NATURAL FOOD PIGMENTS APPLICATION IN FOOD PRODUCTS

BARWNIKI NATURALNE W ŻYWNOŚCI

Summary
Natural pigments, food compounds, are responsible for the colour of the products. These additives can impart, to deepen or renew the colour of the product, if it has been lost while processing. They also improve the taste of the product and facilitate its identification. It is hard to imagine today’s food industry without the use of pigments. Presently, more and more conscious consumers are demanding products to be coloured with natural pigments or any pigment added to the final product. Artificial pigments are considered to be harmful and undesirable so food manufacturers focus on the use of natural colour substances. 16 natural pigments are presently permitted to be used. These compounds are: betalains – betanin, quinones – cochineal, flavonoids – anthocyanins, isoprenoids – carotene, annatto (bixin, norbixin), paprika extract, lutein, canthaxanthin, porphyrins – chlorophylls and chlorophyllins and copper complexes of these compounds, and others: caramels, curcumin, or plant coal.

Key words: natural pigments, chlorophyll, carotenoids, betalain

Introduction

Food industry has developed very dynamically, and the consumers’ knowledge concerning food has significantly increased, owing to the fact the communication between the producer and the consumer has become possible. One of the topics rising numerous controversices is the problem of colour additives application in food. The additives obtained synthetically do not find any approval on the market and thus the producers, meeting the customers expectations, do concentrate on natural pigments use. Nowadays, there is an observable need to consume food not containing colour additives or pre-
servatives, i.e. ecological food. But the food is fairly expensive and not easily accessible on the market, and usually consumed without any further processing where a colour additive would be needed (Rembiałkowska et al., 2013).

Pigments are mainly applied to give colour to the products which do not have it, or have lost it in the course of processing. Thanks to it food sensory attributes are increased, and resulting from it – satisfaction of a majority of customers which plays a key role in the food branch. The application of pigments aims at emphasizing the taste and the colour of the products. Though it needs to be remembered that a pigment must not be added to conceal the symptoms of food deterioration or worsened food quality. The European Parliament and the Council have precisely defined which pigments are allowed to be used. In the group of food additives there are as many as 40 commonly used compounds, including 16 natural ones. Despite their popularity the application brings about a sequence of problems. The reason is the compounds are not stable which is caused by various environmental factors.

**Definition and functions of pigments**

Food pigments are compounds (added to food) which are responsible for the colour of products. The additives may give, deepen, or renew the food colour if it has been lost in the course of technological processing. They also improve the product’s taste and facilitate its identification. Their range comprises natural material sources and natural compounds that are not consumed separately as food. On the other hand, raw materials which contain pigments but possess nutritional, smell or taste properties, are not classified as pigments. Saffron and dried paprika can be mentioned here (European Parliament..., 1994; Gajda, 2006; Solymosi et al., 2015).

Pigments can be divided, in respect of their origin, into: natural, identical to natural and synthetic ones (Fig. 1). The natural ones are obtained from plant or animal material mainly in the process of extraction, unfortunately they perform a low stability away from the system they have been derived from. Synthetic pigments are products of the chemical synthesis. Originally, they were made from coal-tar. When compared to the natural ones they show a high stability and capability of occurring in high concentrations. Moreover, they can be divided into water-soluble and insoluble in respect of their solubility (Fig. 1). The latter ones may be divided further into organic and inorganic (Amchova et al., 2015; Solymosi et al., 2015).

EFSA defines the application of food colours to be permitted for the following purposes:

- “to restore the original exterior appearance of food whose colour changed as a result of processing, storing, packaging and distribution,
- for improving the visual food attractiveness,
- to add colour to food which otherwise would be colourless” (European Commission..., 2011).

Food is pigmented basically to give colour to colourless products (e.g. beverages); to give or strengthen the colour of products (e.g. candies, drinks, desserts); bring back the original colour lost during processing (e.g. compote), balance and give the same colour
to all the products of a given batch (e.g. sauces); provide an intensive colour to products meant for dilution (e.g. syrups, fruit condiments for yoghurts) (Galaffu et al., 2015; Scotter, 2015).

