meat technology
Founder and publisher:
Institute of Meat Hygiene and Technology, Belgrade

UDK: 637.057
ID: 158114569
https://doi.org/10.18485/meattech.2024.65.2.6

Original Scientific Paper

Evaluation of food additive analyses based on five years of food safety and quality controls

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ARTICLE INFO

Keywords:
Food
Food additives
Animal feed
Food colourants
Vitamins
Preservatives
Antioxidants
Food quality
Food safety

ABSTRACT

Analyses of eight groups of additives in food and animal feed for nearly five years were included in this research. Food samples were grouped according to EU directive 1333/2008 and national regulation 53/2018 into 18 food categories. A total of 4539 samples was analysed, of which the most numerous categories were meat and dairy products, with 2833 (62.4%) and 649 (14.3%) samples, respectively, and a total of 8203 analyses. Over 90% of all analyses were determinations of food colourants, inorganic anions and preservatives & sweeteners, accounting for 3478 (42.4%), 2937 (35.8%) and 1122 (13.7%) of the analyses, respectively. The least common were tartaric and fumaric acid determinations, and the food categories with the lowest numbers of analyses were: food supplements (rarest), fats and oils and fat and oil emulsions (second rarest), and sugars, syrups, honey and table-top sweeteners (third rarest). The analyses of additives are unevenly represented in food and animal feed and it is necessary to balance and harmonise them with legislative requirements. Adequate control of food additives is an important part of the regulatory requirements and can only be fulfilled by continuous monitoring of additive use in food and animal feed.

1. Introduction

Regulation (EC) No 1333/2008 of the European Parliament and of the Council describe food additives as "substances that are not normally consumed as food itself but are added to food intentionally for a (certain) technological purpose" (*European Union*, 2008). They have various roles in food preparation and are commonly used to improve some quality attributes, from acceptability to the safety of food, as well as prolong shelf-life of food commodities etc. Current food industry practices and manufacturing would not be possible without the use of food additives.

On the other hand, animal feed additives are defined by Regulation (EC) No 1831/2003 as "substances, micro-organisms or preparations, other than feed material and premixtures, which are intentionally added to feed or water in order to perform, in particular, one or more of the functions", such as to: favourably affect the characteristics of feed and animal products; change/enhance the colour of ornamental fish and birds; positively impact animal production, performance or welfare, particularly by affecting the gastrointestinal flora or digestibility of feeding stuffs; mitigate the environmental consequences of animal production; satisfy the nutritional needs of animals; have a coccidiostatic or histomonostatic effect (*European Union*, 2003).

Both regulations set the terms of the categories of additives, permitted amounts and authorise additive usage in particular food and feed. Setting such

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Paper received: September 24th 2024. Paper accepted: November 12th 2024.
Published by Institute of Meat Hygiene and Technology — Belgrade, Serbia.

conditions requires the development of a reliable methodology for determining the correct amounts of additives in food and animal feed. The existing techniques of chemical analysis of food, especially based on chromatography (*Bajcic et al.*, 2021, *Petronijevic et al.*, 2021, *Petronijevic et al.*, 2023), more or less successfully satisfy this requirement. The greatest problems are determining the content of additives that are naturally present in a particular form in the raw materials in the food and animal feed industry, because it is often impossible to determine to what extent they originate from the raw material, and how much comes from the additive itself (*Petronijevic et al.*, 2023).

At the end of the last century, risk assessment of food and animal feed additive usage became especially relevant due to the general increase in consumption of packaged and processed foods rich in additives. Possible connections of chronic consumption of food additives to adverse effects on human and animal health are described (*Polak et al*., 2018; *Bajcic et al*., 2018). In order to accurately estimate food additives' impacts on health based on the results of new scientific research, the European Union (EU) set up a programme for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 (*European Union*, 2008) under the jurisdiction of the European Food Safety Authority (*European Union*, 2010).

National regulation in Serbia on food additives (*Serbia*, 2018) is mostly harmonised with EU legislation, and usage of additives in animal feed is authorised by the Serbian Regulation on animal feed quality (*Serbia*, 2017; this regulation refers to the *Official Gazette of the Republic of Serbia 4/2010, 113/2012, 27/2014, 25/2015, 39/2016*). Therefore, continuous monitoring of the use of additives in food and animal feed is not a matter of good will but a legal obligation that must be systematically implemented and controlled at the state level. The results presented in this research are the consequence of the implementation of monitoring of particular additive groups in food and feed produced in or imported into Serbia. The data provides the possibility to determine the type of additives and their quantity introduced through the diet, individually or in total, as well as what kind of products have a greater impact on the increased intake of additives. Consequently, these data are significant for reliable risk assessments of additive consumption in human and animal nutrition.

