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COLORANTS IN FOODS – FROM PAST TO PRESENT

BARWNIKI W ŻYWNOŚCI – OD PRZESZŁOŚCI DO TERAŹNIEJSZOŚCI

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Summary: Consumer expectations toward food products are changing due to the development of the food industry and new technologies. The growing knowledge in the field of health impact of natural and artificial colorants added to food results in an awaking interest of natural dyes among researchers, producers and consumers. Food colour plays a fundamental role in identifying the quality of food, so it is associated with the sensory quality of food products. In this paper the different types of colorants, their production methods and the evolution of these methods were characterized.

Keywords: colorants, food colouring, natural pigments, synthetic dyes.

Streszczenie: Oczekiwania konsumentów wobec produktów spożywczych zmieniają się z powodu rozwoju przemysłu spożywczego i nowych technologii. Rosnąca wiedza w dziedzinie wpływu na zdrowie naturalnych i sztucznych barwników dodawanych do żywności skutkuje wzbudzeniem zainteresowania naturalnymi barwnikami wśród badaczy, producentów i konsumentów. Kolor żywności ma zasadniczą rolę w identyfikacji jakości żywności, gdyż jest wiązany z jakością sensoryczną produktów spożywczych. W niniejszym artykule opisano różne rodzaje barwników, metody ich produkcji oraz ewolucję tych metod.

Słowa kluczowe: barwniki, barwniki spożywcze, naturalne pigmenty, barwniki syntetyczne.

1. Food colorants

The colour perception by humans influences the efficiency of the food market, especially The market of colorants used for food pigmentation. Colours basically neither improve taste or smell, nor extend the shelf-life and in most cases do not bring any nutritional value. However, from the food industry point of view, food colorants belong to the key food additives, next to taste improving substances. Their ability for food pigmentation is used to reproduce the original colours of food lost during processing. Food colorants are therefore applied to regain previously existing colour.

Among the natural organic colorants are: annatto (bixin, norbixin), anthocyanins, betaine, chlorophylls, caramel, cochineal, curcumin, lycopene or carotenoids. This group of colorants has a lower pigmentation capacity compared to synthetic dyes. Research conducted on natural pigments showed their high sensibility to light, temperature, pH, oxidative and reducing agents [Baranowska 2005].

In contrast, synthetic dyes like azo, indigo, quinoline and triarylomethane dyes, exhibit such features as higher durability and a wider colour spectrum in comparison to natural pigments, moreover they are also much cheaper. Synthetic dyes are widely used in the food industry also due to low prices [Carocho et al. 2014; Baranowska 2005].

In recent years the discussion has arisen on the harmfulness of synthetic dyes, which has encouraged the growth of interest in natural colorants, both on the part of food producers and consumers. Natural colorants are obtained by a multi-step process of extraction from the biological material of plants or insect-origin, while those identical with natural pigments are obtained by chemical synthesis [Stolarzewicz et al. 2012].

1.1. Colouring food in the past

Food colouring has been known already for a long time. Several thousand years ago dyes of natural origin – animal, vegetable and mineral – were used. Spice plants, like curry, saffron or paprika, beside aroma contain large amounts of colorants. Although food pigmentation is not necessary, a number of studies underline the importance of colour in the quality reception of the product [Zawirska-Wojtasik 2005].

Natural colorants are now much more appreciated than synthetic dyes due to their health safety, but they are characterized by a number of features reducing their industrial suitability. In addition to higher prices, the natural dyes are usually characterized by their smaller colouring ability, lower resistance to temperature, light, oxygen, metals, much narrow spectrum of colours, non-uniform composition and often by a distinctive aroma. Natural cochineal, considered one of the best food colours, has been substituted in the food industry by much cheaper synthetic dyes. In nature there is still, however, a variety of other colorants used before as the purple orceina, orange alizarin, indigo blue, which currently, due to the proven negative or toxic impact, have been banned for food production [Oplatowska-Stachowiak, Elliott 2017; Zawirska-Wojtasik 2005].

