v. 1, n. 2, 2021

International Journal of Health Science

BETANIN, MORE THAN A FOOD COLORANT

Rogério Côrte Sassonia
Center for Integrated Sciences, Federal University of Tocantins
Araguaína, Tocantins, Brazil
http://lattes.cnpq.br/9341522545622587

Abstract: Betalains are natural pigments found in approximately 17 families of vegetables of the order *Caryophyllales*, such as beet, and some basidiomycete fungi. In addition to finding application as a colorant in the food industry, interest in the biological activity of betalains and its use as a functional food for health promotion and disease prevention has grown in recent years. "Red beet" (INS 162) is a colorant allowed for use in food in Brazil and its main component is a betalain called betanin. Betalains have significant antioxidant properties through the direct elimination of free radicals and in the restoration of the balance of redox processes in the body. Recent results show that such properties may be related, in part, to the effect of betanin in the signaling pathways that mediate the transcription of antioxidant genes such as the Nrf2-Keap1 pathway and the NF-kB pathway, responsible for triggering the inflammatory response. In this work, significant results are presented that demonstrate the great potential for the inclusion of betalains, especially betanin, in processed foods due to its complementary role in the treatment of various clinical pathologies associated with oxidative stress and inflammation.
Keywords: Beet, Betalains, Antioxidants, Oxidative Stress, Inflammation, Obesity.

THE CHEMICAL STRUCTURE OF BETALINS AND THEIR APPLICATION AS FOOD COLORANT

Color is undoubtedly one of the most important factors related to the acceptability of food by consumers and, since 1960, the Food and Drug Administration (FDA) has considered the colorants desirable and necessary [1]. Food colorants are substances that have the property of conferring, intensifying, or standardizing the coloring of food and beverages. In Brazil, resolution CNS/MS nº 4 classifies food colorants as synthetic, natural, synthetic identical to natural, inorganic, and caramel [2]. "Red beet", also known as "beet colorant" or "betanina" is among the natural colorants. Current legislation in Brazil does not specify limits for its use in food and can be used in sufficient quantity to achieve the desired effect *(quantum satis*, q.s.p.) [2].

Colorants found in beets belong to a class of natural colorants known as betalains. These compounds occur in approximately 17 vegetable families of the order *Caryophyllales*, an order of vegetables that includes beetroot (*Beta vulgaris*) [3], red pitaya (*Hylocereus polyrhizus)* [4], prickly pear (*Opuntia spp.*) [5], and ornamental plants such as great bougainvillea *(Bougainvillea spectabilis)* [6], and amaranth *(Amaranthus tricolor*)[7]. Betalains also occur in some fungal species such as the fly amanita *(Amanita muscaria), Hygrocybe* and *Hygrophorus*[8]. Several processes of isolation of betalains from their natural sources are described in the literature. A noteworthy process, however, is a new method that precipitates betalains using anhydrous ethanol, which generates a product without nitrates and with high concentration of betalains[9, 10].

In nature, plants containing betalains have colors similar to plants containing anthocyanins, however, these two classes of colorants are mutually exclusive [11, 12]. Plants that produce betalains do not contain anthocyanins[13]. Erroneously, betalains were known in ancient literature as "nitrogen anthocyanins" [8]. Today, anthocyanins and betalains are easily differentiated through their chemical structures that are identified by modern chromatographic and spectrometric techniques. However, simple tests can be used to differentiate them using the color displayed at different pHs and temperatures[14]. Anthocyanins are easily extracted with acidified methanol (0.001% HCl) and hardly

with water, while the opposite is valid for betalains. In electrophoresis with weakly acidic medium, anthocyanins go to the cathode while betalains go to the anode [13, 15].

There are approximately 78 known betalains [12] and all have the same basic structure, Figure 1, formed by condensation of an amine or amino acid with bethalamic acid[16], where R1 and R2 can be hydrogen or an aromatic substitute [13].

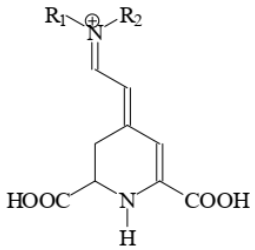

Figure 1: Fundamental structure of betalains.