2. Materials and Methods

2.1 Chemicals

All standard chemicals and reagents were purchased from Merck KgaA, Darmstadt, Germany. Ultrapure water, ≥18 MΩ, was obtained from ELGA Ultrapure (LabWater, Lane End, High Wycombe, UK).

Samples

Food and feed samples were part of regular control of quality and safety parameters, obtained from retail, producers and importers.

Sample preparation

Solid food and feed products were ground and homogenised prior to analysis. Depending on the applied determination technique and the type of additive, the samples were prepared according to appropriate procedures.

2.2 Methods

Antioxidants

An in-house, validated method of high-performance liquid chromatography with UV/VIS detection via photodiode array (HPLC-PDA) was used for the determination of butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHT), and propyl-, octyl- and dodecyl-gallate. The chromatographic system was an Alliance 2695 separation module with photodiode array detector 2996 (Waters, Milford, Massachusetts, USA). Antioxidants were extracted from the samples with methanol and centrifuged, and after filtration, the supernatants were submitted for analysis. Identification of each analyte was based on retention time (RT) and UV/VIS spectra.

Food colourants

Determination of 13 synthetic food dyes was performed in accordance to the reference method (*ISO*, 2021): Tartrazine, E 102, Sunset yellow FCF, E 110, 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, Indigotine, E 132, Brilliant Blue FCF, E 133, Green S, E 142 and Brilliant Black BN, E 151. An identical chromatographic system as for antioxidant determination was used.

Carminic acid, E 120, was determined by liquid chromatography with mass spectrometry (LC-MS/MS) on a triple quadrupole mass spectrometer, LCMS-8050 CL (Shimadzu Corporation, Japan). Preparation of sample for analysis included extraction of colourants in acidified ethanol, centrifugation and filtration. MS detection was in MRM mode, and 491.1 to 446.75 transition was used for quantification.

Fumaric acid

Fumaric acid analysis was carried out by IFU method Nr. 72 (*IFU*, 1998).

Hydrosoluble vitamins

Analyses of vitamins C, B2 and B6 were performed in accordance with reference methods (*ISO*, 2018; *SRPS EN*, 2014; *SRPS EN*, 2008b).

Inorganic anions

For determination of inorganic anion additives (mainly phosphoric acid and mono-, di-, tri- and polyphosphates, nitrites and nitrates, and sulphites) in food and animal feed, IC with conductometric detection was used. The IC system consisted of an 858 Professional Sample Processor, 930 Compact IC Flex with Oven/SeS/PP, and Conductivity Detector, (Metrohm AG, Herisau, Switzerland). The separation column was Metrosep A Supp 7 250/4.0 (Metrohm), and separation of anions was achieved by a mobile phase gradient in accordance with the original method provided by manufacturer (*Metrohm*, 2019).

Liposoluble vitamins

Vitamins A and E were determined by reference methods (*SRPS EN ISO*, 2011; *SRPS EN ISO*, 2008) based on HPLC.

Preservatives

Determination of sorbate and benzoate additives was according to the procedure described in the reference method (*SRPS EN*, 2008a). The chromatographic system was the same as was used for determination of antioxidants and artificial colourants.

Tartaric acid

The reference method (*SRPS EN*, 2008c) was applied for determination of tartaric acid.

2.3 Statistics

Food samples were strictly categorised into 18 groups according to food categories in EU directive 1333/2008 (*European Union*, 2008) and national regulation 53/2018 (*Serbia*, 2018). The meat category refers not only to raw meat, but also to meat products and all other products covered by this category, including meat casings, etc.

MS Office 2016 Excel software was applied for data preparation. Contingency analysis of categorical data was performed in JMP Statistical Discovery 10 (SAS Institute Inc. NC, USA https://www.jmp.com).