1.2. Synthetic dyes

The wide colour spectrum of synthetic dyes enables their application in different food products. Organic synthetic dyes, in comparison with natural colorants or identical with natural pigments, are:

- more efficient thanks to the high concentration of the dye substance;
- characterized by high purity of colour.

Formerly used natural pigments were not stable, however the formulations of natural colorants used nowadays exhibit good quality and stability. Numerous studies have shown that synthetic organic dyes are more susceptible to different factors than the currently used natural colorants [Gasik, Mitek 2007]. Synthetic organic dyes used as food colouring ranks among the five classes depending on the chemical structure (Table 1).

Table 1. Groups of synthetic organics dyes. Chromophore group – group of atoms in molecule of organic compound, which absorbs radiation in range of visible light – caused compound pigmentation – and ultraviolet light
Tabela 1. Grupy syntetycznych barwników organicznych. Grupa chromoforowa – grupa atomów w cząsteczce związku organicznego pochłaniająca promieniowanie w zakresie światła widzialnego – powoduje zabarwienie związku – i światła ultrafioletowego

Groups of organic synthetic dyes with their Colour Index (CI)	Group characteristics
Azo-dyes	11 dyes from monoazo-dyes and biazo-dyes are used in food colouring. Chromophore group is an azo-group. Colour: depending on the connection between azo-group and aromatic-group in molecule these compound have a yellow, orange, red and brown colour
Monoazo-dyes (no 11 000-19 000)	
Biazo-dyes (no 20 000-29 999)	
Triazo-dyes (no 30 000-30 999)	
Polyazo-dyes (no 31 000-30 999)	
Triarylmethane dyes (CI no 42 000-44 999)	For food colouring are used only three triphenylmethyl-dyes. Chromophore group is a chinoide and iminochinoide group. Colour: blue and green in a bright range of tones
Quinoline dyes (CI no 47 000-47 999)	For food colouring only one dye is allowed – quinoline yellow. Colour: green-yellow
Xanthenes dyes (CI no 45 000-45 999)	These compounds belong to xanthenes derivatives. Only erythrosine is allowed in food colouring. Colour: cherry red
Indigo dyes (CI no 73 000-73 999)	Only indigotin is allowed in food colouring. It is disulfones derivative of natural indigo pigment. Colour: blue

Source: own study based on [Carocho et al. 2014; Gasik, Mitek 2007; Janiak 2006].
Źródło: opracowanie własne na podstawie [Carocho et al. 2014; Gasik, Mitek 2007; Janiak 2006].

The colour and the stability of the dyes depend on their chemical structure and chromophore group. Synthetic food dyes are mainly acidic sodium salts, except for patent blue, used in the form of a calcium salt. Their good solubility in water results from the presence of at least one molecule of acidic group (sulfone group – SO_3H, carboxylic group – COOH). They also dissolve in propylene glycol, glycerine and sorbitol, although less than in the water. This feature is used in recipes of products without water [Carocho et al. 2014; Gasik, Mitek 2007; Janiak 2006].

The azo dyes are the ones most used among synthetic food dyes. The azo colorants include tartrazine, sunset yellow, carmoisine, amaranth, cochineal red, 2G red, Allura red AC, brilliant black BN, HT bronze. They are prone to loss of colour and precipitation in the presence of reducing agents, such as polysaccharides, aldehydes, ketones or ascorbic acid. Azo dyes are reduced to hydrazine compounds, and even to aromatic amines of proven carcinogenic activity [Rollas 2010]. This phenomenon also occurs as a result of the activity of baking and brewing yeasts. Azo compounds can form in the human body linkages with some of the components of blood, mainly erythrocytes distorting them and thus causing anaemia. Some of them, for example tartrazine, are known allergens, similar properties are also revealed by quinoline dyes [Vojdani, Vojdani 2015].