Betalains are formed by red colorants called betacyanins, which comprise about 90% of betalains in beet, and by yellow colorant called betaxanthins [17, 18]. Its color is due to the formation of the resonance structures shown in Figure 2. Betaxanthins are characterized by substituents that do not extend the resonance system, the compounds exhibit UV-Vis spectra with a maximum absorption of around 480 nm. Betacyanin, on the other hand, have aromatic rings as substituents, such as cycle-3,4-dihydroxyphenylalanin (cycle-DOPA), or some of its glycosylated derivatives, which extend resonance and change the maximum absorption from 480 nm to 540 nm [13, 15]. The presence of amine groups or amino acids linked to the chemical structure of betaxanthins can displace its maximum absorption peak in UV-Vis spectra (batochromic/hipsochromic displacement) [5]. The same effect occurs with glycosylated betacyanins [19].

Figure 2: Resonance structures responsible for the color of betalains.

Within the order *Caryophyllales,* there is a large variety of betaxanthins, however, in beets, there are only two, known as vulgaxanthins I and II [3], both soluble in ethanol, whose structures are represented in Figure 3.

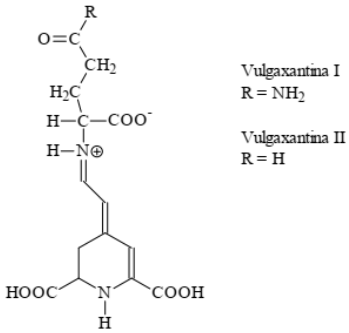

Figure 3: Betaxanthins from beetroots.

Betacyanins are optically active due to chiral carbons C-2 and C-15. Isomerization occurs under acid and/or heating conditions, increasing the concentration of iso compounds[9], Figure 4.

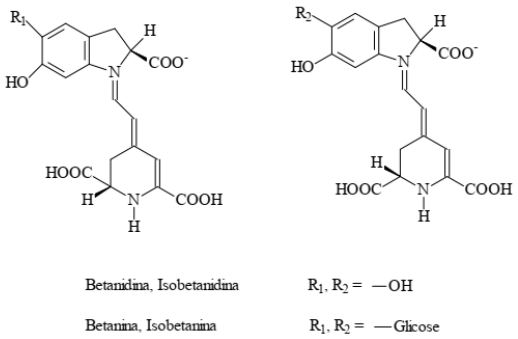

Figure 4: Structure of betacyanins.

Both betacyanins and betaxanthins are soluble in water and can be found in the ionic form in the vacuoles of plant cells[7]. Betacyanins are the most important colorants within

the betalain group, from the perspective of their industrial application as food colorant. Betanin is the main component of betacyanins in beets and its structure is showed in Figure 5. This colorant contributes to approximately 75% to 95 % of the total red color in beetroots.

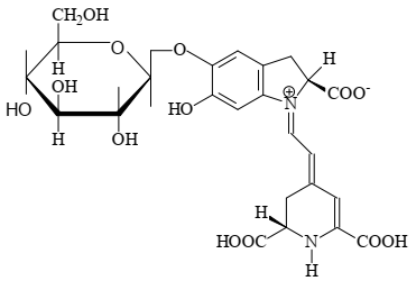

Figure 5: Betanin structure.

Betanin has greater coloring power than many synthetic colorants [16]. Table 1 shows comparative data of the coloring power of betanin.

Colorant	Specific absorbability $\varepsilon^{1\%}_{1cm}$	$\lambda_{max.}$ (NM)
Betanin	1220	537
Amaranth	438	523
Carmoisine	545	515
Ponceau 4R	431	505

Table 1: Comparative data of the coloring power of betanin and some equivalent synthetic colorants [17].

BIOAVAILABILITY

After ingestion, bioactive compounds must be absorbed into the gastro-intestinal tract and made available in circulation in sufficient quantity to be used by cells. In the case of betacyanins, studies indicate that although in small amounts, they can be absorbed by the human body. Kanner *et al.*[20] evaluated the amount of betacyanin (betanin and isobetanin) absorbed in four healthy volunteers after consuming 300 mL of beet juice, containing 120 mg betanin. Betacyanins were absorbed from the gut into the blood system, and it was possible to identify them in urine after 2-4 h in amounts of ~1% of those ingested. Frank *et al.*[21] reported a lower result. After providing six healthy participants with 500 mL of commercial beet juice, they identified betacyanins in urine in amount equivalent to 0.3 ± 0.1% of the ingested dose over a 24-hour period. It should be considered, however, that these results may be underestimated since betacyanins should not be eliminated exclusively by renal route. Other bioavailability values in humans for betanin are available in the literature and have been compiled by Khan, M. I. (2016) [22]. The values range from 0.28 ± 0.08% (n = 6) to 3.7 ± 0.2% (n = 8).

