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Betanin: A Red-Violet Pigment - Chemistry and Applications

Deepak Devadiga and T.N. Ahipa

Abstract

Nowadays, the demand for eco-friendly/nontoxic natural colorants is growing as an essential alternative to potentially harmful synthetic dyes. Betanin is the chief red pigment of beetroot, and it is the only betalain approved for use in food and pharmaceutical products as a natural red colorant. This chapter is mainly dealing with the betanin pigment, and also the chapter is subdivided into six sections covering the chemistry of betanin, extraction of color using various novel techniques (like microwave- and ultrasonic-assisted extraction) from raw plant material, bio-synthesis of betanin followed by chemical synthesis of betanidin, and also the effect of pH, temperature, and light on the stability of betanin followed by its applications.

Keywords: beetroots, betanin, red pigment, extraction, chemical synthesis, stability

1. Introduction

Vegetable beetroot (*Beta vulgaris* L.) has the notable scientific interest, because it is a rich source of nitrate (NO_3^-), a compound with advantageous cardiovascular health effects through the endogen production of nitric oxide (NO) [1, 2]. There are two classes of pigment in plants, i.e., betalains and anthocyanins. Beetroots are the chief sources of betalains which is a water-soluble nitrogen pigment with heterocyclic ring, which can be further subdivided into two classes depending on chemical structure: betaxanthins comprising indicaxanthin; vulgaxanthin I and II, accountable for orange-yellow coloring; and betacyanins, such as betanin, isobetanin, neobetanin, and prebetanin, accountable for red-violet coloring [3, 4]. The most abundant betacyanin is betanin (betanidin 5-O- β -D-glucoside) and is the only pigment which is an approved natural colorant for the use in food products prescribed by the Food and Drug Administration (FDA) in the United States [5, 6]. **Figure 1** represents the chemical structure of betanin.

According to experimental studies, raw beetroot generally contains water (87.1%), carbohydrate (7.6%), protein (1.7%), fat (0.1%), and betanin (0.03–0.06%) [7]. In addition to natural food colorant property, betalain also exhibits antimicrobial, antiviral, and antioxidant activities [8]. Moreover, beetroot dye has nutrient value along with nontoxic nature; therefore, it even finds application in dyeing industry where the health aspect is a foremost criterion. Also, this natural dye extracted from beetroot is eco-friendly in nature and does not cause any environmental problems in contrast to the commercially available synthetic dyes [7].

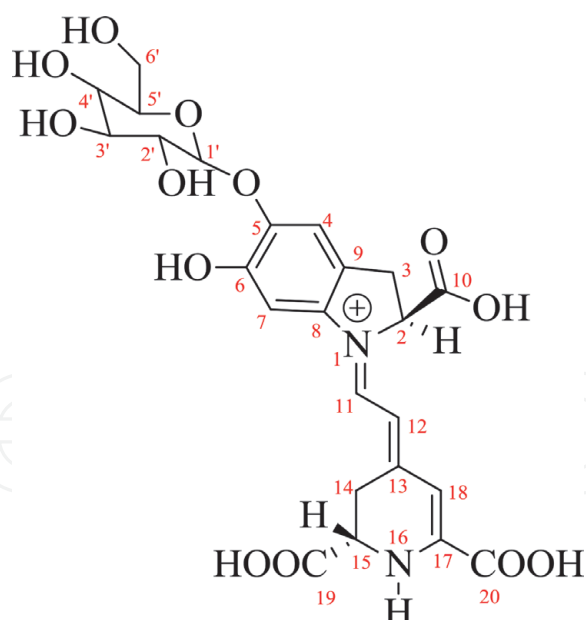


Figure 1.
Chemical structure of betanin.

2. Chemistry of betanin

The first report on the crystallization of betanin was communicated by two independent groups Schmidt and Schonleben [9] and Wyler and Dreiding [10]. These two groups employed an electrophoretic strategy for betanin purification. Wyler and Dreiding [11] recognized three products which were formed by the alkaline degradation of betanidin (**Figure 2**); these were 4-methylpyridine-2,6-dicarboxylic acid, formic acid, and S-cyclodopa(5,6-dihydroxy-2,3-dihydroindole 2-carboxylic acid). When these three products are placed in correct relationship with one another, they form the betanidin's carbon skeleton and also the configuration of the second carbon [12].

2.1 Position of the β -D-glucosyl group

To identify the position of β -D-glucosyl group, betanin was reacted with diazomethane in the presence of air yielded tetramethyl derivative of neobetainidin which was then converted to 6-methoxy-neobetainidin-trimethylester by hydrolysis and acetylation [13, 14]. In the alkali condition, neobetainidin cleaved to yield 5-hydroxy-6-methoxyindole 2-carboxylic acid. Its methyl ester was prepared from

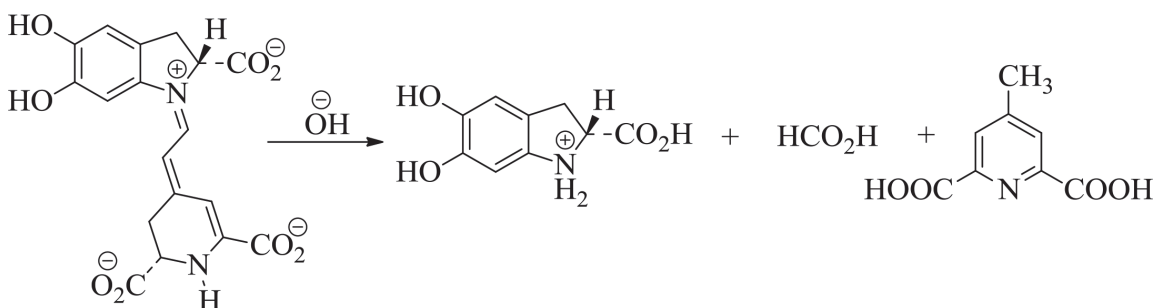


Figure 2.
Alkaline degradation of betanidin.

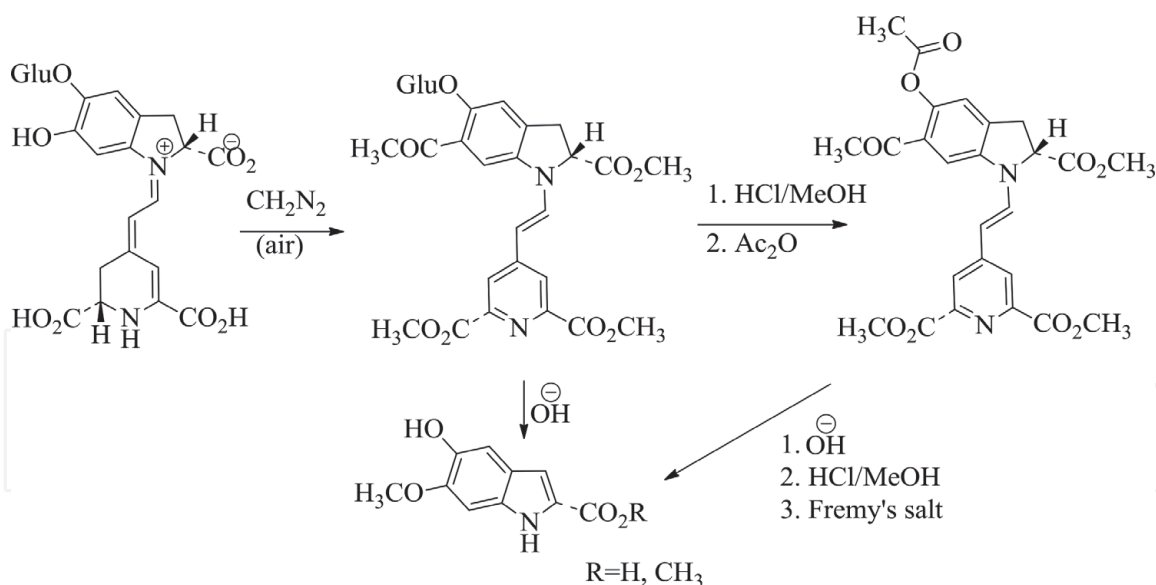


Figure 3.
 Reactions that revealed the glucosyl residue position in betanin.