3. Results and Discussion

Analyses of eight important groups of additives in food and animal feed for almost five years were included in this research. A total of 4539 food/feed samples was analysed, of which 224 (5%) were animal feed. The most numerous categories were meat products and dairy products and analogues, with

Table 1. Number of samples per food category

Food categories	Samples
Additives	36
Animal feed	224
Bakery wares	34
Beverages	105
Cereals and cereal products	10
Compound food	15
Confectionery	42
Dairy products and analogues	649
Edible ices	62
Eggs and egg products	21
Fats and oils and fat and oil emulsions	2
Fish and fishery products	124
Food supplements	1
Fruits and vegetables	284
Meat	2833
Ready-to-eat savouries and snacks	25
Salts, spices, soups, sauces, salads and protein products	69
Sugars, syrups, honey and table-top sweeteners	3
Total	**4539**

Table 2. Contingency analysis of additive group by food category

Food category	Antioxidants	Food colourants	Fumaric acid	Hydrosoluble vitamins	Inorganic anions	Liposoluble vitamins	Preservatives & sweeteners	Tartaric acid	Total
Additives		87				10	10		107
Animal feed	95	39		90	22	403	1		650
Bakery wares		17			43		19		79
Beverages		4	3	18	214	1	9	1	250
Cereals and cereal products					11		5		16
Compound food					14		6		20
Confectionery		97	5	1	1		36		140
Dairy products and analogues		338	4		488		547		1377
Edible ices		169			51		17		237
Eggs and egg products		11			8		26		45
Fats and oils and fat and oil emulsions		27							27
Fish and fishery products		205		5	123		27		360
Food supplements					2				2
Fruits and vegetables		67		2	477		116		662
Meat		2318		3	1346	3	267	2	3939
Ready-to-eat savouries and snacks		9			17		12		38
Salts, spices, soups, sauces, salads and protein products	20	90			115		24		249
Sugars, syrups, honey and table-top sweeteners					5				5
Total	**115**	**3478**	**12**	**119**	**2937**	**417**	**1122**	**3**	**8203**

2833 (62.4%) and 649 (14.3%) samples, respectively (Table 1). Fewer analyses were conducted on fruits and vegetables, animal feed, fish and fishery products and beverages (Table 1).

As shown in Table 1, some food categories had only a few requests for additive analysis (< 10 samples per year) in the research period. This group included food categories that are widely consumed (bakery wares, confectionery, cereals and snacks) or mainly imported or exported (additives, egg products, supplements) as raw materials for use in the food industry. Therefore, with respect to their demand and presence on the market, the lack of extensive control of additive content in these food categories is surprising. This is especially the case considering that some of those categories are highly processed foods with significant quantities of one or more additives.

Table 2 presents a comprehensive overview of the results, showing individual numbers and totals of analyses by food categories and in each additive group. In almost five years, 8203 analyses were performed. The most common analyses were determinations of food colourants, inorganic anions and preservatives & sweeteners, accounting for 3478 (42.4%), 2937 (35.8%) and 1122 (13.7%) of analyses, respectively. In fact, 91.9% of all analyses were for these additives. On the other hand, the least common analyses performed were determinations of tartaric and fumaric acids, 3 and 12 times, respectively. Analyses of hydrosoluble and liposoluble vitamins, as additives in food and feed samples, made up less than 10% of all determinations.

Following the nature of the obtained data, since they consisted of a large number of results that could be classified into several categories and groups based on frequency, contingency analysis was chosen. The uneven number of analyses per sample and the large disparity in the number of samples per food category was the main obstacle in presenting and interpreting results. Hence, to enable their distinct presentation, results had to be divided into two groups based on the number of additive analyses performed in the correspondent food categories. One group consisted of the most common determinations: food colourants, inorganic anions, vitamins, preservatives & sweeteners and antioxidants in the following food categories: additives; animal feed; bakery wares; beverages; confectionery; dairy products and analogues; edible ices; fish and fishery products; fruits and vegetables; meat; and salts, spices, soups, sauces, salads and protein products. A second group contained less frequent analyses of fumaric acid, tartaric acid, food colourants, inorganic anions and preservatives & sweeteners in the following food categories: beverages; cereals and cereal products; compound food; confectionery; dairy products and analogues; eggs and egg products; fats and oils and fat and oil emulsions; food supplements; meat; ready-to-eat savouries and snacks; and sugars, syrups, honey and table-top sweeteners. Visual representations of additive analyses by food category for each of the groups are given in Figures 1 (main, common analyses) and 2 (infrequent analyses).

Determinations of food colourants, inorganic anions and preservatives & sweeteners accounted for most of the analyses performed in the main (commonly analysed) food categories. This is certainly a consequence of legal requirements, because additives from these three groups are permitted and regulated in most food categories. However, the category of animal feed differed, as determinations of liposoluble vitamins, followed by antioxidants and hydrosoluble vitamins were more common, mainly due to the specific requirements of the corresponding regulations. An equally significant contribution was requests from animal feed manufacturers to control and validate the composition and quality of their products. However, analysis of permitted preservatives in feed was performed only once in the 5-year period, compared to other food categories where this is one of the most common determinations.

