# Food colorants used in meat processing

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#### Abstract

Meat products or meat preparations play virtually no role for humans as a source of synthetic colorants. Of natural dyes, curcumin (E 100) in a maximum quantity of 20 mg/kg, cochineal (E 120) at a maximum dose of 100 mg/kg, carotenes (E 160a) with a limit of 20 mg/kg and paprika extract (E160c) with a limit of 10 mg/kg are permitted for cooked sausages. Caramel and betalain red in quantum satis can be used. No azo-dyes must be added to conventional cooked meat products prepared in the Czech Republic. Dyes are subjected to a number of tests before they can be used in the food industry. Acute, subchronic and chronic toxicity, carcinogenic effects, mutagenicity, teratogenicity, reproductive toxicity, accumulation in the body, effects on the immune system are monitored. On the basis of constantly new knowledge, it is necessary to regularly evaluate the potential toxicity of food colors and subsequently revise the rules for their application.

natural dyes, synthetic dyes, ADI, allergic reaction

## Introduction

The addition of colors to foods during their processing aims to increase the visual appeal of the final product. With the exception of water, each food has its own color due to its chemical composition and physical properties. Dyes are substances whose presence determine a characteristic color based on the selective light absorption (Velíšek and Hajšlová, 2009). Dyes also belongs to the group of food additives. European food law regulates which substances and to which foods, dyes can be added and improve food color tone.

Fresh meat belongs to the basic foods and additives are not allowed to be added. An exception is the possibility of surface application of three synthetic dyes (Allura Red AG, brilliant blue FCF and brown HT) only for the purpose of health marking (Regulation, 2008).

Dyes can be used in meat semi-finished products only in defined products, where they have been historically added. Out of the natural dyes, curcumin (E 100) can be added in a maximum amount of 20 mg/kg for *merguez* type of products, as well as for *salsicha fresco*, *butifarra fresca*, *longaniza fresca* and *chorizo fresco*. Betalain red (E 162 in *quantum satis* mode) can also be added to these products, and also to *bifteki*, *soutzoukaki* and *kebap*. Pepper extract (E 160c) is allowed to be added to the same products at a maximum level of 10 mg/kg (Regulation, 2008).

Cochineal (E 120) is another natural coloring that is permitted to be added to the *breakfast sausages* (they can have this name if they contain at least 6% cereals), *burger meat* (with a minimum proportion of vegetables and/or cereals 4%) and *merguez* type products, in *salsicha fresco*, *mici*, *butifarra fresca*, *longaniza fresca*, *chorizo fresco*, *cevapcici* and *pljeskavice*. E 120 can be only used in a maximum amount of 100 mg/kg.

Natural dyes also include caramels. Coded E 150a-d, they may be added to the same meat products as cochineal, except for *cevapcici* and *pljeskavice*, where they are not permitted. Out of the synthetic dyes, only AC red (Allura Red AC, E 129) belongs to the azo dyes and can be used in meat semi-finished products. Its maximum amount is limited to 25 mg/kg. These dyes are allowed only in two types of meat products - *breakfast sausages* and *burger meat* (under the same conditions as the use of cochineal).

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Curcumin (maximum amount 20 mg/kg), cochineal (max. 100 mg/kg), caramel (quantum satis) and other natural dyes: E 160a (carotenes) with a limit of 20 mg/kg, E 160c (pepper extract) with a maximum of 10 mg/kg and E 162 (betalain red) also in quantum satis mode can be added to minced meat (Regulation, 2008). None of the azo dyes can be used in commonly produced heat-treated meat products in the Czech Republic. For pasturmas (uncooked meat product) curcumin, riboflavin (E 101) or cochineal may be used in quantum satis. In addition to cochineal (maximum addition of 200 mg/kg), ponceau 4R (cochineal red A, E 124) with a limit of 50 mg/kg can also be used for Spanish chorizo or salchichon meat products. The application of the azo dye E 110 with the designation SY (Sunset Yellow) with a maximum of 15 mg/kg is permitted in the sobrasada product.

Similar requirements apply to minced heat-treated meat products (defined by the terms "sausages, pâtés and terrines"). E 100 is permitted in a maximum amount of 20 mg/kg, E 120 in a maximum dose of 100 mg/kg, E 160a with a limit of 20 mg/kg and E 160c 10 mg/kg. Caramel and betalain red in *quantum satis* mode. The use and dosage of the dyes mentioned in the previous sentence do not differ from the milled uncooked products. Out of the synthetic dyes, only AC red (E 129) can be used in the *luncheon meat* product in a maximum amount of 25 mg/kg (Regulation, 2008).

