



Betalain as a Food Colorant: its Sources, Chemistry and Health Benefits

Sumra Naseer¹, Shabbir Hussain^{1*} and Amin Abid²

¹Department of Chemistry, Lahore Garrison University, DHA Phase VI, Lahore, Pakistan

²Department of Chemistry, University of Sahiwal, Pakistan

Abstract: Food colors are essential ingredients of modern processed foods. Betalain can be used as a natural color source for various foods due to its nutritional and safety value. This natural and water soluble dye occurs either as red-violet betacyanin or the yellow-orange betaxanthins. Betalain plays a vital role in human health due to its pharmacological activities such as antioxidant, anti-lipidemic, antimicrobial, antitumor, antiviral and anticancer potential. There are many eatable sources of betalain e.g., *Opuntia stricta* (cactus fig), *Opuntia ficus-indica* (barbary fig), *Beta vulgaris* (red beet), *Amaranthus tricolor* (Amaranth), *Celosia argentea* (silver cock's comb) and *Chenopodium quinoa* Wild. Red beetroot provides carotenoids, glycine, betanin, saponins, betacyanin, folates, polyphenols and flavonoids. *Amaranthus* is an important source of vitamin C, vitamin B, soluble fiber, betacyanin and shows significant antimalarial and antimicrobial activity. *Celosia argentea* Linn provides carbohydrates, vitamins, dietary minerals and high amount of betalain. *Chenopodium quinoa* contains high content of vitamins and minerals. Betalain of *Opuntia ficus-indica* fruits (barbary fig) possesses strong anti-proliferative activity. Extract of prickly pear (cactus fig) pulp which contains betalain, can act as an antibiotic agent in cases of wounds, digestive and urinary tract infections and inflammations. It also has significant inhibitory effect against humanoid ovarian tumor and cervical cancer cells. Optimum pH for maximum stability of betanin lies between pH 5.5 and 5.8. Betalain stability is affected by long term exposure to oxygen/air, light in the presence of oxygen, high temperature and water activity (aw) but it is highly stable in the presence of low moisture.

Keywords: Food color, Betalain, Betacyanin, Betaxanthins, Pharmacological, Health

1. INTRODUCTION

Color is the most important feature which may effect on the acceptability of a consumer for food items [1, 2]. To enhance the visual attraction of food products, many artificial colors are used in processed foods e.g., candies, soft drinks, sweets and in some prepared dairy products such as cheese and butter [3]. However, some of these artificial food colors may cause allergic responses in children while their long term use results in carcinogenic diseases [4]. Consequently, the consumers have started to avoid the commodities containing synthetic colors while the plant pigments are gaining special attraction [5]. Pigments are the plant compounds which are perceived by human beings due to their specific colors [6]. Their varieties of colors and structures have also attracted the attention of biologists and

chemists [7]. In addition to their fascinating colors, the plant pigments also possess nutritional value and health promoting affects [8, 9] and are also considered environment friendly [10]. Betalains are water soluble nitrogenous pigments of plant nature [4, 11,12] and exist mostly in florets, fruits and in vegetative tissues of plants [13]. They are comprised of either yellow (betaxanthins) or red (betacyanins) pigments and their main sources are the beetroots [14, 15]. They contain a group of molecules which are responsible for the red hue of red beet juice [16] and are currently gaining popularity in the food industry [17]. Betanin (a betalain in beetroot) has been recommended as a natural red colorant for use in pharmaceutical and food items. This pigment also possesses health-promoting properties and antioxidant potential; it also displays peroxy-radical scavenger potential in

Received: May 2018; Accepted: June 2019

* Corresponding Author: Shabbir Hussain<shabchem786@gmail.com>

pork meat and can be used as an attractive colorant in frozen or refrigerated foods. Its high antioxidant ability is retained even after simulated digestion in small intestine. Organisms can be protected from oxidative stress by long-term supplementation from beetroot [18]. The present studies focus on the chemistry of betalain and its uses as a natural colorant in food products.