The type and number of analyses from the category of additives as raw materials for the food and animal feed industries (the additives category in the tables and Figure 1) is primarily a consequence of import controls. No conclusion can be made or generalised because of the relatively small number of samples analysed (36), but results can be considered indicative.

Figure 2 shows the results for food categories that either had few samples or few additive analyses. The results presented for this group should be taken with caution due to the small numbers of determinations and samples, and so could be regarded as inconclusive. The only unmistakable conclusion is that these food categories should be given greater importance regarding their additive content analysis, both in terms of the number of samples and the types of additives. For example, according to official statistical data (*Serbia*, 2023), the import of animal and vegetable oils and fats in Serbia over a 5-year period (2018–2022) was worth US$394 million. A significant part of that was frying oils and fats for fast-food restaurants and the confectionery industry. Considering the amounts of fast-food, fried food and confectionery products that are now consumed, especially

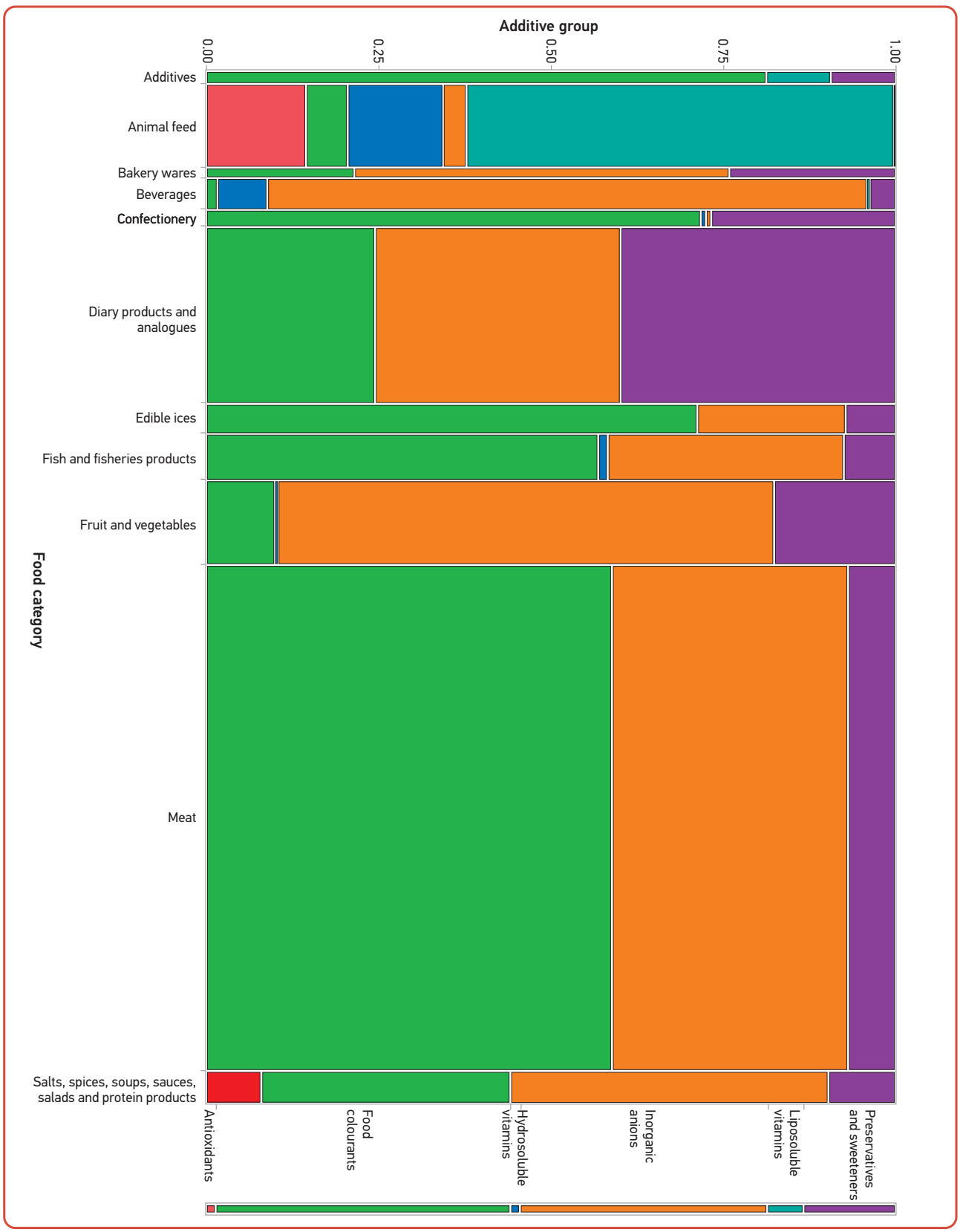

Figure 1. Graphic representation of the food categories vs. main analyses. The X-axis represents the relative ratios of the number of samples by food category, and the Y-axis shows the relative ratios of the food additive analyses within each food category. The blocks depicted show the relative proportions of the performed analyses within the entire population. Each additive determination is marked with a different colour. The side bar shows the overall ratios of food additive analyses within the group of more commonly analysed food and feed products.