Table 2. Quality parameters of synthetic organic dyes according to their resistance against light and temperature with ADI and colour index
Tabela 2. Parametry jakościowe syntetycznych barwników organicznych według ich odporności na światło i temperaturę z ADI i wskaźnikiem barwy

Pigment	Resistance		ADI [mg/kg body weight]	Colour Index
	light	temperature		
Azorubine	Very good	Good	0-4.0	14720
Brilliant black PN	Very good	Medium	0-12.5	28440
Brilliant blue FCF	Good	Good	0-12.5	42090
Allura red Ac	Good	Good	0-7.0	16035
Tatrazine	Good	Good	0-7.5	19140
Amaranth	Good	Good	0-0.5	16185
Cochineal red	Good	Good	0-4.0	16225
Brown HT	Good	Good	0-1.5	20285
Quinoline yellow	Good	Good	0-0.5	47005
Red 2 G	Good	Good	0-0.1	18050
Patent blue V	Good	Good	-	42051
Sunset Yellow	Medium	Very good	0-2.5	15985
Green S	Low	Good	-	44090
Erythrosine	Low	Good	0-0.1	45430
Indigotin	Very low	-	0-1.5	73015

Source: own study based on [Gasik, Mitek 2007; Janiak 2006].
Źródło: opracowanie własne na podstawie [Gasik, Mitek 2007; Janiak 2006].

Triarylomethane dyes (S green, FCF brilliant blue, patent blue V) are characterized by a clean, brilliant characteristic of several colours, while the xanthene dye (erythrosine) is cherry red and is not resistant to the light. Indigoine dye (Indigo Carmine) is an equivalent of the natural indigo colorant and exhibit poor resistance to light exposure, which causes fading. Quinoline dye (quinoline yellow) is yellow-green and weakly resistant to physico-chemical factors in comparison with its yellow counterpart from the azo group – tartrazine [Janiak 2006].

The parameter characterizing dyes and other food additives in terms of their toxicity is acceptable daily intake (ADI value). The ADI is determined on the basis of toxicological studies carried out by the FAO/WHO Expert Committee on Food Additives and by the EFSA organisation. Table 2 shows the most important physical and chemical properties of organic synthetic food colours together with the values of the ADI [Martins et al. 2016; Rutkowski et al. 2003].

EFSA recommended new tests related to the possible genotoxicity of Allura Red AC. After several trials, EFSA concluded that Allura Red AC is not genotoxic for dogs and cats, neither demonstrates damage to the DNA of individual, nor shows other evidence of genotoxicity (*in-vivo* micronucleus test) [The EFSA Website 2017].

The synthetic organic dyes approved to food pigmentation include: azorubine, patent blue V, brilliant blue PCF, HT brown, Allura red AC, cochineal red, brilliant black PN, Indigo Carmine, tartrazine, S green, sunset yellow, quinoline yellow, amaranth, erythrosine, 2G red, FK brown, litholrubine BK, gentian (the latter for marking slaughtered animals, bacon and coatings for cheese ripening). Synthetic inorganic pigments are titanium dioxide, calcium carbonate, iron oxides and hydroxides, aluminium, silver (in leaves) and gold (in leaves) [Regulation EC No. 1333/2008; Baranowska 2005].

1.3. Synthetic dyes – identical with natural ones

The nature-identical colourings are synthetic compounds with the same structure as their natural equivalents. Synthetic dyes identical with natural ones include: riboflavin, beta-carotene, the ethyl ester of beta-apo- 8’-carotenic acid and canthaxanthin.

Consumers are prejudiced against such food additives. Regarding the economical background, synthetic dyes are often applied into food products. The appropriate dosage of pure chemical substances is easier than natural extracts, because natural colourings contain other compounds or by-products generated during the extraction [Carocho et al. 2014; Alvarez et al. 2014; Zawirska-Wojtasik 2006].