2. SOURCES OF BETALAIN

There are many eatable sources of betalain (Fig. 1) [19] which include red and yellow beetroot, leafy amaranth, cactus fig, Barbary fig, silver cock's comb etc. *Phytolacca americana* Linn (pook weed) is another source of betalain, but it has been banned as a food colorant due to the presence of toxic saponins and lectins. Betalain pigment is not only found in edible plants but it can also be obtained from leaves, pulp and stem of plants [20]. Betalain plays a vital role in human health due to its pharmacological activities such as anti-cancer, antimicrobial, anti-lipidemic and antioxidant activities.

Red beetroot is grown all over the world as a source of food and red dye. The color of red beetroot is due to the presence of red betacyanin and yellow

betaxanthin pigments which are placed in betalain or betanin compounds group [21]. Red beet provides carotenoids, glycine, betanin, saponins, betacyanin, folates, polyphenols and flavonoids [22]. Betalain pigments display an excellent antioxidant and antimicrobial potential [23].

Amaranthus is an herbaceous plant which possess very high amount of betalain pigments. It belongs to family Amaranthaceous; the word *Amaranthus* is derived from a Greek word which means flower. This plant is also an excellent source of vitamin C, vitamin B, soluble fiber and betacyanin; it shows antioxidant activity higher than ascorbic acid [24].

Celosia argentea commonly known as silver cock's comb, is an herbaceous plant which belongs to tropical regions. The word *Celosia* was derived from a Greek word *Kelos* which means "burned, burning"; this name was given due to the color of inflorescences (orange, red and yellow), specially for *P. plumosa*, which resembles flames erupting from the stems [24, 25]. *Argentea celosia* provides carbohydrates, vitamins, dietary minerals and huge amount of betalain. This plant is commonly grown for ornamental purposes and finds applications in medicinal field as well. The color of *C. Argentea*

