SCOPE OF BETALAINS AS A FOOD COLORANT

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Abstract: Consumers are avoiding foods containing synthetic colorants, which lead food industries to replace them by natural pigments. Food colorants may be classified into synthetic, nature-identical, inorganic and natural colorants. Natural colorants for food are made from renewable sources. Generally, the colorants are extracted from plant material, but other sources such as insects, algae, cyanobacteria and fungi are used also. Natural colorants are normally extracted and concentrated using either water or lower alcohols for water-soluble pigments and organic solvents for lipophilic pigments. But Legislation restricts which colorants are allowed, what sources may be used for that particular colorant, what solvents may be used to extract it and the purity of the pigment. Colorants are formulated to make them more suitable for a variety of foods and drinks and to increase their stability.

Key words: Food colorants, Natural colorants, Pigments, stability.

INTRODUCTION: Color is an important characteristic of food. First impressions are based on the color of the food. Human beings, like most animals, come in contact with their surroundings through color, and things can or cannot be acceptable based on their color characteristics Is the fruit immature, ripe, or overripe? Is the food fresh? Based on these first impressions, a judgment is made whether the food is safe to eat or not and whether it can be good in taste or not. Since color is closely associated with expectations, the addition of color to food is a way to fulfill these expectations. Color is

added to food for one or more of the following reasons: (1) to replace color lost during processing, (2) to enhance color already present, (3) to minimize batch-to batch variations, and (4) to color otherwise uncolored food. Food colors can be divided into four categories: (1) natural colors, (2) nature-identical colors, (3) synthetic colors, and (4) inorganic colors. Natural colors are pigments made by living organisms. Nature-identical colors are man-made pigments which are also found in nature. Examples are β-carotene, canthaxanthin, and riboflavin. Synthetic colors are man-made colors which are not found in nature—these are often azo-dyes. Examples of inorganic colors are titanium dioxide, gold, and silver.

The terms "pigment" and "dye" are often used interchangeably. Strictly speaking, a pigment is insoluble in the given medium, whereas a dye is soluble. This distinction may be difficult to maintain if nothing is assumed about the medium, and in the following the term "pigment" will be used for colored substances in general.

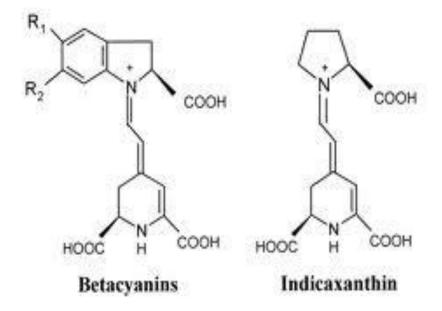
NECESSITY OF FOOD COLORANTS:

The Natural and synthetic pigments are used in medicines, foods, clothes, furniture, cosmetics, and in other products. However, natural pigments have important functions other than the imparted beauty. Development of foods with an attractive colors appearance is an important goal in the food industry. Increasingly, food producers are turning to natural food colors, since certain artificial color additives have demonstrated negative health issues following their consumption. Because of the deficiencies of existing natural food colorants, the demand for natural colorants is repeatedly raised by the food industry. This demand can be fuelled by research to offer a more natural-

healthy way of coloring foods and provide a clean label declaration. Therefore, part of plant pigment research is seeking new sources of pigments. This will not only directed in finding natural alternatives for synthetic dyes but also discover new procedures for the pigment production. The most common plant pigments are carotenoids, chlorophylls, anthocyanins and betalains. Most research has been focused on carotenoidsand and anthocyanins but betalains have recently gained interest in food science. Compared to anthocyanins, betalains are ideal for coloring low-acid foodstuff as they maintain their color over a wide array of pH, from 3 to 7.