It is inadmissible though to conceal food deterioration processes by adding pigments. A change of the shade may imply a development of microorganisms since the naturally occurring food colours belong to unstable compounds (Florowska et al., 2013; Gajda, 2006; Regulation (EC)...., 2008).

A particularly important group are natural pigments. They can be obtained both from leaves, flowers and plant fruits, as well as from animal blood and muscle tissue. The compounds that are not met in fresh products, yet rising as a result of a combination of colourless food components during the process of food production (European Parliament..., 1994; Gajda, 2006; Solymosi et al., 2015), are also numbered among the substances of the group.

The most frequent method of obtaining natural pigments is their extraction in the liquid-solid system from the matrix (leaves, roots etc.), and ethanol, acetone is used as a solvent (Presilski et al., 2016; Silva et al., 2015). This type of extraction uses a selected solvent which will separate either the pigment or the undesired substance. Next, the separation process of the solid and liquid phases takes place via either filtration or decantation. If the pigments occur in chloroplasts, the cell structure has to be destroyed before the extraction. This is done, among others, by the application of pulsatory electric field, microwaves or ultrasounds (Silva et al., 2015). Another rationalization of the method of obtaining natural pigments is extraction provided in the supercritical conditions of carbon dioxide, the method is safe both for the final product and the environment (Silva et al., 2015).
The pigment is already present in the material it is obtained from, in each of the presented methods. Thus, it is not indispensable to conduct chemical analyses, or microbiological or enzymatic processes from crude oil products targeting at creating a colour compound, as it is in the case of artificial colour additives (Gajda-Wyrębek et al., 2011; McCann et al., 2007).

It is also the source of the consumers’ trust towards the natural pigments compared to the synthetic ones. Natural pigments are believed to be more favourable or less harmful for the organism than their synthetic counterparts. It is so especially after the research carried out by McCann et al. (2007) on the influence of selected colour additives on children’s oversensitivity (the so-called Southampton six).

The natural pigments, despite the recognition they have, present some disadvantages such as: low susceptibility to a higher temperature, oxygen, light, metal ions, low coloring capacity, changeable composition, low variety of colours, higher costs, giving a specific smell and taste, problems with colour standardization (Scotter, 2011; Zawirska-Wojtasiak, 2005).

**Legal regulations and limitations**

The most crucial criterium of applying additives is human life and health safety. That is why the EU strongly stresses the problem of unification of the compounds application by the member states, introducing adequate regulations (Florowska et al., 2013; Scotter, 2015).

The use of colour additives is regulated by the following acts of law:
- Regulation of the Minister of Health of 22 November 2010 on the permissible additional substances (Rozporządzenie Ministra..., 2010) – in the domain of detailed terms of the application of additional food substances in compliance with the EU corresponding directives.
Regulation (EU) No 231/2012 as regards the specifications for riboflavins (E 101) (Commission Regulation..., 2014b).


Pigments permitted for use in Poland in accordance with the above documents are: curcumin, cochineal, carminic acid, carmine, chlorophylls, and chlorophyllins, copper complexes of chlorophylls and chlorophyllins, caramel, sulphur carmine, ammonium-sulphur carmine, plant coal, carotenes, annatto (bixin, norbixin), ammonium carmine, paprika extract, capsanthine, capsorubin, lycopene, lutein, betalain, anthocyanins.

The pigments are permitted on the principle quantum satis, i.e. the lowest dose necessary to achieve the set technological aim, in accordance with good production practice (Florowska et al., 2013; Scotter, 2015).