Food legislation in different countries treats differently additional substances identical to natural ones. Basically the regulations in all countries mainly differentiate between natural and synthetic. However, nature-identical substances are treated as natural in Germany, while the FDA in the United States classifies as natural only the substances derived from natural sources. Due to the limited precision in the definition of nature-identical substances, lots of doubts regard the food additives origin. However, the toxicity of a substance is associated primarily with its dosage, and both synthetic and natural substances can be toxic for humans [Wrolstad, Culver 2012].

Following a series of food crises, the European Food Safety Authority (EFSA) was funded by the European Union – in order to be a source of scientific advice and communication on the risks associated with the food chain. EFSA advises on food safety and supports the legislation process of synthetic dyes. In cases of allergies, the experts of EFSA recommend the possible inclusion of food colours in the list of food allergens in Annex IIIa of Directive 2000/13/EC on the labelling, presentation and advertising of foodstuffs [The EFSA Website 2017].

The biological activity of nature-identical colouring can differ from their natural equivalents, due to the chirality. Chiral compounds are called optical or configuration isomers or enantiomers, and are substances of the same chemical structure, but showing different spatial organization, such as right and left hand. Only living organisms are the source of optically active compounds and mostly one of the isomers in greater quantities is preferred to synthesize. Natural compounds are most often homochiral, i.e. have the same spatial configurations. Proteins are made up of L-amino acids, and carbohydrates with D-saccharides. The presence in nature of other enantiomers (D-amino acid or L-Saccharides) is extremely rare or the result of an awkward or artificial transformation. Homogeneous compounds are synthesized during a biological process, while as a result of chemical synthesis a mixture of enantiomers (racemate) is produced [Fontan et al. 2013; Britton et al. 1996].

The optical isomerism also applies to certain dyes. A naturally occurring astaxanthin is found in the form of isomers, of which the most popular is the 3S, 3 'S-astaxanthin. Although a variety of carotenoids can be synthesized chemically, in this case, an identical compound cannot be obtained. Because of this, a lot of attempts are undertaken to obtain chiral compounds in their natural configuration by the biosynthesis. The application of the biotechnological methods is possible because the reactions catalysed by microbial enzymes are stereo specific. Pigments produced by biotechnological methods are natural. The discussion arises whether natural means can also be obtained by biosynthesis. It is difficult to determine the identity of synthetic substances compared to natural counterparts. It is necessary to set the safety and security of borders for all substances, regardless of the classification [Zawirska-Wojtasik 2006].

1.4. Natural colorants

Natural colorants are obtained from biological material like vegetables, animals, microorganisms and minerals (Table 3).

Natural colorants are applied in the food industry in the form of extracts or concentrates containing natural raw materials of vegetable origin. The extraction of colouring matters is based on their isolation from the cells, tissues or organs (roots,

Table 3. Natural pigments
Tabela 3. Barwniki naturalne

Group of natural dyes	Occurrence	Group characteristic	Application
Isoprenoids Carotenoids	Fruits, vegetables and other plants with red colour, orange and yellow	A great amount is fat-soluble. Resistance against heat and pH changes. Orange-red colour	Dairy products Margarine Non-alcoholic drinks
Porphyrin Chlorophyll	All green leafy plants	Green colour. Fat-soluble and water-soluble. Chlorophyll derivatives – chlorophyllin. Enough resistance against heat and light	Dairy products Confectionery
Flavonoids	Flowers, fruits and vegetables with blue-red-violet colour	*Anthocyanins are water-soluble.* Blue-red-violet colours depending on pH, in strong acid environment are intensive red. Increase of pH causes blue pigmentation	Non-alcoholic drinks Jam Confectionery
Chinoline dyes – Cochineal red	Cochineal – raw product of animal origin. Roots and wood	Main component – carminic acid. Water-soluble. Resistance against heat, light and oxygen. Carmin red colour	Alcoholic-drinks Processed meat products
Betalains	Pure beetroot, amaranth, opuntia ficus-indica	Water-soluble. Sensitivity to heat, light and oxygen. Yellow-red-violet colour	Frozen food Dried food Product with short shelf-life, like yoghurt
Others			
Riboflavin	Green parts of plants, beans, soybeans, milk, yeast, walnuts	Vitamin B2 used for food enrichment and pigmentation. Water-soluble. Resistance against heat	Dairy products Cereal products Desserts
Curcumin	Curcuma	Fat-soluble. Turn pale under the light exposure. Resistance against heat. Lime yellow colour	Curry flavour Soups Confectionery
Carbon	A porous solid product containing 85-98% carbon and produced by heating carbonaceous materials such as cellulose, wood or peat at 500-600 C° in the absence of air	Charcoal. Resistance against heat, light and oxygen. Black pigment	Confectionery