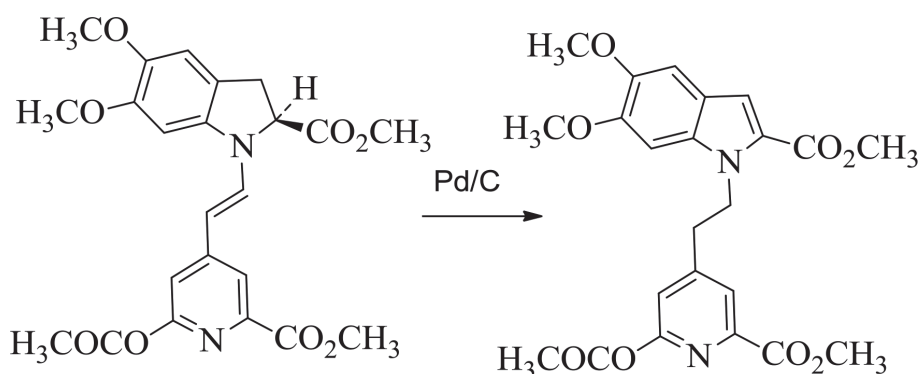


Figure 4.
 Reaction which confirmed the presence of vinylene connecting group.

acetylated molecule by degrading in basic condition, and further it was esterified and oxidized. Further, analysis of all these reactions together revealed the glucosyl residue position (**Figure 3**). In addition, NMR of betanin in trifluoroacetic acid and hydrolytic studies of β -glucosidase [15] revealed that betanin was an O- β -D-glucopyranoside.

The yellow-colored compound, i.e., 5,6-di-O-methylneobetanidin trimethyl ester, converted into colorless compound, i.e., 5,6-di-O-methyl-2,3-dehydro-11,12-dihydro-betanidin trimethyl ester, by the palladium-catalyzed disproportionation reaction which confirmed the existence of vinylene connecting group (**Figure 4**) in the derivatives of neobetanidin [12].

Further, betanin can also be interconverted into betanidin. To prepare betanidin, betanin is initially reacted with the excess of L-proline in the presence of dilute ammonia which results in the formation of indicaxanthin which can later be converted into betanidin by reacting it with excess S-cyclodopa [12].

3. Extraction techniques

The drawbacks of conventional approaches, such as time-consuming methods, safety risks with some hazardous solvent systems, contaminated product, and comparatively less yields, have increased the demand for the novel processing methods

Variants	Solvents
V ₁	Distilled water
V ₂	1% citric acid
V ₃	0.5% citric acid
V ₄	0.2% citric acid
V ₅	0.1% ascorbic acid
V ₆	50% ethanol
V ₇	20% ethanol
V ₈	0.5% citric acid + 0.1% ascorbic acid
V ₉	0.2% citric acid + 0.1% ascorbic acid
V ₁₀	20% ethanol + 1% citric acid
V ₁₁	20% ethanol + 0.5% citric acid

Table 1.
Betanin extraction from beetroot using different solvent systems.

[16, 17]. To improve the betalain extraction, some of the pretreatment methods have been proposed, such as pulsed electric fields [18], gamma irradiation [8], and low-direct current electric fields [19]. But, such methods are quite expensive when compared to the solvent extraction techniques. Further, certain nonthermal techniques, like ultrasound (sonication) processing, and microwave-assisted extractions are also significantly productive in order to enhance the extraction yields of bioactive molecules with minimum degradation [17].

Neagu and Barbu [20] studied the betanin extraction from beetroot using different solvent systems by solid-liquid extraction technique (liquid/solid ratio is 5:1). **Table 1** presents the different extraction solvents used in this study. Results revealed that the highest betanin content of about 20 mg/g of beetroot was obtained with the use of weak acid solution (i.e., V₈, using 0.5% citric acid +0.1% ascorbic acid). Also, they extracted the considerable amount of betanins by using ascorbic acid added solutions. Thus, it is clear that the acidic medium influences positively during the low-temperature extraction process.

In case of ultrasound (sonication) processing, ultrasonic-assisted extraction approach requires the use of ultrasound (with 20–2000 kHz frequencies range) which generally increases the cell wall permeability and generates the cavitations. Because of cavitations, cell membrane breaks down, and internal materials (color and oil) come out [21].

On the other hand, microwave-assisted extractions are also contributed significantly to speed up the sample digestion and extraction of bioactive molecules from matrices. Here, the microwave energy has been utilized and employed in this extraction process. Moreover, this microwave energy induces molecular motion by the rotations of dipoles and migration of ions without varying the structure of the molecules provided the temperature of the system is not too high [21].

4. Biosynthesis of betanin

The biosynthetic pathways for the betanin molecule are depicted in **Figure 5** [22]. Three enzymes such as 4,5-DOPA (dihydroxyphenylalanine)-extradiol-dioxygenase, tyrosinase, and betanidin-glucosyltransferase were involved in the biosynthesis of betalains in the cytoplasm [23]. From arogenic acid, the amino acid L-tyrosine was

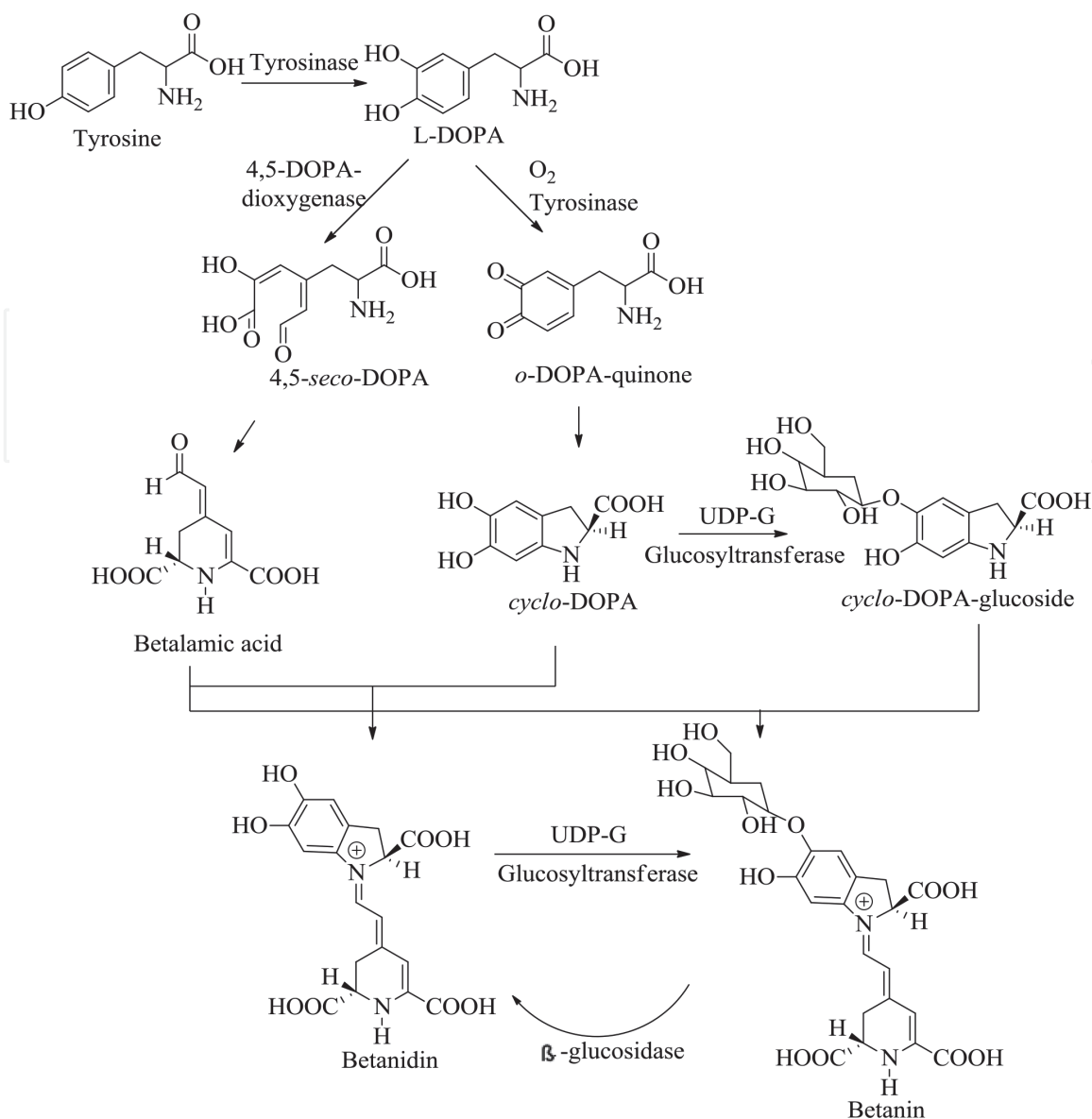


Figure 5.
 Biosynthesis of betanin.

formed enzymatically over the shikimate pathway [24], which is the starting material for the biosynthesis of L-DOPA [25]. During this conversion, tyrosinase enzyme helped to convert tyrosine to DOPA through hydroxylation. Further, between carbons 4 and 5 of the L-DOPA cyclic ring was opened by 4,5-DOPA-extradiol dioxygenase enzyme to give 4,5-seco-DOPA [26–28] which is then converted into betalamic acid by intramolecular condensation between aldehyde and amine groups [25]. Further, L-DOPA is transformed into o-DOPAquinone in the presence of molecular oxygen [29]. Furthermore, it was spontaneously cyclized due to the nucleophilic attack of amino group on the ring system to yield *cyclo*-DOPA [23]. However, *cyclo*-DOPA can also be obtained from the cyclization of L-DOPA in the presence of cytochrome P450 (CYP76AD1) [30].

