NATURAL FOOD COLORANTS

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Keywords: Pigment, fermentation, riboflavin, carotenoid, astaxanthin, phycobiliprotein, indigoid, anthraquinone, *Phaffia*, *Monascus,*

Contents

Summary

Despite the enormous economic potential of microbial pigments, few known microorganisms lend themselves to a commercial exploitation. It is clear that the vast range of microorganisms constitutes a storehouse of different molecules that vary in their color and stability. However, only β–carotene and riboflavin are produced from microbial sources. In general, microbial pigments are not approved for use in the food industry, although they do appear to be non–toxic. Thus, even though species of *Monascus*, for example, have been eaten in the Far East for many years, this does not help the pigment to gain approval in the EU or the US.

Another interesting aspects of biotechnology is the transfer of biosynthetic capability from one microorganism to another. The genes for pigment production can now be isolated and transferred to another microorganism (which may be generally recognized as a safe microorganism (GRAS)). The use of bacterial strains as recipient cells is advantageous because of the ease of genetic manipulation in bacteria as well as their high biosynthetic rates. But, this possibility is directly linked to a general acceptance of genetically modified organisms (GMO).

Indeed, the dislike of modern consumers for novel ingredients is likely to be the biggest obstacle for expansion of the colorant list in the near future. The main restriction facing industries which wish to investigate new colorant sources is the expense of carrying out the compulsory toxicology testing, which requires a major investment without the certainty that money will eventually be made or, worse, that their rivals will get the benefits of this investment.

So many authors have expressed doubts about the successful commercialization of fermentation–derived food colorants because of the high capital investment requirements for fermentation facilities and the expensive and lengthy toxicity studies required by regulatory agencies. Nevertheless, world trade favors cultural exchanges, and the demonstration that biotechnology–derived food colorants used elsewhere and consumed here in exotic dishes will certainly be instrumental in the acceptance of these ingredients by both the public and regulators

1. Introduction

There has been much interest in the development of new colorants for use in the food industry, which is apparently due to strong consumer demand for more natural products in the E.U. and the USA. But, even if it is technologically feasible to prepare new natural colorants from plants or microorganisms, considering economic, legislative and consumer acceptance aspects, their introduction to the market looks less promising. Penetration in the food industry by fermentation–derived pigments is limited at present to β–carotene and riboflavin markets (see also food industry). This is mainly due to the higher production cost of fermentation pigments compared to those of synthetic origin or extracted from natural sources.

Moreover, as long as current food legislation remains unchanged, it is unlikely that it will be possible to introduce completely new natural colorants (even more so those which are fermentation–derived) into the food manufacturing industry. Furthermore, newly discovered pigments will not be any more resistant to processing conditions than those that are already available.

2. Current Natural Colorants

Currently, 43 colorants are authorized as food additives by the EU and have been assigned an E number. Sixteen of these are of plant origin (Table 1). Juices or extracts from some fruit and vegetables are also used for coloring purposes (for example fruit juices containing carotenoid and anthocyanin pigments, gardenia extract, grape skin extract, green color extract from spinach or alfalfa). In the US, two categories of colorants are permitted: "certified color additives" and "colorants exempt from certification" (turmeric oleoresin, annatto extract, paprika oleoresin, β–carotene, cochineal extract, vegetable and fruit juices, grape skin extract, caramel color, and titanium oleoresin).

Colorant	**E number**	**Design features**
Anthocyanin	E163	Red to blue pigments found in mature fruits (strawberries, blueberries, cherries, grapes),

		vegetables, seeds and flowers
Betanin	E162	Major pigment in red beet
Caramel color	E150	Obtained by careful heating of food-grade carbohydrates
Carminic acid	E120	Extract of the female cochineal insect
Carotenoids	E160	Pigments extracted mainly from plants (carrots, tomatoes)
β-carotene	E160a	
Bixin, norbixin, or annatto extract	E160b	Extract from a seed (*Bixa orellana*)
Lycopene	E160d	Pigment found in a variety of fruit (tomatoes, pink grapefruit, watermelons)
Lutein	E161b	
Canthaxanthin	E161g	Pigment that occurs naturally in salmon, shrimp and feathers of flamingos
Chlorophyll	E140	Natural green pigment participating in the photosynthetic process
Chlorophyllin	E141	Natural green pigment
Curcumin	E100	Major pigment of turmeric extracted from a plant rhizome (*Curcuma longa*)
Indigotin	E132	Pigment found in the plants *Indigofera tinctoria* or *Isatis tinctoria* (woad)

β-Carotene : Pigments extracted mainly from carrots
Lutein : Pigments extracted from alfalfa, corn, marigold flowers or maïze

Table 1. Natural food colorants currently authorized in the EU(Wissgott and Bortlik, 1996)

In 1994, the international natural colorant market was valued at US$ 250 million and a recent Frost and Sullivan study (Table 2) has shown that, from 1998 to 2005, the natural food colorant industry in the EU is experiencing an annual average growth rate of 2.7 percent.