THE POTENTIAL BENEFITS OF BETALAIN SUPPLEMENTATION FOR HEALTH PROMOTION AND DISEASE PREVENTION

Chronic inflammation is often involved in the development of various diseases such as obesity, liver disease, cancer, and heart disease [23]. Consequently, attention has been paid to obtaining compounds able to restore the balance between the generation of oxidizing compounds and the action of the body's antioxidant response elements (AREs) as a complementary treatment in various clinical pathologies associated with oxidative stress and inflammation such as atherosclerosis and Alzheimer´s disease [24]. In this sense, betalains have significant antioxidant properties and act in the restoration of the balance of redox processes in the body[25].

A recent study showed that the administration of betalains obtained from beets relieved inflammation and pain in patients suffering from osteoarthritis, with doses ranging from 35 mg to 100 mg per day after 10 days of treatment[26]. Supplementation

with betalain-rich extract also brought benefits for a patient with cutaneous sarcoidosis[27]. In another study, beet juice (8 mL/kg pc/day for 28 days) also demonstrated a protective effect against N-nitrosodiethylamine (NDEA)-induced liver injury [28]. Beet juice reduced DNA damage as well as liver injury biomarkers increased by treatment with NDEA. Pretreatment with beet juice before NDEA administration resulted in a significant reduction in DNA damage (20%) in the liver of animals [28].

El Gamal *et al*. showed that oral doses of dry beet extract, obtained by alcoholic extraction (70% ethanol), provided renal protection on gentamicin-induced nephrotoxicity in rats. Beet extract attenuated the increase of urea, uric acid, and creatinine levels in serum in a dose-dependent manner [29]. Urea, uric acid and creatinine are markers of renal function, and their serum levels increase because of the toxic effects of gentamicin. Rats treated with 250 or 500 mg/kg pc/day for 28 days showed lower concentrations of the pro-inflammatory mediators IL-6 and TNF-α. The concentrations of myeloperoxidase (MPO) and renal nitric oxide level were also reduced. The decreased activity of MPO enzyme (which represents neutrophil infiltration) and nitric oxide levels (indicator of damage caused by oxidative stress) in the tissue demonstrate the antioxidant and anti-inflammatory activity of beet extract, that was also observed in the restoration of histological changes [29]. Other studies in the literature confirm these results [30, 31] and attribute these properties to betalains [32].

El Gamal *et al*. also showed a reduction in oxidative stress and increased endogenous antioxidant activity in rats treated with alcoholic beet extract. Beet extract reduced malondialdehyde (MDA) levels (reduction of lipid peroxidation) and maintained catalase (an endogenous antioxidant) close to its normal level [29]. Many biomarkers have been used to evaluate oxidative stress; however, MDA is one of the most widely used biomarkers because it is one of the most well-known secondary lipid peroxidation products [33]. Catalase acts in the decomposition of hydrogen peroxide (H_2O_2) in water and oxygen, thus decreasing its cellular concentration. It is an essential enzyme in the protection against cellular damage caused by reactive oxygen species (ROS).

Studies indicate that the anti-inflammatory action of betalains occurs mainly due to their regulatory activity in the signaling pathway of Nuclear Factor Kappa B (NF-κB), a pro-inflammatory mediator [34, 25]. This family of transcription factors regulates the expression of essential genes in the inflammatory process and, consequently, their activity plays a central role in the inflammatory process that manifests itself in chronic diseases [35]. NF-κB acts on genes for pro-inflammatory cytokines, chemokines, immunoreceptors, and adhesion molecules. They also play an important role in the recruitment of leukocytes in the site of inflammation. According to Blanco and Neto (2003), many research groups have been explored the modulation action of new bioactive compounds on the transcription factor NF-κB in the control of the inflammatory process[36]. Betalains also exert regulatory activity on the nuclear factor erythroid 2 related to factor 2 (Nrf2). Nrf2 factor acts in the expression of more than 200 cytoprotective genes related to the neutralization or detoxification of endogenous metabolites and environmental toxins [37].