Finally, betanidin was formed by the formation of imine bond between *cyclo*-DOPA and betalamic acid which is then converted to betanin using betanidin-5-O-glucosyltransferase enzyme by connecting glucose unit of uridine diphosphate-glucose (UDP-G) to the hydroxyl group in position 5 [31]. But this reaction can be reversed in the presence of β-glucosidase [32]. Additionally, it is also concluded that enzyme *cyclo*-DOPA-5-O-glucosyltransferase catalyzes the transport of glucose molecule on *cyclo*-DOPA, by which the *cyclo*-DOPA-glucoside condense with betalamic acid to yield the betanin [33].

5. Chemical synthesis of betanidin

The chemical synthesis of betanidin is illustrated in **Figure 6** [34]. For the synthesis of betanidin, 4-hydroxypyridine-2,6-dicarboxylic acid is used as the starting material which upon hydrogenation and followed by esterification yields all products in *cis* form. Further, Pfitzner-Moffatt oxidation reaction converted the secondary hydroxyl group to a ketone. In the next step, it is converted to semicarbazide using Horner-Wittig reagent. Then, the obtained semicarbazide is further hydrolyzed to give unsaturated ketone, which is converted to betalamic acid by Pfitzner-Moffatt oxidation. Reacting betalamic acid with L-cyclo-DOPA methyl ester yielded betanidin trimethyl ester which is then converted to betanidin through acid hydrolysis by using concentrated hydrochloric acid.

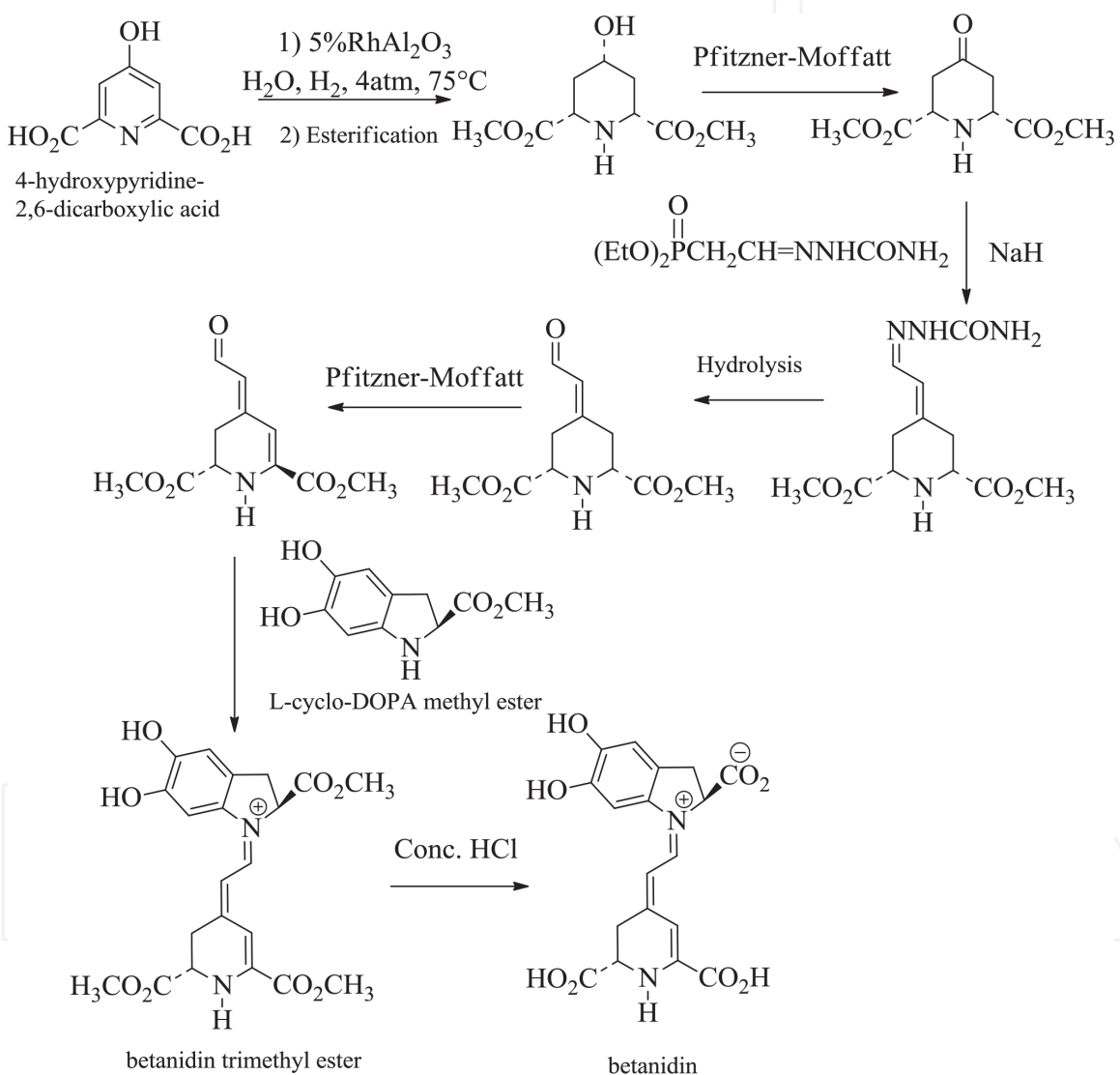


Figure 6.
Chemical synthesis of betanidin.

6. Characterization of betanin

6.1 UV-vis absorption spectra of beetroot extracts

The UV-vis spectra of beetroot extracts in different solvents (ethanol, methanol, and water) are depicted in **Figure 7** [35]. Strong absorption band was observed at

around 530 nm in the visible range for the red beet juice which was attributed to the betanin pigment. As the solvent changed from ethanol (532 nm) to water (542 nm) and to methanol (544 nm), absorption maximum shifted towards longer wave-lengths. These results showed that methanolic and aqueous extracts mainly contain

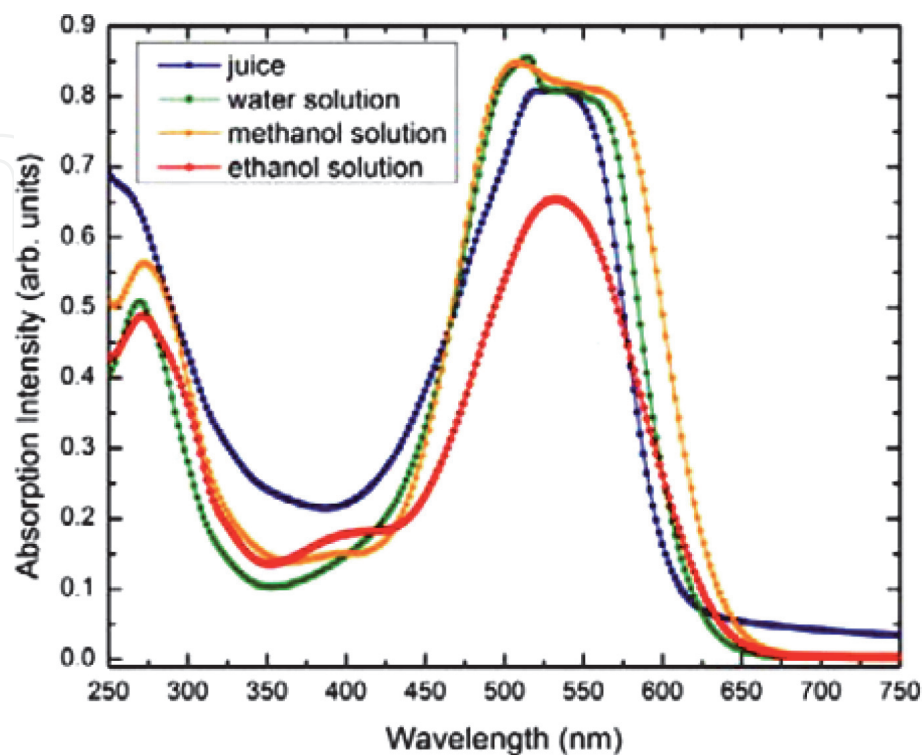


Figure 7.
The UV-vis spectra of beetroot extracts in different solvents (ethanol, methanol, and water).

