



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Directorate F - Health, Consumers & Reference Materials (Geel/Ispra)
European Union Reference Laboratory for Feed Additives

 Ref. Ares(2019)4959308 - 29/07/2019

JRC F.5/CvH/ZE/AS/Ares

**Evaluation Report on the Analytical Methods submitted
in connection with the Application for Authorisation of a
Feed Additive according to Regulation (EC) No 1831/2003**

Caramel colours E150 b, E150c and E150d
(FAD-2010-0249; CRL/100279)



**Evaluation Report on the Analytical Methods submitted
in connection with the Application for Authorisation of a
Feed Additive according to Regulation (EC) No 1831/2003**

Dossier related to: **FAD-2010-0249 - CRL/100279**

Name of Feed Additive: ***Caramel colours E150b, E150c and E150d***

Active Agent (s): **-**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
JRC Geel, Belgium**

Report prepared by: **Zigmas Ezerskis**

Report checked by: **María José González de la Huebra**
Date: **26/07/2019**

Report approved by: **Christoph von Holst**
Date: **29/07/2019**

EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 10 for *caramel colours E150b, E150c and E150d* under the category / functional group 2 (a) "sensory additives" / "colourants" according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additives* for all animal species.

Caramel colours E150b, E150c and E150d are redish-brown to brown-black viscous liquids or hygroscopic powders composed of complex mixture resulting from the reaction of caramelisation of sugars under controlled heat treatment in the presence of sulfite or/and ammonium compounds.

The Applicant states that the specific purity criteria set in the Commission Directive 2008/128/EC amended by the Commission Regulation (EU) 231/2012 for the use of *Caramel colours E150b, E150c and E150d* as food additives are also applicable when using them as *feed additives*.

The *feed additives* are intended to be included into *feedingstuffs* through *premixtures* with no minimum or maximum dose indicated by the Applicant.

For the identification/characterisation of the *feed additives*, the Applicant referred to the various methods described in the FAO JECFA '*caramel colours*' monograph and the '*volume 4*' of the FAO JECFA combined compendium for food additives specifications. These methods are used for checking the compliance with the criteria specified in Commission Regulation (EU) 231/2012 for *caramel colours E150b, E150c and E150d*.

The EURL recommends for the identification/characterisation of the *feed additives* the above mentioned methods described in the FAO JECFA '*caramel colours*' monograph and the '*volume 4*' of the FAO JECFA combined compendium for food additives specifications.

As the accurate quantification of *caramel colours E150b, E150c and E150d* added to *premixtures* or *feedingstuffs* is not achievable experimentally the EURL cannot evaluate nor recommend any method for official control to quantify *caramel colours E150b, E150c and E150d* in *premixtures* or *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Caramel colours E150b, E150c and E150d, sensory additives, colourants, all animal species

1. BACKGROUND

In the current application authorisation is sought under Article 10 (re-authorisation of existing feed additives) for *Caramel colours E150b, E150c and E150d* under the category / functional group 2 (a) "sensory additives" / "colourants" according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1]. Specifically, authorisation is sought for the use of the *feed additives* for all animal species [2].

Caramel colours E150b, E150c and E150d are redish-brown to brown-black viscous liquids or hygroscopic powders composed of complex mixture resulting from the reaction of caramelisation of sugars under controlled heat treatment in the presence of sulfite or/and ammonium compounds [3].

The Applicant states [3] that the specific purity criteria set in the Commission Directive 2008/128/EC [4] amended by the Commission Regulation (EU) 231/2012 [5] for the use of *Caramel colours E150b, E150c and E150d* as food additives are also applicable when using them as *feed additives*.

The *feed additives* are intended to be included into *feedingstuffs* through *premixtures* with no minimum or maximum dose indicated by the Applicant [3].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Caramel colours E150b, E150c and E150d* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water (section 2.6.1 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

Identification/Characterisation of the feed additive (section 2.6.3 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

For the identification/characterisation of the *feed additives*, the Applicant referred to the methods described in the FAO JECFA '*caramel colours*' monograph [6] and the '*volume 4*' of the FAO JECFA combined compendium for food additives specifications [7]. These methods are used for checking the compliance with the criteria specified in Commission Regulation (EU) 231/2012 [5] for *caramel colours E150b, E150c and E150d*.

For the characterisation of *caramel colours E150b, E150c and E150d* the determination of colour bound by diethylaminoethyl (DEAE) cellulose, colour intensity, total nitrogen and total sulfur has to be performed. In addition, the determination of sulfur dioxide, sulfur bound by DEAE cellulose, colour bound by phosphoryl cellulose, ammoniacal nitrogen, nitrogen/sulfur ratio of alcohol precipitate, 4-methylimidazole, 2-acetyl-4-tetrahydroxybutylimidazole and absorbance ratios of i) colour bound by DEAE cellulose; ii) colour bound by phosphoryl cellulose; iii) alcohol precipitate; and iv) the caramel colours at 280 and 560 nm for one or two of the *feed additives* have to be followed [5].

For the determination of colour bound by DEAE, the caramel colour is mixed with diluted hydrochloric acid and centrifuged or filtered if needed. The aliquot of the obtained mixture is treated with DEAE cellulose, mixed thoroughly, centrifuged or filtered and clear supernatant is collected. The absorbance of the caramel colour solution and one of the supernatant is measured by spectrophotometry at 560 nm, and the percentage of colour bound by DEAE cellulose is calculated [6].