As far as some food agents are concerned, only selected pigments means are added either jointly or individually at a maximum defined dose, and a part of pigments is allowed exclusively for some defined purposes (Gajda, 2016; Rozporządzenie Ministra..., 2010). It is prohibited to directly sell the E 123, E 127, E 160b, E 173 and E 180 pigments to the consumer in accordance with the Commission Regulation (EU) No 1129/2011 (Commission Regulation..., 2011). Owing to the fact that the Scientific Food Committee analysed the pigments at the earliest, the European Parliament and the Council decided they should be evaluated again. In 2008 lycopene (E 160d) was subjected to analyses, in 2014 some changes were introduced, among others, concerning the use of aluminium lakes of riboflavins (E 101), cochineal (E 120a), carminic acid (E 120b), carmines (E 120c) or caramel pigments (E 150 a-d), as well as certain colours in flavoured ripened cheeses (Florowska et al., 2013; Scotter, 2015). In 2015, in accordance with the designations (Commission Regulation..., 2014a, 2014b, 2014c; European Parliament..., 1994), successive pigments were analysed including chlorophyllin (E 141), betalain (E 162), cochineal (E 120), paprika extract (E 160c), anthocyanins (E 163), as well as chlorophyll (E 140) (EFSA, 2015a, 2015b, 2015c, 2015d, 2015e, 2015f). All the sweeteners and the other pigments not evaluated by December 2015 will have been evaluated by December 2020.

Moreover, the law precisely defines in which food agents the transfer principle of pigments is not permitted. The transfer principle refers to multi-components mixtures to which a pigment addition is introduced along with one of the mixture components. They are: unprocessed food in the light of Article 3 of Regulation (EC) No 1333/2008 (Regulation (EC)..., 2008), water, milk, buttermilk, cream, fats and oils, ripened and non-ripened cheeses, bread, sheeps milk and goats milk butter, eggs, egg products, starch, flour, sugar, salt and its substitutes, tea and its extracts, burnt coffee, tomato-based sauces, tomato paste, jarred tomatoes, tinned tomatoes, pasta, vegetables, fruit and vegetable juices and nectares, jellies and extra jams, crème de pruneaux, shellfish, crustaceans, fish, meat, venison and poultry including their preserves (excluding the ready-to-use ones containing the components), cacao products and chocolate components in chocolate products, spices and their mixtures, herbal and fruit infusions, as well as their extracts, chicory, cereal preparations for infusions, grape vinegar, spirit drinks, aqua
vitae, wine, Clarea, Zurra, Sambuca, Maraschino, Marasquino or Maraskino, Mistrà, London gin, honey, malt, and malt products, as well as baby and infant food (Commission Regulation..., 2011).

**Division of natural pigments**

Five basic groups of natural pigments can be distinguished. They are: betalains, chinoids, flavonoids, isoprenoids and porphyrins. Apart from them there are other natural pigments, equally significant in the food industry, such as: caramel, plant coal and curcumin, which are commonly applied in the today’s food. Besides their basic function, the pigments possess very vital health-promoting properties (Florowska et al., 2013; Galaffu et al., 2015; Solymosi et al., 2015).

**Betalain pigments**

The group includes red betacyanins, as well as yellow-orange betaxanthins. The latter ones are not utilized as food pigments. Betacyanins are the yellow betalamic acid, as well as cyclo-3,4-dehydroxyphenylalanine. The combination of the acid with a molecule of cyclo-DOPA-5-O-Glc is decisive in prolonging the conjugated double bonds system which results in an emergence of red-violet betalain. It is the decisive factor in creating the final colour of betacyanins and is the focus of the food industry (Klewicka, 2012; Solymosi et al., 2015).

Betalains are oil-insoluble but water-soluble. Depending upon the pH of the environment, they take the tawny (pH > 8.5), blue-violet (6.5–8.0), red-purple (3.0–6.5) or red-violet colour (pH < 3.0), but their highest stability is observed at the pH 4–5 range. Other decisive factors concerning their stability are: the concentration of pigments in the solution, the presence of iron ions, water activity, the occurrence of antioxidative properties compounds, or temperature. Betalains are weakly resistant to light and temperature above 70°C. The colour may then change from red to yellow. And this is the reason why the compounds are applied to fresh products exclusively, to the modified atmosphere packaged food, or products not thermally processed. They are used to colour e.g. frozen food, ice-creams, flavoured milk drinks, yoghurts, powdered desserts, gels, sauces, jams, jellies, candies (Delgado-Vargas et al., 2000; Galaffu et al., 2015; Janiszewska, 2014; Klewicka, 2012).