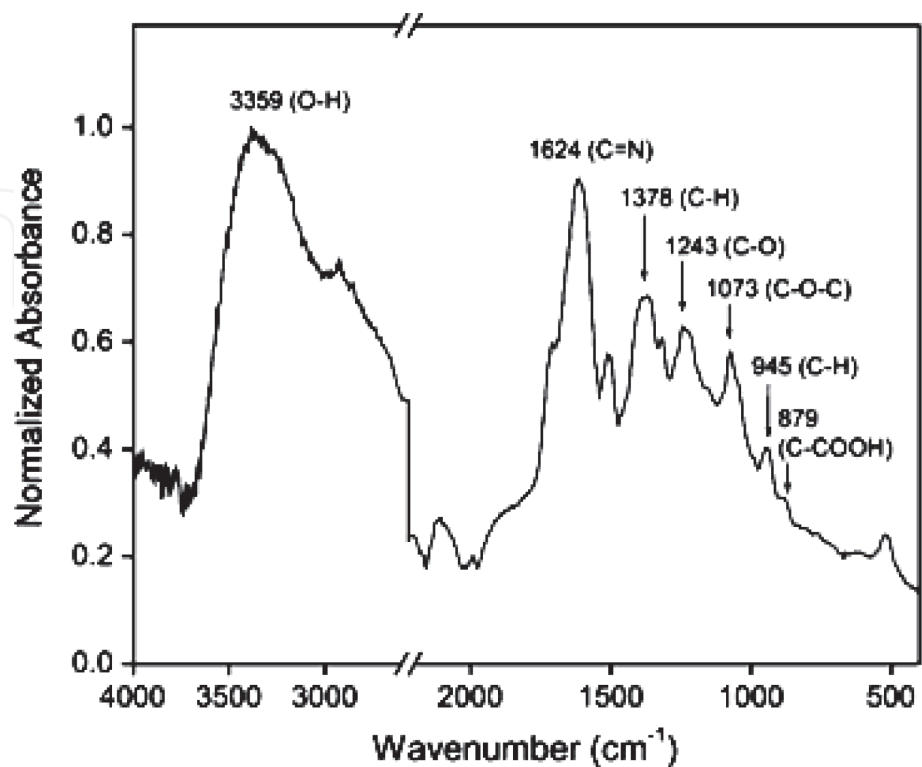


Figure 8.
FT-IR spectrum of betanin (scale range, 400–4000 cm^{-1}).

betanidin, whereas ethanolic extract mainly contains betanin. The intensity of absorption maxima for the aqueous and methanolic extracts is approximately equal, higher than the ethanolic extract.

Further, partially overlapped two absorption bands were observed in case of aqueous and methanolic extracts. For aqueous extract the second band has an absorption maximum at 515 nm and for aqueous extract at 509 nm. These bands are only observed in high concentrated extract, and it vanishes as the solution diluted. Hence, it can be attributed to the formation of supramolecular structure in the concentrated solutions.

6.2 FT-IR data of betanin

Different characteristic absorption bands corresponding to the functional groups of betanins were observed, and its FT-IR spectrum is shown in **Figure 8** [36]. The absorption band around 3359 cm^{-1} was ascribed to the —OH bond stretching vibration [37]; on the other hand, the absorption band around 1624 cm^{-1} was ascribed to the $\text{C}\equiv\text{N}$ bond stretching vibration [38, 39]. The next absorption band located at 1378 cm^{-1} was ascribed to the C—H bond extension stretching vibration, while the absorption band at 1243 cm^{-1} was ascribed to the C—O bond of the carboxylic acid stretching vibration [38, 39]. Another absorption band centered at 1073 cm^{-1} was ascribed to the C—O—C linked symmetric stretching vibration [40], the absorption band at 945 cm^{-1} was ascribed to the C—H bond deformation, and lastly the absorption band at 879 cm^{-1} was ascribed to the C—COOH bond stretching vibrations [41].

6.3 ^1H , ^{13}C , and $\text{LC-}^1\text{H}$ NMR data of betanin

The ^1H and ^{13}C NMR data of betanin was obtained by dissolving it in D_2O , and its $\text{LC-}^1\text{H}$ NMR data was also obtained by dissolving it in acetonitrile (MeCN)/ D_2O /0.05% trifluoroacetic acid (TFA) using 500 MHz frequency at 25°C . **Figure 9** represents the $\text{LC-}^1\text{H}$ NMR spectrum of betanin and followed by **Table 2** which represents the ^1H , ^{13}C , and $\text{LC-}^1\text{H}$ NMR data of betanin [42].

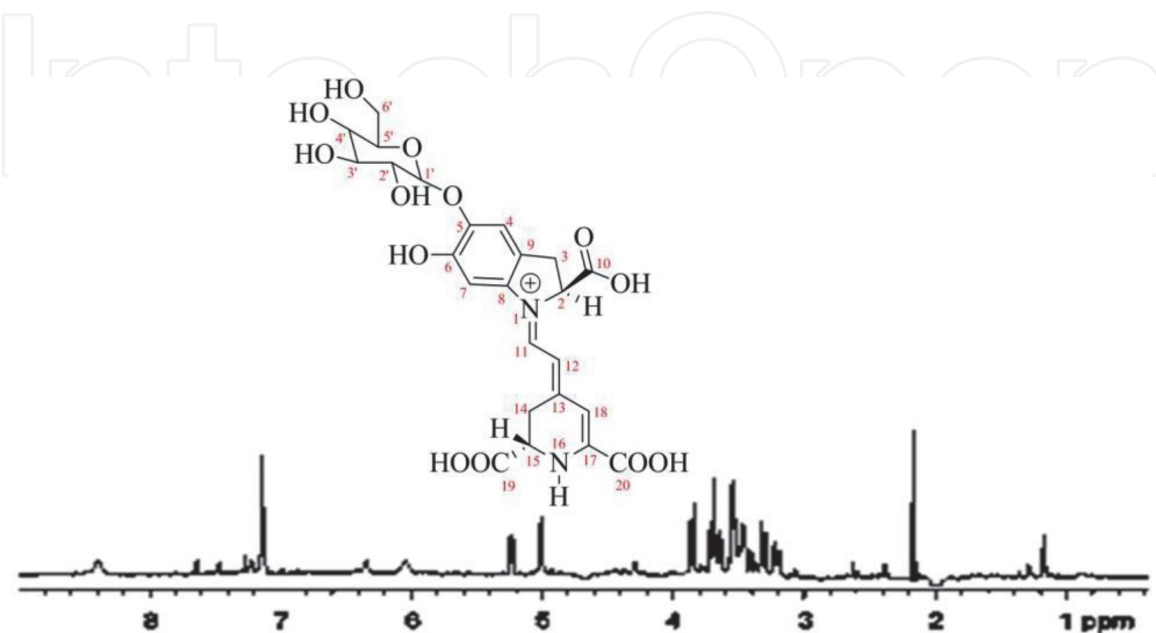


Figure 9.
LC- ^1H NMR spectrum of betanin.