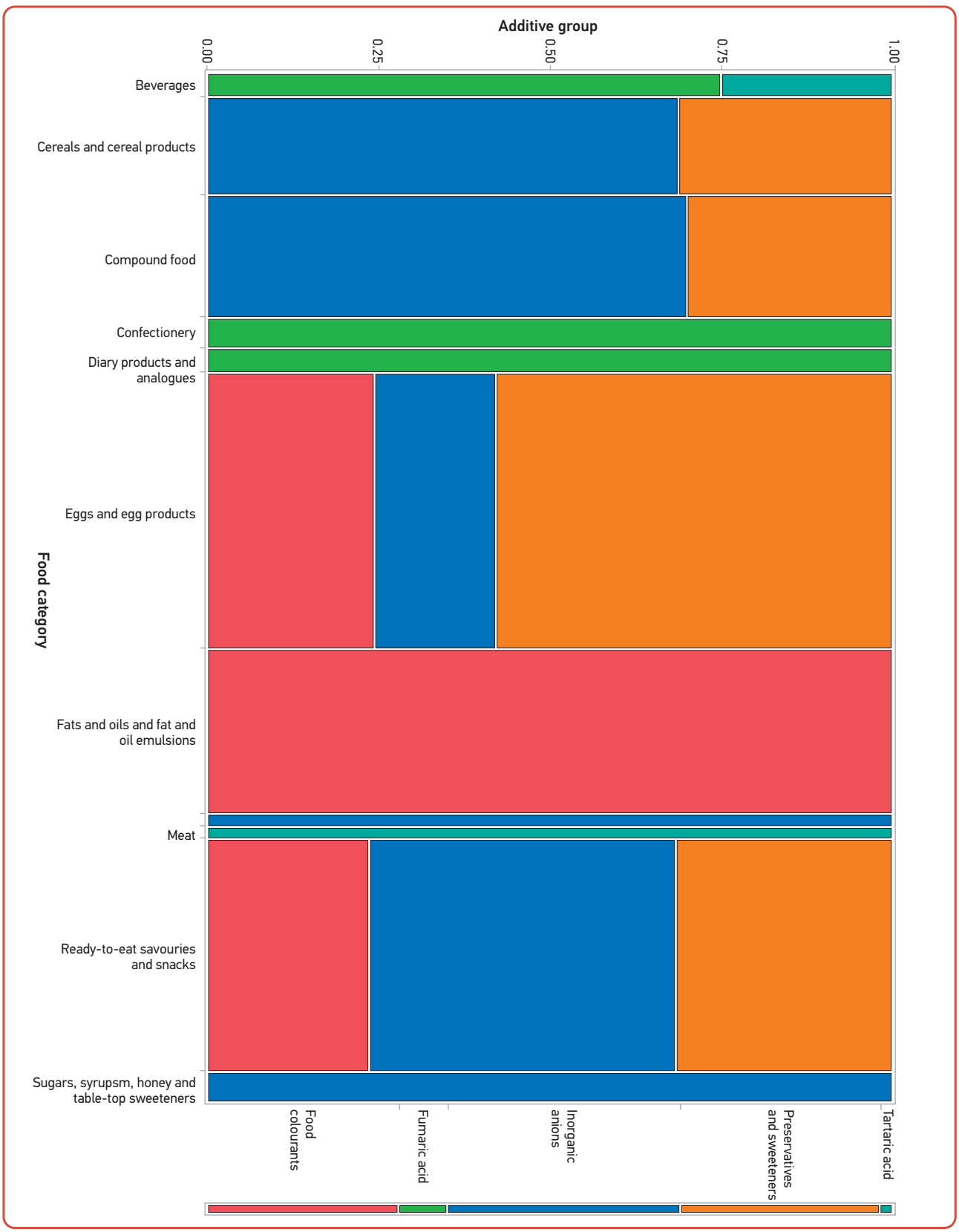

Figure 2. Graphic representation of the food categories vs. infrequent analyses. The X-axis represents the relative ratios of the number of samples by food category, and the Y-axis shows the relative ratios of the food additive analyses within each food category. The blocks depicted show the relative proportions of the performed analyses within the entire population. Each additive determination is marked with a different colour. The side bar shows the overall ratios of food additive analyses within the group of infrequent analyses.

by the young population, it is unnecessary to underline the relevance of determining the chemical safety of imported fats and oils, which includes the analysis of additives. Consequences and implications of inadequate control, along with other health issues related to fast food and confectionery consumption, could have great and long-term adverse impacts on public health.

4. Conclusion

Food additives have gained a lot of attention in recent decades. On the one hand, they have become an irreplaceable factor in food production today, but on the other hand, their use is, from time to time and justifiably or not, associated with controversies regarding their adverse impact on human or animal health. In addition, a negative side of the food additives can be their use to mask food frauds and adulterations.

The processing of the results of 5-year additive analyses in food and animal feed at the national level showed that the control of additives is carried out regularly in some food categories, while in others it is not. Also, in some cases, in the categories in which regular control is performed, analyses of all relevant additives are not included.

From the results, it can be concluded that the most common determinations were for food colourants, inorganic anions, preservatives & sweeteners, which made up almost 92% of the analyses performed. Among the food categories, the largest number of analyses were for meat, while four times fewer analyses were conducted for dairy products and analogues, followed by fruits and vegetables, animal feed, fish and fishery products and beverages.

In animal feed, the main determinations were for liposoluble vitamins, followed by antioxidants and hydrosoluble vitamins. Analysis of permitted preservatives in feed was performed only once in the observed period.

