Effects of regular control of food colours content in meat products in Serbia

R B Petronijevic, D Trbovic and M Sefer

1 Institute of meat hygiene and technology, Kacanskog 13, Belgrade, Republic of Serbia

E-mail: radivoj.petronijevic@inmes.rs

Abstract. Research of added food colours presence in meat products was carried out for a period of almost six years, as a continuance of previous study on market in Serbia. Improved method of high performance liquid chromatography was applied for identification and quantification of added colours in meat products. The colours were determined in variety of meat products, smoked meat and bacon, fermented sausages and heat-treated dry sausages, boiled sausages, cooked sausages, canned meat and meat meals, meat semiproducts and mechanically separated meat and, as a separated category, sea fish pastes, both from domestic market and from import. Over the 1400 products were analysed. The research results showed great improvement both in content of colours and labelling of products of meat products in Serbia.

1. Introduction

Colour is, in general, the most important sensory attribute of food and might sometimes have dramatic impact on the expectations, as well as on the subsequent taste/flavour experiences of consumers. Better understanding of the consumer’s sensory expectations caused by the food colour, lead to better understanding of the ways in which what we see can attune our perception of flavour, and, as a consequence, alter our food behaviours [1].

The use of food additives, and therefore food colours in meat products, is regulated in Serbia as well as in EU [2-5]. Like as in other categories of food, colours affect the visual experience of the products, give them a characteristic appearance and thus directly affect the acceptability of meat products by consumers [6,7]. Recent studies show that the appearance of a meat product is the first and, most often, crucial sensory factor that influences the consumer's decision to choose a particular product, and one of the most significant contributions to the appearance is its colour. Taste, smell, texture and other sensory factors are of secondary significance for choice and more important to the decision whether the consumer will buy the same product again. Product presentation, which is greatly contributed by the appearance of the product to the appearance of the packaging, can often be of more influence than the imperfection of other sensory qualities of the product.

However, the use of food colours in meat products has its good and bad sides. On the one hand, they overcome the difficulties associated with manufacturing technology and achieve uniformity of product appearance [8], and on the other hand, their use can mask intentional changes in product composition and use of inferior raw materials [9]. Also, some of the colours used in meat products can cause adverse health reactions in children and some population groups [10]. Unauthorised use of colours for adulteration affects the nutritional properties of the product and directly deceive consumers [11,12]. Adverse health effects of colour use are one of the reasons why the European Food Safety Authority
(EFSA) periodically evaluates food safety risks and issues scientific opinions on the use of certain colours in food and suggests values for their acceptable daily intake (ADI) [13].

From 2012 to 2014, the Institute of Hygiene and Meat Technology organized a study of the red food colours presence in meat products that were in stores in Serbia. Laboratory of the Institute for these purposes, developed a method of high-performance liquid chromatographic (HPLC) method for the simultaneous qualitative and quantitative determination of the added colour in meat products [14].

Results of that study showed that most products on the domestic market had a legal irregularity, whether it was a misdeclaration, addition of food colours to products in which their use are not allowed, or addition of forbidden colours. The results indicated that adequate control was not being carried out in Serbia and that it was necessary to constantly monitor the use of food colours in meat products on the market [14]. In accordance with this conclusion and new legal regulations for the safety of the additives use and labelling of meat products [3,11], as well as special requirements of the meat industry, continuous control of the presence and content of colours in meat products has been performed since then. The original liquid chromatographic method was improved both in the efficiency of sample preparation and in number of analytes. This review presents the results of the application of the improved HPLC method for colour control in meat products in the period from 2016 to the first half of 2021.

2. Materials and methods

2.1. Reagents
Colour standards purity ≥98% (Tartrazine, E 102, Sunset yellow FCF, E 110, Carminic acid, E 120, Azorubine, E 122, Amaranth, E 123, Ponceau 4R, E 124, Erythrosine, E 127, Red 2G, E 128, Allura Red AC, E 129, Patent Blue V, E 131, Indigo Carmine, E 132, Brilliant Blue FCF, E 133, Green S, E 142 and Brilliant Black BN, E 151), were purchased from MERCK (Darmstadt, Germany). Other chemicals and preparation of reagent solutions, buffers and mobile phase were the same as previously described [14].

2.2. Meat products
Meat products are obtained as part of regular control of food quality and safety, from domestic market, and, directly, from producers and importers. The research included over a thousand samples from the vast variety of meat products. For the presentation purpose, products of similar properties and production technologies were associated in larger groups, to avoid scarcity of samples in some groups which is, on the other hand, important for reliability of statistical analysis results. The samples were grouped in following categories: smoked meat and bacon, fermented sausages and heat-treated dry sausages, boiled sausages, cooked sausages, canned meat and meat meals, meat semiproducts and mechanically separated meat and, as a separated category, sea fish pastes.