Atom numbering	¹ H NMR δ-[ppm], mult, J [Hz]	¹³ C NMR δ-[ppm], mult, J [Hz]	LC- ¹ H NMR δ-[ppm], mult, J [Hz]
2	4.92, <i>dd</i> , 3.1, 10.3	65.0	5.23, <i>dd</i> , 2.0, 10.2
3a/b	3.53, <i>dd</i> , 11.5, 16.9 3.10, <i>dd</i> , 4.3, 16.8	32.7	3.66, <i>dd</i> , 10.4, 16.7 3.31, <i>dd</i> , 2.0, 16.7
4	7.06, <i>s</i>	113.9	7.13, <i>s</i>
5	—	144.0	—
6	—	146.1	—
7	6.98, <i>bs</i>	100.0	7.12, <i>bs</i>
8	—	137.4	—
9	—	124.1	—
10	—	175.8	—
11	8.19, <i>bs</i> (<i>d</i> , 12.6) ^b	144.4	8.38, <i>bd</i> , ≈11
12	5.84, <i>bs</i> (<i>d</i> , 12.6) ^b	106.9	6.04, <i>bd</i> , ≈11
13	—	— ^a	—
14a/b	3.20, <i>bm</i> 3.12, <i>bm</i>	26.5	3.21, <i>dd</i> , 7.4, 17.2 3.41–3.59 (overlap)
15	4.40, <i>bt</i> , 7.1	53.1	Overlapped by D ₂ O
17	—	— ^a	—
18	6.22, <i>bs</i>	— ^a	6.34, <i>bs</i>
19	—	— ^a	—
20	—	— ^a	—
1'	4.98, <i>d</i> , 7.4	101.4	5.00, <i>d</i> , 6.9
2'	3.55 (overlap)	75.7	3.41–3.59 (overlap)
3'	3.55 (overlap)	73.9	3.41–3.59 (overlap)
4'	3.41 (overlap)	69.3	3.41–3.59 (overlap)
5'	3.52 (overlap)	76.2	3.41–3.59 (overlap)
6'a/b	3.85, <i>dd</i> , 1.6, 12.3 3.70, <i>dd</i> , 5.3, 12.3	60.6	3.86, <i>dd</i> , 1.4, 12.6 3.70, <i>dd</i> , 5.5, 12.6

^aChemical shifts were not observable.
^bAfter acidification (TFA) to pH 2, *s* = singlet, *d* = doublet, *dd* = doublet of doublet, *bs* = broad singlet, *bd* = broad doublet, *bt* = broad triplet, *bm* = broad multiplet.

Table 2.
¹H, ¹³C, and LC-¹H NMR data of betanin.

6.4 Mass spectrum of betanin

The mass spectrometry of betanin in the positive ionization mode exhibited a molecular ion (*m/z* 551, [M+H]⁺, 100%). **Figure 10** represents the obtained mass spectrum of betanin molecule [43].

6.5 Thermogravimetric (TG) analysis

The dynamic thermogravimetric (TG) analysis was conducted on fresh betanin and dried betanin (**Figure 11a** and **b**) [44]. Since fresh betanin contains water, immediate mass loss is noted in the temperature range of 40–100°C. Further, the degradation temperature of betanin dye is noted at the temperature of about 204°C.

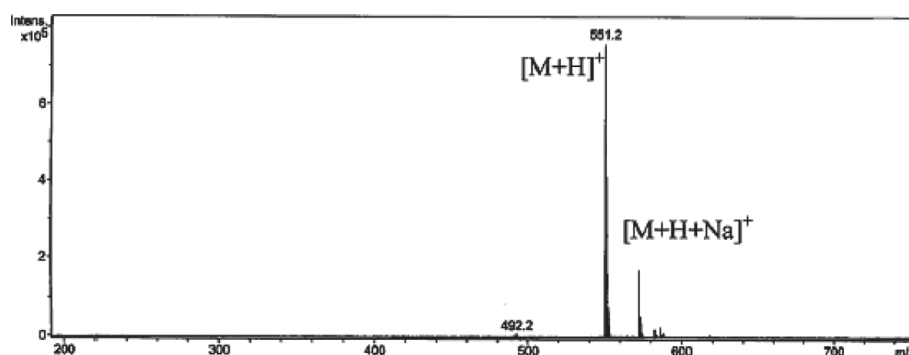


Figure 10.
Mass spectrum of betanin.

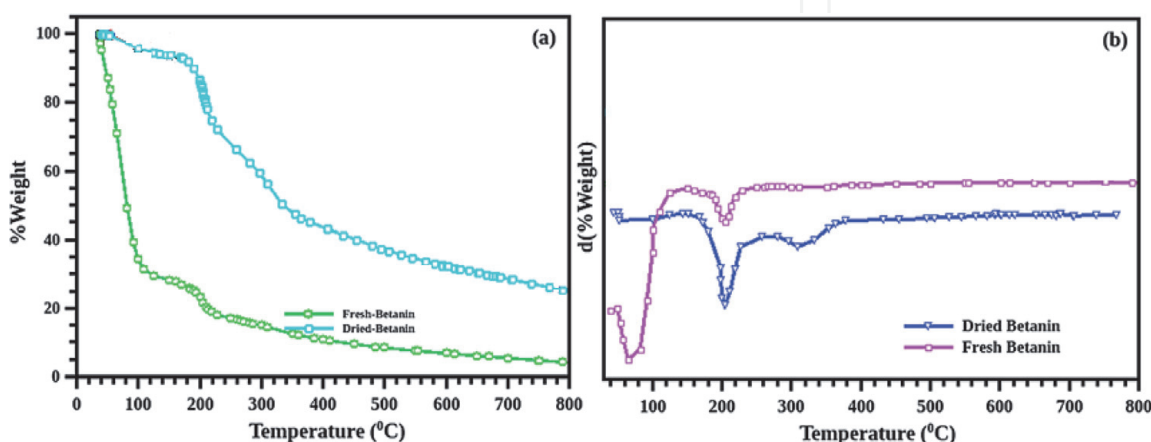


Figure 11.
(a) TG curves and (b) derivative TG curves of fresh and dried betanin.

Furthermore, the dried sample has also exhibited similar behavior except the mass loss at the beginning (in the temperature range of 40–100°C).

7. Factors effecting the stability of betanin

- **pH:** in the buffers of pH 2–9, betanin was stored at 4°C for 7 days and measured the visible spectra at both starting and end of this time span. No shifts in the absorption maxima were noted in between pH 4 and pH 7. A shift of about 2 nm towards a shorter wavelength with a decreased absorbance intensity was observed in case of the buffer which has pH less than 4. In the 575–650 nm region, the spectrum has slightly increased absorbance, and the solution color changed to red-violet from red. While in case of the solutions which has pH value of above 7, i.e., at pH 9, the absorption maximum moved to a longer wavelength region (544 nm) by decreasing the intensity. In the 575–650 nm and 400–450 nm wavelength regions, the absorbance increased to a considerable extent, and the solution color changed to violet from red. These results showed that between pH 3 and pH 7, storage had no effect on betanin solutions, and above and below of these pH values causes the considerable losses of betanin. Visible spectra of betanin compound at pH 2, 5, and 9 are illustrated in **Figure 12** [12, 45].
- **Temperature:** on heating the red color of betanin solutions starts to diminish, and finally it turns to brown color. The color loss was followed by the betanin assay, and the rate indicates that it follows first-order kinetics. The graph indicates that at 100°C, the degradation rate at pH 5 is still less than it is at pH 3

and pH 7. Further, the betanin compound is more stable between pH 4 and 5. However, betanin in beet juice is far more stable at pH 5, which reveals a protective effect by the constituents of juice. The rates of degradation for

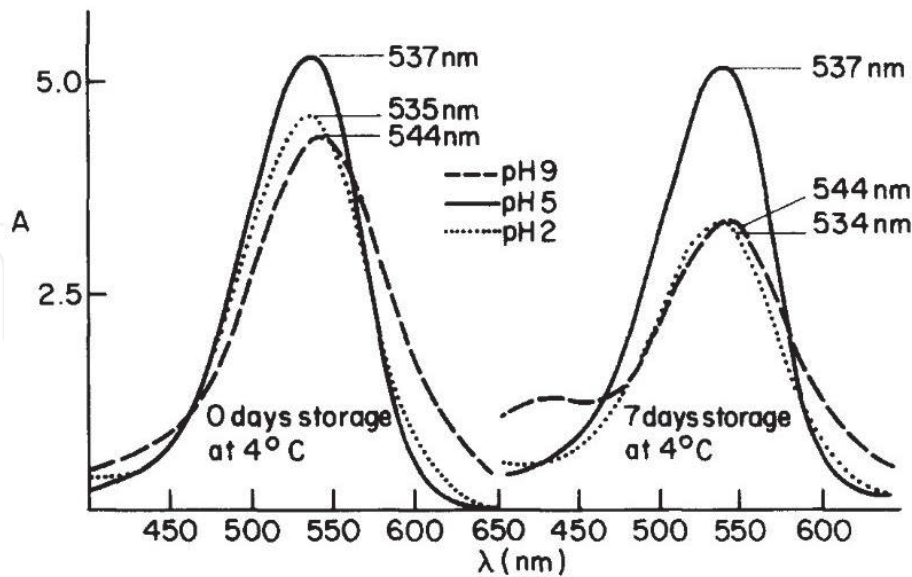


Figure 12.
Visible spectra of betanin compound at pH 2, 5, and 9.