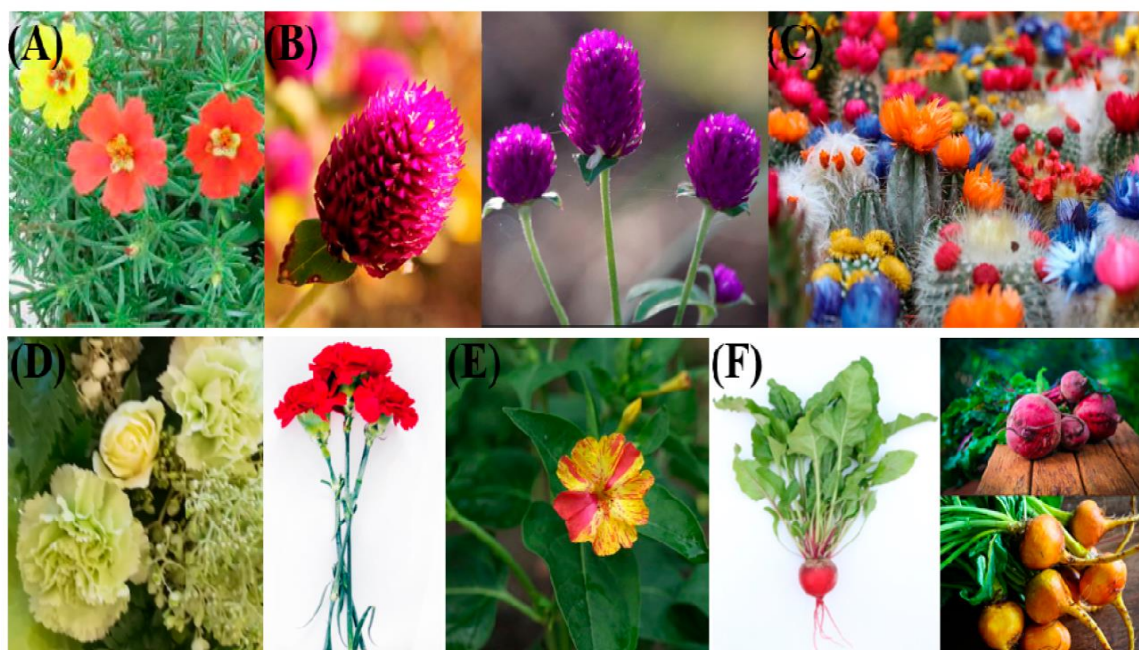


Fig. 1. Several plants containing betalains. (A) *Portulaca oleracea* L. flowers (B) *Gomphrena globosa* L. flowers (C) *Echinopsis tubiflora* flowers (D) *Dianthus caryophyllus* L. flowers and stems (E) *Mirabilis jalapa* L. flowers (F) *Beta vulgaris* L. whole plants (some pictures from pexels.com) [19]

varies from yellow to many other shades like violet and red, especially for the garden *Celosia* (plumose and cristata); these colors are owed to the presence of betalains [24, 26].

Chenopodium quinoa is herbaceous and flowering plants which belong to Amaranthaceae family. The word *Chenopodium* was derived from the Greek words *chenos* (meaning goose) and *podos* (meaning foot). This plant contains vitamin B, dietary minerals and has very high amount of betacyanin and betaxanthins pigments. All these plants have medicinal applications in inflammation, digestive and urinary tract [24].

3. CHEMISTRY OF BETALAIN

Betalains may either be in the form of betacyanin (red-violet pigment) or betaxanthins (yellow-orange pigment). It is discovered that betalains may act against microbes/viruses and also hinder the growth of tumor cells in human. Betalain obtained by extraction from beetroot is also known as “beetroot red”; however, it may be degraded soon after its

extraction. There are several factors which influence the pigment stability for example; temperature, enzymes, pH and oxygen [27]. Betalain pigments show more stability towards pH and temperature while they are suitable to those foods in which anthocyanin cannot be used as a coloring agent due to low-acid conditions [28].

Betalains are derived from betalamic acid (Fig. 2) [29]. Betalamic acid and amino acids or amines are condensed to form betaxanthins; glutamine-betaxanthins (vulgaxanthin) is the most common betaxanthin which occurs in red beet (*Beta vulgaris* Linn). While in yellow cactus pears (*Opuntia* sp.) indicaxanthin is present dominantly. Betacyanins, due to their deep violet color, are considered to be the condensation products of betalamic acid and cyclo-dopa [cyclo-3-(3,4-dihydroxyphenylalanine)]. Various betacyanin structures can be obtained as a result of glycosylation with one or two monosaccharides and as a result of acylation of the resulting 5-O- or 6-O-glucosides [30].