For the determination of colour bound by phosphoryl cellulose a similar procedure as the one used for the determination of colour bound by DEAE has to be performed [6].

For the determination of colour intensity, the sample (0.1 g) is mixed with water and the solution is centrifuged if needed. The absorbance of the supernatant is measured at 610 nm. The colour intensity is calculated taken into account the percentage of content of solids, which is determined following the procedure described in the monograph [6].

For the determination of the total nitrogen content, the sample (1 g) is mixed with potassium or sodium sulfates, cupric sulfate and sulfuric acid in a Kjeldahl flask. The content is heated for 30 min, cooled down and sodium hydroxide solution and granulated zinc are added to the reaction mixture. A distillation of the latter mixture is performed and the distillate is titrated with sulfuric acid in the presence of a methyl red/methylene blue indicator. The content of total nitrogen is derived by calculation [7].

For the determination of ammoniacal nitrogen, the sample (2 g) is mixed with magnesium oxide and water in a Kjeldahl flask, connected through a condenser to the receiving flask filled with sulfuric acid, and heated to boiling. The content of the receiving flask (the formed

ammonium sulfate) is titrated with sodium hydroxide in the presence of a methyl red indicator. The content of ammoniacal nitrogen is calculated as mass fraction [6].

For the determination of total sulfur, the sample (5 to 10 g) is mixed with magnesium oxide or nitrate, sucrose and nitric acid. The mixture is heated in an oven, cooled down, dissolved in hydrochloric acid, filtered, heated again to the boiling point and a solution of barium chloride is added. The reaction mixture is left overnight, filtered, washed, ignited and the residue of barium sulfate is weighed. The content of total sulfur is calculated as mass fraction [6].

For the determination of sulfur dioxide, the sample (25 g) is mixed with water and concentrated hydrochloric acid in a modified Monier-Williams apparatus and heated/refluxed for more than 2 h. The distillate is titrated with sodium hydroxide in the presence of a methyl red indicator. The content of sulfur dioxide is calculated as mass fraction [6].

For the determination of 4-methylimidazole and 2-acetyl-4-tetrahydroxy-butylimidazole, the samples are analysed after appropriate treatment by gas chromatography coupled to flame ionisation detection (GC-FID) and high performance liquid chromatography (HPLC) equipped with UV detection at 385 nm, respectively [6].

The EURL recommends for the identification/characterisation of the *feed additives* the above mentioned methods described in the FAO JECFA '*caramel colours*' monograph [6] and the '*volume 4*' of the FAO JECFA combined compendium for food additives specifications [7].

As the accurate quantification of *caramel colours E150b, E150c and E150d* added to *premixtures* or *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control to quantify *caramel colours E150b, E150c and E150d* in *premixtures* or *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for the identification/characterisation of the *feed additives* the above mentioned methods described in the FAO JECFA '*caramel colours*' monograph and the '*volume 4*' of FAO JECFA combined compendium for food additives specifications.

Since the accurate quantification of *caramel colours E150b, E150c and E150d* added to *premixtures* or *feedingstuffs* is not achievable experimentally the EURL cannot evaluate nor recommend any method for official control to quantify *caramel colours E150b, E150c and E150d* in *premixtures* or *feedingstuffs*.

Recommended text for the register entry (analytical method)

For the identification/characterisation of *caramel colours E150b, E150c and E150d* in the *feed additives*:

- The FAO JECFA '*caramel colours*' monograph and the '*volume 4*' of FAO JECFA combined compendium for food additives specifications

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *caramel colours E150b, E150c and E150d* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

- [1] *Application, Reference SANTE_E5_FWD. APPL. 1831-0027-2019 & Annex I – submission number 1288278379442-1262
- [2] *Application, proposal for Register entry – Annex A
- [3] *Technical dossier, Section II: Identify, characterisation and conditions of use of the additive; methods of analysis
- [4] Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs
- [5] Commission Regulation (EU) No 231/2012 of 9 March 2012, laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council
- [6] FAO JECFA Combined Compendium of Food Additive Specifications, '*caramel colors*', Monograph No. 11 (2011)
http://www.fao.org/fileadmin/user_upload/jecfa_additives/docs/monograph11/additive-102-m11.pdf
(last visited on 20/06/2019)
- [7] FAO JECFA Combined Compendium for Food Additive Specifications - Analytical methods, test procedures and laboratory solutions used by and referenced in the food additive specifications, Vol. 4
<http://www.fao.org/docrep/pdf/009/a0691e/a0691e.pdf> (last visited on 20/06/2019)

*Refers to Dossier no: FAD-2010-0249

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

The following National Reference Laboratories contributed to this report:

- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
- Istituto Superiore di Sanità. Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare, Roma (IT)
- ¹Wageningen Food Safety Research (WFSR) (NL)
- Instytut Zootechniki — Państwowy Instytut Badawczy, Krajowe Laboratorium Pasz, Lublin (PL)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, PESCA, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft. Geschäftsbereich 6 — Labore Landwirtschaft, Nossen (DE)
- Univerza v Ljubljani. Veterinarska fakulteta. Nacionalni veterinarski inštitut. Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)

¹ Name and address according to according COMMISSION IMPLEMENTING REGULATION (EU) 2015/1761: RIKILT Wageningen UR, Wageningen.