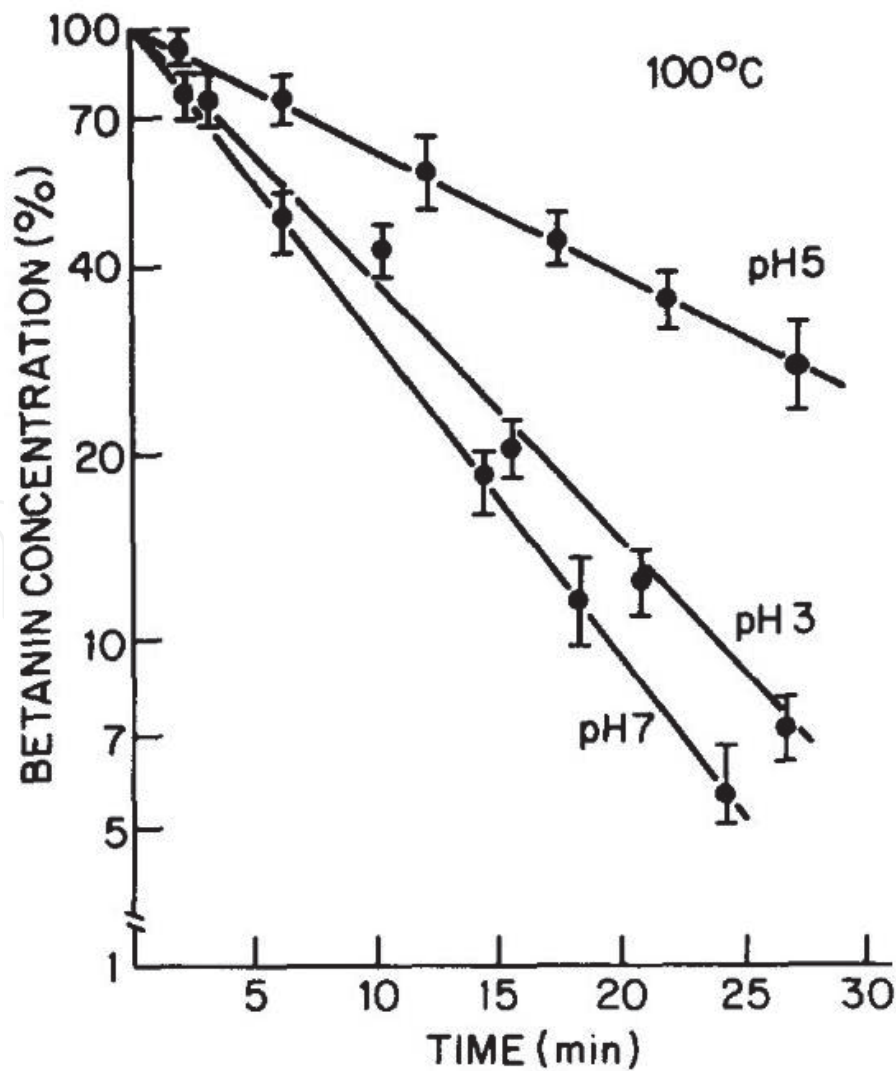


Figure 13.
Rates of degradation for betanin molecule in a system at 100°C at pH 3, 5, and 7.

betanin molecule in a system at 100°C at pH 3, 5, and 7 are depicted in **Figure 13** [12, 45].

- *Light*: at 15°C and pH 7, the presence of light increased the rate of degradation by 15.6% and air (rather than nitrogen) by 14.6%. Both light and air together increased the rate by 28.6% [12, 42].

8. Applicability of betanin

8.1 Dyeing acrylic fabric

Pure betanin dye can compete with synthetic dyes in color depth shade properties and in color fastness properties. Guesmi et al. [43] studied the dyeing of betanin on modified acrylic fabrics and evaluated the effect of dye bath pH, salt concentration, dyeing time, and temperature on dyeing. In a dye bath having sodium chloride (0–15 g/L) and a dye of 30 mg/L concentration with the 40:1 liquor ratio, modified acrylic fabric was dyed using conventional heating.

8.1.1 Effect of pH on dyeing

Over the pH range 1–5, increase in pH increases the adsorption of betanin onto acrylic fabric. Color strength decreases as the pH increased above 5. Generally, amino functional groups of acrylic fibers get protonated as the pH value decreases. Thus, ion-ion forces induced with ionized carboxyl groups in betanin. Betanin may exist in cationized or on monoanion form in a strongly acidic environment which results in the lower depth of dyeing at pH less than 4, and also it is due to the betanin stability loss at low pH [43].

At pH 5 maximal color strength was observed, whereas at pH 4, little decrease in color strength was observed; this is attributed to the increased carboxyl groups in this range and to the high thermal stability of betanin molecule. The number of protonated terminal amino functional groups of fabric decreases at pH > 5, which causes the decreased ionic interaction between the carboxylate anion of the dye and acrylic fibers, thus lowering its dye ability. The structures of betanin molecule as a pH varied are depicted in **Figure 14** [43].

8.1.2 Effect of salt addition

Color strength decreases as the salt concentration increases, hence dyeing without salt addition is the best condition [43].

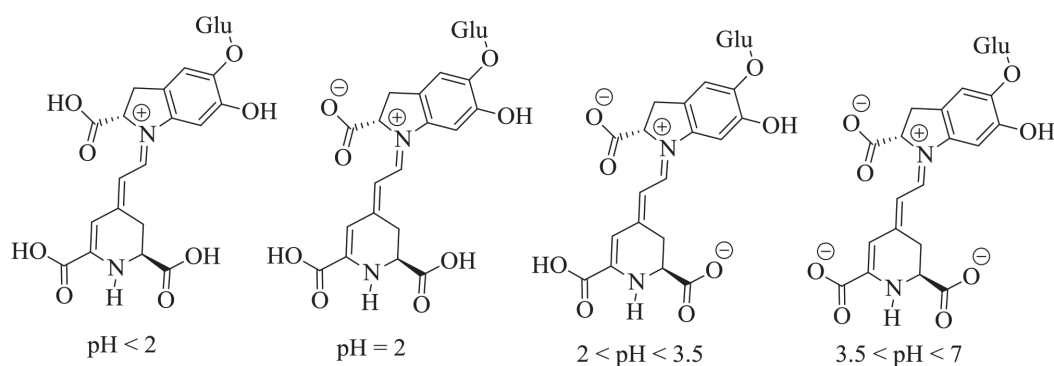


Figure 14. Structures of betanin molecule as a pH varied [46].

8.1.3 Effect of temperature on dyeing

As the temperature of dyeing increases, the color strength increases up to 50°C, and further by increasing the dyeing temperature, the color strength decreased slowly which is attributed to the decrease in stability of dye at higher temperatures [43].

8.1.4 Effect of time span on dyeing

Color strength increases as the dyeing time increases up to 30 min; from 30 to 45 min, there is no change in color strength, and then it started to decrease as the time increases [43].

8.1.5 Color fastness

The fastness properties of betanin-dyed acrylic fabrics are shown in **Table 3**. The fastness properties of the dyed samples were examined according to ISO standard methods, the specific tests conducted for color fastness to rubbing is as per ISO 105-X12:1987, the color fastness to water is as per ISO 105-E01:1989, the color fastness to washing is as per ISO 105-C02:1989, and the color fastness to light is as per ISO 105-B02:1988 (carbon arc) [43]. It was noted that rubbing, washing, and water fastness of unmordanted acrylic fabrics exhibited significantly good property. But, the light fastness of unmordanted acrylic fabrics was found to be bad. However, light fastness was found to increase from rating 3* to 4* in premordanted fabrics using manganese sulfate and ferrous sulfate, and light fastness was increased from rating 3* to 5* by using cobalt sulfate. Nevertheless, the other mordants did not affect the light fastness of premordanted fabrics. It was found that for the improvements of color strength and light fastness, cobalt sulfate was established as the best mordant [43].