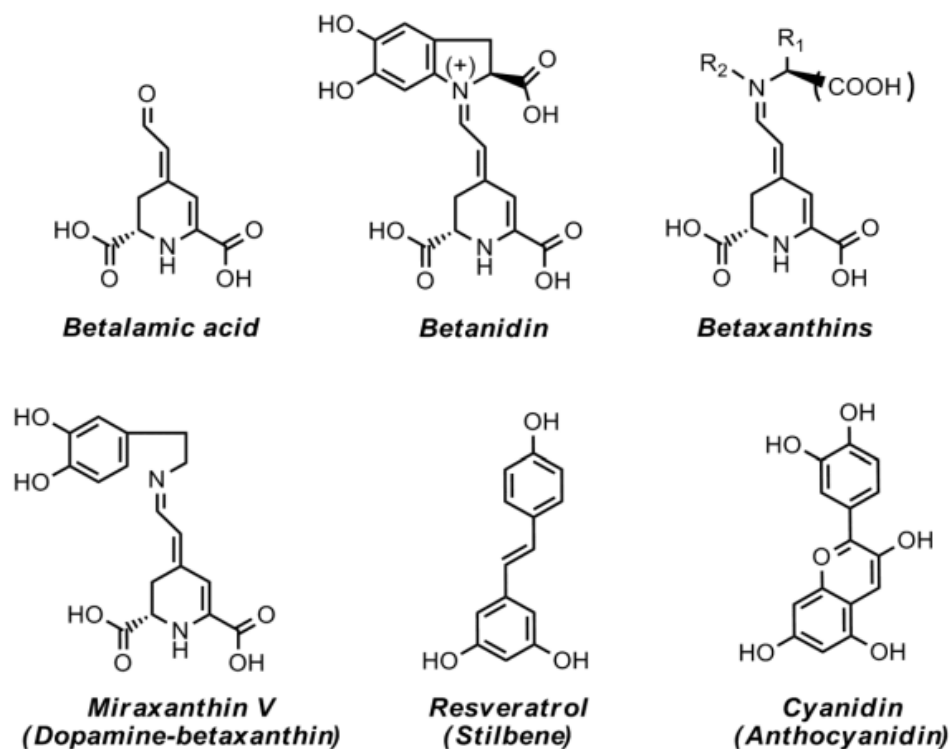


Fig. 2. Structures for *betalamic acid*, the *betacyanins aglyca (betanidin)*, and the general structure for *betaxanthins*. R_1 and R_2 are lateral residues present in amines or amino acids. For comparative purposes, the structure for the *diphenolic betaxanthin Miraxanthin V* is also shown together with *resveratrol* and *cyaniding* [29]

4. BETALAINS AS COLORANTS

Betalains are produced from amino acids (tyrosine) and occur in two structural forms; the red-violet betacyanin and the yellow-orange betaxanthins. All betalain pigments contain a common chromophore betalamic acid which is used to classify betalain pigment into betacyanin and betaxanthins on the basis of other structural differences. Betacyanin contains a cyclo-3, 4-dihydroxyphenylalanine residue. The condensation with the closed structure of cyclo-dopa extends the electronic resonance to the phenolic aromatic ring. This extra conjugation shifts the absorption maximum from 480 nm (yellow, betaxanthins) to 540 nm (violet, betacyanin). The betacyanin is more brighter in color as compared to the red radish anthocyanin and have almost the same color stability during the initial 4-weeks storage ($\leq 25^{\circ}\text{C}$) or during 20-weeks storage ($\leq 14^{\circ}\text{C}$). However, less stability of betacyanin was observed at 37°C as compared to the red radish anthocyanin [31].

5. MICROENCAPSULATION OF BETALAIN

A bioactive compound could be protected from oxygen and water and its stability could be improved by using microencapsulation technique. There are several benefits of encapsulation such as; easier handling, prevention of lumping, compression and mixing properties, improvement in flow ability, reduction in core particle dinginess and modification of atom concentration. It is also helpful in increasing shelf life of some natural dyes like betalain [32].

6. FACTORS AFFECTING BETALAIN STABILITY

Stability of betalain pigments is of great importance. There are some factors which affect the color stability of betalain

6.1 pH

Optimum pH for maximum stability of betanin lies between pH 5.5 and 5.8 in the presence of oxygen. Betalains extracted from *Amaranthus* species exhibit maximum stability at pH 5.0-7.0 at 25°C [33]. Betacyanin of *Opuntia* shows thermal stability at pH 5 [32] and those obtained from beetroot are

normally stable at pH 5.5. The betanin solution in the presence of nitrogen has highest stability at pH 4.0-5.0. The degradation of betanin solution is reversible [34]. There were investigations on the partial regeneration of betanin after heating and the results showed that amount of regeneration of the pigment depends upon the pH of the sample [35]. The maximum regeneration of betanin is affected by the storage temperature and the type of buffer solution. Under low oxygen levels, the heated betalain solution (pH 4.75, 130 minutes, 15°C) has shown the increase in betanin retention from 54% to 92% [36].