Source: own study based on [Carle, Schweiggert (eds.) 2016; Sigurdson et al. 2017; Hendry, Houghton 1996].
Źródło: badania własne na podstawie [Carle, Schweiggert (eds.) 2016; Sigurdson et al. 2017; Hendry, Houghton 1996].

root, flowers, fruits) and transferred to the solvent [Hendry, Houghton 1996]. The extraction yield usually oscillates around 20%. For colours associated with the process of photosynthesis in chloroplasts (chlorophyll), it is necessary to destroy the cell structures before the extraction process. The disintegration of the cell walls should be carried out with the use of physical methods (homogenization, temperature changes, and sonification) and/or chemical methods (solvents or enzymes). The obtained extracts are concentrated and cleaned by appropriate methods [Hendry, Houghton 1996].

2. Bioactivity of food colorants

The application range of synthetic dyes is limited due to their non-safety. In some cases these compounds are technologically not necessary [Carle, Schweiggert (eds.) 2016; Sikorski (ed.) 2002; Gawęcki, Hryniewiecki 2003]. The application need of the synthetic organic dyes divides public and scientific opinion, although they have been tested much more than natural colours. The harmful effects often depend on the metabolites or impurities of the colourings. Some dyes are attributed to strong allergy (e.g. tartrazine) and cause allergic reactions, like a rash or asthma in about 0.01-0.1% of the population [Gasik, Mitek 2007].

It was also found that the complexes of dyes and proteins are an important element of carcinogenesis, as it can be quite easily linked to the rest of the protein containing tyrosine, methionine or tryptophan. In the synthesis of dyes, there are used salts and oxides of metals (copper, lead, selenium and others) that have catalytic activity and are not always completely removed; also unreacted organic contaminants or the substrates residues cannot be sufficiently recovered. The changing conditions and possibilities of synthetic organic dyes' applications, and the development of new research techniques result in toxicological testing of these compounds, in order to ensure their safety [Carle, Schweiggert (eds.) 2016].

Because of the fear of the harmful effects of synthetic food additives there is an increasing trend to replace them with natural colorants. However, synthetic dyes in comparison with natural have many advantages: better ability to dissolve, higher resistance to physical and chemical agents, are characterized by a high dye concentration and a large variety of colours [Janiak 2006].

Based on the results of toxicological tests, the safe amount permitted for food additive synthetic dyes is determined by WHO and FAO. Allowable quantities of dyes in food are regulated in all the countries in which they are used [Baranowska 2005].

Regardless the search for effective natural additives, it is recommended to develop an appropriate and effective control system. Another aspect is to optimize the dyes doses as a result of creating an appropriate compositions of additives, natural and synthetic; another optimization possibility is to broaden the technological functions of colourings [Carle, Schweiggert (eds.) 2016].