Similarly, Guesmi et al. [46] in the year 2013 studied the dyeing of wool fabric using betanin and chlorophyll-a as biomordant. In a dye bath having sodium chloride (0–5 g/L) and a dye with 40:1 liquor ratio, wool fabric was dyed using conventional heating. Results revealed that the increase in the concentration of biomordant increases the color strength values. They also investigated the effect of variables on the color of dyed fibers and noted that from pH 3.5 to pH 4.5, the color strength considerably increases, the color strength was found to be better without salt than with salt, and the color strength of dyed wool increases as the increase in temperature was up to 40°C and starts to decrease slowly till 50°C. Further increase in temperature, the color strength decreases in pronounced manner. According to the authors, color strength increases with the time span (up to 45 min) of dyeing, and

	Fastness to dry rubbing	Fastness to wet rubbing	Fastness to washing	Fastness to water	Fastness to light
None	5*	5*	4*–5*	4*–5*	3*
Manganese sulfate	5*	5*	4*	4*	4*
Ferrous sulfate	4*	4*	3*	3*	3*
Zinc sulfate	5*	5*	4*–5*	4*–5*	4*
Aluminum potassium sulfate	4*	4*	3*	3*	3*
Cobalt sulfate	5*	5*	4*–5*	4*–5*	5*

Table 3.
The fastness properties of betanin-dyed acrylic fabrics represented with the rating scale using star (*).

then it starts to decrease because betanin losses thermal stability, and also it starts to escape from the fiber. Dye exhaustion was examined in both ultrasonic and conventional dyeing approaches. It was exhibited that in a shorter time span of dyeing, sonication increases the dye exhaustion from rating of 30% to rating of 60%. The fastness properties of dyed wool were studied against wet rubbing, light, washing, and dry rubbing. Unmordanted and mordanted samples have good fastness properties too.

8.2 Medicinal application

Antioxidant activity of betanin in biological lipid domain has been exhibited in human macromolecules, like lipoproteins of low density, whole cells, and membranes [2]. Moreover, betanin has attracted researchers because of its anti-inflammatory activities and hepatic safety activities in whole human cells [47]. In cultured endothelia cells, this molecule regulates the redox-mediated signal transduction pathways which is required in responses during inflammation, and betanin also showed antiproliferative effects on tumor cell lines in human [48–50]. In both tumoral and healthy hepatic cell lines in the human body, betanin translocates the antioxidant response element (erythroid 2-related factor 2 (Nrf2)) from the place of cytosol to the place of nuclear domain, which regulates m-RNA and protein levels of antioxidant/detoxifying enzymes, which includes heme oxygenase-1, NAD(P)H quinone dehydrogenase-1, and glutathione S-transferase and, in these cells, bears anticarcinogenic and hepatoprotective effects [51]. Also, it exhibits antidiabetes properties by controlling the activities of liver markers enzymes [52–54].

8.3 Betanin as food colorant

Betanin is the oldest and most abundant red food colorant which has been established in the market, which is noted as E-162 in the European Union and in the United States; it is known as 73.40 in the twenty-first chapter of the Code of Federal Regulations (CFR) section of the Food and Drug Administration [2, 5, 6].

Betanins are most commonly used for coloring of ice cream and powdered soft drink beverages. Additionally, betanin is used in some of the sugar confectionery, like sugar coatings, fruit or ice cream fillings, fondants, and sugar strands. At the final part of the processing, it can be added while preparing hot processed candies. Also, it is used in soups as well as bacon and tomato.

9. Summary

In this chapter, the first and second section covered the chemistry of betanin which contains reactions that revealed the glucosyl residue position and presence of vinylene connecting group in betanin. Further, the third section narrated the extraction techniques which mainly included the microwave- and ultrasonic-assisted extraction method. Furthermore, the fourth and fifth sections elucidated the biosynthesis of betanin molecule and chemical synthesis of betanidin molecule, respectively. In addition, different characterization techniques were also explicated in the sixth section which includes UV-Vis, FT-IR, ^1H NMR, ^{13}C NMR, LC- ^1H NMR, mass spectrum, and thermogravimetric analysis of betanin. Also, the factors effecting the stability of betanin were explained in the seventh section which covers the effect of pH, temperature, and light on the stability of betanin. Lastly, the applicability of betanin was taken into account in the eighth section which comprised of dyeing of acrylic fabric, dyeing of wool fabric, and medicinal and food colorant applications of betanin.

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References

- [1] Baião DDS, Silva DVTD, Aguila EMD, Paschoalin VMF. Nutritional, bioactive and physicochemical characteristics of different beetroot formulations. In: Food Additives. InTech; 2017. pp. 1-24
- [2] da Silva DVT, dos Santos Baião D, de Oliveira Silva F, Alves G, Perrone D, Del Aguila EM, et al. Betanin, a natural food additive: Stability, bioavailability, antioxidant and preservative ability assessments. *Molecules*. 2019;**24**(3):458
- [3] Azeredo HMC. Betalains: Properties, sources, applications, and stability—A review. *International Journal of Food Science and Technology*. 2009;**44**(12): 2365-2376
- [4] Tumbas Šaponjac V, Čanadanović-Brunet J, Četković G, Jakišić M, Djilas S, Vulić J, et al. Encapsulation of beetroot pomace extract: RSM optimization, storage and gastrointestinal stability. *Molecules*. 2016;**21**(5):584
- [5] EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food). Scientific opinion on the re-evaluation of beetroot red (E 162) as a food additive. *EFSA Journal*. 2015; **13**(12):4318. Available from: <http://doi.wiley.com/10.2903/j.efsa.2015.4318>
- [6] Nemzer B, Pietrzkowski Z, Spórna A, Stalica P, Thresher W, Michałowski T, et al. Betalainic and nutritional profiles of pigment-enriched red beet root (*Beta vulgaris* L.) dried extracts. *Food Chemistry*. 2011;**127**(1):42-53
- [7] Sivakumar V, Anna JL, Vijayeeswarri J, Swaminathan G. Ultrasound assisted enhancement in natural dye extraction from beetroot for industrial applications and natural dyeing of leather. *Ultrasonics Sonochemistry*. 2009;**16**(6):782-789
- [8] Nayak CA, Chethana S, Rastogi NK, Raghavarao KSMS. Enhanced mass transfer during solid-liquid extraction of gamma-irradiated red beetroot. *Radiation Physics and Chemistry*. 2006; **75**(1):173-178
- [9] Schmidt OT, Schonleben W. Notizen: Zur Kenntnis der Farbstoffe der Roten Rübe (*Beta vulgaris*) II. *Zeitschrift für Naturforsch B*. 1957;**12**(4):262-263
- [10] Wyler H, Dreiding AS. Kristallisiertes Betanin. Vorläufige Mitteilung. *Helvetica Chimica Acta*. 1957;**40**(1):191-192
- [11] Wyler H, Dreiding AS. Darstellung und Abbauprodukte des Betanidins. 3. (vorläufige) Mitteilung. Über die Konstitution des Randenfarbstoffes Betanin. *Helvetica Chimica Acta*. 1959; **42**(5):1699-1702
- [12] Harmer RA. Occurrence, chemistry and application of betanin. *Food Chemistry*. 1980;**5**(1):81-90
- [13] Mabry TJ, Wyler H, Sassu G, Mercier M, Parikh I, Dreiding AS. Die Struktur des Neobetamidins. 5. (vorläufige) Mitteilung. Über die Konstitution des Randenfarbstoffes Betanin. *Helvetica Chimica Acta*. 1962; **45**(2):640-647
- [14] Mabry TJ, Wyler H, Parkih I, Dreiding AS. The conversion of betanidin and betanin to neobetainidin derivatives. *Tetrahedron*. 1967;**23**(7): 3111-3127
- [15] Wilcox ME, Wyler H, Mabry TJ, Dreiding AS. Die Struktur des Betanins. 7. Mitteilung. Über die Konstitution des Randenfarbstoffes Betanin. *Helvetica Chimica Acta*. 1965;**48**(1):252-258
- [16] Wang L, Weller CL. Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science and Technology*. 2006;**17**(6):300-312