6.2 Water Activity

The stability of betalain is affected by water activity (wa) which is considered to be a primary factor in color degradation. The pigment stability is exponentially related with moisture content and water activity (aw); the low aw means higher stability of pigment. The stabilities of beet pigments (vulgaxanthine I and betanin) were affected by moisture content and water activity ("dry"– 0.84) in the beet powder stored at 35°C . When aw was increased from 0.32 to 0.75, the pigment stability was decreased by one order of magnitude. However, the betalain pigments showed no degradation when the beet powder was stored at aw 0.12 or below for even a period of several months [37].

6.3 Oxygen

The oxidation is an important reason of darkening and color loss of fruits and vegetables. Oxygen causes the degradation of color pigments and hence the loss of original color of a product. Buffered betalain solutions were kept at pH 7 in the presence of nitrogen and air for 6 days at 15°C . It was concluded that about 15% degradation of color was occurred only due to the presence of air [35]. The results were further verified when the pigments were degraded in the saturated solutions of air due to the reaction of betalain with molecular oxygen. Betalain and betalain pigments are considered to be unstable in the presence of oxygen. The betalain stability is decreased linearly when oxygen concentration is increased. In addition to oxygen, hydrogen peroxide is also a cause of pigment degradation. However, the betalain stability is considered to be increased in the presence of nitrogen atmosphere [35].

6.4 Light

Light is another important factor which effects on the degradation of betalain pigment [36]. Presence of light shows disastrous effects on betalain degradation at temperature lower than 25°C while there was no effect of light above 40°C. Light-induced betalain degradation was occurred after absorption of light in UV and visible range; this absorption resulted in electron excitation of betalain pigment to a more excited state and caused higher reactivity and lower activation energy of the molecule. The combined effects of oxygen and light were also observed. Light and oxygen independently have shown the degradation of betanin up to 15.6% and 14.6%, respectively. However, the combined availability of both light and oxygen has shown about 28.6% degradation of the pigment [36]. The degradation of pigments by light depends on the presence of oxygen because this degradation is very low in the absence of oxygen even when light is present [35].

6.5 Temperature

Temperature plays a vital role in the stability of betalain as far as food processing and storage is concerned [33]. Betalains are heat sensitive pigments so they lose their stability at higher temperatures. The degradation of betalain is increased with increase in temperature and time period of heating. A considerable decrease in betalain stability was observed at 50-60°C and also between 70 to 80°C [38]. Thermal degradation of betalain is of great concern because many heat treatment processes are being used to ensure the safety and quality of various food products. Thermal stability of betalain depends on heating time and temperature as well as some other factors such as light concentration and structure of the pigment [33].

6. SOCIAL AND ECONOMIC USES OF BETALAIN

Betalain can be used as a food color in food industry and agro industry. *Beta vulgaris* (red beet), *Amaranthus tricolor* (Amaranth) and *Celosia argentea* (silver cock' scomb) are those plants which produce betacyanin commonly used in food, medicine and also in oil for diet supplementary and soap. They are also used as decorative plants [39].

7. HEALTH BENEFITS OF BETALAIN

The betalain as a food colorant may have high beneficial effects on health due to its valuable ingredients and natural resources. The pharmaceutical and nutritional value of extracts of plants has been well recognized in early reported literature [40-42]. Betalain also shows pharmacological activities including antioxidant activity, antimicrobial activity, anti-cancer activity as well as other activities. The antioxidant activity of betalain can be verified through different chemical and biological methods. Betanin obtained from red beet is about 1.5-2.0-folds more active as compared to the some other anthocyanins which are considered excellent free radical scavengers as determined by Trolox equivalent antioxidant capacity (TEAC) assay at pH>4. This exceptionally higher antioxidant potential of betanin is due to increase of its electron-donation and H-donation ability in going from the cationic form to mon-, di- and tri-deprotonated states present in basic solutions [43]. Betalain from Amaranath shows strong antioxidant activity than ascorbic acid as determined by DPPH assay. Betalain obtained from *Opuntia ficus-indica* fruits (Barbary fig) has strong anti-proliferative activity against cancer cells. Extract of prickly pear (cactus fig) pulp containing betalain can be used as an antibiotic agent in digestive and urinary tracts, wounds and inflammation. It also shows considerable inhibitory effect on humanoid ovarian tumor and cervical cancer cells. Betalain from Amarnath shows significant antimalarial and antimicrobial activity [20]. It is worth mentioning that it is highly important to implement stricter quality control measures, regulatory initiatives, technical assistance and hygienic precautions in order to ensure the nutritional value of the food [44].