2.3. Preparation of samples for HPLC
Homogenize part of the sample for determination. Weigh 5 g of the sample to the nearest 1 mg. If sample contain more than 10 % of fat, remove the fat with 2-3 portion of light petroleum. Extract colours from sample with ethanol-water solution. Place in the ultrasonic bath for 30 minutes. Add Carrez I and Carrez II solutions, stir on a vortex mixer to homogenize the mixture and centrifuge at 3000 rpm. If needed, repeat the extraction until the supernatant is colourless. Combine the supernatants. Transfer to a centrifuge cuvette and centrifuge for 10 minutes at 3000 rpm. Transfer the supernatant to a 10 ml measuring flask and fill it to the mark with deionised water. Filter the prepared samples through 0.45 µm pore size membrane filters into autosampler vials.

2.4. Chromatographic determination
Condition for HPLC determination were the same as previously described [14].
3. Results and discussion
The initial chromatographic method described in previous study [14] has been greatly improved in two ways. First, number of analytes has been increased from initial 7 red food colours to 14 colours. Second, preparation of samples has been optimised for extraction and rapid determination from large number of diverse samples, not just meat and meat products, with minor modifications of the preparation procedure.

![Figure 1. Number of samples per year.](image1)

![Figure 2. Number of analyses per year.](image2)

The optimized method was applied to determine the presence and content of these colours in meat products, as well as to check the safety of products and their compliance with legislation. The research
included the period from the beginning of 2016 to the first half of 2021. In the mentioned period, 1402 samples were examined and 1563 analyses were performed. The distribution of the number of samples and analyses by years, as well as the number of non-compliant samples and analyses are shown in Figures 1 and 2.

It is evident that number of analysed samples has been increased in the period of observation, as well as number of analyses, but number of noncompliant samples decreased, and in last two and half year not a single case of irregularity considering food colours content in meat product has been recorded.

As it previously noted, the samples were grouped in following categories: smoked meat and bacon, fermented sausages and heat-treated dry sausages, boiled sausages, cooked sausages, canned meat and meat meals, meat semiproducts and mechanically separated meat and, as a separated category, sea fish pastes. Distribution of samples and analyses by categories are rendered in Figure 3.

![Figure 3. Total number of samples and analyses per meat product categories.](image)

Apparently, fermented and boiled sausages are the two categories with greatest number of analysed samples, namely, over the 80 % of total samples and over the 75 % of total analyses. This is a consequence of regular safety control of these categories of meat products, and comprehensibly regulated added colours content in them [3,4,5]. It is surprising that a relatively small samples number of cooked sausages has been analysed in the research period, considering that the use of colours in them is also regulated [3,4,5] as in the two mentioned groups of products.

### 3.1. Compliance with legislation

The study published in 2015 concluded, based on the results of testing 74 meat products from three categories over a period of two and a half years, that close to 10% of products contained unpermitted added colours. In regard to product labelling, in slightly more than half (52.63%) of the products in which colour was not declared presence of added colour was confirmed. Added colour was labelled only in 23% of the total number of analysed products [14].

Current study, as it can be seen, was more comprehensive, including longer period of observation and largely greater number of samples. Number of analysed samples has been increased from year to year, as a consequence of the adoption of new legislation related to the safety of the use of food additives.
and food labelling [3,11] and as well as the need of the meat industry to control raw materials and retail chains to control safety of meat products of their brands.

It is evident that despite the huge increase in the number of examined samples, the share of non-compliant analyses is declining. While a couple of sporadic cases were recorded in 2017 and 2018, there were no such occurrences in the later period. In 2017, there were three cases of adding cochineal, E 120, to canned meat, and in 2018, one case of unpermitted use of E 120 and two cases of Allura Red AC, E 129, and E 120 in the same samples.

The results of the added colours content in analysed meat products considering food labelling are shown in Table 1. Comparing the presented results with the results of the previous study, a great improvement of the conformity of the product declaration is noticed. While previously more than half of the tested samples were mislabelled, now that percentage is far lower and about one fifth of the samples with confirmed colour content, and less than 9% of the total samples. Nevertheless, although the results are promising, they show that it is necessary to continue with the control and influence the producers and distributors of meat products to direct additional attention to the declaration of products.

Table 1. Share of samples with labelled and unlabelled colour in the total number of analysed samples with the presence of colour confirmed

| Samples with added colour | Number | %     |
|--------------------------|--------|-------|
| Total                    | 602    | 100.00|
| Declared colour          | 482    | 80.07 |
| Nondeclared colour       | 120    | 19.93 |

3.2. Self-control of raw materials at the request of the manufacturer

Some producers in the meat industry introduced the practice of periodically checking the input raw materials for meat products because there was a reasonable suspicion that dyes were added to some of the raw materials. Such raw materials contaminated the products, although the manufacturer did not add colour in his original recipe or used some of the colours that are allowed in quantum satis quantities. For the purposes of raw material control, the described method was used to determine the added colours in meat, mechanically separated chicken and pork meat.