**Chinoide pigments**

The group of chinoide compounds includes dark-red cochineal. It is the only animal-origin pigment applied in food. It is obtained via extraction from female insects Dactylopius coccus (Galaffu et al., 2015). Cochineal is abundant in carminic acid, that is why the pigment is also frequently referred to as carminic acid. It is also added to food in the form of red powder. It is stable in the acidic environment and shows resistance to light, oxygenation, SO₂. The pigment belongs to compounds insoluble in organic solvents, oil and ethanol and weakly soluble in water. It takes colours depending on the pH value: orange – pH value lower than 5, red – pH in the range 5–7, above 7 – cochineal’s solubility drops and it becomes violet. The cochineal pigment with aluminium makes lakes,
i.e. colour complexes. They may take the colours from yellow to violet (Galaffu et al., 2015; Solymosi et al., 2015).

Cochineal is used to colour milk desserts, alcoholic and soft drinks, jams, tomato preserves, and also to various confectionery and bakery products (Galaffu et al., 2015; Solymosi et al., 2015).

**Flavonoid pigments**

The best known representative of flavonoids are anthocyanins. Approximately 500 compounds from the group have been researched so far. They occur in nearly all plants apart from mushrooms and algae. Depending upon the cell juice they are in, they may take the following colours: red – pH value lower than 4, red-violet – pH 4–6 or blue – when the indicator pH is higher than 7. The presence of metal ions and enzymes, as well as plant species will significantly influence their colour, too. Apart from giving colours to plants they protect them against the negative ultraviolet rays activity. Anthocyanins are first of all obtained from the pomace of: red grapes, elder, aronia, red currant, purple carrot, as well as hibiscus flowers (Galaffu et al., 2015; Solymosi et al., 2015).

Anthocyanins belong to glycosides i.e. organic compounds consisting of sugar and glycon parts. The following can be distinguished among the aglycons i.e. the non-sugar components of glycosides: delphinine, malvin, pelargonin, as well as cyanidin, whereas the sugar part is built of galactose, rhamnose or glycose (Kozłowski, 2002). The pigments are water and ethanol soluble, but cannot be dissolved in oil. They show amphoteric properties – they can combine with acids and react as they do via reactions with iron ions and magnesium. They belong to weakly stable compounds and their decomposition may be caused by the presence of oxygen, temperature increase, a longer period of heating or storing. The factors bring about forming of yellow or colourless chalcones, and finally cause browning (Sadilova et al., 2006).

The key issue for the compounds group is their capability of minimalizing the activity of some enzymes and elimination of superoxide free radicals. The agents decrease the problems with sclerosis and arterial hypertension, improve metabolism, are a significant vitamins source, also show antialergic and sometimes anti-tumorous activity. They may positively influence the cardio-vascular system, as well as eyesight (Kozłowski, 2002; Zafra-Stone et al., 2007).

The above properties prove how important it is for people to consume anthocyanins. Application of the group of flavonoids by the food industry is a common practice and they can be met e.g. in fruit, alcoholic and non-alcoholic drinks, sauces, cheeses, milk desserts, jellies, jams, candies (Galaffu et al., 2015; Solymosi et al., 2015).

**Isoprenoid pigments**

Carotenoids which are divided into carotenes and xanthophylls are numbered among the isoprenoid pigments. Approximately 600 of the compounds have been recognised so far. They are derivatives of tetraterpenes. The molecule is built of 40 atoms of coal and 8 units of isoprene. They have an acyclic system with a long central chain of double conjugated bonds.

Carotenoids are susceptible to oxygenation which is accelerated by the presence of peroxides, lipoxidases or ions of metals. The activity is prevented by an addition of
ascorbic acid as an antioxidant. Moreover, the pigments are susceptible to light and that is why they are stored in dark packaging. Carotenoids are resistant to heating, sterilization and freezing processes. They are well soluble in oils but insoluble in water (Delgado-Vargas et al., 2000; Galaffu et al., 2015; Solymosi et al., 2015).

