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**European Union Reference Laboratory for Feed Additives**

JRC F.5/CvH/SB/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

**Monosodium L-glutamate**
produced by fermentation with
Corynebacterium glutamicum KCCM80188
***(FAD-2018-0090; CRL/180070)***

European Union Reference Laboratory
Feed Additives

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| Dossier related to: | **FAD-2018-0090 - CRL/180070** |
| Name of Product: | ***Monosodium L-glutamate produced by fermentation with Corynebacterium glutamicum KCCM80188*** |
| Active Agent: | **Monosodium L-glutamate** |
| Rapporteur Laboratory: | **European Union Reference Laboratory for Feed Additives (EURL-FA)**<br>**JRC Geel, Belgium** |
| Report prepared by: | **Stefano Bellorini** |
| Report checked by:<br>Date: | **Zigmas Ezerskis**<br>**28/06/2019** |
| Report approved by:<br>Date: | **Christoph von Holst**<br>**28/06/2019** |

## EXECUTIVE SUMMARY

In the current application authorisation is sought under Article 4(1) for *monosodium L-glutamate (MSG) produced by fermentation with Corynebacterium glutamicum KCCM80188*, under the category/functional group 2(c) 'sensory additives'/flavouring compound', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species.

According to the Applicant *MSG* has a minimum purity (mass fraction) of 99 %. The *feed additive* is intended to be added directly into *feedingstuffs* (or through *premixtures*) and *water* for drinking. The Applicant proposed a maximum content of *MSG* in *feedingstuffs* of 25 mg/kg.

For the quantification of *MSG* in the *feed additive* the Applicant submitted an in-house validated analytical method based on reversed phase high performance liquid chromatography coupled with ultraviolet detection (HPLC-UV). While in the frame of the validation study satisfactory performance characteristics were derived, the Applicant did not present a verification study or any additional test performed by a second independent laboratory applying the above mentioned method.

For the quantification of *MSG* in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated European Union method (Commission Regulation (EC) No 152/2009) based on ion exchange chromatography coupled to photometric detection (IEC-VIS). This method, designed only for the analysis of amino acids in *premixtures* and *feedingstuffs*, does not distinguish between the salts (*MSG*) and the amino acid enantiomers. The method was further ring-trial validated resulting in the EN ISO 13903:2005 method. The following performance characteristics were reported for the quantification of glutamic acid: $RSD_r$ ranging from 0.9 to 2.7 % and $RSD_R$ ranging from 6.2 to 9.1 %. However, while the lowest limit of quantification (LOQ) of 30 mg/kg has been reported for the analysis of certain amino acids, a specific LOQ for glutamic acid has not been indicated. Therefore the method does not ensure the accurate determination of *MSG* when added into feed at the proposed maximum content (i.e. 25 mg/kg *feedingstuffs*). Hence, the EURL recommends for official control the European Union method based on IEC-VIS for the quantification of *MSG* in *premixtures* only.

The Applicant did not provide any experimental data to determine *MSG* in *water*. Nevertheless, as concluded in previous EURL reports on amino acids, the EURL recommends for official control the procedure based on IEC-VIS and described in the ring-trial validated European Union method (or in equivalent ring trial validated methods e.g. VDLUFA Method 4.11.6.) to quantify *MSG* in in the *feed additive* and *water*.

In addition, the EURL recommends the "Monosodium L-glutamate monograph" of the Food Chemical Codex (FCC) for the identification of the *feed additive*.

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**

*Monosodium L-glutamate (MSG) produced by fermentation with Corynebacterium glutamicum KCCM80188*, sensory additives, flavouring compounds, all animal species and categories, glutamic acid.

## 1. BACKGROUND

In the current application authorisation is sought under Article 4(1) (authorisation of a new feed additive) for *monosodium L-glutamate (MSG) produced by fermentation with Corynebacterium glutamicum KCCM80188*, under the category/functional group 2(c) 'sensory additives'/flavouring compound', according to Annex I of Regulation (EC) No 1831/2003. Authorisation is sought for all animal species [1-2]. Synthetically produced *MSG* is already authorised as sensory *feed additive* [3-4].

According to the Applicant, *MSG* (monosodium salt of glutamic acid) is an off-white crystalline powder with a minimum purity (mass fraction) of 99 % [5]. The *feed additive* is produced by fermentation with a non-genetically modified strain of *Corynebacterium glutamicum* [6]. The production strain is deposited in the "Korean Culture Centre of Microorganisms" (KCCM) with the accession number *80188* [7].

The *feed additive* is intended to be added directly into *feedingstuffs* or through *premixtures* and *water* for drinking [8]. Furthermore, the Applicant proposed a maximum content of *MSG* in *feedingstuffs* of 25 mg/kg [1,8].