Betalains as a pigment-Beet (Beta vulgaris subsp. vulgaris) has been bred to produce a number of varieties, some of which are used as fodder and some of which are used for human food consumption: chard (var. cicla), sugar beet (var. altissima), and beetroot (var. rubra). Beetroot is a variety with a strongly colored root. The purple root owes its color to the presence of betalains. Betalains are found in other plants than beetroot, but beetroot is the only allowed source of betalain colorant in the EU and the USA [2]. Betalains are actually comprised of two groups of pigments: the red-purple betacyanidins and the yellow betaxanthins (Fig. 1), both of which are water-soluble. Betacyanidins are conjugates of cyclo-DOPA and betalamic acid, and betaxanthins are conjugates of amino acids or amines and betalamic acid. Just as with anthocyanins, the betacyanidins (aglycones) are most often glycosylated, in which case they are called betacyanins, and the sugar residue may also be acylated. The red-purple betacyanins comprise the major part of pigments in beetroot, and of these a single one, betanin, comprises 75-95 % [2]. Compared to anthocyanins, beetroot color is more purple and brighter, and the color hue does not change with pH in the range 4–7. The major

disadvantage of beetroot color is its low heat stability.



Betanidin: R_1 and R_2 =OH Betanin (5-O-glucose betanindin): R_1 =glucose; R_2 =OH

Fig.1 Structure of Betalains

PLANT SOURCES OF BETALAINS

Betalains can be found in roots, fruits and flowers [15]. They absorb visible radiation over the range of 476–600 nm with a maximum at 537 nm at pH 5.0. The few edible known sources of betalains are red and yellow beetroot (Beta vulgaris L. ssp. vulgaris), coloured Swiss chard (Beta vulgaris L. ssp. cicla), grain or leafy amaranth (Amaranthus sp.) and cactus fruits, such as those of Opuntia and Hylocereus genera [3,11,16.20]. The major commercially exploited betalain crop is red beetroot (Beta vulgaris), which contains two major soluble pigments, betanin (red) and vulgaxanthine I (yellow). According to Nilsson (1970), the betacyanin and betaxanthin contents of red beetroots vary within the ranges 0.04–0.21% and 0.02–0.14%, respectively, depending on the

cultivar[21], although some new varieties produce higher betalain contents[6,7,14] analysed five red beet varieties ('Bonel', 'Nero', 'Favorit', 'Rubin' and 'Detroit') in terms of their pigment composition. In all cases, the major red-violet pigments were betanin, isobetanin, betanidin and isobetanidin, and the major yellow components were vulgaxanthin I and vulgaxanthin II. 'Bonel', 'Favorit' and 'Rubin' exhibited the highest betacyanin contents (around 0.08%), while the 'Nero' variety showed the poorer betacyanin content (near 0.06%). The variety 'Rubin' showed the highest betacyanin / betaxanthin ratio (2.08), so was considered as the most suitable for food colourant production.

BIOTECHNOLOGICAL PRODUCTION OF BETALAINS

Some authors have investigated beet cell cultures for producing betalains [1,12]. With this technology, it would be easier to control quality and availability of pigments independently of environmental changes[4]. However, these cultures are unable to compete with the beetroot, which is an abundant and inexpensive crop which may produce up to 0.5 g of betanin per kg of roots [7]. The low productivity of the current bioreactor systems available and the high cost of the process impair its economic feasibility. Then, the selection of a bioreactor and cultivation techniques for optimal culture growth and betalain production is one of the most important issues to be solved [9].

CLASSIFICATION

They can be divided into two structural groups, the yellow betaxanthins (from Latin *beta*, red beet and Greek *xanthos*, yellow) and red-purple betacyanins (*kyanos*, blue color), depending on R1-*N*-R2 moieties(fig.1).

Table1: Some Fully Identified Naturally Occurring Betalains[5]

Betalain[A]*1	Residue[B]*2	
1.Aglycones		
Betanidin	-	
2. Betanin group		
Betanin	5-O-Glc	
Phyllocactin	5-O-Glc	
Lampranthin-I	5-O-Glc	
3. Amaranthin group		
Amaranthin	5-O-Glc-2-O-GlcU	
Celosianin II	5-O-Glc-2-O-GlcU	
4. Bougainvillein		
Bougainvillein	5-O-Glc-2-O-Glc	
5. Gomphrenin group		
Gomphrenin-I	6-O-Glc	
6. Betaxanthins		
DOPAxanthin	DOPA	
Indicaxanthin	Proline	

Portulaxanthin-II	Glycine
Vulgaxanthin-I	Glutamic acid

^{*1} A Names were standardized by Strack et al.