They are used in the production of e.g.: butter, margarines, oils and fats, cheese spreads, non-alcoholic drinks, fruit juices, confectionery baked goods, ice-creams, yoghurts, desserts, jams, creams, pastries, jellies. The best known carotenoids are: β-carotene, annatto (bixin, norbixin), capsanthin, lutein, canthaxanthin (Galaffu et al., 2015; Solymosi et al., 2015; Szterk and Lewicki, 2007).

- β-carotene – is susceptible to the presence of oxygen (oxygenation) and long-time heating. Yet, it performs a high resistance to the activity of sunrays and higher temperature, and is stable at pH values from 2 to 7. It is water insoluble and difficult to dissolve in oils and ethanol.

- Annatto (bixin, norbixin). Bixin: a powder of yellow-brown colour, which stains yellow-orange. It is a pigment soluble in oil but insoluble in water and weakly soluble in ethanol. It shows resistance in the acidic environment, but is susceptible to the process of saponification in the basic environment. Norbixin: red-ginger powder or extract. The pigment is water-soluble, partly in ethanol, totally insoluble in oil. It may give orange-red colour. Similarly to bixin – susceptible to the reaction with light.

- Capsanthin – crystals or oily extract of reddish colour and hot taste. Resistant in a wide pH range and weakly susceptible to heating, yet it can easily undergo oxygenation. It is well dissolved by oils and organic solvents, weakly by ethanol. It shows no water-solubility. It is frequently obtained by extraction, presently also in supercritical CO2 conditions.

- Lutein – yellow-brown extract insoluble in water, but soluble in organic solvents and oil. Its colour is weakly resistant to temperature, light, SO2, pH. It undergoes oxygenation and is susceptible to microorganisms.

- Canthaxanthin – takes the form of crystals or crystal powder of dark violet colour. After dissolving gives orange to red colour. It is weakly water and oil-soluble. It shows a weak resistance to light and oxygen. The colour it gives is resistant in changeable pH conditions and shows sufficient resistance to the presence of oxygen, sunrays and microorganisms.

**Porphyrin pigments**

The group of natural porphyrin pigments includes chlorophylls and chlorophyllins. The green chlorophylls can be found in cell chloroplasts of autotrophic plants, and their presence facilitates the assimilation of CO2 by plants during the vegetation period. They undergo the colour change from red to yellow, which makes it possible to observe the occurrence of other pigments such as carotenoids or flavonoids. Chlorophylls are built of 4-pyrene core with a centrally located atom of magnesium and can be numbered among metalloporphyrins. Their structure is similar to the one of the hemoglobin molecule, yet it can be found magnesium occurring in them instead of iron (Galaffu et al., 2015; Kozłowski, 2002; Solymosi et al., 2015).

Chlorophyll is olive-green to dark green. It is obtained via extraction from leaves it occurs in (peppermint, pistachio, common or stinging nettle). It shows the lowest stabil-
ity of all the natural pigments and low resistance to a higher temperature, light activity, oxygenation and pH change. It hydrolyses in the acidic environment giving a brown colouring. It is an oil- and ethanol-soluble compound but water-insoluble. Two types of chlorophyll can be distinguished: a and b. Chlorophyll a consists of the methyl group and gives the green-blue colour, while chlorophyll b has the aldehyde group and gives the yellow-green colour. Chlorophylls are used in beverages, jellies and jams, sweets, chewing gums, soup concentrates, cheese spreads, preserves and vegetable pickles. Besides they show medicinal properties since they improve human metabolism, being a source of magnesium (Galaffu et al., 2015; Solymosi et al., 2015).