3.3. Product authenticity and branding

The production of traditional meat products often completely prohibits the use of added colours, or the use is strictly limited and regulated. Authentic products are highly demanded and appreciated by consumers and must meet strict legal criteria. A large number of traditional products are exported, and the ban on exports due to security reasons caused by the unpermitted use of food colours causes great damage to both industry and the state.

On the other hand, many large retail chains require the meat industry to deliver products that are proven safe, without added colours or other additives. Also, in case of adding allowed colours, a valid certificate is required, including confirmation that their quantity is within the allowed limits. The use of HPLC method for simultaneous determination of food colours in meat products enables control and reliable confirmation of the colours content in products obtained in the traditional way or produced for the needs of trade brands or, for example, to obtain the label of authentic "Serbian quality".

4. Conclusion

Continuous determination of the content of food additives ensures the protection of consumer safety. Also, these analyses confirm that the food is prepared in accordance with good manufacturing practice and confirm that it is suitable for distribution through the sales network. This implies the application of adequate and reliable analytical techniques for qualitative and quantitative determination of the content of added additives in food. Food colours are additives that can be added in accordance with legal
regulations and must be clearly declared on the product. These same principles have been applied to the use of food colours in meat products.

The results of the research conducted with the aim of re-evaluating the situation on the Serbian market six years after the initial study performed in the period from 2012 to 2015 show that the state of the use of food colours in meat products according to legislative has changed significantly. While in previous study [14], far more irregularities were noticed on a smaller number and in several categories of samples, now it can be said that the situation is completely under control. Except for a few sporadic cases of unpermitted use of colours in previous years, not a single case has been reported in recent years.

A great improvement of the compliance in the product labelling is noticed. Previously in more than half of the tested samples were observed lack of added colour labelling or a non-present colour is declared, now that percentage is far lower and about 20% of the samples with confirmed colour content, and less than 9% of the total samples. These results show that it is necessary to continue with the control and additional attention need to be paid to the declaration of colours in meat products.

Regular control of the colour content in meat products has achieved that today we have a more auspicious situation and safer products on the domestic market, that our traditional and other meat products are easier to export and to acquire nutritional declarations and prestigious quality labels. In this way, better consumer protection is provided, as well as better sales of products of the domestic meat industry.

Acknowledgments
This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, according to the provisions of the Contract on research financing in 2021 (No 451-03-9/2021-14/200050 dated 05.02.2021).

References
[1] Spence C and Piqueras-Fiszman B 2016 Food Color and Its Impact on Taste/Flavor Perception _Multisensory Flavor Perception From Fundamental Neuroscience Through to the Marketplace_ (Cambridge: Woodhead Publishing) chapter 6 pp 107–32
[2] Pravilnik o kvalitetu usitnjenog mesa, poluproizvoda od mesa i proizvoda od mesa, _Službeni glasnik RS_ 50/2019
[3] Pravilnik o prehrambenim aditivima, _Službeni glasnik RS_ 53/2018
[4] Regulation (EC) No 1333/2008, _OJ L_ 354
[5] Regulation (EU) No 1129/2011, _OJ L_ 295
[6] Lukić M, Vranić D, Turubatović L, Petrović Z, Milićević D, Kran D and Milijašević M 2013 Comparison of results of sensory and chemical and physico-chemical investigations of fresh chilled beef packaged in vacuum during storage in retail conditions _Tehnol. mesa_ 54 (1) 21–32
[7] Vesković Moračanin S, Kran D, Trbović D, Okanović D, Džinić N and Jokanović M 2013 Colour and texture characteristics of “Užička” fermented sausage produced in the traditional way _Tehnol. mesa_ 54 (2) 137–43
[8] Knecht D, Duziński K and Jankowska-Mąkosa A 2021 Bloom Time Effect Depends on Muscle Type and May Determine the Results of pH and Color Instrumental Evaluation _Animals_ 11 (5) 1282
[9] Teixeira A and Rodrigues S 2021 Consumer perceptions towards healthier meat products _Curr. Opin. Food Sci._ 38 147–54
[10] Petronijević R., Matekalo-Sverak V, Spirić A, Vuković I, Babić J, Milijašević M and Trbović D 2014 The chemometric approach in development of the colorimetric method for the estimation of food colorants in meat products _Hem. Ind._ 68 6 781–91
[11] Pravilnik o deklarisanju označavanju i reklamiranju hrane, _Službeni glasnik RS_ 19/2017, 16/2018, 17/2020
[12] Regulation (EU) No 1169/2011 _OJ L_ 304
[13] EFSA 2016 Re-evaluation of food colours: EFSA completes major programme, https://www.efsa.europa.eu/en/press/news/160914-0

[14] Petronijević R, Lukić M, Karan D, Vuković I, Parunović N, Spirić A and Trbović D 2015 The use of colours in meat products in Serbia – state of the market and compliance with legal regulations. 26th Conf. of veterinar. of Serbia, September 2015, Zlatibor, Serbia. Conf. proceed. 299–303