3. Methods of obtaining natural colorants

Microorganisms produce various colouring compounds. However due to difficulties with the introduction of microbial pigments on the market (because of the possibility of mycotoxin) only a small number of these colorants are produced industrially. The first European success in the area of pigment biosynthesis was to the application of *Blakeslea* mould in the production of β-carotene. Currently *Blakeslea trispora* is also used for the production of lycopene [Batt, Tortorello 2014].

The industrial production of lycopene (orange-red colorant) is based on the chemical synthesis or extraction from natural sources such as the tomato (*Solanum lycopersicum*). The growing interest with this colorant results from its medical importance. Plants and algae produce only a mixture of carotenoids, therefore numerous research, mostly based on the biosynthesis method, focus on pure lycopene production. Lycopene yield in biosynthesis by *Blakeslea trispora* achieves about 5% of the dry matter [López-Nieto et al. 2004].

Another example of microbial biosynthesis is the production of yellow colorant riboflavin (vitamin) by yeasts *Eremothecium ashbyii*, *Ashbya gossypi*, *Candida guilliermudii* or *Debaryomyces subglobosus* [Dufossé 2006].

On the one hand, the colourings of microbial origin are expected to be more economically competitive than dyes extracted from vegetables and fruits, and on the other hand, to have more valuables properties compared to their synthetic equivalents [Stolarzewicz et al. 2012].

Microorganisms effectively produce pigments located in biomass. These pigments have slight sensitivity to heat and pH. Metabolites of fungi provide different functions in the cell, like protection against photo oxidation (carotenoids) and environmental stress (melanin) or participation as cofactors in enzyme catalysis (flavins) [Mapari et al. 2005]. Pigments derived from filamentous fungi have been known for a long time and serve as identifying substances in taxonomic studies, however no consideration was given to their use in food technology [Pitt 1979].

Microorganisms can produce quinones such as anthraquinones [Duran et al. 2002] and naphthoquinones [Baker, Tatum 1998; Firn, Jones 2003; Langfelder et al. 2003], dihydroxynaphthalene and melanin [Butler, Day 1998] and flavins derivatives such as riboflavin [Baker, Tatum 1998]. *Monascus spp.* is the best known fungus producing pigments such as monascorubrin and rubropunctatine. In the presence of different amino acids in cultivation medium, the mould of *Monascus* produces pigments from orange-yellow to purplish-red; also a third red colorant has been isolated and described *Monascus spp*., which has antibacterial properties to Gram-positive bacteria [Mukherjee, Singh 2011].

Aprink RedTM (ASCOLOR BIOTEC, Czech Republic) is an example of a pigment produced by moulds and allowed as food colouring. The red pigment is produced by *Penicillium oxalicum var. Armeniach CCM 8242* as extracellular metabolite of anthraquinones. Aprink RedTM is stable at pH higher than 3.5, and with pH neutral maintains colour for 30 minutes of cooking. Moulds *Penicillium aculeatum* and *P. pinophilum* produce azaphilone polyketide pigments “Monascus-like”, which are not toxic for humans [Mapari et al. 2010].

Naphthoquinones are another group of pigments with high commercial potential, as they exhibit a wide range of colours and are soluble in water. The chemical structure of the naphthoquinones is similar to the structure of vegetable or animal compounds. Therefore the naphthoquinones do not have to be chemically modified, stabilized or embedded. The chemical structure of naphthoquinones produced by *C. unilateralis* is similar to commercially available red dyes such as shikonine and allkanine (metabolites of plant roots *Alkanna tinctoria* and *Lithospermum erythrorhizon)*. Shikonine has a strong antibacterial action and is widely used in the pharmaceutical industry. This colorant is also applied in the textile, cosmetic and food industries [Unagul et al. 2005].