8. CONCLUSIONS

The color of food is a significant factor in determining its acceptance. However, to ensure the food safety, it is important to add up safe and healthy ingredients into the food products. The synthetic colors may cause some allergic reactions; their long term use is considered to be a cause of carcinogenesis. Betalains have some properties which are beneficial to our health due to which they

can be used to enhance the flavor and color of ice cream, jellies, jams, desserts, sauces, sweets, tomato paste and breakfast cereals. Betalain contains antioxidants, antimicrobial agents and antiradical compounds which show the nutritional and safety significance of red dye as a natural source of color. There are many eatable sources of betalain e.g., cactus fig, barbary fig. Betalain occurs either as red-violet betacyanin or the yellow-orange betaxanthins and can be extracted from *Beta vulgaris* (red beet), *Amaranthus tricolor* (Amaranth), *Celosia argentea* (silver cock' scomb) and *Chenopodium quinoa* wild. Betalain stability is affected by long term exposure to oxygen/air, light in the presence of oxygen, high temperature and water activity (wa) but it is highly stable in the presence of low moisture. Its pigments show more stability towards pH and temperature and are suitable for those foods in which anthocyanin cannot be used as a coloring agent.

9. REFERENCES

1. Chattopadhyay, P., S. Chatterjee & S.K. Sen. Biotechnological potential of natural food grade biocolorants. *African Journal of Biotechnology*, 7(17): 2972-2985 (2008).
2. Rymbai, H., R. Sharma & M. Srivastav, Bio-colorants and its implications in health and food industry—a review. *International Journal of Pharmtech Research*, 3(4): 2228-2244 (2011).
3. De Azeredo, H. M. C., A. C. Pereira, A. C. R. De Souza, S. T. Gouveia & K. C. B. Mendes, Study on efficiency of betacyanin extraction from red beetroots. *International Journal of Food Science & Technology*, 44(12): 2464-2469 (2009).
4. Benson L. *Family 7 Amaranthaceae (amaranth family)*. In: Benson L (Ed.), Plant classification. D.C. Heath and Company, Boston. 196-198 (1957).
5. Aberoumand, A. A review article on edible pigments properties and sources as natural biocolorants in foodstuff and food industry. *World Journal of Dairy and Food Sciences*, 6(1): 71-78 (2011).
6. Tanaka, Y., N. Sasaki & A. Ohmiya. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *The Plant Journal*, 54(4): 733-749 (2008).
7. Shamina, A., K. Shiva & V. Parthasarathy. Food colors of plant origin. CAB Reviews: Perspectives in Agriculture, Veterinary Science. *Nutrition and Natural Resources*, 2(1-25): (2007).
8. Rivera-Madrid, R., M. Aguilar-Espinosa, Y. Cárdenas-Conejo & L. E. Garza-Caligaris. Carotenoid derivatives in achiote (*Bixa orellana*) seeds: synthesis and health promoting properties. *Frontiers in plant science*, 7, 1406 (2016).
9. Santos-Buelga, C., N. Mateus & V. De Freitas. *Anthocyanins. Plant pigments and beyond*. ACS Publications (2014).
10. Adeel, S., F.-U. Rehman, S. Rafi, K. M. Zia & M. Zuber. *Environmentally Friendly Plant-Based Natural Dyes: Extraction Methodology and Applications*, in: *Plant and Human Health*, Springer (2019).
11. Esquivel, P. *Betalains*, in *Handbook on Natural Pigments in Food and Beverages*. Elsevier. (2016).
12. Esatbeyoglu, T., A. E. Wagner, V. B. Schini-Kerth & G. Rimbach. Betanin—A food colorant with biological activity. *Molecular Nutrition & Food Research* 59(1): 36-47 (2015).
13. Strack, D., T. Vogt, & W. Schliemann. Recent advances in betalain research. *Phytochemistry*, 62: 247-269 (2003).
14. Azeredo, H. M. Betalains: properties, sources, applications, and stability—a review. *International Journal of Food Science & Technology*, 44(12): 2365-2376 (2009).
15. Šaponjac, V. T., J. Čanadanović-Brunet, G. Četković, M. Jakišić, S. Djilas, J. Vulić & S. Stajčić. Encapsulation of beetroot pomace extract: RSM optimization, storage and gastrointestinal stability. *Molecules*, 21(5): 584 (2016).
16. Choo, W. S. Betalains: application in functional foods. In book: *Bioactive Molecules in Food*. In: Mérillon J. M., Ramawat K. (eds). *Bioactive Molecules in Food*. Reference Series in Phytochemistry. Springer, Cham (2017).
17. Kezi, J. & J. Sumathy. Betalain-a boon to the food industry. *Discovery*, 20(63): 51-58 (2014).
18. Vieira Teixeira da Silva, D., D. dos Santos Baião, F. de Oliveira Silva, G. Alves, D. Perrone, E. Mere Del Aguila & V. M. Flosi Paschoalin. Betanin, a Natural Food Additive: Stability, Bioavailability, Antioxidant and Preservative Ability Assessments. *Molecules*, 24(3): E458 (2019).
19. Li, G., X. Meng, M. Zhu & Z. Li, Research Progress of Betalain in Response to Adverse Stresses and Evolutionary Relationship Compared with Anthocyanin. *Molecules*, 24(17): 3078 (2019).
20. Gengatharan, A., G. A. Dykes & W. S. Choo. Natural plant pigments with potential application in functional and food. *Food Science and Technology*, 64: 645-649(2015).