Chlorophyllins are compounds with a slightly higher stability compared to chlorophyll. It results from the ester bonds hydrolysis in the basic environment without magnesium removal. They are susceptible to acids and higher temperature and present a moderate resistance to light and can be water-dissolved (Galaffu et al., 2015; Kozłowski, 2002; Solymosi et al., 2015). Low resistance of chlorophyll to an increased temperature resulted in the fact that its degradation products and their derivatives find a broader application. Copper chloropyllin complexes whose popularity results from their good water-solubility and light and heating resistance belong to the above-mentioned group. The complexes show a high stability and are not regarded as harmful since the copper ions are not released in the digestive tract. Copper complexes of chlorophyllins create a green solution in water. They are not resistant to acids but show a high resistance to bases, temperature and light. They are fairly easily soluble in water, less in ethanol and totally insoluble in oil, whereas, the chlorophylls complexes take the blue-green to dark green colour and are water-insoluble. They can be, though, dissolved in oils, methanol and ethanol. The above complexes are found, in the food industry, in vegetable and fruit preserves, alcoholic drinks, or cheese spreads (Galaffu et al., 2015; Solymosi et al., 2015).

**Caramel**

Heated sugar of a tawny colour and specific taste and smell is called caramel. It is used in food as a natural pigment, despite the fact it does not occur in plants or animals but is obtained by heating of sugars. Glucose, fructose, saccharose, polymers of the substances and starch syrup are used. Adequate pressure and temperature conditions must be provided in the production process of caramel. The whole process may be accelerated by the application of phosphates, carbonates and sulfites. Caramels are marked by symbols E 150a-d. Various types of caramel are used for dying in Poland (Galaffu et al., 2015; Mitka and Nowak, 2005; Solymosi et al., 2015).

**Plant coal**

Plant coal is derived from the wood charing process. It is used as a black pigment in the food industry. It possesses neither smell nor taste. It is highly resistant to light activity, as well as SO₂, heating, chemical and physical agents. It is stable in the wide range pH: from 2 to 10. The coal can be dissolved neither in organic solvents nor in water. The pigment is used to either giving colouring or shading. It is also used in wine, vinegar, and juices purification process (Solymosi et al., 2015).
Curcumin

Curcumin is derived from *Curcuma longa* L. Its characteristic feature is the fluorescence, yellow colour, spicy smell and taste. It shows a high resistance to the temperature below 120°C. It is stable in the acidic environment (pH 2.5–6.5), but susceptible to the activity of light and SO₂. Curcumin can be dissolved in the acetic acid, oil and alcohol. It is water-insoluble but becomes suspended in the medium creating dispersed systems. It can create complexes with proteins. Curcumin is used in dairy products, frozen desserts, ice-creams, confectionery, mustards, meal concentrates. It is also added to margarines and seasoning and mayonnaise sauces as a mixture with annatto (Solymosi et al., 2015).

Conclusions

Historically it has been claimed that natural food is the healthiest. Nowadays, due to the fact that there is a necessity to provide a distant consumption expiry date, the food needs to be processed which is connected with the thermal treatment frequently degrading the natural pigments or flavours. Thus, it is impossible to imagine the contemporary food industry without food additives including natural pigments. The food colour as the main and frequently first attribute of the purchased food is particularly desirable to consumers whose opinion becomes the most important to the producers. More and more aware consumers regard artificial pigments as harmful and undesirable. That is why the food producers focus on applying natural pigments substances. Natural pigments find a wide application in the food industry, yet the producers are bound to keep in mind that adding them to food has to be strictly controlled in accordance with the regulations.

The natural pigments have been defined in the Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008, together with the Annexes (Regulation (EC)..., 2008). The documents include a detailed information on the application of individual pigments in defined food products, their doses, and limitations of use. All the aspects have to be absolutely observed by the producers.

Presently 16 natural pigments are permitted. These compounds are: betalains – betanin, quinones – cochineal, flavonoids – anthocyanins, isoprenoids – carotene, annatto (bixin, norbixin), paprika extract, lutein, canthaxanthin, porphyrins – chlorophylls and chlorophyllins and copper complexes of these compounds, and others: caramels, curcumin, or plant coal.

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Streszczenie

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