The current consumer preference for natural colorants is associated with their image of being healthy and of good quality. They have become increasingly popular with consumers because synthetic colorants tend to be perceived as undesirable and harmful; some are considered to be responsible for allergenic and intolerance reactions. So new opportunities exist for fermentation–derived pigments such as phycocyanins, xanthophylls (astaxanthin) or *Monascus* pigments.

Year	**Volume (tons)**	**Value (millions US$)**	**Growth in value (%)**
2000	324.9	197.8	4.4
2001	331.8	205.6	4.0
2002	339.4	208.8	1.6
2003	346.5	213.8	2.4

2004	353.0	219.6	2.7
2005	359.5	225.5	2.7

Table 2. Development of the natural colorants market in EU(source Frost and Sullivan, cited in Arômes, Ingrédients Additifs, January 2000).

3. Colorants Obtainable by Biotechnology Routes

With the growing interest worldwide in developing food colorants from natural sources, novel organisms from which colorants can be extracted are being evaluated. This section focuses on those obtainable by biotechnology processes (fermentation, plant cell and algae cultures). It examines the efforts being made in the development of fermentation processes to produce pigments that are competitive with extracted natural pigment or nature–identical pigments, with a special focus on *Monascus* pigments, which are of great interest in the EU even if legal approval has still not been obtained.

The organisms under examination produce flavonoids, carotenoids, phycobiliproteins, and other pigments which can range in color from yellow to orange and red to purple and blue. Some authors emphasize that the only food–approved colors produced by fermentation are β–carotene and riboflavin.

3.1 Riboflavin

Riboflavin (Vitamin B2) is used as a yellow food colorant. (Figure 1).

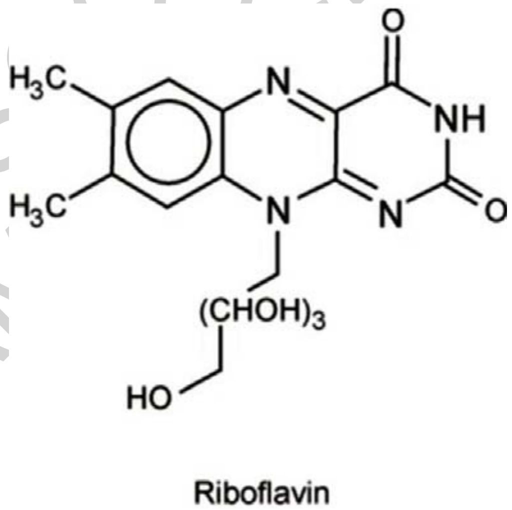

Figure 1. Structure of riboflavin.

It is produced both by fermentation and synthetically and is permitted in most countries. Numerous microorganisms produce riboflavin, being classified as weak, moderate and strong producers. In Table 3, bacteria, yeasts, and fungi able to produce riboflavin are classified according to their production yields.

Categories of	**Microorganisms**	**Production g L^{-1}**

producers		
Weak	*Clostridium acetobutylicum*	0.1
Moderate	*Candida flareri* *Candida guilliermondii* *Debaromyces subglobosus*	0.6 " "
Strong	*Eremothecium ashbyii* *Ashbya gossypi* *Candida flareri* *Saccharomyces cerevisiae* *Bacillus subtilis*	5 15 21 2 15

Table 3. Biotechnology ways to produce riboflavin (Jacobson and Wasileski, 1994). Fermentation with *Ashbya gossypii* is preferred because of its higher yields and greater genetic stability; riboflavin levels of over 15 g L^{-1} have been reported. Nevertheless, *Bacillus subtilis* is also able to produce 15 g L^{-1} of riboflavin.

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Bibliography

Blanc P. J., Laussac J. P., Le Bars J., Le Bars P., Loret M.O., Pareilleux A., Promé D., Promé J. C., Santerre A. L. and Goma G. (1995). Characterization of monascidin A from *Monascus* as citrinin. *International Journal of Food Microbiology* **27**, 201–213. [This presents the characterization of the so–called secondary metabolite monascidin A as citrinin.]

Blanc P. J., Loret M. O., Santerre A. L., Pareilleux A., Promé D., Promé J. C., Laussac J. P. and Goma G. (1994). Pigments of *Monascus*. *Journal of Food Science* **59**(4), 862–865. [This presents the chemical structures of the free and complexed pigments of *Monascus*.]