21. Gasztonyi, M. N., H. Daood, M. T. Hajos & P. Biacs. Comparison of red beet (*Beta Vulgaris* Var *conditiva*) varieties on the basis of their pigment components. *Journal of the Science of Food and Agriculture*, 81: 932- 933 (2001).
22. Singh, B. & B.S. Hathan. Chemical composition, functional properties and processing of Beetroot—a review. *International Journal of Scientific & Engineering Research*, 5(1): 679-684 (2014).
23. Gentile, C., L. Tesoriere, M. Allegra, M. A. Livrea, & P. Dalesio. Antioxidant betalains from Cactus Pear (*Opuntia ficus-indica*) inhibit endothelial ICAM-1 expression. *Annals of the New York Academy of Sciences*, 1028(1): 481-486 (2004).
24. Miguel, M. G. Betalains in Some Species of the Amaranthaceae Family: A Review. *Antioxidants*, 7(4): 53(2018).
25. Surse, S., B. Shrivastava, P. Sharma, P. Gide & S. Attar. *Celosia cristata*: Potent pharmacotherapeutic herb—A review. *International journal of pharmaceutical and phytopharmacological research*, 3: 444-446 (2014).
26. Schliemann, W., Y. Cai, T. Degenkolb, J. Schmidt & H. Corke, Betalains of *Celosia argentea*. *Phytochemistry*, 58(1): 159-165 (2001).
27. Ravichandran, K., R. Palaniraj, N. M. M. T. Saw, A. M. Gabr, A. R. Ahmed, D. Knorr & I. Smetanska. Effects of different encapsulation agents and drying process on stability of betalains extract. *Journal of Food Science and Technology*, 51(9): 2216-2221(2014).
28. Stintzing, F. C. & R. Carle. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends in Food Science & Technology*, 15(1): 19-38(2004).
29. Gandía-Herrero, F., J. Escribano & F. García-Carmona, Biological activities of plant pigments betalains. *Critical reviews in food science and nutrition*, 56(6): 937-945 (2016).
30. Scotter, M. J. Emerging and persistent issues with artificial food colors: natural color additives as alternatives to synthetic colors in food and drink. *Quality Assurance and Safety of Crops & Foods*, 3(1): 28-39 (2011).
31. Cai, Y. & H. Corke. *Amaranthus* betacyanin pigments applied in model food systems. *Journal of Food Science*, 64: 869–873(1999).
32. Bolkent, S., R. Yanardag, A. Tabakoğlu-Oğuz & O. Özsoy-Saçan. Effects of chard (*Beta vulgaris* L. var. *cicla*) extract on pancreatic B cells in streptozotocin-diabetic rats: a morphological and biochemical study. *Journal of Ethnopharmacology*, 73(1): 251-259 (2000).