More than 50 betalains are well known, and all of them have the same basic structure in which R1 and R2 may be hydrogen or an aromatic substituent. Their color is attributable to the resonating double bonds. Conjugation of a substituted aromatic nucleus to the 1,7-diazaheptamethinium chromophore shifts the absorption maximum from 480 nm in yellow betaxanthins to 540 nm in red-purple betacyanins.[19] . Betacyanin structures show variations in their sugar (e.g., 5-O-D-Glucose) and acyl groups (e.g., feruloyl), whereas betaxanthins show conjugation with a wide range of amines (e.g., glutamine) and amino acids (e.g., tyrosine) in their structures. Table 1 shows some well-studied betalains.

Up to date more than 50 structures of naturally occurring betalains have been identified.

IMPORTANCE OF BETALAINS AS FOOD COLORS

(a)Stability of betalains

When betalains are used as food colorants, color stability is a major concern. There are several factors that have been recognized to affect the stability of these pigments:

^{*2} B Abbreviations: Glc β-D-Glucose; GlcU β-D-Glucoronic acid; DOPA 3,4-dihidroxyphenylalanine.

- **1. pH.** The hue of betalains is unaffected at the pH between 3.5 to 7; the values of most foods are in this range. Betalain solutions in this pH range showed a similar visible for betacyanins and betaxanthins.
- **2. Temperature.** Temperature also shows a clear effect on betalain stability. Thermal kinetic degradation of betanin has been evaluated by several authors. It has been reported that thermostability of betanin solutions is pH dependent and partially reversible. Heating of betanin solutions produces a gradual reduction of red color, and eventually the appearance of a light brown color.
- **3. Light.** Von Elbe et al. found that rate of betanin degradation increased 15.6% after pigment daylight exposure at 15°C. Degradation of light-exposed betalains followed a first-order kinetic. In addition, it was observed that degradation was higher at pH 3.0 (k = 0.35 days-1) than at pH 5.0 (k = 0.11 days-1), when betacyanins were exposed to fluorescent light.
- **4. Water activity.** Recognizing the importance of water in many degradation reactions, it is not surprising that water activity (aw) is included among the primary factors affecting the betalain stability and/or color of a food product containing these pigments. Because the degradation reaction does involve water, the greatest stability of betalains has been reported in foods or model systems of low moisture and aw.
- **5. Oxygen.** Oxygen causes a product darkening and loss of color. Von Elbe et al stored buffered betanin solutions at pH 7 under atmosphere of air and nitrogen for 6 days at 15°C; it was observed that color degradation increases up to 15% due to air conditions. Betanin reacts withmolecular oxygen, producing pigment degradation in air-saturated solutions.

Several methods have been reported to prevent the destruction or to improve the stability of pigments, including degassing, addition of antioxidants and stabilizers, control of pH, minimal heat treatment and these efforts have been directed to their application in food products.

(b) Processing and Stability in Foods

The sensitivity of betalains to different factors suggests that their application as food colorants is limited. Based on these properties, betalains can be used in foods with a short shelf-life, produced by a minimum heat treatment, and packaged and marketed in a dry state under reduced levels of light, oxygen, and humidity. Betalains have several applications in foods, such as gelatins desserts, confectioneries, dry mixes, poultry, dairy, and meat products. Table 2 summarizes some applications of betalain pigments in food products. The amount of pure pigment required in these foods groups to obtain the desired color is relatively small and for most applications does not exceed 50 ppm of betalains, calculated as betanin. Problems associated with betalain degradation and pigment recovery during the processing operations are of economic importance and must be solved to betalains displace the application of synthetic dyes in some food products.