In this sense, studies in Huh7 cells culture have shown that betanin (TCI Europe N. V., CAS-Nr.: 7659-95-2) increased the cellular protein concentration of the heme oxygenase-1 (HO-1), paraoxonase-1 (PON1) and glutathione (GSH) in a dose-dependent manner through the signaling pathway of

transcription factor Nrf2 [38]. PON1 prevents HDL (high density lipoprotein) oxidation and GSH is an important cytosolic antioxidant. HO-1 is an antioxidant enzyme with cytoprotective properties (anti-apoptotic). It is important to mention that heme oxygenase system interacted with other systems to minimize the deleterious effect of oxidative stress in obesity and cardiovascular diseases [39]. A clinical study with obese women (n = 15) corroborates the cytoprotective effect caused by the bioactive compounds found in beets by inhibiting the oxidative metabolism of neutrophils and it indicates that these compounds can be used as an adjunctive in the treatment of obesity [40]. Neutrophils from the obese group had a significantly higher production of reactive oxygen species (ROS) compared with controls. Oxidative stress results from an imbalance between the generation of oxidizing compounds and the performance of antioxidant defense systems [41].

Another important characteristic of betalains is their ability to decrease COX-2 expression *in vitro*. Cyclooxygenase enzymes, COX-1 and COX-2, catalyze the conversion of arachidonic acid result in chemical mediators of inflammation. Reddy *et al.* found that betanin inhibits the enzymatic capacity of COX-2 in 97% with IC_{50} of 100 µg/mL [42]. Under normal metabolic conditions, oxidizing agents are in equilibrium with reducing agents in the biological environment of a cell. Phagocytic cells produce superoxide, an oxidizing species, as part of the immune defense mechanism to eliminate pathogenic microorganisms; however, in chronic inflammatory diseases, this production becomes excessive, causing tissue lesions. In this sense, *in vitro* studies have shown that betalains protect cellular components from lesions caused by oxidizing agents [28, 30]. Wootton-Beard *et al.* suggest that the main mechanism by which beet juice exerts its antioxidant effect is through the elimination of radical species[43]. A systematic review of anti-inflammatory activity of betalains was published recently. It summarizes the current research on the anti-inflammatory properties of betalains describing the role of betalains in various physiological systems [44].

CONCLUSION

Due to its toxicological safety, accessibility, low price, biodegradability, and significantly health-advantageous biological effects, the food industry's greater commitment to incorporate betalains as a natural colorant in an increasing number of its products could pave the way to overcome current concerns about the health risks of artificial colorants and cochineal (carmine). It would also provide the population with functional foods as an adjunctive therapy of various chronic diseases associated with oxidative stress and inflammation. In this sense, new processes to extract betalains from their natural sources have been proposed and should assist the industry in its technological challenges [10].

REFERENCES

1. Froes, N.C., Garcia, M.V., Silva, A. A., 1992. Use of red beet colorant as food additive, Food and Nutrition, 4, 33.

2. BRAZIL. National Health Council. (1988) RESOLUTION CNS/MS nº. November 4, 1988.

3. Kujala, T.S., Vienola, M.S., Klika, K.D., Loponen, J.M., Pihlaja, K., 2002. Betalain and phenolic compositions of four beetroot (Beta vulgaris) cultivars. Eur. Food Res. Technol. 214, 505–510.

4. Wybraniec, S., Platzner, I., Geresh, S., Gottlieb, H.E., Haimberg, M., Mogilnitzki, M., Mizrahi, Y., 2001. Betacyanins from vine cactus Hylocereus polyrhizus. Phytochemistry 58, 1209–1212.

5. Stintzing, F.C., Schieber, A., Carle, R., 2002. Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear [*Opuntia ficus-indica* (L.) Mill.] by high-performance liquid chromatography–electrospray ionization mass spectrometry. J. Agric. Food Chem. 50, 2302–2307.

6. Kugler, F.; Stintzing, F.C.; Carle, R., 2007. Characterisation of betalain patterns of differently coloured inflorescences from *Gomphrena globosa* L. and *Bougainvillea* sp. By HPLC-DAD-ESI-MSn. Anal. Bioanal. Chem., 387, 637–648.

7. Michael M.G., 2018. Betalains in sOme species of the aMaranthaceae family: a review. Antioxidants, 7, 53, 1-33.

8. Slimen, I.B., Najar, T., Abderrabba, M., 2017. Chemical and antioxidant properties of betalains. J. Agric. Food Chem., 65, 675–689.