Other microorganisms – algae – belong to the autotrophic group of organisms producing different compounds like carbohydrates, proteins, amino acids, vitamins and fatty acids [Perez-Garcia et al. 2011]. The main colours of algae are chlorophylls a, b and c, β-carotene, phycocyanin, xanthophylls and phycoerythrin, and all show the high potential for application in the food, pharmaceutical and cosmetic industries. The trend of turning in the direction of natural colorants has become the reason for extensive research on these organisms. *Spirulina* genus producing blue phycocyanin, *Dunaliella* synthesizing β-carotene and the genus *Haematococcus* producing red astaxanthin are key microorganisms attracting the greatest attention [Dufossé et al. 2005; Raja et al. 2007].

Carotenoid pigments are produced in the chloroplasts of algae in the form of a mixture typical for each of their classes. *Rhodophyta* (red alga) produces α and β-carotene and its hydroxyl derivatives, *Chloromonadophyta* produces diadinoxantin and heteroxantin, while in the *Chlorophyta* are present acetylene derivatives of carotenoids called alloxantin, monadoxantin and crocoxantin. *Dunaliella salina* is a highly efficient manufacturer producing up to 400 mg of β-carotene/m^2 of field cultivation that can be safely used in the food industry because of its GRAS status (Generally Recognized As Safe) [Dufossé et al. 2005; Zhi-Wei et al. 2008]. *Porphyridium* (red microalgae) is a manufacturer of compounds of various therapeutic and nutrition values; among them are zeaxanthin and fluorescent phycobiliprotein. Red phycobiliprotein – phycoerythrin and blue phycocyanin dissolve in water and have the potential to be applied in food, cosmetic and pharmaceutical industries [Cohen 1986]. One of the species of the microalgae produces fluorescent pink pigments, B-phycoerythrin and b-phycoerythrin. They belong to the group phycobiliprotein. This colour enables their application in confectionary, dairy and jelly products. In addition, the red phycoerythrin shows a yellow fluorescence, which can be used for colouring special food products like transparent lollipops, candy, soft and alcoholic drinks.

Porphyridium aerugineum is a microalgae, which is the source of a blue colorant called Marine blue. This species of microalgae does not produce red phycoerythrin and therefore cell extract of *P. aerugineum* is blue [Glazer 1999].

Bacteria and yeast are unfortunately of less interest as potential producers of colorants. Species able to conduct pigments synthesis in the bacteria kingdom are, for example *C. violaceum*, producing violacein (a purple-violet), *Flavobacterium sp*., producing yellow zeaxanthin [Dufossé 2006] or *Agrobacterium aurantiacum* producing pinky red astaxanthin [Yokoyama et al. 1995; Yokoyama, Miki 1995]. Photosynthesizing bacteria, *Bradyrhizobium sp.* [Lorquin et al. 1997] and halophile *Halobacterium sp.* [Asker, Ohta 1999] produce the dark red canthaxanthin while the yeast produce primarily astaxanthin and carotenoids. *Phaffia rhodozyma* is the most potential producer of astaxanthin, while *Rhodotorula* is the most efficient producer of carotenoids [Simova et al. 2004].

The genetic modification of bacteria and yeast, which originally did not produce coloured substances, can be another effective method of acquiring these colourful metabolites. Lastly, a very large number of crt genes encoding carotenoid synthesis have been cloned. Heterologous expression allows the design of carotenoid biosynthesis pathways in yeast and bacteria. The expression of specific combinations of genes and mixing the tracks of the biosynthesis of the cellular level have both created the possibility of obtaining new and valuable carotenoids [Sandmann 2001]. The genes encoding the enzymes of early carotenoid biosynthetic pathway such as: GGDP synthase, phytoene synthase desaturase, are more than half of all the presently cloned genes of the crt. The successes of the crt genes recombination and new biosynthesis pathways creation diversify the production capacity of structurally different carotenoids. Thus used the potential of genetic engineering allows obtaining the new carotenoids, applicable in the food, pharmaceutical and cosmetic industries and medicine [Stolarzewicz et al. 2012].

The market of the natural colouring is characterized by high prices, the difficulty of their acquisition and the convenience of use. This opens up tremendous prospects for the application of biotechnology in the production of additives. Genetic modifications and the use of enzymes or cell cultures provide opportunities for specific substances [Martins et al. 2016].