33. Herbach, K. M., F. C. Stintzing & R. Carle. Betalain stability and degradation—structural and chromatic aspects. *Journal of Food Science*, 71(4): R41-R50 (2006).
34. Castellar, R., J. M. Obon, M. Alacid & J. A. Fernández-López. Color properties and stability of betacyanins from *Opuntia* fruits. *Journal of Agricultural and Food Chemistry*, 51(9): 2772-2776 (2003).
35. Huang, A.S.& J.V. Elbe. Effect of pH on the degradation and regeneration of betanin. *Journal of Food Science*, 52(6): 1689-1693(1987).
36. Elbe, J. H., S. J. Schwartz & B.E. Hildenbrand. Loss and regeneration of betacyanin pigments during processing of red beets. *Journal of Food Science*, 46(6): 1713-1715 (1981).
37. Cohen, E. & I. Saguy. Effect of water activity and moisture content on the stability of beet powder pigments. *Journal of Food Science*, 48(3): 703-707 (1983).
38. Havlíková, L., K. Miková & V. Kyzlink. Heat stability of betacyanins. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 177(4): 247-250 (1983).
39. Wettasinghe, M., B. Bolling, L. Plhak, Xiao, H. and K. Parkin. Phase II enzyme-inducing and antioxidant activities of beetroot (*Beta vulgaris* L.). Extracts from phenotypes of different pigmentation. *Journal of Agricultural and Food Chemistry*, 50: 6704-6709 (2002).
40. Naseer, S., S. Hussain, N. Naeem, M. Pervaiz & M. Rahman. The phytochemistry and medicinal value of *Psidium guajava* (guava). *Clinical Phytoscience*, 4:1-8 (2018).
41. Rehman, A., S. Hussain, M. Javed, Z. Ali, H. Rehman, T. G. Shahzady, A. Zahra. Chemical composition and remedial Perspectives of *hippophae rhamnoides* linn. *Postepy Biologii Komorki*, 45:199-210 (2018).
42. Naseer, S., S. Hussain, and Z. Zahid. Nutritional and antioxidant potential of common vegetables in Pakistan. *RADS Journal of Biological Research & Applied Sciences*, 10(1): 36-40 (2019).
43. Gliszczyńska-Świgło, A., H. Szymusiak & P. Malinowska. Betanin, the main pigment of red beet: Molecular origin of its exceptionally high free radical-scavenging activity. *Food additives and contaminants*, 23(11): 1079-1087 (2006).
44. Tahir, N.I., S. Hussain, M. Javed, H. Rehman, T. G. Shahzady, B. Parveen & K.G. Ali. Nature of aflatoxins: Their extraction, analysis, and control. *Journal of Food Safety*, 38: (2018). doi.org/10.1111/jfs.12561.