9. Martinez, R. M., Hohmann, M. S.Longhi-Balbinot, D. T., Zarpelon, The. C., Baracat, M, M., Georgetti, S. R., Vincentian, F. T. M. C., Sassonia, R. C., Verri, W. A. Jr, Casagrande, R., 2020. Analgesic activity and mechanism of action of a *Beta vulgaris* enriched colorant in betalains in inflammatory models in mice. Inflammopharmacology, 28, 6, 1663-1675.

10. Camas, E.M., Matos, S.C. G., Sassonia, R.C.. Production process of beet colorant. 1998, Brazil. Patent: Innovation Privilege. Registration number: PI98021486, title: "Production process of beet colorant", Registration institution: INPI - National Institute of Industrial Property. Deposit: 03/06/1998; Concession: 06/05/2008.

11. von Elbe, J. H., 1998. Betalains: Past and Future, Third International Symposium on Natural Colorants. Princeton N. J. April 19 – 22, 397-408.

12. Gandía-Herrero, F., García-Carmona, F., 2020. The dawn of betalains. New Phytol., 227, 3, 664-666.

13. Stafford, H.A., 1994. Anthocyanins and betalains: evolution of the mutually exclusive pathways. Plant Science, 101.2, 91-98.

14. Delgado-Vargas, F., Jiménez, The. R, Paredes-López, Or., 2000. Natural pigments: carotenoids, anthocyanins, and betalains--characteristics, biosynthesis, processing, and stability. Crit Rev Food Sci Nutr.,40, 3,173-289. Note: information on tests that differentiate between anthocyanins and betalains can be found in Table 9, page 263.

15. Fennema, O. R., 1985. Food Chemistry, 2nd ed., Marcel Dekker: New York, 568 –569.

16. Quina, F. H., Bastos, E. L., 2018. Chemistry Inspired by the Colors of Fruits, Flowers and Wine. An. Acad. Bras. Cienc., 90, 1, 681-695.

17. Hendry, G. A. F., Houghton, J. D., 1992. Natural Food Colorants, Blackie: Glasgow, 59 – 63 e 217 - 231.

18. Coultate, T. P., 1984. Alimentos, Química de sus Componentes, Editorial Acribia, Zavagoza, Espanha, 115 – 117.

19. Azeredo, H.M.C., 2009. Betalains: properties, sources, applications, and stability – a review. Int. J. Food Sci. Technol., 44, 2365-76.

20. Kanner, J., Harel, S., Granit, R., 2001. Betalains--a new class of dietary cationized antioxidants. J. Agric. Food Chem., 49, 5178-85.

21. Frank, T., Stintzing, F.C., Carle, R., Bitsch, I., Quaas, D., Strass, G., Netzel, M., 2005. Urinary pharmacokinetics of betalains following consumption of red beet juice in healthy humans. Pharmacol. Res., 52, 290–297.

22. Khan, M. I., 2016. Plant Betalains: Safety, Antioxidant Activity, Clinical Efficacy, and Bioavailability. Compr. Rev. Food Sci. Food Saf., 15, 316-330.

23. Monteiro, R., Azevedo, I., 2010. Chronic inflammation in obesity and the metabolic syndrome. Mediators Inflamm., 2010, 289645.

24. Calixto, J. B., Campos, M. M., Otuki, M. F., Santos, A. R., 2004. Anti-inflammatory compounds of plant origin. Part II. modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta Med., 70, 2, 93-103.

25. Clifford, T., Howatson, G., West, D. J., Stevenson, E. J., 2015. The potential benefits of red beetroot supplementation in health and disease. Nutrients,7, 2801-22.

26. Pietrzkowski, Z., Nemzer, B, Spórna, A., Stalica, P., Tresher, W., Keller, R., Jimenez, R., Michałowski, T., Wybraniec, S., 2010. Influence of betalain-rich extract on reduction of discomfort associated with osteoarthritis. New Med., 1, 12-17.

27. Barrick, B. J., Bruce, A. J., Kalaaji, A. N., Bauer, B. A., 2013. Clinical improvement of recalcitrant cutaneous sarcoidosis with regular nutritional supplementation with extract of the prickly pear cactus. Explore (NY), 9, 6, 377-8.

28. Krajka-Kuźniak, V., Szaefer, H., Ignatowicz, E., Adamska, T., Baer-Dubowska, W., 2012. Beetroot juice protects against N-nitrosodiethylamine-induced liver injury in rats. Food Chem. Toxicol., 50, 2027-33.