# Azo dyes from a food safety point of view

Therefore, no azo dyes may be added to ordinary heat-treated meat products that are produced in the Czech Republic. However, their use in the food industry is relatively common. About seventy percent of all dyes used in food processing are azo dyes (EFSA, 2010).

Before dyes can be used in the food industry, they are subjected to a number of tests. Acute, subchronic and chronic toxicity, carcinogenic effects, mutagenicity, teratogenicity, reproductive toxicity, accumulation in the body, effects on the immune system are monitored (Amchova et al., 2015).

The available toxicological data must be evaluated and subsequently confirmed in more than one animal species. Toxicity is monitored in six species, of which at least three must be mammals. Most tests use small rodents (mice, rats, guinea pigs, etc.), as well as special breeds of rabbits, dogs, cats or pigs, which are physiologically closer to the human body.

From animals outside the mammal class, nematodes (e.g. nematodes), two-winged insects (octopuses) or fish (striped dane) are used.

According to the European legislation, ponceau 4R and cochineal can be used in the chorizo product (On the Czech market, however, this meat product can also be purchased without added dyes. The typical appearance of chorizo is provided by the addition of dried peppers). (Plate VII, Fig. 1)

To evaluate the potential toxicity of an additive, preclinical studies are performed to determine the No-Observed-Adverse-Effect-Level (NOAEL), which is the highest concentration or amount of the substance used in the experiment that does not cause any detectable negative change in morphology, functional capacity, growth, development or lifespan of the organism; used under defined conditions of exposure to this substance (Amchova et al., 2015). E.g. for Allura Red AC (E 129) reported Bastaki et al. (2017) NOAEL is 695 mg/kg of body weight per day (a toxicity study performed with rats).

For clinical recommendations, the NOAEL is usually divided by a safety factor of 100 (in the case of toxic substances with a serious effect, this safety factor may increase a thousand fold). Due to this factor, differences in the extrapolation of animal models to humans and individual differences in the human population with respect to the response to a given substance are taken into account (Amchova et al., 2015). The value obtained is referred to as the "Acceptable Daily Intake" (ADI) expressed in mg per kg of body weight per day. It expresses the amount of food additive that can be taken daily for the rest of

his life without resulting in a significant risk for the health of the consumer. The specific dietary habits of certain groups of consumers (eg children, vegetarians, etc.) are also taken into account to ensure that the ADI values are not exceeded.

Feketea and Tsabouri (2017) reported ADI values for E 110 (Sunset Yellow) 2.5 mg/kg of body weight per day, for E 124 (Ponceau 4R) 4.0 mg/kg per day, for E 129 (Allura Red AC) 7.0 mg/kg of body weight per day (these limits are valid in the European Union). In the United States of America (USA), ADI values may or may not be the same as in the European Union. According to the above authors, the ADI value is the same for E 129 (ie 7.0 mg/kg per day), but for E 110 it is 3.75 mg/kg per day. E.g., for the other synthetic dye tartrazine (E 102), they have a lower ADI in the USA (5.0 mg/kg/day) in comparison with European regulation (7.5 mg/kg/day; Feketea and Tsabouri, 2017).

The values set by the responsible authorities cannot fully reduced the risks of the risk of adverse reactions to the allergic compound, especially for sensitive groups or hypersensitive individuals. However, even in these cases, life should not be endangered, with the exception of rare anaphylactic reactions, which, however, are unlikely to occur after ingestion of a food color (Amchova et al., 2015).

