was prepared in pH 3-8 and all samples (with and without citric acid) were heated to 85˚C for 60 minutes. The results showed the stability of betalain pigments at pH of 4-5. Also, the addition of citric acid was not effective in extraction from red beet (Skopinska *et al*., 2015). This results were in agreement with the pH rang in the present work (2-6).

In this work, high betalains concentration was found in citric and water extract at 40 and 50°C, respectively, which were stable between 25 - 60 °C. Another study examined the effect of temperature on the stability of pigments, using ethanol (acidified with 2% citric acid) to extracted betalain pigments from red beet. Then, the influence of different temperatures (40 to 100˚C) for 30 minutes on the stability of betalain was determined. Tthe results revealed no degradation of pigments in moderate temperatures (40 to 50˚C; Attia *et al*., 2013). This results agreed with present research.

Kadian and Sharma (2013) used mixture of aqueous and ethanol at 50:50ratio to extracted betalains pigments from red beet in chilling conditions, and concentrated the pigments in rotary evaporator at 35˚C. Then, they evaluated the effect of different times (5, 10, 15 and 20 minutes) with dissimilar temperatures (40, 50, 60, 70 and 80˚C) on stability of betalains. The results showed that, pigments were lost by increasing time and higher temperature. The results were similar to this work, as in red beet pigments were lost in higher temperatures and longer times.

Conclusion

The concentration of total red beet betalains in water extract was more than citric extract. Also, the pigments extracted by water solvent were more stable with increasing pH values and increasing temperature compare with red beet pigments extracted by citric acid solvent. The concentration of red beet pigments was reduced by increasing pH values, extraction temperature and time of extraction. Finally, vulgaxanthin-1 pigment concentration extracted from red beet by water and citric solvents was not affected by changes in pH values, extraction temperature and time of extraction.

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