- [17] Bastos EL, Gonçalves LCP. Microwave-assisted extraction of Betalains. In: Water Extraction of Bioactive Compounds: From Plants to Drug Development. Elsevier; 2017. pp. 245-268
- [18] López N, Puértolas E, Condón S, Raso J, Alvarez I. Enhancement of the extraction of betanine from red beetroot by pulsed electric fields. *Journal of Food Engineering*. 2009;**90**(1):60-66
- [19] Zvitov R, Schwartz A, Zamski E, Nussinovitch A. Direct current electrical field effects on intact plant organs. *Biotechnology Progress*. 2003;**19**(3): 965-971
- [20] Neagu C, Barbu V. Principal component analysis of the factors involved in the extraction of beetroot betalains. *Journal of Agroalimentary Processes and Technologies*. 2014; **20**(4):311-318
- [21] Amlepatil NM, Miraje SY, Patil PD, Sahoo AK, Mote GV. Natural color extraction from Amaranth and beetroot: A review. *Indian Journal of Applied Research*. 2015;**5**(5):19-20
- [22] Esatbeyoglu T, Wagner A, Schini-Kerth VB, Rimbach G. Betanin—A food colorant with biological activity. *Molecular Nutrition & Food Research*. 2015;**59**(1):36-47
- [23] Gandía-Herrero F, García-Carmona F. Biosynthesis of betalains: Yellow and violet plant pigments. *Trends in Plant Science*. 2013;**18**(6):334-343
- [24] Tzin V, Galili G. New insights into the shikimate and aromatic amino acids biosynthesis pathways in plants. *Molecular Plant*. 2010;**3**(6):956-972
- [25] Fischer N, Dreiding AS. biosynthesis of betalains. On the cleavage of the aromatic ring during the enzymatic transformation of dopa into betalamic acid. *Helvetica Chimica Acta*. 1972; **55**(2):649-658
- [26] Christinet L, Burdet FX, Zaiko M, Hinz U, Zryd J-P. Characterization and functional identification of a novel plant 4,5-extradiol dioxygenase involved in betalain pigment biosynthesis in *Portulaca grandiflora*. *Plant Physiology*. 2004;**134**(1):265-274
- [27] Takahashi K, Takamura E, Sakuta M. Isolation and expression analysis of two DOPA dioxygenases in *Phytolacca americana*. *Zeitschrift fur Naturforsch C*. 2009;**64**(7-8):564-573
- [28] Gandía-Herrero F, García-Carmona F. Characterization of recombinant *Beta vulgaris* 4,5-DOPA-extradiol-dioxygenase active in the biosynthesis of betalains. *Planta*. 2012;**236**(1):91-100
- [29] Gandía-Herrero F, Escribano J, García-Carmona F. Betaxanthins as substrates for tyrosinase. An approach to the role of tyrosinase in the biosynthetic pathway of betalains. *Plant Physiology*. 2005;**138**(1):421-432
- [30] Hatlestad GJ, Sunnadeniya RM, Akhavan NA, Gonzalez A, Goldman IL, McGrath JM, et al. The beet R locus encodes a new cytochrome P450 required for red betalain production. *Nature Genetics*. 2012;**44**(7):816-820
- [31] Hans J, Brandt W, Vogt T. Site-directed mutagenesis and protein 3D-homology modelling suggest a catalytic mechanism for UDP-glucose-dependent betanidin 5-O-glucosyltransferase from *Dorotheanthus bellidiformis*. *The Plant Journal*. 2004;**39**(3):319-333
- [32] Zakharova NS, Petrova TA. β -Glucosidases from leaves and roots of the common beet, *Beta vulgaris*. *Applied Biochemistry and Microbiology*. 2000; **36**(4):394-397
- [33] Sasaki N, Adachi T, Koda T, Ozeki Y. Detection of UDP-glucose:cyclo-DOPA 5-O-glucosyltransferase activity in four

- o'clocks (*Mirabilis jalapa* L.). FEBS Letters. 2004;**568**(1-3):159-162
- [34] Hermama K, Dreiding AS. Totalsynthese von betalainen. Helvetica Chimica Acta. 1975;**58**(6):1805-1808
- [35] Dumbrava A, Enache I, Oprea CI, Georgescu A, Girtu MA. Toward a more efficient utilisation of betalains as pigments for dye-sensitized solar cells. Digest Journal of Nanomaterials and Biostructures. 2012;**7**(1):339-351
- [36] Aztatzi-Rugerio L, Granados-Balbuena SY, Zainos-Cuapio Y, Ocaranza-Sánchez E, Rojas-López M. Analysis of the degradation of betanin obtained from beetroot using Fourier transform infrared spectroscopy. Journal of Food Science and Technology. 2019;**56**(8):3677-3686
- [37] Kumar SNA, Ritesh SK, Sharmila G, Muthukumaran C. Extraction optimization and characterization of water soluble red purple pigment from floral bracts of Bougainvillea glabra. Arabian Journal of Chemistry. 2017;**10**:S2145-S2150
- [38] Cai Y, Sun M, Wu H, Huang R, Corke H. Characterization and quantification of betacyanin pigments from diverse Amaranthus species. Journal of Agricultural and Food Chemistry. 1998;**46**(6):2063-2070
- [39] Molina GA, Hernández-Martínez AR, Cortez-Valadez M, García-Hernández F, Estevez M. Effects of tetraethyl orthosilicate (teos) on the light and temperature stability of a pigment from *Beta vulgaris* and its potential food industry applications. Molecules. 2014;**19**(11):17985-18002
- [40] Sengupta D, Mondal B, Mukherjee K. Visible light absorption and photo-sensitizing properties of spinach leaves and beetroot extracted natural dyes. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015;**148**:85-92
- [41] Dong J, Ozaki Y, Nakashima K. Infrared, Raman, and near-infrared spectroscopic evidence for the coexistence of various hydrogen-bond forms in poly(acrylic acid). Macromolecules. 1997;**30**(4):1111-1117
- [42] Stintzing FC, Conrad J, Klaiber I, Beifuss U, Carle R. Structural investigations on betacyanin pigments by LC NMR and 2D NMR spectroscopy. Phytochemistry. 2004;**65**(4):415-422
- [43] Guesmi A, Ladhari N, Ben Hamadi N, Sakli F. Isolation, identification and dyeing studies of betanin on modified acrylic fabrics. Industrial Crops and Products. 2012;**37**(1):342-346
- [44] Susha N, Nandakumar K, Nair SS. Enhanced photoconductivity in CdS/betanin composite nanostructures. RSC Advances. 2018;**8**(21):11330-11337
- [45] Von Elbe JH, Young Maing I, Amundson CH. Color stability os betanin. Journal of Food Science. 1974;**39**(2):334-337
- [46] Guesmi A, Ladhari N, Ben Hamadi N, Msaddek M, Sakli F. First application of chlorophyll-a as biomordant: Sonicator dyeing of wool with betanin dye. Journal of Cleaner Production. 2013;**39**:97-104
- [47] Khan MI. Plant betalains: Safety, antioxidant activity, clinical efficacy, and bioavailability. Comprehensive Reviews in Food Science and Food Safety. 2016;**15**(2):316-330
- [48] Gentile C, Tesoriere L, Allegra M, Livrea MA, D'Alessio P. Antioxidant betalains from cactus pear (*Opuntia ficus-indica*) inhibit endothelial ICAM-1 expression. Annals of the New York Academy of Sciences. 2004;**1028**:481-486
- [49] Kapadia GJ, Azuine MA, Rao GS, Arai T, Iida A, Tokuda H. Cytotoxic

effect of the red beetroot (*Beta vulgaris* L.) extract compared to doxorubicin (Adriamycin) in the human prostate (PC-3) and breast (MCF-7) cancer cell lines. *Anti-Cancer Agents in Medicinal Chemistry*. 2011;**11**(3):280-284

[50] Reddy MK, Alexander-Lindo RL, Nair MG. Relative inhibition of lipid peroxidation, cyclooxygenase enzymes, and human tumor cell proliferation by natural food colors. *Journal of Agricultural and Food Chemistry*. 2005; **53**(23):9268-9273

[51] Krajka-Kuźniak V, Paluszczak J, Szaefer H, Baer-Dubowska W. Betanin, a beetroot component, induces nuclear factor erythroid-2-related factor 2-mediated expression of detoxifying/antioxidant enzymes in human liver cell lines. *The British Journal of Nutrition*. 2013;**110**(12):2138-2149

[52] Dhananjayan I, Kathirolu S, Subramani S, Veerasamy V. Ameliorating effect of betanin, a natural chromoalkaloid by modulating hepatic carbohydrate metabolic enzyme activities and glycogen content in streptozotocin—Nicotinamide induced experimental rats. *Biomedicine & Pharmacotherapy*. 2017;**88**:1069-1079

[53] Sutariya B, Saraf M. Betanin, isolated from fruits of *Opuntia elatior* Mill attenuates renal fibrosis in diabetic rats through regulating oxidative stress and TGF- β pathway. *Journal of Ethnopharmacology*. 2017;**198**:432-443

[54] Amjadi S, Ghorbani M, Hamishehkar H, Roufegarinejad L. Improvement in the stability of betanin by liposomal nanocarriers: Its application in gummy candy as a food model. *Food Chemistry*. 2018;**256**: 156-162