Nowadays, beet roots represent the main commercial source of betalains (concentrates or powders). The average pigment content of beets is approximately 130 mg/100 g fresh weight, but new red beet varieties produce around 450 to 500 mg/100 g fresh weight. Furthermore, this value is increasing as advanced selection is developed.

On a laboratory scale, betalains can be obtained by employing reverse osmosis, ultrafiltration solid-liquid extraction, and diffusion.

FUTURE TRENDS

Since the 1960s people have shown a clear preference for natural products, including pigments, because more nutritious and healthy characteristics have been associated with them. Moreover, one of the main problems to be solved, that natural colors approved by FDA and European Union do not cover all ranges of colors (e.g., blu

TABLE 2: Applications of Beet Root Powder as Natural Color in Food Products

Food products	Shade	Level
1. Dairy products:		
(a) Strawberry yogurt	Rose-pink	0.08%
(b) Ice creams	Pink	0.27%
	Rose-pink	0.25%
2. Meat products:		
·(a)Sausages	Pink	550 mg/100 g
·(b) Cooked ham	Pink–brown	0.15%
3. Dry powder beverages	Strawberry	1.1%
	Raspberry	1.5%
	Blackcurrant	1.0%
4. Water ices	Strawberry-red	0.8 to 1.0%
	Raspberry	0.5%
5. Marzipan	Pastel-red	0.45%
	Bluish-red	1.5 mg/cm2
6. Biscuit creams	Pink	0.27%
7. Baked goods	Pink-brown	2.5%

8.Hard candies	Pink	0.15%
9. Fruit cocktails	Raspberry-red	1.5%
10. Jellies	Raspberry–red	0.18%

Thus, many research groups are looking for new sources of natural pigments; however, these efforts have vanished, because under the current legislation the FDA or the European Union approval of new natural sources of pigments is very difficult. Consequently, it is expected that the world global market must contribute to the implementation of more realistic laws.

On the other hand, it has been clearly established that technology development has introduced new methodologies or processes to avoid the intrinsic instability and solubility problems of natural pigments, and today most of these problems can be solved through technological processes. Thus, a more adequate and updated legislation will provide a broad range of natural colors, and importantly with better stability characteristics.

Anthocyanins are the most widespread and most used natural pigments covering the red-purple colour range. However, the instability of anthocyanins at pH values above 3 [17] makes betacyanins the natural pigments of choice to provide red-purple colour shades to low acid foods. Moreover, betalains can effectively be stabilised by ascorbic acid, which on the other hand, impairs anthocyanin stability [15]. Hence, application of betalains instead of anthocyanins for colouring foods with high ascorbic acid contents may be interesting[9].

The demand for better natural- colored foods by an important sector of the society will be increased because it is highly likely that future studies will increase people's conscience about the positive health benefits of natural pigment consumption. Remarkably, the most impressive advances in these aspects have been reached using molecular biology techniques, and this will be so in the near future. Interestingly, model systems for pigment production under controlled conditions are now available, but production at the industrial level has not been feasible yet. Thus, carotenoid production by yeasts, bacteria and fungi, and anthocyanin and betalain production by plant tissue cultures require the development of better biotechnological approaches. Production of colors by fermentation has a number of advantages: cheaper production, possibly easier extraction, higher yields, no lack of raw materials, and no seasonal variations. Fermented colors are already used today: D. salina, B. trispora, spirulina, and monascus. It is not unlikely that new, fermented colors such as lycopene from B. trispora will become allowed in the near future. A giant leap forward in color production could be achieved by combining genetic manipulation and fermentation.

Finally, new colorants would have to be approved by the authorities, which is very costly because of the various toxicological studies needed to confirm the safety of a new food additive.