Note: The EURL has previously evaluated the analytical methods for the determination of *MSG* as sensory *feed additive* in the frame of the "Chemical Defined flavouring Group 34" [9].

## 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 *monosodium L-glutamate* 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)***

For the quantification of *MSG* in the *feed additive* the Applicant submitted an in-house validated analytical method based on reversed phase high performance liquid chromatography (HPLC) and ultraviolet detection (UV) [10,11].

The *feed additive* is diluted in distilled water and an aliquot is directly injected into the HPLC system equipped with a C18 column. The analyte is eluted via a potassium phosphate buffer. *MSG* is detected with a photodiode array detector (PDA) at 210 nm and quantified via a calibration curve. In the frame of the validation study, the Applicant reported relative standard deviations for repeatability ($RSD_r$) and intermediate precision ($RSD_{ip}$) below 3 %, and a recovery rate ($R_{rec}$) ranging from 98.9 to 105.4 % [11]. While in the frame of the validation study satisfactory performance characteristics were derived, the Applicant did not present a verification study or any additional test performed by a second independent laboratory applying the above mentioned method.

For the quantification of the *MSG* content in *premixtures* and *feedingstuffs* the Applicant submitted the ring-trial validated European Union method [10,12]. This method applies for the determination of free (synthetic and natural) and total (peptide-bound and free) amino acids (including glutamic acid), using an amino acid analyser or HPLC equipment with an ion exchange column (IEC). The method is intended for *premixtures* and *feedingstuffs*, it does not distinguish between the salts of amino acids and it cannot differentiate the amino acid enantiomers.

The *free* amino acids are extracted with diluted hydrochloric acid. Co-extracted nitrogenous macromolecules are precipitated with sulfosalicylic acid and removed by filtration. The solution is filtered and adjusted to pH 2.2. The amino acids are separated by IEC and *free* glutamic acid is determined after post-column derivatisation with ninhydrin by spectrophotometric detection at 570 nm (visible – VIS).

The procedure chosen for the determination of the *total* amino acids depends on the amino acids under investigation. Glutamic acid can be determined in either oxidised or non-oxidised samples. Oxidation is performed at 0 °C with a performic acid/phenol mixture. The excess of the oxidation reagent is decomposed with sodium disulfite. The oxidised or non-oxidised

sample is hydrolysed with hydrochloric acid (6 mol/l) containing 1 g phenol/l for 23 hours. The hydrolysate is adjusted to pH 2.2. The amino acids are separated by IEC and total glutamic acid is determined by post-column derivatisation with ninhydrin and photometric detection at 570 nm.

As a general rule, in order to avoid an overestimation of added *MSG* content, the procedure based on quantification of *free* amino acids content in *premixtures* and *feedingstuffs* has to be followed. When the procedure for the quantification of *total* amino acids is applied on *premixtures* and *feedingstuffs* containing proteins, an overestimation of *MSG* may occur.

The European Union method was further ring-trial validated by twenty-three laboratories for the determination of total glutamic acid in *feed* and resulted in the equivalent standard method EN ISO 13903:2005 [13]. The reported performance characteristics are listed in Table 1.

While limits of quantification (LOQ) ranging from 30 to 200 mg/kg have been reported for the analysis of free amino acids, a specific LOQ for glutamic acid has not been indicated [13]. However, according to the EURL's former evaluations, this method does not ensure the accurate discrimination between *MSG* as flavouring added at low concentration to the *feedingstuff* (i.e. 25 mg/kg) and endogenous *MSG*/glutamic acid possibly present at higher level of concentration in the *feed* matrix [9]. Therefore the EURL is unable to recommend a method for the official control to determine *MSG* added in *feedingstuffs*.

Based on the performance characteristics presented, the EURL recommends for official control the ring-trial validated European Union method based on IEC-VIS to quantify *MSG* in *premixtures*.

The Applicant did not provide any experimental data to determine *MSG* in *water* [10]. Nevertheless, based on NRL's experience, as concluded in previous EURL's amino acids reports even if the determination of *MSG* in the *feed additive* and *water* is not explicitly stated in the scope of the European Union method (or similar ones e.g. VDLUFA Method 4.11.6.), the IEC-VIS procedure described above is considered fit for purpose for the determination of *MSG* in these matrices [14].