29. El Gamal, A. A., AlSaid, M. S., Raish, M., Al-Sohaibani, M., Al-Massarani, S. M., Ahmad, A., Hefnawy, M., Al-Yahya, M., Basoudan, O. A., Rafatullah, S., 2014. Beetroot (Beta vulgaris L.) extract ameliorates gentamicin-induced nephrotoxicity associated oxidative stress, inflammation, and apoptosis in rodent model. Mediators Inflamm., 2014, 983952.

30. Wang, J., Zhang, D., Cao, C., Yao, J., 2020. Betalain exerts a protective effect against glaucoma is majorly through the association of inflammatory cytokines. AMB Express.;10, 1, 125.

31. Martinez, R. M., Longhi-Balbinot, D. T., Zarpelon, A. C., Staurengo-Ferrari, L., Baracat, M. M., Georgetti, S. R., Sassonia, R. C., Verri, W. A. Jr, Casagrande, R., 2015. Anti-inflammatory activity of betalain-rich dye of Beta vulgaris: effect on edema, leukocyte recruitment, superoxide anion and cytokine production. Arch Pharm Res., 38, 4, 494-504.

32. Lee, C. H., Wettasinghe, M., Bolling, B. W., Ji, L. L., Parkin, K. L., 2005. Betalains, phase II enzyme-inducing components from red beetroot (Beta vulgaris L.) extracts. Nutr Cancer., 53, 1, 91-103.

33. Esterbauer, H., Schaur, R. J., Zollner, H., 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med., 11, 81-128.

34. Tan, D., Wang, Y., Bai, B., Yang, X., Han, J. 2015. Betanin attenuates oxidative stress and inflammatory reaction in kidney of paraquat-treated rat. Food Chem. Toxicol., 78, 141-6.

35. Baker, R. G., Hayden, M. S., Ghosh, S., 2011. NF-κB, inflammation, and metabolic disease. Cell Metab., 13, 1, 11-22.

36. Blanco, M. L., Neto, A. C., 2003. O fator nuclear Kappa B: uma nova perspectiva para o estudo de drogas antiinflamatórias. Rev. Ciênc. Med. 2003, 12, 4, 341-349.

37. Copple, I. M., 2012. The Keap1-Nrf2 cell defense pathway: a promising therapeutic target? Adv. Pharmacol., 63, 43-79.

38. Esatbeyoglu, T.; Wagner, A. E.; Motafakkerazad, R.; Nakajima, Y.; Matsugo, S.; Gerald Rimbach, G., 2014. Free radical scavenging and antioxidant activity of betanin: Electron spin resonance spectroscopy studies and studies in cultured cells. Food Chem. Toxicol., 73, 119-126.

39. Abraham, N. G., Junge, J. M., Drummond, G. S., 2016. Translational significance of heme oxygenase in obesity and metabolic syndrome. Trends Pharmacol Sci., 37, 1, 17-36.

40. Zielińska-Przyjemska, M., Olejnik, A., Dobrowolska-Zachwieja, A., Grajek, W., 2009. In vitro effects of beetroot juice and chips on oxidative metabolism and apoptosis in neutrophils from obese individuals. Phytother Res., 23, 1, 49-55.

41. Barbosa, K. B. F., Costa, N. M. B., Alfenas, R. de Cássia G., De Paula, S. O., Minim, V. P. R., Bressan, J., 2010. Estresse oxidativo: conceitos, implicações e fatores modulatórios. Rev. Nutr., 23, 4, 629-643.

42. Reddy, M. K., Alexander-Lindo, R. L, Nair, M. G., 2005. Relative inhibition of lipid peroxidation, cyclooxygenase enzymes, and human tumor cell proliferation by natural food colors. J. Agric. Food Chem., 53, 9268-73.

43. Wootton-Beard, P. C., Moran, A., Ryan, L., 2011. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion measured by FRAP, DPPH, ABTS and Folin–Ciocalteu methods. Food. Res. Int., 44, 217–224.

44. Moreno-Ley C, Osorio-Revilla G, Hernández-Martínez D, Ramos-Monroy O, Gallardo-Velázquez T. Anti-inflammatory activity of betalains: A comprehensive review. Human Nutrition & Metabolism. 2021 Jul 5:200126.