REFERENCES

[1] Akita, T., Hina, Y. & Nishi, T. (2000). Production of betacyanins by a cell suspension culture of table beet (Beta vulgaris L.). Bioscience, Biotechnology, and Biochemistry, 64, 1807–1812

- [2] Alan Mortensen(2006) Carotenoids and other pigments as natural colorants *Pure Appl. Chem.*, Vol. 78, No. 8, pp. 1477–1491
- [3] Cai, Y., Sun, M. & Corke, H. (1998). Colourant properties and stability of Amaranthus betacyanin pigments. Journal of Agricultural and Food Chemistry, 46, 4491–4495.
- [4] Do rnenburg, H. & Knorr, D. (1997). Challenges and opportunities of metabolite production from plant cell and tissue culture. Food Technology, 51, 47–54.
- [5] F. Delgado-Vargas,1 A. R. Jiménez,2 and O. Paredes-López3*(2000) Natural pigments:carotenoids,anthocyanins and betalains- characteristics, biosynthesis ,processing and stability *Critical Reviews in Food Science and Nutrition*, 40(3):173–289
- [6] Gaertner, V. & Goldman, I.L. (2005). Pigment distribution and total dissolved solids of selected cycles of table beet from a recurrent selection program for increased pigment. Journal of the American Society for Horticultural Science, 130, 424–433
- [7] Gasztonyi, M.N., Daood, H., Hajos, M.T. & Biacs, P. (2001). 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
- [8] Han, D., Kim, S. J., and Kim, D. M.(1998) Repeated regeneration of degraded red beet juice pigments in the presence of antioxidants, *J. Food Sci.*, 63(1): 69–72

- [9] Herbach, K.M., Stintzing, F.C. & Carle, R. (2006b). Betalain stability and degradation – structural and chromatic aspects. Journal of Food Science, 71, R41–R50.
- [10] Jimenez-Aparicio, A. & Gutierrez-Lopez, G. (1999). Production of food related colourants by culture of plant cells. The case of betalains. Advances in Experimental Medicine and Biology, 464, 195–210.
- [11] Kugler, F., Stintzing, F.C. & Carle, R. (2004). Identification of betalains from petioles of differently coloured Swiss chard (Beta vulgaris L. ssp. cicla [L.] Alef. cv. Bright Lights) by highperformance liquid chromatography–electrospray ionization mass spectrometry. Journal of Agricultural and Food Chemistry, 52, 2975–2981.
- [12] Leathers, R.R., Davin, C. & Zryd, J.P. (1992). Betalain producing cell cultures of Beta vulgaris L. Var. Bikores Monogerm (red beet). In vitro Cellular & Developmental Biology, 28, 39–45.
- [13] Nilsson, T. (1970). Studies into the pigments in beetroot (Beta vulgaris L. ssp. vulgaris var. rubra L.). Lantbrukhogskolans Annaler, 36, 179–219
- [14] Pszczola, D.E. (1998). Natural colours: pigments of imagination. Food Technology, 52, 70–76
- [15] Shenoy, V.R. (1993). Anthocyanins Prospective food colours. Current Science, 64, 575–579.
- [16] Stintzing, F.C., Schieber, A. & Carle, R. (2002b). 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 spectroscopy. Journal of Agricultural and Food Chemistry, 50, 2302–2307.
- [17] Stintzing, F.C., Conrad, J., Klaiber, I., Beifuss, U. & Carle, R. (2004). Structural investigations on betacyanin pigments by LC NMR and 2D NMR spectroscopy. Phytochemistry, 65, 415–422.
- [18] Strack, D., Vogt, T. & Schliemann, W. (2003). Recent advances in betalain research. Phytochemistry, 62, 247–269.
- [19] Strack, D., Steglich, W., and Wray, V.(1993) Betalains. In: *Methods in Plant Biochemistry Vol. 8*, Academic Press, Orlando, 421–450.
- [20] Vaillant, F., Perez, A., Davila, I., Dornier, M. & Reynes, M. (2005).

 Colourant and antioxidant properties of red-purple pitahaya (Hylocereus sp.)

 Fruits, 60, 1–10.
- [21] von Elbe, J.H. (1975). Stability of betalaines as food colors. Food Technology, 5, 42–44.