In recent years, increased attention has been paid to the toxicity of food additives, especially azo dyes. On the one hand, their potential carcinogenic effects are being monitored, however, adverse effects on human health are unlikely due to the low rate of absorption in the gut. E.g. for Allura Red AC, the proportion of absorption after oral ingestion is reported to be about 5 % (Bastaki et al., 2017). However, based on new knowledge, it is necessary to regularly assess the potential toxicity of food colors and then revise the rules for their application. In 2010, EFSA published a scientific opinion on the use of 10 food azo dyes (EFSA, 2010), including those that may be used in meat processing (E 110, E 124 and E 129). The reason was to investigate the possible adverse effect on human health in terms of intolerance or allergic reaction. Based on the analyzes of the conducted studies, only isolated cases of disability were reported, that in susceptible persons included urticaria, difficulty breathing or allergic vasculitis (note: vasculitis is inflammation of the vascular wall). However, EFSA's view was that serious adverse reactions in humans are unlikely to occur following consumption of food colors under the conditions. Though, cases of allergies or intolerances to some of the used dyes are described in the literature. Voltolini et al. (2014) reported the case of a 32-year-old woman who developed hypersensitivity to IgE antibodies to cochineal. The woman had not previously suffered from allergies and arrived at a clinic in Genoa, Italy, with generalized urticaria, swelling of the eyelids and a cold after ingesting a dietary supplement containing proteins and vitamins. The addition of carmine was indicated on food labeling. A few months after this event, the woman experienced another, more serious reaction a few hours after eating a chicken burger, for the preparation of which carmine was also used (Voltolini et al., 2014).

Bastaki et al. (2017) published the results of genotoxicity tests of allura red AC without proven negative effect in the bone marrow, liver, stomach and colon of laboratory animals. The authors also mentioned similar negative results of parallel studies performed with the azo dyes tartrazine (E 102) and ponceau 4R (E 124).

As a source of dyes for humans, meat products or meat semi-finished products play only a completely secondary role. In this regard, bakery products and desserts, confectionery and beverages are important. The most common sources of food colors for children are drinks and confectionery (Feketea and Tsabouri, 2017; Stevens et al., 2015). In the diet of Americans, beverages are mentioned as one of the largest sources of artificial colors, due to the large volume that is consumed in the intake portion (Stevens et al., 2015). The amount of dyes that children consume daily affects the incidence of possible adverse reactions. Due to the higher sensitivity of children, they could be more often affected than adults. Individuals with atopic dermatitis are more susceptible.

# Determination of azo dyes present in meat products

Although food colors are thoroughly tested for their safety and food law clearly defines the requirements for their application, there is a risk to consumers. The reason may be the illegal use of approved dyes, eg in high doses in permitted foods or in products in which they are not allowed to be used, according to the legislation. The possibility of illegal addition of unapproved and therefore unauthorized dyes must also be taken into account. Iammarino et al. (2019) stated that some dyes which have been shown to have a carcinogenic effect or are of unknown origin have been used in spices intended for meat processing. As part of their supervisory activities, the control authorities also monitor the presence of food additives, including colors. Therefore, a suitable laboratory method must be available. The laboratory that is able to detect with a certain sensitivity the levels of dyes present in samples.

Iammarino et al. (2019) published an article in the journal Food Chemistry on the development and use of an analytical method based on the principle of high performance liquid chromatography with UV-diode field detection. The method was verified by the detection of 12 food dyes (amaranth, Ponceau 4R, carmine, Ponceau SX, Ponceau 3R, Allura Red AC, azorubin, erythrosine, Sudan I, Sudan II, Sudan III and Sudan IV) in meat products. The detection limits of the developed method ranged from 1.4 and 7.5 mg/kg for the type of dye and the limits of quantification between 4.2 and 22.9 mg/kg. The sensitivity of the method complies with the legislative requirements, where it is able to detect the permitted dyes used in lower concentrations than the maximum permitted amounts in meat preparations or meat products. It appears to be suitable for use in the laboratories of control authorities.

### Conclusion

Meat products or meat semi-finished products are given their natural color by the muscle dye myoglobin influenced by the addition of nitrite and nitrate. In some products, the addition of peppers, or natural dyes such as betalain red, paprika extract or cochineal are used. The application of synthetic dyes is limited by the European legislation to only a few traditional, for us "exotic" products. It can be stated that at the present time the use of synthetic dyes is not appreciable by consumers. New natural resources are always being sought to create or maintain the color that customers expect. Increasingly, there are demands not to use anything that must be declared as a colorant on the packaging. The modern motto has become "from food for food".

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Fig. 1. Spanish paprika salami. Meat product not heat treated - sliced. 100 g of salami was made from 135 g of pork meat. Ingredients: Salami: pork, table salt, 2.4% dried pepper, glucose syrup, garlic; antioxidant: sodium ascorbate starter cultures; preservatives potassium nitrate, sodium nitrite