Another method of obtaining natural colorants is their recovery from waste. Fruit and vegetable waste are very rich in chemical substances. This type of raw material is cheap and available. A good example is juice pressing. The colorants contained in the skin pass significantly less to juice than to pulp, and the majority of the skin colorants remain in the residues. The economic dimension of obtaining natural colorants depends on their concentration in the raw material, therefore for their production mostly used are pomace from elderberry, cherry, blackberry, chokeberry and blackcurrant. The most common colours obtained by this method are: anthocyanins, carotenoids and chlorophylls. Chokeberry pomace is used for anthocyanins production as fresh fruits of chokeberry contain 0.3-0.65%

anthocyanins. Chokeberry juice contains only 34 – 40% of the total quantity of anthocyanins contained in these fruits, so most of them remain in the product. In turn, products made from rosewood are the raw material for the production of procyanidin oligomers and carotenoid colorants, however so far rarely used [Nawirska 2007].

The concept of a so-called "clean label" is increasingly important in the discussion about food components. Consumers' awareness about natural food has caused a greater interest in food without food additives. Since information about the product composition is placed on the packaging the consumers' attention is drawn to the following phrases: "no artificial additives" or "does not contain dyes" or "contains only natural ingredients" [Martins et al. 2016].

Colouring food is an alternative to many dyes. Colour concentrates are produced from fruits, vegetables and edible plants, as they are a natural source of colour and allow providing the desired colour to the product. This is an old method using for example the very colourful spices.

4. Natural food colorants market

The market size of natural colorants is estimated to reach 1.7 billion US dollars in 2020, while the CAGR index (compound annual growth rate) is up to 7.0%. It should be noted that 20% of its whole use will be by the beverage industry [*The World Coloring Market Report* 2015].

The main growth factors in the market of natural colorants are: consumers' awareness of artificial flavourings' and colourings' effects on human health caused by their application in food products, and strict regulations concerning the inclusion of synthetic substances for food production. Among the basic limiting factors one can include smaller stability of natural colorants and higher production costs compared to artificial colourings and flavourings. The lingering health concerns of consumers regarding artificial colours used in food, combined with the demand for a "clean label" contribute to the strong growth of the natural colorants market [*The World Coloring Market Report* 2015].

The market experts predict that the global marketplace of natural colorants will develop fast in 2016, compared to the previous year, and then achieve slightly lower but sustainable growth over the next decade. In particular, an increase to the level 6.8% CAGR is predicted to achieve 1.3 billion US dollars in 2016, before slowing slightly to an annual rate of growth of the component at 5.4% by 2026, which will bring this category an income of 2.2 billion US dollars [*The World Coloring Market Report* 2015; Crawford 2016]. The biggest gains on natural colorants will be provided by the sale of extract from Spirulina, for which the annual growth rate for such substances is predicted at 11%. This microalgae is a source of many colours like green and blue. These tints are most difficult to create in food [Crawford 2016].

5. Summary

The oldest method of dye production is the extraction from plant material. Extraction was the basic method of colourings' production until the 20th century. In the fifties the development of the chemical synthesis began, nowadays there is growing interest in the biotechnological methods of pigments production due to their natural properties.

The division into colourings groups depends on the production method and on the chemical structure of the dye. Each group differs in the physical and chemical properties. Natural pigments exhibit high bioactivity and are applied in medicine. Synthetic dyes are resistant to high temperatures, have longer durability and exhibit a wide range of colours.

The market authorisation of food colouring depends on their production method, however consumer awareness is an influencing factor of market growth. The development of dyes' production technology results from market trends and consumer behaviour. Nowadays the synthetic colourings are mostly applied in food products due to their technological properties and low price. Regarding the health promoting properties of natural pigments, the market experts predict the growth of the natural colorants market.

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