**Table 1:** Method performance characteristics obtained in the frame of EN ISO 13903:2005 [13] for the determination of total *glutamic acid* in *feed*.

| Ring-Trial | Matrix | *Glutamic acid* content g/kg | $RSD_r$ % | $RSD_R$ % |
|---|---|---|---|---|
| [13] | Poultry meal | 79.7 | 2.7 | 9.1 |
| | Broiler finisher feed | 32.5 | 1.6 | 7.0 |
| | Broiler starter feed | 40.4 | 1.8 | 8.4 |
| | Corn | 15.1 | 2.4 | 6.2 |
| | Fishmeal | 73.7 | 0.9 | 4.7 |

$RSD_r$, $RSD_R$ - relative standard deviation for *repeatability* and *reproducibility*, respectively

Therefore, the EURL recommends for official control the procedure based on IEC-VIS and described in the ring-trial validated European Union method to quantify *MSG* in in the *feed additive* and *water*.

***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)***

The 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 of the *feed additive* the EURL recommends the "Monosodium L-glutamate monograph" of the Food Chemical Codex (FCC) where a test based on infrared absorption is described [15].

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 official control (i) the "Monosodium L-glutamate monograph" of the Food Chemical Codex (FCC) based on infrared absorption for the identification of *MSG* in the *feed additive*; (ii) the European Union method based on IEC-VIS or equivalent for the quantification of *MSG* in the *feed additive* and *water*; and (iii) the European Union method based on IEC-VIS for the quantification of *MSG* in *premixtures*.

***Recommended text for the register entry (analytical method)***

For the identification of *monosodium L-glutamate* in the *feed additive*:

- Food Chemical Codex "Monosodium L-glutamate monograph"

For the quantification of *monosodium L-glutamate* in the *feed additive* and *water*:

- ion-exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS), as described in Commission Regulation (EC) No 152/2009 (Annex III, F)

For the quantification of *monosodium L-glutamate* in *premixtures*:

- ion-exchange chromatography coupled with post-column derivatisation and photometric detection (IEC-VIS), Commission Regulation (EC) No 152/2009 (Annex III, F)

## 5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *monosodium L-glutamate produced by fermentation with Corynebacterium glutamicum KCCM80188* 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, Proposal of Registry Entry – Annex A

[2] *Application, Reference SANTE/E5: Forw. Appl. 1831/0002-2019 & Annex I – submission number 1544782598492-2336

[3] Commission Implementing Regulation (EU) 2018/249 of 15 February 2018 concerning the authorisation of taurine, beta-alanine, L-alanine, L-arginine, L-aspartic acid, L-histidine, D,L-isoleucine, L-leucine, L-phenylalanine, L-proline, D,L-serine, L-tyrosine, L-methionine, L-valine, L-cysteine, glycine, monosodium glutamate and L-glutamic acid as feed additives for all animal species and L-cysteine hydrochloride monohydrate for all species except cats and dogs, O.J. L 53/134, 23.2.2018

[4] Commission Implementing Regulation (EU) 2018/1567 of 18 October 2018 correcting Implementing Regulation (EU) 2018/249 concerning the authorisation of taurine, beta-alanine, L-alanine, L-arginine, L-aspartic acid, L-histidine, D,L-isoleucine, L-leucine, L-phenylalanine, L-proline, D,L-serine, L-tyrosine, L-methionine, L-valine, L-cysteine, glycine, monosodium glutamate and L-glutamic acid as feed additives for all animal species and L-cysteine hydrochloride monohydrate for all species except cats and dogs

[5] *Technical dossier, Section II: II.2.1.1. Chemical substances

[6] *Technical dossier, Section II: II.2.1.2. Micro-organisms

[7] *Technical dossier, Section II: II.1.1. Name of the additive

[8] *Technical dossier, Section II: II.5.1. Proposed mode of use in animal nutrition

[9] FAD-2010-0107, Chemically defined flavourings from Chemical Group 34, Ref. Ares(2011)301126 - 18/03/2011 https://ec.europa.eu/jrc/sites/jrcsh/files/FinRep-FAD-2010-0107.pdf

[10] *Technical dossier, Section II: II.6.1. Methods of analysis for the active substance

[11] *Technical dossier, Section II: Annex_II_6_01 CJ MSG Method validation report.pdf

[12] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed, O.J. L 54, 26.02.2009

[13] EN ISO 13903:2005- Animal feeding stuffs – Determination of amino acids content

[14] FAD-2018-0045, L-arginine produced by fermentation with Corynebacterium glutamicum KCCM80182, Ref. Ares(2019)758065 - 08/02/2019 https://ec.europa.eu/jrc/sites/jrcsh/files/finrep_fad-2018-0045_l-arginine.pdf

[15] Food Chemical Codex monograph "monosodium L-glutamate", FCC 7 (2010), p.698

*Refers to Dossier no: FAD-2018-0090

## 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

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- Państwowy Instytut Weterynaryjny, Pulawy (PL)
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