In conclusion, the results indicate that the control of additives in food and animal feed is uneven. Whatever the reasons for this situation, it is necessary to balance the control of additives in some food categories, and harmonise them to legislative requirements, deriving the assessment from the needs of the national market, the import of raw materials and the export of food products. Adequate control of food additives is an important part of the fulfilment of the legal regulation requirements that ensure better quality and safer food.

Procena učestalosti analize prehrambenih aditiva na osnovu petogodišnje kontrole bezbednosti i kvaliteta hrane

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INFORMACIJE O RADU

Ključne reči:
Hrana
Prehrambeni aditivi
Hrana za životinje
Prehrambene boje
Vitamini
Konzervansi
Antioksidansi
Kvalitet hrane
Bezbednost hrane

APSTRAKT

Istraživanje je obuhvatalo analize nekoliko grupa aditiva u hrani i hrani za životinje u toku 5 godina. Uzorci su grupisani prema kategorijama hrane definisanim u EU direktivi 1333/2008 i Pravilniku o prehrambenim aditivima, Službeni glasnik br. 53/2018. Analizirano je ukupno 4539 uzoraka, od kojih su najbrojnije grupe bile meso, 2833 (62,4%), i mlečni proizvodi, 649 (14,3%) uzoraka, sa ukupno 8203 izvršene analize. Preko 90% svih analiza odnosilo se na određivanje boja, anjona i konzervanasa, 3478 (42,4%), 2937 (35,8%) i 1122 (13,7%) analize, redom. Najmanje učestale analize su bile određivanje sadržaja vinske i fumarne kiseline, a najmanji broj uzoraka bio je u tri kategorije namirnica: dodaci ishrani, masti i ulja i emulzije masti i ulja i šećeri, sirupi, med i stolni zaslađivači. Analize aditiva su neravnomerno zastupljene u hrani i hrani za životinje i potrebno ih je izbalansirati i uskladiti sa zakonskom regulativom. Adekvatna kontrola aditiva u hrani je važan deo ispunjavanja regulatornih zahteva kontinuiranim praćenjem upotrebe aditiva u hrani i hrani za životinje.

Disclosure statement: No potential conflict of interest was reported by authors.

Funding: The research results presented in this paper were financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, and based on the Agreement on the implementation and financing of scientific research work of the NIO in 2024 no. 451-03-66/2024-03/200050 from 05.02.2024.

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