Chen M. H. and Johns M. R. (1993). Effect of pH and nitrogen source on pigment production by *Monascus purpureus*. *Applied Microbiological Biotechnology* **40**, 132–138. [This works presents the effect of various carbon sources on individual pigment concentrations.]

Fabre C. E., Santerre A. L., Loret M. O., Baberian R., Pareilleux A., Goma G. and Blanc P. J. (1993). Production and food applications of the red pigments of *Monascus ruber*. *Journal of Food Science* **58**(5), 1099–1102, 1110. [This work provides extensive data on the stability of *Monascus* pigment in meat products.]

Hajjaj H., Blanc P. J., Groussac E., Goma G., Uribelarrea J. L. and Loubiere P. (1999). Improvement of the red pigment/citrinin production ratio as a function of environmental conditions by *Monascus ruber*. *Biotechnology Bioengineering* **64**(4), 497–501. [This work presents the growth and metabolic behavior of *Monascus ruber* in submerged cultures.]

Hajjaj H., Klaebe A., Loret M.O., Goma G., Blanc P.J. and François J. (1999). The biosynthetic pathway of citrinin in the filamentous fungi *Monascus ruber* as revealed by ^{13}C–NMR. *Applied Environmental Microbiology* **65**, 311–314. [This works shows that the production of polyketide red pigments and citrinin by *Monascus ruber* is regulated at the level of the tetraketide branch point.]

Hamdi M., Blanc P. J. and Goma G. (1995). A new process for red pigment production by *Monascus purpureus*. Culture on prickly pear juice and the effect of Partial Oxygen Pressure. *Bioprocess Engineering* **17**, 75–79. [This works presents the optimization of the formation of red pigment by *Monascus purpureus* based on inoculum preparation and culture medium.]

Jacobson G. and Wasileski J. (1994). Production of food colorants by fermentation. *Bioprocess production of flavor, fragrance and color ingredients*, (ed. Alan Gabelman), pp. 205–237. New York: John Wiley and Sons. [This is a review of fermentation–derived colorants usable by the food industry.]

Juzlova P., Martinkova L., and Vren V. (1996). Secondary metabolites of the fungus *Monascus*: a review. *Journal of Industrial Microbiology* **16**, 163–170. [This review deals with polyketides produced by *Monascus*.]

Kusdiyantini E., Gaudin P., Goma G. and Blanc P. J. (1998). Growth kinetics and astaxanthin production of *Phaffia rhodozyma* PR 190 on glycerol as a carbon source during batch fermentation. *Biotechnology Letters* **20**(10), 929–934. [This presents a strategy of astaxanthin production in fed batch culture or chemostat at a specified growth rate.]

Lee Y. K., Chen D. C., Chauvatcharin S., Seki T. and Yoshida T. (1995). Production of *Monascus* pigments by a solid–liquid state culture method. *Journal of Fermentation Bioengineering* **79**(5), 516–518. [This presents the production of the pigments of *Monascus* on starch.]

Nelis H.J. and De Leenheer A.P. (1991). Microbial sources of carotenoids used in foods and feeds. Journal of Applied Bacteriology **70**, pp. 181-191. [This review presents the sources of carotenoids pigments produced by microorganisms.]

Sabater–Vilar M., Maas R. F. M. and Fink–Gremmels J. (1999). Mutagenicity of commercial Monascus fermentation products and the role of citrinin contamination. *Mutation Research.* **444**, 7–16. [This presents the mutagenicity of citrinin and of some commercial samples of *Monascus* pigment.]

Wissgott U. and Bortlik K. (1996). Prospects for new natural food colorants. *Trends in Food Science and Technology* **7**, 298–302. [This presents the latest development in new natural colorants in the food industry.]

Xu G., Lu C., Mu X., Chen J., Chen Y., Gu Y., Wu Y., Sheng F. and Wu M. (1999). A study on the production of citrinin by *Monascus* spp. *Archiv für Lebensmittelhygiene* **50**, 88–91. [This paper discuss aspects of citrinin determination methods for high color value.]

Biographical Sketch

Philippe J. Blanc is a French scientist. He has a Ph.D. in Microbiology from INSA of Toulouse in 1986 under the guidance of Professor Goma. Since 1986, he has been a scientist in the Biotechnology–Bioprocess Laboratory at INSA Toulouse where he is in charge of the technology transfer unit of the laboratory (realization of research programs in fermentations applied to the food and health industries, techno–economic information for the scientific teams, and realization of prospective studies for industrialists, responsibility for MIRCEN–UNESCO (Toulouse), and representation of the laboratory mainly in European symposia). His present areas of investigation are the metabolism of fungi (synthesis of pigments, flavors, mycotoxins and other secondary metabolites). He has been managing a program of research on *Monascus* for 11 years, and one on the yeast *Phaffia* for 5 years.